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Primary production of protein: I. Comparison of net cellular carbon and protein synthesis with ^{14}C-derived rate estimates in steady-state cultures of marine phytoplankton

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ABSTRACT: Measurements of total inorganic ^{14}C-carbon fixation and ^{14}C-carbon incorporation into protein were compared with calculated net synthesis of total cellular carbon and protein carbon respectively in NH_4^+-limited continuous cultures of 4 marine phytoplankton species: the marine chlorophyte *Nannochloris atomis*, the diatom *Chaetoceros simplex*, the chrysophyte *Chattonella luteus*, and the cryptomonad *Chroomonas salina*. Net synthesis rates were estimated from dilution rates and steady-state concentrations of total cellular carbon and protein in the chemostat cultures. For 3 out of the 4 species, there was good agreement between the increase in total fixed ^{14}C-carbon over 3 h and the calculated net synthesis of newly fixed cellular carbon. Observed increases in fixed ^{14}C-carbon in cultures of *C. salina* exceeded calculated values indicating degradative loss of unlabeled cell material. Extracellular release of fixed ^{14}C-carbon was significant for *C. luteus* as shown by a higher rate of production of acid non-volatile ^{14}C relative to total particulate ^{14}C (i.e. retained on Whatman GF/F glass fiber filter). As a result of incomplete equilibration of protein precursors with the exogenous ^{14}C-labeled inorganic carbon pool, incorporation of ^{14}C-carbon into protein of all species was less than calculated net synthesis of protein carbon for up to 3 h after isotope addition. Comparisons of net synthesis and incorporation of ^{14}C-carbon into individual protein amino acid residues revealed that precursor sources of protein-bound glycine and alanine rapidly attained isotopic equilibrium with the ^{14}C-labeled inorganic carbon pool for all growth conditions and in all species examined. Precursor equilibration dynamics of protein-bound glutamate and aspartate were generally slower than glycine and alanine. Rates of incorporation of ^{14}C-carbon into protein of *N. atomis* decreased more rapidly in response to interruption of the supply of fresh media to the chemostat culture than did rates of total ^{14}C-carbon fixation. *C. simplex* was also sensitive to interruption of media inflow, as indicated by continuous decreases in ^{14}C-carbon incorporation into protein-bound glycine and alanine. These results emphasize the importance of time-course sampling and keeping incubations as short as possible for measurements of ^{14}C-carbon incorporation into protein. Characteristics of incorporation of ^{14}C-carbon into protein-bound glycine and alanine make them select tracers for measuring protein synthesis in mixed autotrophic populations using short-term incubations. The relative uniformity of the amino acid composition of proteins provides a basis for extrapolating synthesis rates of glycine and alanine to total protein production rates, thereby leading to a determination of the proportion of primary production associated with protein synthesis. This will provide information about the physiological state of phytoplankton populations, and their role in influencing the trophodynamic flux and composition of particulate organic matter.

INTRODUCTION

Protein is nutritionally essential for growth at higher trophic levels in the marine food chain, and phytoplankton are the major primary source of protein. Knowledge about factors which influence the proportion of primary production allocated to protein synthesis will help in understanding marine ecosystem trophodynamics, and the role of phytoplankton in influencing the flux and composition of particulate organic matter. In addition, the ability to trace accurately autotrophic production of protein in relation to total primary production may be useful in assessing the

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physiological state of phytoplankton populations (e.g. Morris 1981). A number of investigators have sought to physiologically characterize natural phytoplankton populations by fractionating ^{14}C labelled cellular materials using sequential solvent extraction procedures (e.g. Morris & Skea 1978, Li et al. 1980, Smith & Morris 1980a,b, Morris 1981, Morris et al. 1981, Barlow 1982, Li & Harrison 1982, Li & Platt 1982, Priscu & Goldman 1983). However, the interpretation of results from such studies have been complicated because not enough is known about physiological and biochemical factors which may influence observed patterns of incorporation in the field and their relationship to *in situ* rates of biosynthesis of cell material. Specifically, there has not yet been a systematic evaluation of conditions required for the precise and accurate quantitation of protein synthesis by marine phytoplankton based on the incorporation of ^{14}C-carbon.

Radioisotopic tracers other than ^{14}C associated with distinct types of algal cell material have been used to monitor specific biochemical activities. These include ^{3}H-adenine (Karl 1982, Winn & Karl 1984) and $^{32}PO_4$ (Cuhel & Waterbury 1984) to assess nucleic acid biosynthesis, and $^{35}SO_4$ to trace the production of protein (Bates 1981, Cuhel 1981, Cuhel et al. 1984). However, inorganic ^{14}C is attractive because it provides an assay of metabolic activity specific to phytoplankton, since contributions by heterotrophs to total carbon fixation will be relatively small. In addition, inorganic ^{14}C can be added in truly tracer amounts to high specific activities without perturbing existing uptake kinetics.

A number of factors may contribute to errors in ^{14}C-derived estimates of protein synthesis. A problem in using any radioisotope to trace biosynthetic activities is the potential for perturbations in physiology as a result of extended sample confinement (Vollenweider & Nauwerck 1961, Venrick et al. 1977, Gieskes et al. 1979, Goldman et al. 1981, Peterson 1980, Ferguson et al. 1984). Different metabolic processes may be differentially sensitive to confinement effects. Incorporation of ^{14}C-carbon into free amino acids and protein decreased more rapidly than total carbon fixation after interruption of media input into NH_4^+-limited continuous cultures of the marine chlorophyte *Nannochloris atomis* Butcher such that the proportion of total fixed ^{14}C-carbon incorporated into free amino acids and protein became lower than their measured proportion of cellular carbon (Lohrenz 1985, Lohrenz & Taylor in press).

Confinement artifacts can be reduced by using short incubation periods. However, short-term ^{14}C distributions are more subject to the effects of isotopic disequilibrium. Hitchcock (1981) noted in dinoflagellate cultures that up to 4 h were required for attainment of uniform radioisotopic composition of the various cellular fractions. Equilibrium distributions of ^{14}C in hot trichloroacetic acid insoluble material (which includes protein) from the cyanobacterium *Synechococcus* sp. (Cuhel & Waterbury 1984) and in *Nannochloris atomis* Butcher (Lohrenz 1985, Lohrenz & Taylor in press) were consistently higher than the short-term distribution of isotopic carbon for the first several hours of incubation. This was a consequence of the relatively slow isotopic equilibration of protein precursors. For incubation periods less than or equal to the time required for isotopic equilibration of precursor pools, true rates of protein synthesis are underestimated by ^{14}C-derived rate measurements.

Errors of this sort should be corrected by monitoring the specific activity of the precursor pools. For amino acids the situation is complicated by the fact that rates of isotopic equilibration differ among the individual free amino acids (Bassham et al. 1964, Larsen et al. 1981, Lohrenz 1985, Lohrenz & Taylor in press). Furthermore, as a result of cellular compartmentalization, the specific activity of the extractable free amino acid pools may differ from that of the immediate precursors to protein synthesis (e.g. Oaks & Bidwell 1970, Bassham et al. 1964, Trewavas 1972, Huffaker & Peterson 1974, Wheeler & Stephens 1977, Lohrenz 1985).

Our approach in this study was to examine the kinetics of radiocarbon incorporation into protein-bound amino acids and identify those whose precursors rapidly attain the specific activity of the exogenous dissolved inorganic carbon pool. Quantification of isotope incorporation into these selected protein amino acids could then serve as an assay of protein synthesis and minimize problems associated with isotopic equilibration. Because of the relative uniformity in the amino acid composition of average cellular protein (Jukes et al. 1975, Doolittle 1981), rates of primary production of individual protein amino acids could be extrapolated to total algal protein synthesis rates. The simplicity of isolating, quantifying, and radioassaying a few selected protein amino acids makes such a technique easily adaptable to field-oriented investigations, and results can be interpreted on unambiguous chemical and physiological bases.

This investigation provides a method for using inorganic ^{14}C to determine the proportion of primary production associated with protein synthesis in natural phytoplankton populations. The incorporation of ^{14}C into protein and total cellular carbon was compared to net synthesis of protein and cellular carbon estimated over a wide range of NH_4^+-limited growth rates in continuous cultures of *Nannochloris atomis*. Interspecific differences were assessed by comparing measurements on 3 other species growing at moderate to high relative growth rates. Time courses of ^{14}C label-

ling of individual protein-bound amino acids were compared to calculated net synthesis in the steady state cultures, leading to the identification of those protein-bound amino acids having rapidly equilibrating precursors in all the species examined. In a subsequent paper (Lohrenz & Taylor unpubl.), this information provides a basis for estimating the proportion of primary production allocated to protein synthesis in natural populations, and comparing it to the measured fractions of particulate organic carbon in protein.

METHODS

Axenic stock cultures of the following species were obtained from the Culture Collection of Marine Phytoplankton, Bigelow Laboratories for Ocean Sciences, West Boothbay Harbor, Maine: *Nannochloris atomis* Butcher (Strain GSBNANNO), *Chattonella luteus* (Strain OLISTH), *Chroomonas salina* (Strain 3C), and *Chaetoceros simplex* (Strain BBSM).

Media composition. Cultures were maintained in a modified artificial seawater medium (McLachlan 1964), consisting of 400 mM NaCl, 20 mM $MgSO_4$, 20 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM KCl, 0.8 mM KBr, and 0.2 mM H_3BO_3. The amounts shown were dissolved in deionized water (Barnstead Nannopure water purification system). Before use, the solution was filtered through 47 mm Whatman GF/F filters and autoclaved in teflon containers. The sterile seawater solution was enriched to 40 µM NaH_2PO_4, 125 µM NH_4Cl, and trace elements and vitamins in a 2-fold dilution of f-medium (Guillard & Ryther 1962). Media for cultures of *Nannochloris atomis* grown in high light intensity (see below) was enriched to 2.0 mM $NaHCO_3$. For all other cultures, a concentration of 3.0 mM $NaHCO_3$ was used. The enrichment solutions had been individually sterilized in teflon containers, with the exception of the vitamin mixture which was filter sterilized (Millipore Millex-GV 0.22 Sterile Filter Unit) and stored at −20 °C in screw-cap pyrex test tubes.

Growth conditions. Cultures were incubated in jacketed flasks of 0.5 l volume (Anderson Glass, Fitzwilliam, New Hampshire) and temperature was maintained at 21 ± 1 °C using a Haake D2 heating-circulation water bath cooled with tap water. Illumination was achieved with Vita-Lite fluorescent bulbs. Unless otherwise specified, data refer to cultures grown at an average quantum density of approximately 176 µE m^{-2} s^{-1} (range 90 to 200 from top to bottom of culture vessel containing deionized water as measured with a Biospherics quantum sensor and a 4 π submersible probe). Experiments were also conducted with batch cultures of *Nannochloris atomis* grown at 29 µE m^{-2} s^{-1} (range 19 to 42) and periodically diluted to reduce self-shading. Cultures were mixed by a combination of aeration (Second Nature Whisper 600 aquarium-type air pump) and magnetic stirring (Fisher Cat. No. 14-511-IV2 magnetic stirrer). Air was sterilized by filtration through a sequence of sterile glass wool and cotton, sterile distilled water, and finally, an additional sterile cotton filter. Media was dispensed to the cultures via Harvard Apparatus Peristaltic Pumps. Tubing was silicon and glass. Sterility was verified by streaking culture samples on Marine Broth 2216 (Difco) in 1 % agar and checking for growth after 48 h, and by inspection under oil immersion (1000× phase).

Chlorophyll *a* analyses. Chlorophyll concentrations were determined by the fluorometric method of Yentsch & Menzel (1963), as described by Smith et al. (1981). Samples were filtered on a Millipore type HA (nominal 0.45 µm pore size) and the filter dissolved in 5 ml 90 % aqueous acetone by vortexing for 1 to 2 min. Samples were allowed to stand in darkness for 10 min, vortexed again, and the fluorescence was read on a Turner designs Model 10-005R fluorometer equipped with a Turner 10-045 blue lamp, a Corning color specification (c/s) 5–60 excitation filter, and a Corning c/s 2–64 emission filter (670 nm cut-off). Sample readings were corrected for phaeopigment fluorescence by subtracting the fluorescence of samples acidified with 2 drops of 0.75 N HCl.

Dissolved inorganic carbon and particulate elemental carbon analyses. Samples were filtered (<150 mm Hg) on combusted (500 °C overnight) Whatman GF/F 25 mm glass fiber filters using a Millipore glass filtration apparatus. The unrinsed filters were dried in an oven at 55 °C for 3 h and stored dessicated until analysis for elemental carbon and nitrogen on a Perkin-Elmer 240 elemental analyzer. Filtrates were stored at 5 °C in 15 ml serum bottles sealed with butyl rubber stoppers with minimal headspace until they could be analyzed for dissolved inorganic carbon.

Dissolved inorganic carbon analyses were performed by placing 3 ml samples in 15 ml serum bottles and sealing the bottles with butyl rubber stoppers. The contents of the bottles were acidified by injecting 0.15 ml 18 N H_2SO_4 through the stopper. The samples were allowed to equilibrate with the headspace, and gaseous CO_2 in the headspace was then analyzed by infrared spectroscopy and corrected for the air blank.

Incubation sampling procedure. For continuous culture experiments, steady state was defined as the time when chlorophyll *a* concentration and cell density remained constant for at least 2 generations. Cells were enumerated using a Spencer Bright-line hemocytometer. Immediately preceding isotope incubations, samples were removed for dissolved inorganic carbon and particulate elemental carbon analyses. These samples accounted for no more than 15 % of the steady

state chemostat volume. Isotope incubations were subsequently begun within 20 min of initial sampling. Inorganic ^{14}C (New England Nuclear, sterile stock solution of 2 mCi ml^{-1} in 0.01 N NaOH) was added to a specific activity of approximately 1 mCi $mmole^{-1}$ HCO_3^-. Immediately before introducing the radiolabel, aeration was stopped. The input of fresh media was interrupted before the volume in the chemostat became significantly reduced due to sample removal, thereby avoiding significant increases in dilution rate and decreases in specific activity.

Time-series sampling during the incubations involved filtration (< 150 mm Hg) of particulates on combusted 13 mm Whatman GF/F glass fiber filters housed in Millipore SX00 13 syringe filter holders. A Gast Manufacturing Corp. Series #1077 vacuum pump was used. In order to avoid rinse-induced losses of labeled cell material (Goldman & Dennett 1985), samples were not rinsed.

Radioassay procedures. For estimates of total particulate ^{14}C fixation, filters were placed in the bottom of a 20 ml scintillation vial, covered with 0.1 ml 1 N HCl, and dried under a gentle air flow at 55 °C. The dried filters were moistened with deionized water, and 10 ml scintillation cocktail, either Aquasol (New England Nuclear) or Scintiverse II (Fisher). Radioactivity was measured using a Beckman LS-100C liquid scintillation counter. Measured radioactivity was corrected for quench by the channels ratio method (Kobayashi & Maudsley 1974). Aquasol and Scintiverse II quench characteristics were quite similar for the materials being counted (data not shown).

Total acid non-volatile ^{14}C was assayed by a method similar to that described by Schindler et al. (1972) and Glibert et al. (1985). One ml of the culture was acidified to a concentration of 0.32 N H_2SO_4 (pH 1.2) in a 7 ml scintillation vial, sparged with air for 15 min, and 6 ml scintillation cocktail added.

Total ^{14}C activity was determined by introducing 50 µl aliquots of the culture into 4 ml scintillation fluor containing 50 µl Protosol (New England Nuclear).

Subcellular fractionation procedure. The fractionation procedure employed for these experiments was modified from procedures previously described by Roberts et al. (1963), Morris et al. (1974), Li et al. (1980), Larsen et al. (1981), Cuhel et al. (1981a), and Hitchcock (1981). Filtered samples for biochemical analyses and determination of subcellular ^{14}C distributions were placed in acid-washed 20 ml glass scintillation vials containing 1.5 ml 80 % aqueous methanol and stored at −6 °C until analysis. Extraction of a sample initially required transferring the filter using a forceps to a Bellco 12 ml glass tissue homogenizer, and grinding in approximately 0.1 ml 80 % aqueous methanol. The liquid remaining in the storage vial was subsequently transferred to the grinder, along with one 1 ml rinse each of 80 % aqueous methanol and 80 % aqueous ethanol. After rinsing, residual label in the storage vials typically accounted for less than 2 % of the total acid non-volatile radioactivity (mean of 9 samples = 1.5 %, range 0.4 to 6.0 %). The resulting suspension was vortexed, and subsequently centrifuged at 4000 g for 20 min at 15 °C in a Sorvall RC2B centrifuge equipped with an HS-4 swinging bucket rotor. The tops of the homogenizer tubes had been removed using a glass saw so that they would fit in the HS-4 rotor. The supernatant was withdrawn and the pellet was resuspended and extracted in 1.5 ml 80 % aqueous ethanol at 60 °C for 20 min. This mixture was again centrifuged, and the supernate removed. The pellet was extracted 2 additional times with a 1:1 mixture of 80 % aqueous ethanol and diethyl ether at 60 °C for 20 min and 10 min respectively.

The remaining pellet could then be subjected to hydrolysis for high pressure liquid chromatographic separation, quantitation, and collection of protein amino acids as described below. Alternatively, the pellet was further extracted to remove hot (95 °C) trichloroacetic acid soluble material (containing carbohydrate and nucleic acid), leaving an insoluble residue (including residual protein) (e.g. Roberts et al. 1963, Cuhel et al. 1981b, Morris et al. 1974, Konopka & Schnur 1980, 1981). For the latter procedure, the pellet was twice resuspended in 1.5 ml trichloroacetic acid and extracted for 20 min and 10 min respectively at 95 °C. The residual pellet was transferred with sequential rinses of 0.5 ml 0.1 N NaOH, 1.0 ml deionized water, and 0.75 ml 1 M Tris (pH 7) into either a 10 ml filmware bag or a 20 ml glass scintillation vial and combined with 6 to 10 ml scintillation cocktail.

Protein hydrolysis. For protein hydrolysis, the pellets remaining after the aqueous alcohol and ether extractions were transferred to a 10 ml ampule along with 0.5 ml doubly distilled 6 N HCl. The ampules were sealed under nitrogen, and incubated at 110 °C for 20 h. The HCl was then evaporated under a flowing stream of air at 55 °C and the residue was stored at −20 °C until analysis of the amino acids as described below.

Amino acid derivatization procedure. Hydrolyzed amino acids were derivatized with dansyl chloride [5-(dimethylamino)-1-naphthalene-sulfonyl chloride] in a procedure modified from Wiedmeier et al. (1982). Dansyl chloride (Sigma) was dissolved in acetonitrile (Spectra UV Grade, Fisher) to a concentration of 6.0 mg ml^{-1} (22 mM). This solution was stored under argon or nitrogen at −20 °C. Distilled H_2O used for the derivatization was specially prepared by distillation from a $KMnO_4$ solution. Protein hydrolysates which had been dried under a flowing stream of air at 55 °C were

resuspended by brief sonication (Branson Instrument Co.) in equal volumes of 0.5 M $NaHCO_3$ (pH 8.5), distilled H_2O, and dansyl chloride solution. The reaction mixture was incubated in darkness for 1 h at 37 °C, and the reaction terminated by addition of distilled H_2O and 0.1 M H_3PO_4 (HPLC Grade, Fisher) in final volume proportions 3:7.5:2 (reaction mixture:H_2O:H_3PO_4). Samples were analyzed within 2 wk of derivatization. Lohrenz (1985) found little or no consistent storage-related losses when derivatives were stored at −6 °C for up to 90 d. DeJong et al. (1982) noted that dansyl-tyrosine was relatively unstable. However, this derivative was not quantified in our study.

Amino acid separation, quantification, and radioassay. The dansylated amino acids were separated by reverse-phased (Nucleosil 5 C18 or Spherisorb S5-ODS) high pressure liquid chromatography (25 cm × 4.1 mm I.D. column protected by a 5 cm guard). The gradient elution system (Waters Associates) consisted of 6000A and M-45 pumps, interfaced with a M660 Solvent Programmer. A Waters UK6 injector was used with a 1 ml sample loop, and detection was by ultraviolet absorbtion at 254 nm on a Waters Model 440 absorbance detector. Integration of peak signals was accomplished by either a Hewlett Packard 3390A or an SP70 Spectrophysics integrator. The gradient conditions consisted of an exponential increase (M660 curve 7) of acetonitrile (12 to 55 % v/v over 75 min for Spherisorb columns and 15 to 55 % v/v over 40 min for Nucleosil columns) in 0.03 M phosphate buffer (pH 6.25).

Individual amino acids were identified by comparison of retention times with authentic standards (Sigma Chemical Co.). An amino acid concentration was determined by dividing its integrated peak area by the mean response factor [(integrated peak area)/(nanomoles injected)] of the corresponding amino acid standard derivatized as previously described. Concentrations were corrected for blanks processed in the same manner as the sample. Response factor errors included those associated with injection variability, storage losses, variations in derivatization efficiency, peak integration, and standard preparation. The 95 % confidence interval was determined for each response factor and propagated in all subsequent calculations. Fractions for isotope quantitation were collected into scintillation vials directly from the detector outflow. A volume of scintillation fluor at least 3 times that of the collected fraction was added immediately.

Recovery of protein. Some loss of protein carbon and nitrogen would be expected during hydrolysis since the amide groups of glutamine and asparagine are lost and tryptophan is destroyed during acid hydrolysis (DeJong et al. 1982). Partial oxidative destruction of the hydroxyamino acids such as serine may also occur. Furthermore, because of low reliability in our measurements of cysteine, methionine and tyrosine, we excluded these amino acids from our results. The extent to which our measurements underestimated protein concentrations were assessed experimentally using 2 different approaches. In the first approach, recoveries were determined for individual free amino acid standards (Sigma) subjected to the previously described conditions for hydrolyzing protein. Based on these determinations (Lohrenz 1985) and the exclusions referred to previously, our recovery of carbon from a mixture of amino acids of the same relative abundance found in average protein (Jukes et al. 1975, Doolittle 1981) was 72 % and recovery of nitrogen was 76 %.

In a second approach, the protein content of an algal culture estimated by dansylation of the protein hydrolysate was compared to measurements of protein by 2 other methods: the Coomassie Brilliant Blue dye-binding method (Bradford 1976), and radioassay of ^{14}C-carbon contained in the hot (95 °C) trichloroacetic acid insoluble fraction of cells labeled with inorganic ^{14}C for 4 generations. Reagents for the dye-binding assay were obtained from Bio-Rad. Standard curves were prepared using egg ovalbumin (Sigma) which has a response factor representative of average cellular protein. Filtered samples were extracted (Rausch 1981) with 0.5 N NaOH twice for 10 min at 80 °C, centrifuged at 4000 g for 20 min, and the protein concentration was assayed in the combined supernatant solution. For the purposes of comparison, a factor of 53 % carbon and 16 % nitrogen by weight in protein was used based on the amino acid residue composition of average protein (Jukes et al. 1975, Doolittle 1981). Values for protein-associated carbon and nitrogen are expressed as a percent of cellular carbon and nitrogen respectively in Table 1. Differences between estimates of the distribution of cellular carbon in protein assayed by the Bradford method indicated little loss of protein during the aqueous alcohol and ether extractions. Dansyl-amino acid carbon recovered in the protein hydrolysate accounted for virtually 100 % of protein-associated carbon measured by the Bio-Rad assay, while recovery of nitrogen was only 74 %. Dansyl-amino acid carbon accounted for 90 % of trichloroacetic acid insoluble carbon.

On the basis of these results, we concluded that losses were comparable to the mean analytical variation in our estimates of protein concentration (18 % for protein carbon, and 23 % for protein nitrogen; Table 1). The data were not corrected, but rather all results were obtained and analyzed in an internally consistent fashion. Our interpretations of the results were based on relative differences which would not be dependent on systematic losses.

Table 1. *Nannochloris atomis*. Comparison of different methodologies for measurement of protein in batch cultures

Method	Cellular distributions (%) in protein	
	Carbon	Nitrogen
Bradford (1976) assay		
Before alcohol and ether extraction:	21.4 (3.4)[1]	57 (12)
After extraction:	22.5 (3.7)	59 (12)
Sum of hydrolyzed protein amino acid residues:	21.9 (3.9)	43 (10)
Proportion of ^{14}C in hot trichloroacetic acid insoluble residue of equilibrium labeled cells:	24.2 (3.8)	–

[1] Values in parentheses represent propagated estimates of 95 % confidence intervals. Mean analytical variation was 18 % for protein carbon and 23 % for protein nitrogen

Calculations of net synthesis in steady-state cultures. In the event of complete and instantaneous equilibration of precursors with an exogenous ^{14}C-labeled dissolved inorganic carbon pool (an unlikely assumption as will be demonstrated), the specific activity of carbon incorporated into protein would be equivalent to that of the ^{14}C-labeled inorganic carbon pool. Hence, the amount of newly fixed carbon entering protein could be determined by dividing the amount of radiocarbon incorporated by the ^{14}C specific activity of the inorganic carbon pool. In our experiments, efforts were made to maintain a constant specific activity of the exogenous inorganic carbon pool during incubations. For long-term incubations, both the chemostat and reservoir were labeled with inorganic ^{14}C to the same specific activity in order to prevent significant dilution of the exogenous inorganic ^{14}C pool. For the short-term incubations (3 h), influent flow was stopped before significant dilution could occur. In subsequent discussion, we refer to observed incorporation of ^{14}C-carbon as the amount of radioactivity incorporated divided by the ^{14}C specific activity of the exogenous inorganic carbon pool.

Observed incorporation was compared to calculated values for which complete equilibration of precursors was assumed. Under circumstances in which metabolic turnover of protein is small relative to net synthesis (i.e. gross synthesis = net synthesis), the rate of accumulation of newly-fixed ^{14}C-labeled carbon in protein of a continuous culture will be equal to the difference between the net rate of protein synthesis and dilution of labeled protein by inflowing media:

$$dP^*/dt = DP_o - DP^*(t) \quad (1)$$

where dP^*/dt = net synthesis or accumulation rate of newly fixed carbon in protein; D = dilution rate of the chemostat; P_o = steady-state protein concentration (in units of carbon concentration); $P^*(t)$ = quantity of newly fixed carbon accumulated into protein at time t (zero time being isotope addition). Equation (1) is a first order linear differential equation, with initial conditions $P^*(0) = 0$. Solving for $P^*(t)$:

$$P^*(t) = P_o(1 - e^{-Dt}) \quad (2)$$

Under conditions where flow is interrupted, the quantity of newly fixed carbon accumulated into protein at time t $[P^{*'}(t)]$ is:

$$P^{*'}(t) = P_o(e^{Dt} - 1) \quad (3)$$

The derivation of this equation is similar to that described by Roberts et al. (1963). It assumes no deviations from the steady-state biosynthetic rates despite the interruption of flow, so that the chemostat is effectively viewed as a batch culture with growth rate D. As will be demonstrated, this assumption is not at all times appropriate.

Equation (2) was used to calculate net synthesis up to the point at which media supply was stopped, after which Equation (3) was used. Under conditions where Dt is small (i.e. incubation times less than 0.05D), $P^*(t)$ approximates $P^{*'}(t)$. Observed incorporation into a cellular constituent with precursors requiring a significant time to reach isotopic equilibrium will be less than predicted by Equations (2) and (3). Calculations of the accumulation of total newly fixed cellular carbon were obtained by substituting the steady state particulate organic carbon concentration for P_o in Equations (2) and (3). Similarly, the calculation of accumulation of newly fixed carbon into individual protein-bound amino acids was made by substituting the individual amino acid concentration (expressed in units of carbon concentration) for P_o.

RESULTS

Intraspecific and interspecific variations in cellular protein content

The percentage of cellular carbon associated with protein of *Nannochloris atomis* was positively correlated with relative growth rate (Table 2). Relative growth rate is defined as the dilution rate of the chemostat (μ, absolute growth rate) normalized to the maximum growth rate (μ_{max}), which is determined as the batch culture growth rate under the same conditions of light and temperature (e.g. Goldman 1980). At low light intensities, the percentage in batch culture ($\mu/\mu_{max} = 1.00$) was slightly lower than at high light intensities, but the difference was not significant. Pro-

Table 2. Percentage of total cellular carbon contained in protein-bound amino acids of batch and NH_4^+-limited steady-state cultures

Species		% (95 % confidence interval)
Nannochloris atomis		
Batch cultures:	High light[1]	21.9 (3.9)
	Low light[2]	15.6 (3.1)
Steady state:	μ/μ_{max}[3] = 0.93	18.9 (4.9)
	0.46	16.8 (3.0)
	0.23	8.8 (2.0)
Chattonella luteus[4]		
Batch culture:	High light	16.8 (4.0)
Steady state:	μ/μ_{max} = 0.54	15.9 (3.0)
Chaetoceros simplex		
Steady state:	μ/μ_{max} = 0.57	15.8 (3.0)
Chroomonas salina		
Steady state:	μ/μ_{max} = 0.80	20.0 (3.6)

[1] 176 μE m^{-2} s^{-1}
[2] 29 μE m^{-2} s^{-1}
[3] Dilution rates of steady state cultures normalized to batch culture growth rate under same conditions of light and temperature. Batch growth rates (95 % confidence intervals in parentheses) were: *N. atomis*, high light 1.30 (0.19) d^{-1}, low light 0.58 (0.05) d^{-1}; *C. luteus*, 0.54 (0.06) d^{-1}; *C. simplex*, 2.3 (0.23) d^{-1}; *C. salina*, 0.52 (0.05) d^{-1}
[4] Total protein of *C. luteus* estimated by assay of Bradford (1976) according to the procedures described in 'Methods'. A factor of 53 % carbon by weight in protein was used for determining the relative abundance of cellular carbon in protein

portions of cellular carbon contained in protein of the other species resembled those observed for *N. atomis* under similar growth conditions. Relative abundances of glycine and alanine in protein were also found to be quite uniform among species, and unaffected by growth conditions (Table 3).

Table 3. Relative abundance of glycine and alanine in protein of different steady-state cultures

Source	Mole % (95 % confidence interval) Alanine	Glycine
Nannochloris atomis		
μ/μmax = 0.93	12.5 (3.2)	13.8 (3.1)
0.23	12.4 (7.0)	14.5 (3.4)
Low light batch	11.2 (2.4)	14.8 (3.4)
Chaetoceros simplex	8.4 (1.8)	12.4 (2.8)
Chroomonas salina	12.2 (2.6)	12.9 (2.9)
Chattonella luteus	12.3 (2.6)	13.0 (2.9)

Kinetics of isotope incorporation: comparison of calculated net synthesis with observed ^{14}C-carbon incorporation

Particulate organic carbon

Time-series measurements of the increase in both total particulate and acid non-volatile ^{14}C were compared to the calculated values from Equations (2) and (3) (Fig. 1). For incubation periods up to 3 h, there was good agreement between the calculated [Eqns (2) and (3)] and ^{14}C-derived rates for NH_4^+-limited steady state cultures of *Nannochloris atomis* growing under high light conditions (Fig. 1a to c) and for the low light batch culture (Fig. 1d). A close correspondence between acid non-volatile and total particulate ^{14}C-carbon indicated

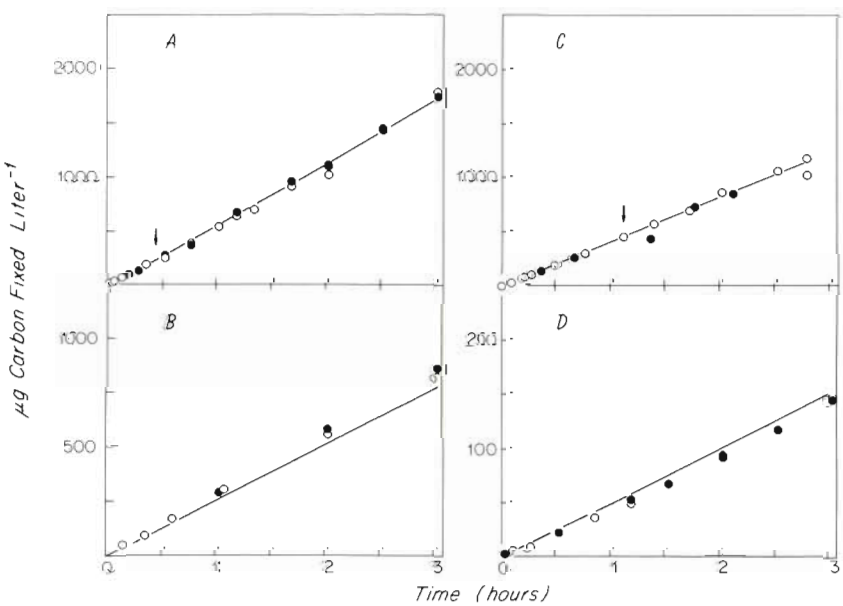

Fig. 1. *Nannochloris atomis*. Time-series measurements of increase in (●) filter-retainable and (○) acid-stable ^{14}C-labeled carbon in high light intensity NH_4^+-limited steady-state cultures and a low light intensity batch culture. Solid line represents net synthesis of newly fixed carbon in steady-state culture as calculated using Eqns 2 & 3 (see text). Arrows in (A) and (C) show the time when media input was stopped. For (A) to (C), light intensity was 176 μE m^{-2} s^{-1} and μ_{max} = 1.3 d^{-1}; μ/μ_{max} = 0.93 (A), 0.46 (B), and 0.23 (C). Culture in (D) was a low light intensity batch culture periodically diluted to reduce self shading. Light intensity was 29 μE m^{-2} s^{-1} and μ_{max} = 0.58 d^{-1}

that extracellular release of newly fixed ^{14}C-carbon was negligible during the incubation period. Media input was interrupted for the experiments shown in Fig. 1a & c at the times indicated by arrows. There was no evidence of nitrogen-depletion-induced decreases in the observed accumulation of fixed ^{14}C-carbon.

Comparisons of 3 other species indicated that the extent to which observed increases in ^{14}C-carbon reflect calculated (Eqn 3) net synthesis of particulate organic carbon is species dependent (Fig. 2). For *Chattonella luteus,* there was good agreement between the observed increases in total particulate ^{14}C-carbon and the calculated values, while production of acid non-volatile ^{14}C-carbon was higher (Fig. 2a). This could have been the result of extracellular release. The increase in particulate and acid non-volatile ^{14}C in the *Chroomonas salina* culture exceeded the calculated increases in newly fixed carbon by approximately 50 % (Fig. 2b). The diatom *Chaetoceros simplex* exhibited the closest agreement between calculated and observed increases in fixed ^{14}C-carbon (Fig. 2c). As in the case of *N. atomis,* total fixation of ^{14}C-carbon by each of the other 3 species remained linear for up to 3 h, despite the interruption of media input at time zero.

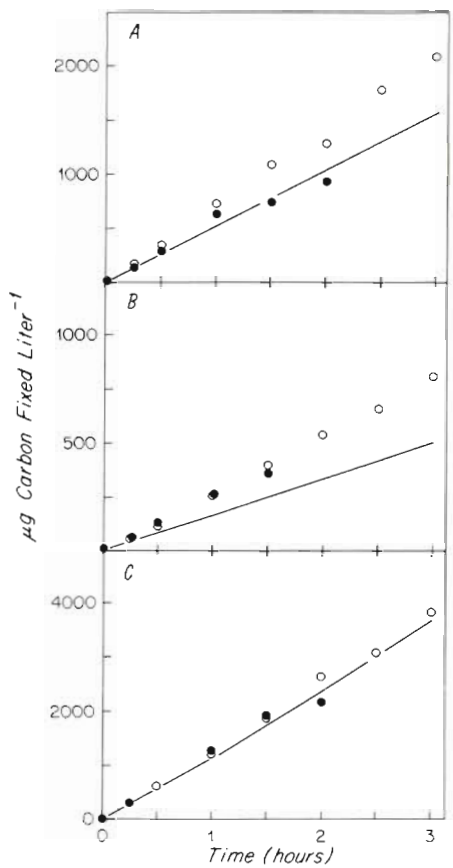

Fig. 2. Time-series measurements of increase in (●) total particulate and (○) acid non-volatile ^{14}C-labeled carbon in NH_4^+-limited steady-state cultures of (A) *Chattonella (Olisthodiscus) luteus;* (B) *Chroomonas salina;* (C) *Chaetoceros simplex.* Growth rates as in Table 2. Solid lines represent net synthesis of newly fixed carbon in steady-state cultures as calculated from Eqn 3 (see text). Media input was stopped at time zero

Particulate protein amino acids

In contrast to the generally close agreement between calculated and observed increases in total fixed ^{14}C-carbon, the observed increase in ^{14}C-carbon content of protein in *Nannochloris atomis* cultures was less than the calculated accumulation for both high and low relative growth rates (Fig. 3a & b respectively). Rates of

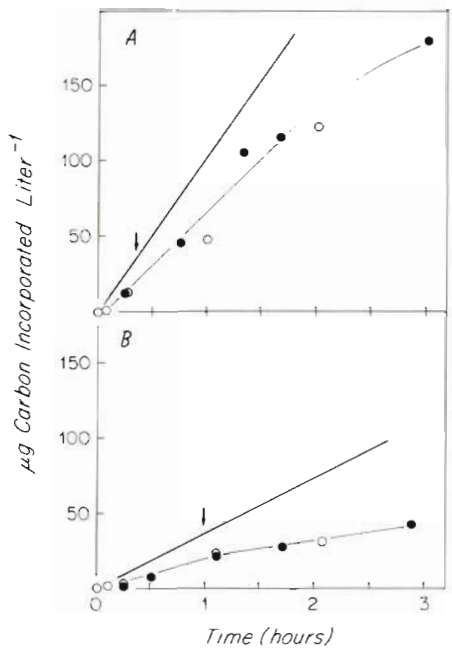

Fig. 3. *Nannochloris atomis.* Time-series measurements of ^{14}C-labeled carbon incorporation into protein of NH_4^+-limited steady-state cultures. (●) Sum of ^{14}C contained in hydrolyzed protein amino acids isolated by HPLC; (○) ^{14}C in hot trichloroacetic acid insoluble material. Heavy solid lines represent calculated (Eqns 2 & 3) net synthesis of new protein carbon. Thin lines drawn by eye. Arrows indicate times when media input was stopped. μ/μ_{max} = (A) 0.93, and (B) 0.23

^{14}C-carbon incorporation into protein also declined towards the latter portion of the incubation period in the flow-interrupted chemostats, suggesting a high sensitivity of protein synthesis to effects of nitrogen depletion. These results are contrasted with observations of a chemostat culture in which flow was not interrupted during the incubation (Fig. 4). In this unperturbed culture, differences between the calculated and observed increases in ^{14}C-carbon in protein

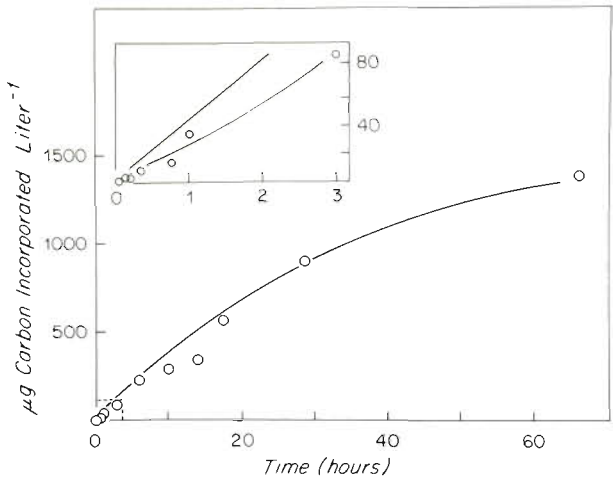

Fig. 4. *Nannochloris atomis*. Time-series measurements of ^{14}C-labeled carbon incorporation into protein of an NH_4^+-limited steady-state culture (μ/μ_{max} = 0.46) where media input was not stopped. Symbols represent ^{14}C in hot trichloroacetic acid insoluble material. Heavy solid lines represent the calculated (Eqn 2) net synthesis of new protein carbon. Inset shows the first 3 h in detail. Thin line in inset drawn by eye

were negligible after several hours and remained so for up to 66 h. However during the first 3 h, incorporation of ^{14}C-carbon into protein was less than the calculated accumulation (inset, Fig. 4), as in the experiments in which the inflow was interrupted (Fig. 3). Hence, the initial discrepancies were not the result of nitrogen depletion, but rather due to the kinetics of equilibration of protein precursors with the exogenous inorganic ^{14}C pool.

Selected protein amino acids

The preceding results indicate that incorporation of ^{14}C into protein underestimates the protein synthesis rate for the first 3 h of incubation, due to incomplete equilibration of precursors with the exogenous inorganic ^{14}C pool. However, precursors for some protein-bound amino acids equilibrate more rapidly than others, and reflect the actual protein synthesis rate sooner. We examined the patterns of ^{14}C incorporation into individual protein amino acids, and present results here for glycine, alanine, glutamate and aspartate. These amino acids were chosen because they are relatively abundant in protein, account for a major proportion of ^{14}C incorporation into protein during the first 3 h, and serve to illustrate differences in isotopic equilibration. Fig. 5 shows time-series measurements of ^{14}C-carbon incorporation into the representative amino acids in relation to their calculated accumulation in cultures of *Nannochloris atomis* growing at high (Fig. 5a) and low (Fig. 5b) relative growth rates, and in

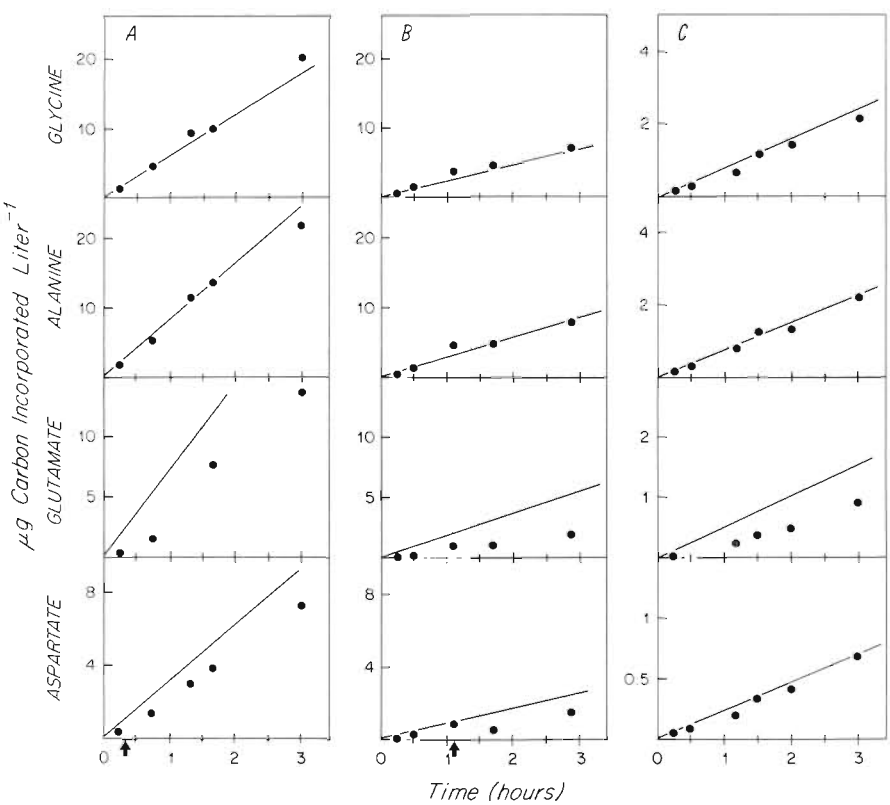

Fig. 5. *Nannochloris atomis*. Time-series measurements of ^{14}C-labeled carbon incorporation into individual protein-bound amino acids of high light intensity NH_4^+-limited steady-state cultures at high (A, μ/μ_{max} = 0.93) and low (B, μ/μ_{max} = 0.23) relative growth rates, and a low light intensity batch culture (C). Solid lines represent the calculated (Eqns 2 & 3) net synthesis of new protein-bound amino acid carbon. Arrows show times when media input was stopped

an exponential phase batch culture at low light intensity (Fig. 5c). In all cases, the observed incorporation of ^{14}C-carbon into protein-bound glycine and alanine was close to calculated net synthesis, indicating that their precursor pools became rapidly equilibrated with the exogenous inorganic ^{14}C pool. In contrast, observed ^{14}C-carbon incorporation into glutamate was lower than calculated values, and hence, contributed to the differences between calculated and observed ^{14}C-carbon incorporation into total protein (Fig. 3). Aspartate equilibration dynamics were intermediate between those of glutamate and glycine (or alanine). Data for other protein amino acids are discussed in Lohrenz (1985). Briefly, other amino acid residues which exhibited slow equilibration dynamics were proline + arginine, valine, and isoleucine + leucine.

Two out of the 3 other algal species exhibited the same general pattern as *Nannochloris atomis* in the relation between calculated and observed values. Differences between calculated net synthesis and observed ^{14}C-carbon incorporation into protein-bound glycine of *Chattonella luteus* (Fig. 6a) were within analytical variation (calculated slope of 2.8 µg C l^{-1} h^{-1} ± 95 % confidence interval of 0.6 versus an observed slope of 2.7 ± 0.3). There was also good agreement between calculated net synthesis and observed incorporation into protein-bound alanine (calculated slope of 3.9 µg C l^{-1} h^{-1} ± 95 % confidence interval of 0.8 versus an observed slope of 3.7 ± 1.1). These results suggest rapid equilibration of precursors to protein-bound glycine and alanine. In contrast, the observed ^{14}C-carbon incorporation into protein-bound glutamate and aspartate was dramatically less than the calculated net synthesis, consistent with patterns observed in *N. atomis*. The slope of ^{14}C-carbon incorporation into glutamate and aspartate continuously increased during the 3 h incubation, a reflection of the gradual equilibration of their precursors with the exogenous inorganic ^{14}C-carbon pool.

The incorporation of ^{14}C-carbon into protein-bound glycine of *Chroomonas salina* (Fig. 6b) was slightly above the calculated values although differences were not significant (calculated slope of 1.8 ± 95 % confidence interval of 0.4 versus an observed slope of 2.2 ± 0.2). Otherwise, the labelling characteristics of individual protein amino acids of *Chroomonas salina* were similar in many respects to those of the other species, supporting the view that basic biosynthetic pathways associated with algal protein metabolism are similar among different taxonomic groups.

Chaetoceros simplex was particularly sensitive to the interruption of steady state which occurred at time zero. Observed ^{14}C incorporation into glycine and alanine was close to that which was expected after the first 15 min, but rates continuously decreased during the incubation (Fig. 6c). Nevertheless, the agreement

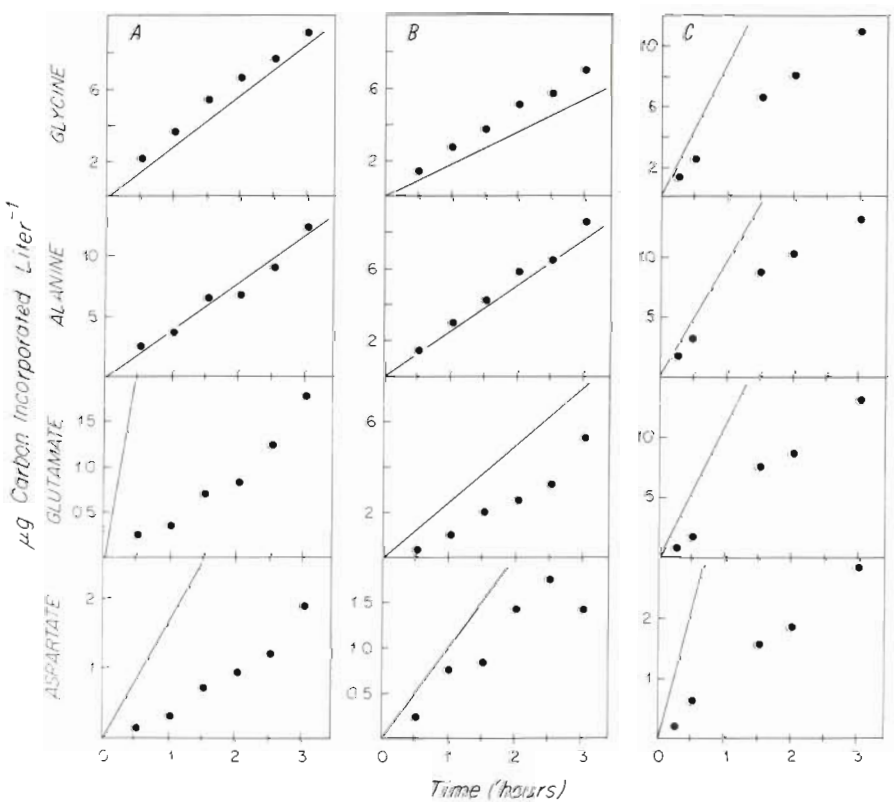

Fig. 6. Time-series measurements of ^{14}C-labeled carbon incorporation into individual protein-bound amino acids of NH_4^+-limited steady-state cultures of (A) *Chattonella luteus*; (B) *Chroomonas salina*; (C) *Chaetoceros simplex*. Solid lines represent calculated (Eqn 3) net synthesis of new protein-bound amino acid carbon. For all 3 cultures, media input was stopped at time zero

between calculated and observed initial rates of incorporation is again consistent with the view that there was rapid equilibration of precursors to protein-bound glycine and alanine. As in the case of the other species, incorporation of ^{14}C-carbon into glutamate and aspartate was significantly less than calculated synthesis at all times throughout the incubation. The apparently linear pattern of accumulation of ^{14}C-carbon in glutamate likely resulted from a combination of effects including the kinetics of saturation of the precursor pool and the nitrogen depletion-induced decrease in protein synthesis.

DISCUSSION

This investigation has provided information about physiological and biochemical factors which influence the incorporation of ^{14}C-carbon into protein and its relation to protein synthesis. A major conclusion of our study is that protein synthesis is underestimated by ^{14}C incorporation for up to 3 h of incubation due to incomplete equilibration of precursors with the exogenous inorganic ^{14}C pool. Examination of the characteristics of ^{14}C-incorporation into selected protein-derived amino acids indicated that precursor pools of protein-bound glycine and alanine consistently exhibited a rapid isotopic equilibration with the exogenous inorganic ^{14}C pool. A potential precursor in the biosynthesis of glycine is 3-phosphoglycerate (3PGA) produced by the RuDP carboxylase reaction of the Calvin cycle (Miflin & Lea 1977). This may explain the apparently close coupling between the supply of photosynthetically fixed carbon and the production of glycine for protein synthesis. Alanine is a product of the reductive amination of pyruvate. Incorporation of photosynthetically-derived ^{14}C into pyruvate is usually proposed to occur via the transport of triose phosphates from the chloroplast into the cytosol (Heber 1974, Leech & Murphy 1976). Once there, the action of the glycolytic enzymes could convert these molecules to pyruvate, which can subsequently reenter the chloroplast (Schulze-Siebert et al. 1984) where it may undergo transamination with glutamate (e.g. Leech & Murphy 1976). However, a direct and possibly more rapid pathway for conversion of 3PGA to pyruvate has been demonstrated to exist within spinach chloroplasts (Schultze-Siebert et al. 1984) and could explain the rapid isotopic equilibrium achieved for alanine.

The carbon skeleton for aspartate is oxaloacetate (OAA) and that of glutamate is alpha-ketoglutarate (KGA). Synthesis of OAA and KGA is catalyzed by tricarboxylic acid cycle enzymes localized in the mitochondria (e.g. Miflin & Lea 1977, Schulze-Siebert et al. 1984). The presence of chloroplast enzymes for their synthesis has not been demonstrated. The cytosol carbon skeletons are able to exchange with the chloroplast pools. Consequently, the nonlinear patterns of ^{14}C incorporation into glutamate and aspartate probably reflect the equilibration characteristics of the 'respiratory' intermediates from which they are derived.

The characteristics of ^{14}C-carbon incorporation into protein-bound glycine and alanine make them select tracers for measuring protein synthesis in mixed autotrophic populations. Their relative abundance in protein was found to be highly consistent, irrespective of growth conditions or species (Table 3). The proportions were similar to those found by other investigators (Table 4). Our estimates did tend to be slightly higher, particularly in comparison to relative abundances determined by sequencing protein. This is a consequence of systematic losses referred to in 'Methods'. Losses of other protein-bound amino acids would increase the apparent relative abundances of glycine and alanine, because their recoveries were nearly quantitative. Despite the differences, our results and those of other studies indicate that glycine and alanine are a highly consistent proportion of protein from a wide range of sources.

Table 4. Relative abundance of alanine and glycine in protein determined in other studies

Source	Mole % Alanine	Glycine
Sequenced protein		
Jukes et al. (1975)	8.7	7.9
Doolittle (1981):		
Newat	7.6	7.9
Atlas	8.2	8.8
Hydrolyzed protein		
Fowden (1954):		
Chlorella vulgaris	9.5	7.6
Anabena cylindrica	7.0	6.5
Navicula pelliculosa	7.6	7.1
Tribonema aequale	10.4	7.6
Cowey & Corner (1963):		
Skeletonema	9.2	11.6
Suspended particulate	8.4 (6.7–11.4)	16.6 (12.5–21.7)
Roberts et al. (1963):		
Chlorella	12.7	7.8
Degens (1970):		
Sargasso Sea (surface)	5.8–6.5	7.8–10.0
Buzzards Bay (surface)	8.8	12.1
Siezen & Mague (1978):		
Suspended particulate	10.0	13.4
Maita & Yanada (1978):		
Suspended particulate	9.0	15.4

An optimal approach towards estimating the proportion of primary production associated with protein synthesis will be to determine the synthesis rates of glycine and alanine, and extrapolate to the total protein synthesis rate using their known average abundance in protein. Extrapolated rates for alanine and glycine are compared to observed and calculated rates of ^{14}C incorporation into total protein in Table 5.

Table 5. Comparison of observed, calculated, and extrapolated rates of incorporation of ^{14}C-carbon into protein-bound amino acids of steady-state cultures. Observed rates represent sum of all measured protein-bound amino acid incorporation rates. Rates determined from 3 h time series incubations ($n \geq 4$). Calculated rates determined using Eqns 2 & 3 (see text). Extrapolated rates determined by dividing rates of ^{14}C-carbon incorporation into protein-bound alanine and glycine by their mean fractions of total protein carbon: alanine, 0.071 (95% confidence interval, 0.016); glycine, 0.055 (0.005)

Species	Observed	Calculated	Extrapolated Alanine	Extrapolated Glycine
	($\mu gC\ l^{-1}\ h^{-1}$)			
Nannochloris atomis				
$\mu/\mu_{max} = 0.93$	85 (34)[1]	111 (11)	106 (35)	125 (21)
0.23	18 (11)	37 (4.8)	40 (26)	45 (28)
Low light batch	6.2 (2.6)	8.9 (1.2)	10 (3.6)	14 (3.7)
Chaetoceros simplex	22 (7.8)	190 (26)	59 (23)	63 (13)
Chroomonas salina	11 (1.2)	34 (3.5)	38 (11)	40 (6.3)
Chattonella luteus	10 (2.0)	50 (4.8)	53 (19)	50 (6.6)

[1] Errors in parentheses represent propagated estimates of 95% confidence intervals

Observed rates were consistently low, while there was closer correspondence between calculated and extrapolated rates. For *Chaetoceros simplex,* both total incorporation of ^{14}C-carbon into protein as well as extrapolated rates were lower than calculated rates, a consequence of nutrient depletion-induced decreases (Fig. 6c). This was not a generalized metabolic response since non-steady-state decreases were not observed in total ^{14}C uptake rate (Fig. 2c), consistent with observations of Li & Goldman (1981). It appears then, that protein synthesis and carbon uptake become uncoupled in *C. simplex* when the supply of ammonium is depleted. *C. simplex* apparently does not accumulate an internal supply of nitrogen sufficient to maintain growth long after the external supply of nitrogen is removed. For the given light intensity and temperature, this species had the highest maximum (batch culture) growth rate of all the species examined in this study. It may be that this higher rate of metabolism contributed to a more rapid manifestation of non-steady-state responses.

The continuously decreasing rate of incorporation into protein-bound glycine and alanine of *Chaetoceros simplex* (Fig. 6c) provided a clear indication that a deviation from initial rates had occurred, emphasizing the value of time-series measurements and the importance of keeping incubations as short as possible. The potential impact of confinement and nutrient depletion on incorporation patterns (Fig. 3; Goldman et al. 1981, Li & Goldman 1981) should be recognized, both for laboratory cultures and natural populations. Li & Goldman (1981) noted that increases in fixed ^{14}C-carbon underestimated net synthesis of particulate organic carbon in 2 species they examined, *Dunaliella tertiolecta* (strain DUN) and *Pavlova lutheri* (strain MONO). They suggested that carbon uptake may have been tightly coupled to ammonium uptake in these species, and was reduced as a result of interrupting steady state. None of the species in our study showed such a decrease in the rate of accumulation of total fixed ^{14}C-carbon for incubation periods up to 3 h. On the other hand, ^{14}C incorporation into protein was sensitive to nitrogen depletion (e.g. Fig. 3). Effects of nutrient depletion may be substantial, and yet not necessarily reflected in an obviously nonlinear pattern of ^{14}C incorporation into total protein carbon due to the mixed kinetics of the different individual amino acids (Fig. 5 & 6). Efforts have been made to measure protein synthesis based on ^{14}C incorporation in nitrogen-limited populations (DiTullio & Laws 1983, Laws et al. 1985). Of course, the environment of a chemostat is very different from a natural system which contains more than just phytoplankton. However, it is important to recognize that long incubation periods without detailed time-series descriptions of carbon incorporation may yield rates which dramatically underestimate the actual protein synthesis rate.

The rapid equilibration dynamics of glycine and alanine will permit accurate determinations of protein synthesis in relation to total primary production for relatively short incubations. For the species examined in this study grown under similar conditions, the proportion of cellular carbon contained in protein was relatively uniform (Table 2). These results are the first, to our knowledge, in which distributions of cellular carbon in protein were compared among different species growing under NH_4^+-limited steady-state conditions. Parsons et al. (1961) compared ratios of protein to carbon in 11 species of marine phytoplankton in exponential phase batch cultures, and also found them to be quite similar. However, estimates of the proportion of cellular carbon in protein from their results were considerably higher than those presented here. Differences in culture conditions may have contributed to the observed differences in cellular protein content. They may also have resulted at least partially because Parsons and coworkers assumed all cellular nitrogen was contained in protein and estimated protein by

multiplying Kjeldahl nitrogen determinations by 6.25 (i.e. protein is 16 % nitrogen by weight). Protein nitrogen generally does not account for all cellular nitrogen (Rhee 1978, Maske 1982, Dortch et al. 1984, Lohrenz 1985, Lohrenz & Taylor unpubl.). Consequently, the assumption that all cellular nitrogen is contained in protein would tend to overestimate true protein content. Finally, we chose not to correct our results for the systematic hydrolysis-related loss of proteinaceous carbon, and the omission of tyrosine, cysteine, and methionine from our calculations due to analytical limitations (see 'Methods'). This may have contributed to observed differences.

Our results and those of Parsons et al. (1961) demonstrate that protein represents a significant proportion of total primary production. In view of the nutritional importance of protein to higher trophic levels, accurate estimates of protein synthesis in relation to total primary production will help in understanding trophodynamic and biogeochemical processes which affect the composition and flux of organic matter. In addition, the fact that the proportion of cellular carbon in protein was positively correlated with the NH_4^+-limited growth rate of *Nannochloris atomis* (Table 2; Lohrenz & Taylor in press) shows promise that assessments of the relation between protein biosynthesis and total primary production can provide information about phytoplankton physiological state.

The accuracy of determinations of the proportion of primary production allocated to protein synthesis will of course depend on the accuracy of the estimate of total primary production in itself. The ^{14}C technique for measuring rates of photosynthetic carbon fixation (Steeman-Nielsen 1952) provides a sensitive and specific assay of phytoplankton activity in units which can be directly compared to biomass. Despite the controversy surrounding the methodology (e.g. Yentsch 1974, Lean & Burnison 1979, Peterson 1980, Dring & Jewson 1982), it remains one of the most powerful and widely applied methods for estimating photosynthetic activity. In 3 out of the 4 species we examined, observed increases in fixed ^{14}C-carbon provided a reasonable estimate of the calculated net synthesis of particulate organic carbon for relatively short incubations (Fig. 1 & 2). *Chroomonas salina* was the exception, showing ^{14}C uptake, estimated either as acid nonvolatile or total particulate ^{14}C, in excess of calculated net synthesis by about 50 %. Li & Goldman (1981) similarly observed an increase in fixed ^{14}C-carbon higher than calculated net synthesis in steady state NH_4^+-limited cultures of *Phaeodactylum tricornutum* (Strain TFX-1). They argued that short-term fixation of ^{14}C in this species represented something between gross and net photosynthesis, although they acknowledged that excretion of dissolved organic carbon or an enhanced growth rate in the assay vessel (samples were removed to separate assay vessels for ^{14}C incubations) could have contributed to the discrepancies observed. The latter problem was eliminated in our study by conducting incubations in the chemostat vessels themselves. In so far as the excretion of dissolved organic carbon may have been important, it should be pointed out that Li & Goldman's estimates of fixed ^{14}C-carbon accumulation were based solely on the time-dependent increase in acid-stable ^{14}C-carbon. Our results with *Chattonella luteus* demonstrated that accumulation of fixed ^{14}C-carbon by this method was higher than the calculated net synthesis, while increases in ^{14}C-carbon retained on filters agreed well with net synthesis for this species. This poses a question as to the extent to which such overestimates of net photosynthesis are a function of extracellular release of ^{14}C-labeled dissolved organic matter. Nevertheless, our results with *Chroomonas salina* clearly indicate that increases in filter-retainable ^{14}C-carbon can in some cases overestimate net synthesis of particulate organic carbon. This tendency is an apparently species-specific phenomenon resulting from the respiratory or excretory loss of cell material.

Although differences were not significant, the incorporation of ^{14}C-carbon into protein-bound glycine of *Chroomonas salina* was slightly higher than its calculated accumulation (Fig. 6b). This might be explained by metabolic turnover of protein, along with other cellular carbon. Protein turnover does occur in higher plants and algae, and varies as a function of physiological state (e.g. Bidwell et al. 1964, Trewavas 1972, Huffaker & Peterson 1974, Bates 1981, DiTullio & Laws 1983, Cuhel et al. 1984). Li & Harrison (1982) suggested that isotope incorporation into protein may be less likely to overestimate net synthesis than total ^{14}C uptake. During the eastern Canadian arctic summer (24 h photoperiod) they found that the time-series increase in particulate ^{14}C-carbon for a single 32 h incubation was non-linear and increasingly lower than the sum of uptake for consecutive 2 h incubations. Discrepancies between the 32 h time course and consecutive 2 h incubations were significantly less for ^{14}C incorporation into the protein fraction. This was interpreted to mean that isotope incorporation into protein is less subject to turnover in excess of net production than incorporation into total cellular carbon. Although our results do not conclusively rule this out, they do suggest an alternative explanation for the results of Li & Harrison. That is, because of incomplete isotopic equilibration of protein precursors in the 2 h incubations, higher ^{14}C incorporation into the protein fraction due to turnover may have been obscured.

The results of this study have demonstrated fundamental similarities in the basic pathways associated

with the supply of photosynthetically fixed carbon for protein synthesis. Precursors to protein-bound glycine and alanine consistently equilibrated rapidly, resulting in a close correspondence between ^{14}C incorporation and estimated net accumulation of fixed carbon in the protein-bound amino acids in short-term incubations. The use of short-term incubations and time-series measurements is important in view of the sensitivity of protein metabolism to nitrogen depletion. In addition, consecutive short-term incubations can be used for precise characterization of diel variations in the relation between protein synthesis and total primary production. Because glycine and alanine are easily and quantitatively recovered from protein hydrolyzates and represent a significant proportion of total protein carbon, they are model protein amino acids for tracing the primary production of particulate protein in relation to total primary production. A subsequent paper (Lohrenz & Taylor unpubl.) describes the use of this approach to determine the allocation of primary production for protein synthesis in natural phytoplankton populations, as part of an investigation of the relation between phytoplankton protein metabolism and natural distributions of particulate organic carbon in protein.

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