Multiphasic uptake of D-glucose by an oligotrophic marine bacterium

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ABSTRACT: Uptake of D-glucose in the range 2.5×10^{-9} M to 4.0×10^{-3} M by an oligotrophic marine bacterium, LNB-155, could be represented by 4 phases of a single, multiphasic mechanism. Other kinetic models did not give a good fit. Sharp transitions occurred at about 1.6×10^{-6} M, 10^{-5} M and 2×10^{-4} M. Values for K_m and V_{max} increased regularly upon transition to higher phases. Multiphasic uptake mechanisms in LNB-155 indicate adaptation to living in environments with fluctuating levels of D-glucose. These observations are consistent with the hypothesis that pelagic marine bacteria experience considerable fluctuations in nutrient concentration in their microenvironment.

INTRODUCTION

Recent demonstration of the quantitative significance of bacterioplankton as a link in pelagic marine foodweb (Hagström et al., 1979; Fuhrman and Azam, 1980, 1982; Williams, 1981; Azam et al., 1982) has generated much interest in the nature of coupling between production of organic matter and its utilization by heterotrophic bacterioplankton. It appears now that this coupling is very tight (Azam and Hodson, 1981; Azam and Ammerman, 1983). The bulk-phase concentrations of bacterial nutrients such as amino acids and saccharides are low and are maintained within narrow limits (Mopper et al., 1980; Burney et al., 1981) apparently due to efficient uptake by marine bacteria. It is of interest therefore to elucidate bacterial uptake mechanisms responsible for this tight coupling.

It had been assumed in the past that pelagic marine bacteria have very low K_m transport systems compatible with the substrate concentration in seawater (Wright and Burnison, 1979). Recently, it was shown that, in addition to the expected low K_m (nanomolar) transport systems, assemblages of marine bacteria express progressively higher K_m transport systems up to 10^{-4} M (Azam and Hodson, 1981). The interpretation of these multiphasic kinetics for species-specific uptake is unclear. There could be 1 uptake system (or mechanism) for each bacterial species or the mul-

tiphasic kinetics could be due to 2 or more independent mechanisms in the same bacterium. At the species level another possibility is that the uptake might be truly multiphasic, i.e. be mediated by a single membrane structure which changes its characteristics in an all-or-none fashion at certain critical external concentrations of the substrate (Nissen, 1971; Linask and Laties, 1973).

To examine the various mechanistic possibilities it was necessary to use pure culture of a marine isolate. We chose an oligotrophic marine bacterium, LNB-155, which had been isolated on unenriched seawater agar (Carlucci and Shimp, 1974). Hodson et al. (1979) discovered a biphasic kinetic pattern for D-glucose uptake in this isolate. We undertook a study of the uptake of D-glucose by LNB-155 over a wide concentration range. Kinetic analyses and statistical comparisons of various kinetic models suggest that uptake is mediated by a single, multiphasic mechanism. We discuss the results in the context of the adaptation of marine bacteria to nutrient uptake in microenvironments where nutrient concentrations may fluctuate.

MATERIALS AND METHODS

Culture conditions

The marine bacterium LNB-155, isolated by Carlucci and Shimp (1974), was kindly provided by Dr. A. F. Carlucci. Cultures were grown in liquid C-P medium

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(Carlucci and Pramer, 1957) at 17 °C with shaking. Cells in late exponential phase were harvested by centrifugation (10,000 g; 5 min), washed with sterile artificial seawater and resuspended in artificial seawater to a density of 0.5–6.7 $\times$ 10^9 cells ml^{-1}. The cells were starved by shaking the suspension for 1 to 2.5 h on a reciprocating shaker at 17 °C.

Uptake measurements

Uptake of D-glucose was initiated by adding 1.0 ml of cell suspension to various amounts of a mixture of ^{3}H-labeled D-glucose and unlabeled D-glucose. Incubation was for 1 or 2 min at 20 °C and was terminated by filtration on membrane filters (0.45 μm, Millipore), followed immediately by 2 rinses with ice-cold sterile seawater. Formalin-killed controls were prepared for all experimental samples. In time-course experiments, 15 ml cell suspensions were added to the ^{3}H-labeled D-glucose at the desired concentration. Samples (1 ml) were withdrawn every 30 s and filtered as above. The wet filters were radioassayed by liquid scintillation counting after addition of 1 ml ethylacetate (to dissolve the filter) and 10 ml Aquasol (New England Nuclear, Boston, MA).

Analysis of data

Isotherms for concentration-dependence of D-glucose uptake were resolved into phases as described previously (Nissen, 1977; see also legend to Fig. 3). Kinetic constants (Table 1) were calculated by means of a Fortran program for minimizing deviations in log v (Cleland, 1963). Kinetic models (Table 2) were fitted to the data using a computer program for nonlinear least squares (GAUSHAUS). The reciprocals of the rates were used for weighting.

Kinetic analysis of transport processes, including model fitting, tests for goodness of fit and comparisons between models, has recently been reviewed by Gardner and Atkins (1982). A comparison between continuous models and the multiphasic model is given by Nissen and Nissen (1983).

RESULTS

Time-course

Uptake of D-glucose by the marine bacterium LNB-155 was linear with time, or nearly so, for at least 5 min both at low, intermediate and high concentrations of the sugar (Fig. 1). The short uptake periods, 2 min at
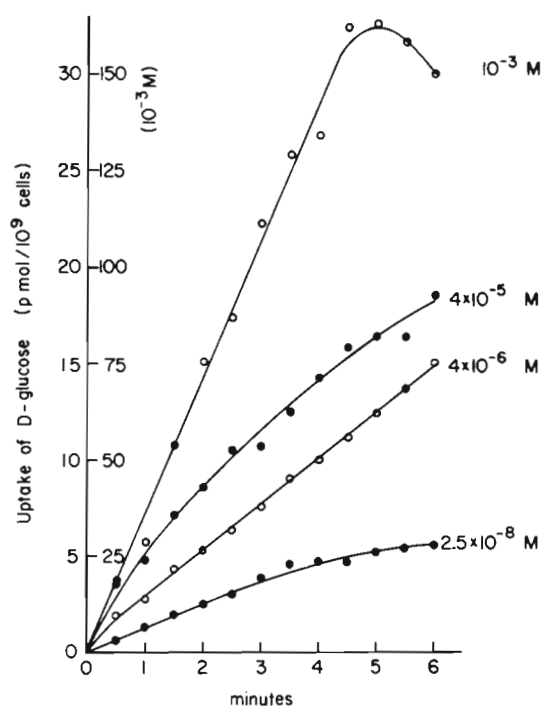

Fig. 1. Time-courses of D-glucose uptake by LNB-155 from 4 different D-glucose concentrations

most, used in the subsequent experiments would therefore tend to preclude complexities due to metabolism of the sugar by the bacteria or to changes in the rate-limiting step.

Concentration-dependence

The dependence of D-glucose uptake upon external concentration is shown in a series of experiments with overlapping concentration ranges (Fig. 2, 3 and 5). Uptake followed simple Michaelis-Menten kinetics at low concentrations (Fig. 2). At 1.6 $\times$ 10^{-6} M a second phase became apparent while yet another transition occurred at or slightly above 10^{-5} M (Fig. 3). Uptake in the range 2.5 $\times$ 10^{-7} M to 4 $\times$ 10^{-5} M can thus be resolved into 3 phases of a single multiphasic mechanism as exemplified in Fig. 4. At still higher concentrations, a third transition occurred at about 2 $\times$ 10^{-4} M (Fig. 5). The fit to adjacent phases (Nissen, 1977) was in all cases better than the fit to a single phase, often highly significantly so (P < 0.001). It may be noted that the transitions were not due to the use of different stock solutions of unlabeled D-glucose or to other experimental artifacts.

Values for K_m increased regularly upon transition to higher phases. K_m for Phase 1 was about 2 $\times$ 10^{-7} M except for the low (but also uncertain) value for experiment C (Table 1). For Phase 2, K_m increased by one
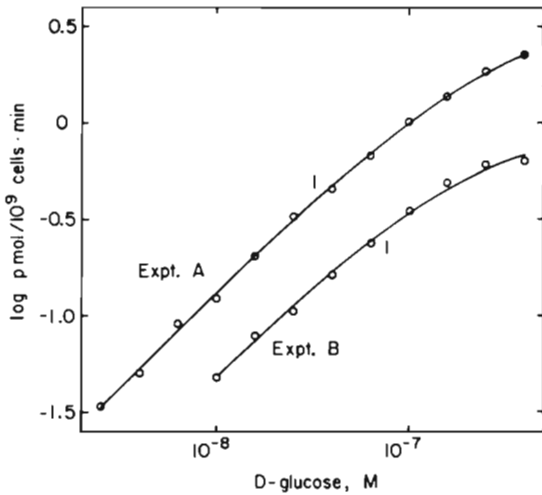
Fig. 2. Uptake of D-glucose by LNB-155. External concentrations: 2.5×10^{-9} M $- 4.0 \times 10^{-7}$ M. Expt. A: average SE $= 11.7\%$ ($n = 3$); Expt. B: average SE $= 13.1\%$ ($n = 3$)
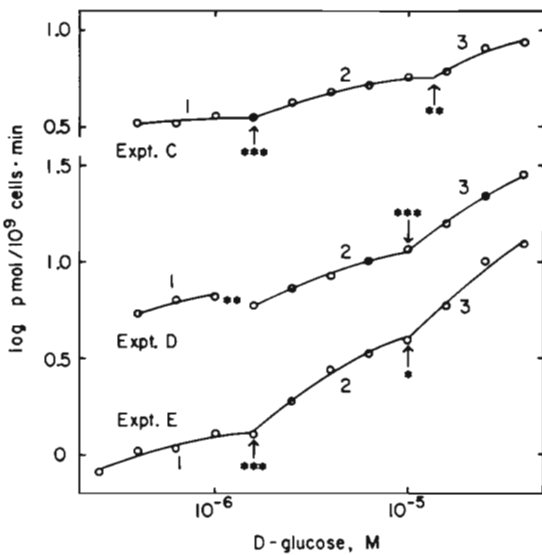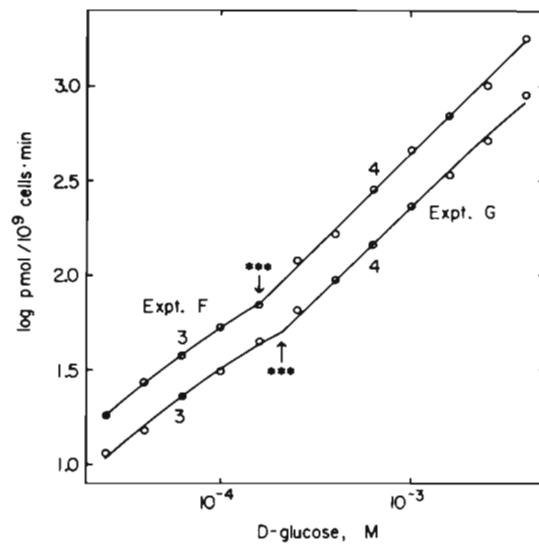

Fig. 3. Uptake of D-glucose by LNB-155. External concentrations: 2.5×10^{-7} M to 4.0×10^{-5} M. Arrows: transition points. Significance of fit to adjacent phases (tested against a single phase): *: $0.01 < P \le 0.05$; **: $0.001 < P \le 0.01$; ***: $P \le 0.001$. See Nissen (1973, 1977) for analytical procedure. Expt. C: average SE $= 8.0\%$ ($n = 3$); Expt. D: average SE $= 5.7\%$ ($n = 6$); Expt. E: average SE $= 11.3\%$ ($n = 4$)

Fig. 4. Eadie-Hofstee plot of the data in Expt. E (Fig. 3)

Fig. 5. Uptake of D-glucose by LNB-155. External concentrations: 2.5×10^{-5} M to 4.0×10^{-3} M. Arrows: transition points. Significance symbols (asterisks): see legend to Fig. 3. Expt. F: average SE $= 5.5\%$ ($n = 3$); Expt. G: average SE $= 7.2\%$ ($n = 3$)

order of magnitude, but there was considerable variation between experiments as apparent from Fig. 3 and shown in Table 1. Similar or even larger increases in K_m occurred upon transition to Phases 3 and 4 (Table 1). For Phase 4 there was little or no indication of saturation at 4×10^{-3} M (Fig. 5), precluding an accurate determination of K_m (Table 1).

Uptake rates varied considerably between experiments (Fig. 2, 3 and 5), as did values for V_{max} (not shown). This may be ascribed to differences in the

starvation of the bacteria prior to uptake (see 'Materials and Methods') and/or to unmonitored differences in the culture conditions. Transition to Phase 2 caused at most a 5-fold increase in V_{max} and transition to Phase 3 at most an 8-fold increase, i.e. somewhat smaller increases than for K_m. In Experiment C, V_{max} only doubled upon transition to a higher phase (Fig. 3). Transition to Phase 4 increased V_{max} by 2 orders of magnitude (Fig. 5). It is notable that the phase pattern remained the same despite the variation in uptake rates and V_{max} (Fig. 3).

Comparison of kinetic models

Various kinetic models were fitted to the data in Fig. 3 as described in 'Materials and Methods'. The

Table 1. K_m-values for the experiments in Fig. 2, 3 and 5

Expt	Phase 1	Phase 2	Phase 3	Phase 4
A	$2.9 \pm 0.2 \times 10^{-7}$ *			
B	$2.1 \pm 0.2 \times 10^{-7}$			
C	$4.0 \pm 4.9 \times 10^{-8}$	$1.3 \pm 0.1 \times 10^{-6}$	$1.5 \pm 3.7 \times 10^{-5}$	
D	$1.9 \pm 2.7 \times 10^{-7}$	$2.2 \pm 0.4 \times 10^{-6}$	$3.7 \pm 0.8 \times 10^{-5}$	
E	$1.8 \pm 0.5 \times 10^{-7}$	$6.6 \pm 1.5 \times 10^{-6}$	$1.8 \pm 1.5 \times 10^{-4}$	
F			$1.8 \pm 0.1 \times 10^{-4}$	$1.6 \pm 4.8 \times 10^{-2}$
G			$2.0 \pm 0.6 \times 10^{-4}$	$2.8 \pm 2.0 \times 10^{-3}$

* All values in M and with 95 % confidence intervals

models include the sum of a Michaelis-Menten term and a term which was taken to be directly proportional to the external concentration ('single + diffusion'), the sum of 2 Michaelis-Menten terms ('dual'), the sum of 3 Michaelis-Menten terms ('triple') and the multiphasic model. The multiphasic model consistently gave the best fit (Table 2). The residual mean sum of squares

Table 2. Goodness of fit of various kinetic models to the data in Fig. 3. The weighted mean square of deviates, or residual mean square, $\Sigma v_{obs}^{-2} (v_{obs} - v_{calc})^2/DF$ is used as measure of fit. Values in table $\times 10^{-3}$

Expt	Kinetic model*			
	Single + diffusion	Dual	Triple**	Multiphasic
C	8.5	5.2	4.2	1.4
D	4.4	4.6	6.2	0.9
E	13.1	12.2	17.3	3.6

* See text
** Constrained to exclude negative values for kinetic constants; see text

was lower for the multiphasic model than for the 3 other models, although only significantly so (P < 0.05) in the case of Experiment D. The triple model, the most complex of the continuous models, was furthermore contraindicated by the finding of negative values for one or more of the kinetic constants.

This comparison is biased against the multiphasic model since only a limited concentration range is considered. In a wider range the marked transition between Phases 3 and 4 (Fig. 5) would clearly make the continuous models untenable. A cooperative model based on the interaction of 4 subunits was also found to give a relatively poor fit (data not given). These models can only give gradual transitions and there are good indications that the transitions are indeed discontinuous (Fig. 4) as predicted by the multiphasic model. The all-or-none nature of the kinetics is especially appa-

rent when a transition is accompanied by a discontiguity in the isotherm (see transition between Phases 1 and 2 – Experiment D in Fig. 3).

DISCUSSION

Our results on LNB-155 extend previous observations of multiphasic uptake of glucose by natural bacterial assemblages (Azam and Hodson, 1981). The finding of multiphasic kinetics in LNB-155 in this study raises the question: How general is this type of transport system in marine bacteria? To answer this question adequately would require work with not only a large number of isolates but also a variety of substrates for each isolate studied. The present study can serve only as a model experimental system for investigating the generality of multiphasic transport systems in marine bacteria.

It is interesting that LNB-155 can grow in particle-free unsupplemented seawater, yet its transport system has K_m values from submicromolar to millimolar. Multiplicity of transport systems is often considered an adaptation to utilizing the nutrient in question in a fluctuating nutrient field. However, the energetic investment in the synthesis and maintenance of multiple transport systems has to be taken into account. Since the average concentration of directly utilizable dissolved organic matter (UDOM) in seawater is quite low, metabolic strategies to forego the need for multiple transport systems, as in the multiphasic transport system here, appear beneficial for the bacterium. The fundamental difference between multiple and multiphasic transport systems should be emphasized in this context. In multiphasic uptake, a single entity (uptake site, carrier, or channel) undergoes abrupt conformational change at certain critical solute concentrations. For ion uptake in higher plants (for which more data are available) it has been demonstrated kinetically that transitions are not caused by interaction of ions with the uptake site but with a separate transition site

accessible only from outside the plasmalemma (Vange et al., 1974; Nissen, 1980). The molecular basis for discontinuous transitions remains unclear, however.

The finding of multiphasic kinetics for D-glucose uptake in an oligotrophic marine bacterium may help explain previously puzzling results for uptake of sugars and amino acids by natural assemblages of aquatic bacteria. On the basis of a simulation of the kinetics of uptake by heterogeneous populations, Williams (1973) concluded that 'departure from the predictions of the [Michaelis-Menten] equation . . . cannot be attributed simply to the fact that the population is heterogeneous'. The departure in question (Vaccaro and Jannasch, 1967; Munroe and Brock, 1968; Hamilton and Preslan, 1970) may, at least in part, have resulted from D-glucose uptake being multiphasic rather than monophasic. Departures from Michaelis-Menten kinetics for uptake of amino acids (Crawford et al., 1974; Barvenik and Malloy, 1979; Geesey and Morita, 1979) may possibly also be explained similarly. Amino acid transport in some bacteria is multiphasic (P. Nissen, unpubl.).

What do these results tell us about the nature of coupling between the production of UDOM and its uptake by bacteria in the seawater? It is reasonable to argue that the high K_m, high V_{max} components of the LNB-155 multiphasic transport system for D-glucose reflect an adaptation to a nutrient field where a broad range of substrate concentrations is encountered by the bacterium. These results thus suggest the existence of microscale variations in nutrient concentrations in seawater. Intracellular concentrations of sugars and amino acids in algae are on the order of millimolar (Dortch, 1982), and similar high nutrient concentrations might exist in microenvironments around sources of sustained production of UDOM such as algae excreting organic compounds or detrital particles undergoing hydrolysis (Azam and Hodson, 1981; Azam and Ammerman, 1983). Multiphasic uptake systems should allow optimum nutrient uptake in microenvironments where the nutrient concentration may vary both in space and in time.

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