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Kinetics of strontium uptake in the scleractinian coral *Stylophora pistillata*

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ABSTRACT: Reef-building corals are very sensitive to changes in their environment and have been identified as potential accurate recorders of environmental changes. They form aragonitic carbonate skeletons that contain relatively high amounts of strontium (Sr^{2+}). The ratio of Sr^{2+}/calcium (Ca^{2+}) has been proved to be useful for paleoclimatic studies since it has been suggested to vary with the seawater temperature. However, no correlation can be established between skeletal Sr^{2+} content and environmental parameters due to the lack of knowledge concerning the extent to which skeletal chemistry is controlled by physiological parameters. In this study, we investigated the pathway of Sr^{2+} incorporation by the scleractinian coral *Stylophora pistillata.* For this purpose, we used ^{85}Sr and a non-destructive NaI γ detector. Sr^{2+} skeletal incorporation was found to be linear during the 9 experimental days of incubation with natural concentrations of Sr^{2+}. The incorporation of Sr^{2+} versus external Sr^{2+} concentration was also linear up to 3.42 mM (i.e. a concentration 37.5 times higher than normal seawater concentration). However, the uptake of Sr^{2+} at high concentrations (>1 mM) decreased with an increase in Ca^{2+} concentration in the seawater. Moreover, Verapamil, a Ca^{2+} channel inhibitor, also inhibits the incorporation of Sr^{2+} with the same I_{c50} (12 µM) as for Ca^{2+}. Incorporation of Sr^{2+} is therefore inversely correlated to the rate of calcification, suggesting interactions between these 2 ions, which should be taken into account during paleoclimatic studies.

KEY WORDS: Corals · Strontium · Calcium · Paleoclimatology

INTRODUCTION

Strontium (Sr^{2+}) is a relatively common constituent of seawater and one of the most abundant trace elements (8 mg l^{-1} or 91 µM). It is chemically similar to calcium (Ca^{2+}) and is generally considered to substitute for Ca^{2+} in the aragonite lattice (Speer 1983) of biogenic carbonate (coral skeletons, mollusc shells, foraminifera and fish bones). The study of Sr^{2+} has several implications.

On one hand, radiostrontium can be very harmful to the environment because of its fission yield (^{90}Sr and ^{89}Sr), long half-life (ca. 20 yr) and accumulation in calcifying tissues. It is one of the major radioelements in the environment, whose level is checked and recorded because high activity was found after the Chernobyl accident in 1986 (Matsunaga et al. 1997). It is also abundant in discharge waters from uranium mines (Nichols & Scholz 1989) and nuclear installations (Spangenberg & Cherr 1996, Noureddine & Baggoura 1997) as well as being detected near oil and gas fields (Olsgard & Gray 1995). Therefore, the processes involved in its incorporation in calcifying tissues are of particular interest.

On the other hand, scleractinian corals form aragonitic carbonate skeletons that also contain relatively high amounts of Sr^{2+} (Milliman 1974). The use of such corals as archives of ocean chemistry and physical properties is one of the most rapidly growing fields of chemical oceanography and paleoceanography (Druffel 1997). Sr^{2+} thermometry has been suggested to be a powerful tool for reconstructing seawater sur-

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face temperature and is based on an inverse relationship between water temperature and Sr^{2+}/Ca^{2+} ratios (Weber 1973). Subsequent studies using mass spectrometry techniques also empirically correlated Sr^{2+}/Ca^{2+} with temperature or a $\delta^{18}O$ estimate of temperature (Swart 1981, Beck et al. 1992, McCullough et al. 1994, DeVilliers et al. 1995, Juillet-Leclerc et al. 1997, Evans et al. 1998), and good relationships were reported between these 2 parameters (Hughen et al. 1999, McCullough et al. 1999), with a precision of ±0.5°C (Beck et al. 1992). Some studies however argued that the Sr^{2+}/Ca^{2+} thermometer is wrought with problems because it was shown to vary widely between or within species (Allison 1996, Rosenthal et al. 1997), or to correlate well with coral extension rates (DeVilliers et al. 1994, 1995). It has also been found within a single *Porites* coral colony that low-density skeleton in depressed valleys had a higher mean Sr^{2+}/Ca^{2+} value than the high-density skeleton on elevated bumps (Alibert & McCulloch 1997). Finally, ion probe microanalyses across coral growth bands have revealed a heterogeneous distribution of Sr^{2+} that is inconsistent with temperature fluctuations (Hart & Cohen 1996). Additionally, the mineral strontianite was also identified in some corals using X-ray analysis (Greegor et al. 1997). These observations all suggest that temperature is not the only factor controlling Sr^{2+}/Ca^{2+} ratio in corals and that biological factors must also play a role (Hart & Cohen 1996, Cohen et al. 2001). Therefore, no clear correlation could be established between skeletal Sr^{2+} content and environmental parameters due to the lack of knowledge concerning the extent to which skeletal chemistry is controlled by physiological parameters (Boiseau et al. 1997).

Few studies have investigated the kinetics of Sr^{2+} uptake by calcifying organisms. In molluscs, it was shown that Sr^{2+} was incorporated into the shells in direct proportion to its level in seawater (Hockett et al. 1997) and that organisms held in Sr^{2+}-enriched seawater partially replaced Ca^{2+} in their shells by Sr^{2+} (Peck et al. 1996). However, Wansard et al. (1999) noticed that the incorporation of Sr^{2+} versus Ca^{2+} in ostracods also largely depended on the calcification processes involved, light-weight shells being depleted in Sr^{2+} content compared to well-calcified valves. Studies performed on corals also gave 2 opposite results for Sr^{2+} uptake. The first mechanism involved similar physiological processes for Ca^{2+} and Sr^{2+} uptake, with competition between these 2 elements (Goreau 1977, Swart 1980, Chalker 1981). In this case, Sr^{2+} is supposed to be deposited via an active process. The second mechanism involved 2 transport systems (Ip & Krishnaveni 1991, Ip & Lim 1991), with discrimination between the 2 elements. In this case, the deposition of Sr^{2+} is supposed to be a diffusive process.

The aim of this study, therefore, is to improve our knowledge of the uptake kinetics of Sr^{2+} in the scleractinian coral *Stylophora pistillata*. For this purpose, corals were incubated with the radiotracer ^{85}S in artificial seawater containing different Ca^{2+} and stable Sr^{2+} concentrations, and the transfer of radioactivity was followed in the different coral compartments. The Ca^{2+} channel inhibitor, Verapamil, was also tested for the Sr^{2+} uptake. An important advance in this research was the utilisation of a non-destructive method, allowing the measurement of Sr^{2+} incorporation into the skeleton.

MATERIALS AND METHODS

Biological material. Experiments were carried out in the laboratory with microcolonies (branch tips 1 cm long, 1 cm wide) of *Stylophora pistillata* (Esper 1797). Colonies were collected in the Gulf of Aqaba (Red Sea, Jordan) from 5 m depth and maintained in open flow aquaria. Microcolonies (n = 180) were prepared by cutting terminal portions of branches of 10 mother colonies with bone-cutting pliers as described earlier (Tambutté et al. 1995). They were then suspended with a nylon mesh in an aquarium supplied with oligotrophic Mediterranean seawater (pumped from 50 m depth and heated to 26°C); 400 W metal halide lamps (Philips, HPIT) provided a constant irradiance of 180 µmol photons m^{-2} s^{-1} on a 12:12 h light:dark regime. After approximately 1 mo of healing, tissue entirely recovered the exposed skeleton, and coral fragments were ready to be used for the uptake experiments. During the whole experiment, microcolonies were fed once a week with *Artemia salina* nauplii.

Experimental protocol. ***Kinetics of Sr^{2+} uptake:*** To assess the rate of Sr^{2+} uptake as a function of time, 3 aquaria containing 20 l of oxygenated seawater were prepared with 3 different concentrations of stable Sr^{2+}, the lowest concentration being similar to the Sr^{2+} level of 8.1 mg l^{-1} observed in the marine environment (Horne 1969), i.e. 10, 20 and 50 mg l^{-1} (or 0.11, 0.23 and 0.57 mM) made up from a stock solution of strontium chloride. Seawater was then spiked in each aquarium with the radiotracer ^{85}Sr (carrier-free, obtained from LEA, $T_{1/2}$ = 64.85 d) to reach an activity of 7.052 KBq l^{-1}. Fifteen coral microcolonies were then randomly divided in each aquarium (5 per tank) and incubated under the same conditions of light and temperature as described above. The incubation medium was changed every 2 d both for the health of the corals and to maintain the ^{85}Sr activity at an approximately constant level. The ^{85}Sr uptake by corals was followed after 2, 7 and then every 24 h over 9 d in the same microcolony and for the 5 colonies incubated in each

tank (a total of 40 measurements for each Sr^{2+} concentration). At each sampling time, all microcolonies were processed as described below.

Process of Sr^{2+} uptake: The second set of experiments was designed to investigate the processes involved in the ^{85}Sr uptake in the coral tissue and skeleton. The first experiment was repeated but with a broader range of Sr^{2+} concentrations. Six 20 l aquaria were spiked with µl quantities of the radiotracer ^{85}Sr solution to reach a final activity of 7.111 Bq l^{-1}. Stable Sr^{2+}, prepared as above, was added to each aquarium to obtain concentrations of 10, 20, 30, 50, 150 and 300 mg l^{-1} (or 0.11, 0.23, 0.34, 0.57, 1.71 and 3.43 mM, respectively). Thirty microcolonies were equally divided into the 6 tanks (5 per tank) and incubated for 3 d under the same conditions of light and temperature as previously described. At the end of the incubation, all corals were processed as described below to measure the amount of ^{85}Sr in the whole colony, tissues and skeleton. The amount of protein was also determined. The experiment was repeated twice.

The uptake of ^{85}Sr was also measured under different concentrations of Verapamil, a Ca^{2+} channel inhibitor (Tambutté et al. 1996). For this purpose, microcolonies were individually pre-incubated for 30 min in 50 ml beakers containing: 0, 1, 5, 10, 50 and 100 µM Verapamil previously dissolved in dimethyl sulfoxide solution (DMSO, 0.1%). Microcolonies were then transferred to another set of 50 ml beakers spiked with 1.200 KBq ml^{-1} of ^{85}Sr and the above concentrations of Verapamil, after which they were incubated for 1 h. Three microcolonies were individually incubated at each Veramapil concentrations and were processed as described below at the end of the incubation to measure the amount of ^{85}Sr in the whole colony, tissues and skeleton.

Sr^{2+}-Ca^{2+} interactions: The third set of experiments was performed to determine the interactions between Sr^{2+} and Ca^{2+} uptake. For this purpose, uptake of ^{85}Sr was measured under different Ca^{2+} concentrations in seawater. Corals were individually incubated for 2 h in a vial containing artificial seawater and 7 different Ca^{2+} concentrations (0, 1, 3, 7, 10, 15 and 20 mM) and µl quantities of the radiotracer ^{85}Sr at a final activity of ca. 1.006 kBq ml^{-1}. Four experiments were performed with 10, 100, 200 and 400 mg l^{-1} of Sr^{2+} in seawater. Free artificial seawater (ASW) was prepared from distilled water (Allemand et al. 1984). NaCl replaced $CaCl_2$ in order to maintain constant osmolarity. All chemicals (Sigma) were of analytical grade and did not contain Sr^{2+} in their composition (or in negligible quantity). At the end of the incubation, microcolonies were processed as described below. This experiment was performed with 1 microcolony for each Ca^{2+} and Sr^{2+} concentration and was repeated 3 times.

Sample processing: At each sampling time, microcolonies were rinsed with normal seawater, blotted dry on absorbent paper to eliminate any adhering radioactive medium and transferred to counting vials containing 50 ml of seawater. γ-emission was counted for 5 min. They were then placed back in their aquarium and the activity of the 50 ml of counting medium was measured to evaluate ^{85}Sr loss during counting. This method is a non-destructive measurement of Sr^{2+} uptake, since coral microcolonies were not sacrificed for the measurement. At the end of the experiment, the whole colony was counted as described above. It was then incubated for 30 min in 50 ml of seawater to measure the efflux, which is the amount of seawater contained in the coelenteric cavity of the corals (Tambutté et al. 1995). The time needed to complete the efflux procedure was determined in previous experiments (Tambutté et al. 1996) and it was shown that 30 min was sufficient to totally renew the coelenteric content. Upon completion of the efflux, tissues were dissolved for 20 min in 1 ml of 1 N NaOH at 90°C. ^{85}Sr activity was determined in the tissue (also referred to as 'NaOH soluble pool') as well as in the skeleton. Protein concentrations were measured in the NaOH soluble pool on a Multiscan Bichromatic system (Labsystem) using the BC assay kit (uptima) with bovine serum albumin as the standard. Skeletons were dried, weighed and their ^{85}Sr activity was evaluated.

The γ-emission of ^{85}Sr (506 KeV) in the whole colony, skeleton, tissue and seawater was determined using 2 well type NaI detectors (3 inch) connected to a multichannel analyser and a computer using a spectral analysis software. The activity of samples was corrected for background, radioactive decay and counting efficiency, and was compared with standards of appropriate geometry. Counting times were adjusted to give the relative propagated error <5% at the 1 SD level, i.e. 5 min for the whole colony and the skeleton, 20 min for the tissues and the efflux seawater.

Results concerning the incorporation of Sr^{2+} in the colony, tissue and skeleton, are expressed as nmol l^{-1} from Bq ml^{-1} and specific radioactivity of the medium (for details see Tambutté et al. 1995).

RESULTS

Results obtained in the first experiment (Fig. 1) showed that the uptake of Sr^{2+} was linear as a function of time over an exposure period of 9 d at 3 different Sr^{2+} concentrations. The same results are obtained when data are normalised per mg protein or per g skeleton (g $CaCO_3$). The slope of the regression lines gave the following uptake rates respectively for 0.11, 0.23 and 0.57 nM of Sr^{2+} in seawater: 28.14, 81.75 and

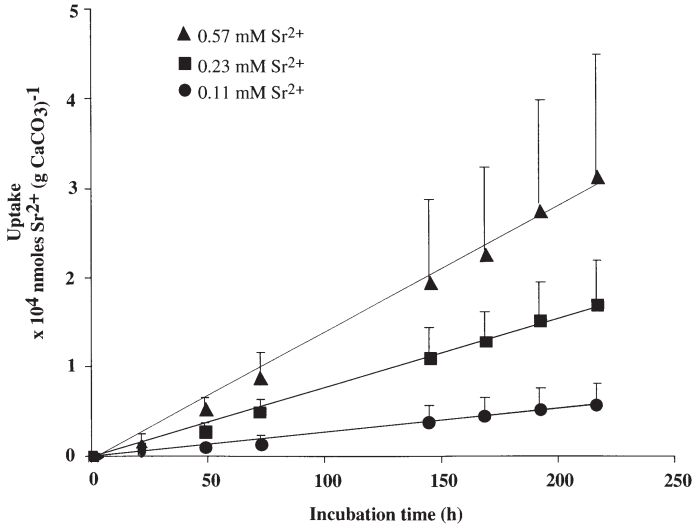

Fig. 1. Sr^{2+} incorporation in the whole colony over 9 d ($\times 10^4$ nmol Sr^{2+} [g $CaCO_3$]$^{-1}$). Uptake of Sr^{2+} in seawater at concentrations of 0.11 (●), 0.23 (■) and 0.57 mM (▲). Data are presented as mean + SD (n = 5)

158.20 nmol Sr^{2+} incorporated g$CaCO_3^{-1}$ h^{-1}, respectively (Fig. 1). Each correlation was highly significant. Radioactive Sr^{2+} was mostly incorporated into the skeleton and less than 1% was found in the tissue. In the following results, therefore, only the incorporation of Sr^{2+} in the skeleton will be presented. The amount of Sr^{2+} contained in the coelenteric cavity varied from 1.8 ± 0.6 to 4.2 ± 1.2 nmol Sr^{2+}(g$CaCO_3$)$^{-1}$ according to the Sr^{2+} concentration in seawater. The time needed to complete the efflux was calculated by measuring the amount of radioactivity released by corals in seawater versus time. It was found that the efflux was completed after between 20 and 30 min.

The kinetics of Sr^{2+} uptake in the skeleton was followed when exposing 5 coral colonies over 3 d to increasing amounts of Sr^{2+} (0 to 300 mg l^{-1} or 0 to 3.42 mM) at normal Ca^{2+} concentration of seawater (Fig. 2). The same results were obtained when data were normalised per g dry weight or per mg protein. Uptake rates (in the whole colony or in the skeleton) were linearly dependent upon the external concentration of Sr^{2+} and were equal to 88 µmol Sr^{2+} incorporated g$CaCO_3^{-1}$ d^{-1} and per mol external Sr^{2+}.

To determine the mechanism of Sr^{2+} transport, the effect of Verapamil, a Ca^{2+} channel inhibitor, was tested. This drug inhibited Sr^{2+} incorporation into the skeleton. The dose response experiments (Fig. 3) showed that half inhibition (I_{c50}) was obtained for a Verapamil concentration of 12 µM.

The kinetics of Sr^{2+} uptake was followed when exposing the corals to a wide range of Sr^{2+} levels at different Ca^{2+} concentrations (Fig. 4). The uptake of Sr^{2+} at high concentrations (>1 mM) decreased with the increase in Ca^{2+} concentration in the experimental medium (Fig. 4). This trend was not apparent for natural Sr^{2+} concentrations (Fig. 4).

DISCUSSION

Calcification in corals has been a topic of interest for over 100 yr (Chalker 1976, Tambutté et al. 1996), but as yet, the processes involved are poorly understood. Most of the papers have related to Ca^{2+} uptake and few have explored the effects of Sr^{2+} on calcification (Chalker 1981, Ip & Krishnaveni 1991, Wright & Marshall 1991). It is however essential for paleontologists to understand calcification processes in corals because

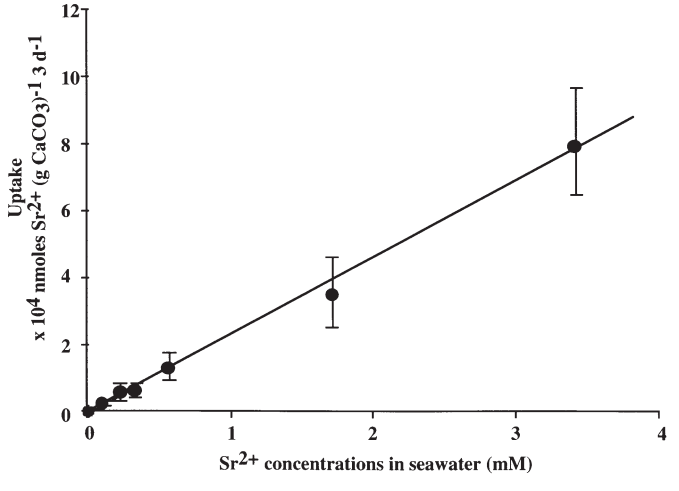

Fig. 2. Sr^{2+} incorporation in coral skeletons according to the Sr^{2+} concentration in seawater (mM) measured after 3 d of incubation ($\times 10^4$ nmol Sr^{2+} [g $CaCO_3$]$^{-1}$ 3 d^{-1}]. Data are presented as mean + SD (n = 10)

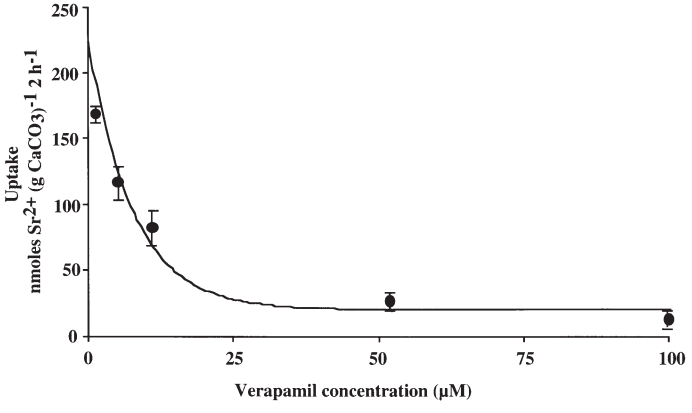

Fig. 3. Sr^{2+} incorporation in coral skeleton according to Verapamil concentrations (µM) in seawater (nmol Sr^{2+} [g $CaCO_3$]$^{-1}$ 2 h^{-1}). Data are presented as mean + SD (n = 3)

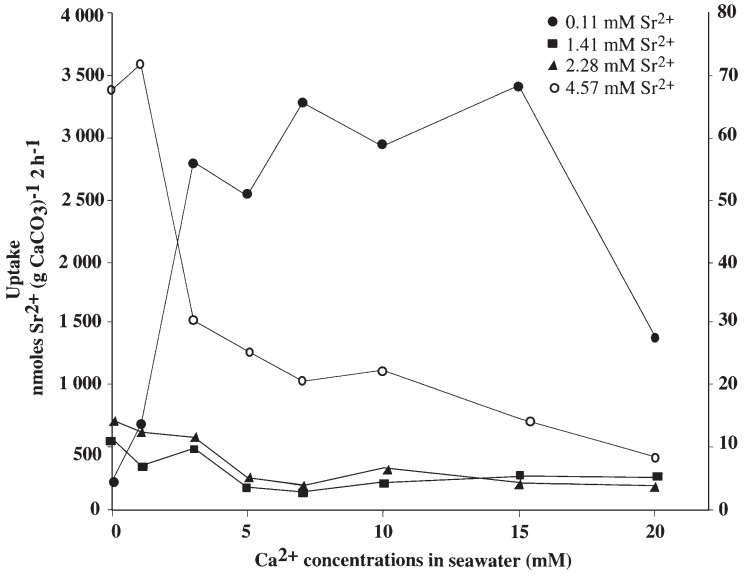

Fig. 4. Sr^{2+} incorporation at different Sr^{2+} (mM) and Ca^{2+} (mM) concentrations in seawater (nmol Sr^{2+} [g $CaCO_3$]$^{-1}$ 2 h^{-1}). Right axis: uptake of Sr^{2+} in seawater at a concentration of 0.11 mM Sr^{2+} (●). Left axis: Uptake of Sr^{2+} in seawater at concentrations of 1.41 (■), 2.28 (▲) and 4.57 mM Sr^{2+} (○). This figure represents the general pattern observed during 3 different experiments

the ratio of Sr^{2+}/Ca^{2+} has been widely used to determine the temperature of the ancient seas (Swart 1981, Aharon 1991). Such determination is important since global climate is strongly influenced by the sea surface temperatures of the tropical oceans (Trenberth & Hurell 1994).

Small coral colonies, prepared as described in Tambutté et al. (1995), were used for our experiments. The whole skeleton was entirely covered by animal tissue in order to avoid any contact with the external seawater containing the radioactive tracer. Therefore, non-specific exchanges between radioactive and stable Sr^{2+} were avoided. For the first time, Sr^{2+} uptake was measured with a non-destructive method, which allowed us to follow its incorporation over 9 d in individual coral colonies. Experiments were performed with *Stylophora pistillata*, which is not a massive species, such as those commonly cored for paleoclimatic studies. The rates of calcification are of course different according to the coral species considered; however, the processes of calcification should be comparable in all corals. Moreover, microcolonies of *S. pistillata* were performed from 10 different parent colonies in order to assess the genetic variability.

By following the same nubbin over 9 d, we found that Sr^{2+} uptake was linear as a function of time at different stable Sr^{2+} concentrations in seawater. Such linear uptake has already been observed for vertebrates (Chowdhury et al. 2000), although for a shorter exposure time (7 h). This result is therefore one of the first showing linear uptake over several days for calcifying organisms such as corals. The rates of Sr^{2+} uptake measured in this study for 0.1 mM Sr^{2+} in seawater varied between 15 and 40 nmol Sr^{2+} g skeleton^{-1} h^{-1}. They are in agreement with those found by Ip & Krishnaveni (1991) at the same seawater concentration (9 to 15 nmol Sr^{2+} incorporated g skeleton^{-1} h^{-1}). These rates are ca. 10 times lower than the rates of Ca^{2+} deposition in *Stylophora pistillata*, estimated to be equal to 550 nmol ^{45}Ca deposited h^{-1} g skeleton^{-1} (Ip & Krishnaveni 1991) or 60 nmol ^{45}Ca deposited h^{-1} mg protein^{-1} (Tambutté et al. 1996). Ca^{2+} concentrations in seawater are however 20 times higher than for Sr^{2+}. This set of experiments also demonstrated the existence of a quick exchangeable Sr^{2+} compartment (efflux). This efflux corresponds to the washout of an extracellular compartment supposed to be the coelenteric cavity. The same conclusions were obtained for Ca^{2+} in scleractinian corals (Tambutté et al. 1996) and octocorals (Allemand & Grillo 1992). The turnover rate of this compartment was estimated to be equal to 4 min in *S. pistillata* (Tambutté et al. 1995).

The incorporation of Ca^{2+} in scleractinian corals has been shown to be saturable and therefore carrier-mediated (Chalker 1981, Tambutté et al. 1996), involving both passive and energy-requiring steps (Marshall 1996, Tambutté et al. 1996). Unlike Ca^{2+}, the processes involved in the deposition of Sr^{2+} remain controversial as contradictory results have been reported. Kinsman (1969) was one of the first to suggest that the incorporation of Sr^{2+} was mediated by a diffusive pathway and that coral aragonite was in chemical equilibrium with seawater. Ip & Krishnaveni (1991) and Ip & Lim (1991) reached the same conclusion. Conversely, Goreau (1977) postulated that Sr^{2+} and Ca^{2+} were transported by the same ion carriers (active transport). This hypothesis was confirmed by the studies of Chalker (1981) and Wright & Marshall (1991) who also suggested that Sr^{2+} was a competitive inhibitor of calcification, being transported to the sites of skeletogenesis by the same mechanisms. This conclusion was based on the observation that Sr^{2+} uptake was affected by the Ca^{2+} concentration in seawater.

The last set of experiments performed in the present work confirmed that Sr^{2+} uptake in corals depends on the Ca^{2+} concentration in seawater since Sr^{2+} uptake rates significantly decreased with an increase in Ca^{2+} concentration in seawater (Fig. 4). This result suggests that Sr^{2+} incorporation into the skeleton is inversely linked to the rate of calcification. Verapamil inhibits

Ca^{2+} uptake through Ca^{2+} channels (Marshall 1996, Tambutté et al. 1996, Zoccola et al. 1999), thereby reducing the rate of calcification. This inhibitor, however, also reduced the rate of Sr^{2+} incorporation. Since this result cannot be interpreted as an effect mediated by the decrease in calcification (which would have stimulated Sr^{2+} incorporation), we can conclude that Verapamil exerts a direct effect on Sr^{2+} uptake. This result therefore strongly suggests a common pathway for Ca^{2+} and Sr^{2+} at the calicoblastic cell entry. This conclusion is strengthened by the similar IC_{50} measured for these 2 ions with Verapamil (ca. 12 µM; Tambutté et al 1996). We can therefore suggest that Sr^{2+} uses a transcellular transport pathway.

However, Fig. 3 showed that uptake of Sr^{2+} versus Sr^{2+} concentration did not display saturable Michaelis-Menten kinetics, as should have been the case if the incorporation of Sr^{2+} was carrier-mediated. The incorporation of Ca^{2+} and Sr^{2+} into the skeletal structures is a multi-step process, involving at least 5 steps: uptake by a Ca^{2+} channel at the cell membrane level, trans-cytotic transport (mediated either by Ca^{2+}-binding proteins or by vesicle shuttle; cf. Johnston 1980, Bronner 1996), exit via a Ca^{2+}-ATPase, binding to organic matrix macromolecules and precipitation with the carbonate moiety. The relationship between the rate of calcification versus ion concentration cannot therefore be interpreted as simple Michaelis-Menten kinetics: a linear dependence between Sr^{2+} incorporation and Sr^{2+} concentration does not mean that a carrier-mediated step is not involved. Furthermore, the behaviour of Sr^{2+} may be different than that of Ca^{2+} for each of these different steps. For instance, while the permeation of Sr^{2+} through the Ca^{2+} channel of the jellyfish is higher than that of Ca^{2+} (Jeziorski et al. 1998), a lower permeation is generally reported in the literature (Estacion et al. 1999). Funamoto & Mugiya (1998) also showed that Sr^{2+} affinity for the plasma protein in the goldfish *Carassius auratus* was 10-fold lower than that of Ca^{2+}. Finally, it has also been demonstrated that the affinity of the Ca^{2+}-ATPase for Sr^{2+} was far lower than the affinity for Ca^{2+} (Yu & Inesi 1995). Therefore, the whole process should adopt the kinetic parameters of the slowest step, thus explaining the linearity observed in the present study as well as that described by Ip & Krishnaveni (1991). Nevertheless, the sensitivity of Sr^{2+} incorporation to Verapamil strongly suggests a common pathway (see above). The suggestion that Ca^{2+} and Sr^{2+} show different molecular behaviours is strengthened by the fact that the solubility of Sr^{2+} in seawater is at least 5-fold lower than that of Ca^{2+}. We were unable to increase the seawater Sr^{2+} concentration up to more than 5 mM because of a strong precipitation of $SrCO_3$ above this concentration.

In conclusion, we have demonstrated that the rate of Sr^{2+} strontium incorporation is constant for at least 9 d. Incorporation of Sr^{2+} is inversely correlated to the rate of calcification, suggesting interaction between these 2 ions. Despite a linear correlation between Sr^{2+} incorporation and Sr^{2+} concentration (up to 3.4 mM), we suggest that Sr^{2+} uptake is mediated by a Verapamil-sensitive Ca^{2+} channel process. Consequently, Sr^{2+} incorporation into the skeleton should be biologically controlled by transcellular mediated steps. All these results should be taken into account before using Sr^{2+} in paleothermometry. Indeed, if the theory of Stoll & Shrag (1998) is correct, i.e. the Sr^{2+} concentration of seawater varies over glacial-interglacial cycles, then the Sr^{2+} concentration in coral skeleton should also be affected and should change according to the external concentration. The Sr^{2+}/Ca^{2+} ratio in molluscs was indeed found to be different between modern and ancient shells (Ragland et al. 1969). Moreover, the Sr^{2+}/Ca^{2+} ratio in coral aragonite skeletons also seems more controlled by calcification processes than by temperature, even if these 2 parameters are closely linked. This conclusion is in agreement with the findings of Cohen et al. (2001) who used an ion microprobe technique. They demonstrated that the Sr^{2+}/Ca^{2+} ratio in the symbiotic coral *Porites lutea* was more related to calcification rates than directly to seawater temperature. They showed that the Sr^{2+}/Ca^{2+} content of the daytime skeleton was always lower than the adjacent nighttime skeleton. The enhancement of calcification in scleractinian corals by photosynthesis has been well documented, with light calcification being 3 to 4 times higher than dark calcification (Barnes & Chalker 1988). According to our results, enhancement of calcification during the day should decrease the rate of Sr^{2+} incorporation, thereby explaining the decrease in the Sr^{2+}/Ca^{2+} ratio observed by Cohen et al. (2001). Therefore, under *in situ* conditions, incorporation of Sr^{2+} should depend on both the coral calcification rate and seawater surface temperature. The same conclusion was reached for mollusc shells, where no correlation between the trace element and the isotope data was found (Leng & Pearce 1999). This suggests that patterns of trace element variations cannot be only related to environmental factors, but that physiological factors affect the incorporation of trace elements into the shells (Leng & Pearce 1999). The relative role of physiological and environmental controls in Sr^{2+} uptake remains to be determined.

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