J. exp. Biol. **159**, 507–513 (1991)
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SHORT COMMUNICATION

ARE CALCIUM AND STRONTIUM TRANSPORTED BY THE SAME MECHANISM IN THE HERMATYPIC CORAL *GALAXEA FASCICULARIS*?

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Accepted 11 June 1991

Information concerning Sr^{2+} content in biogenic carbonates is important for the evaluation of palaeoenvironmental and palaeoecological conditions (Dal Negro and Ungaretti, 1971). Odum (1957) and Goreau (1961) reported that corals were apparently unable to discriminate between Sr^{2+} and Ca^{2+} in the process of skeletogenesis. Later, Kinsman (1969) proposed that reef coral aragonite was in chemical equilibrium with sea water as far as Sr^{2+} was concerned, and suggested the use of the distribution coefficient for Sr^{2+}, K^{Δ}_{Sr}, as an indicator of sediment origin and diagenesis. However, reports from other laboratories indicate significant differences in the relative proportions of Ca^{2+} and Sr^{2+} between coelenterate carbonates and ocean water (Harris and Almy, 1964; Thompson and Livingston, 1970). It is becoming increasingly clear that our earlier expectations for utilizing the chemical composition of biogenic carbonates as an important source of information about palaeoenvironmental conditions are not going to be realized until much is learned about the complex physiological processes involved in skeletal calcification (Weber, 1973).

During calcification, large amounts of Ca^{2+} must be removed from sea water, transported across cells with a typically low intracellular Ca^{2+} concentration (Goreau and Bowen, 1955) and precipitated as an extracellular aragonite skeleton. Chalker (1981), who proved that the deposition of $^{45}Ca^{2+}$ in *Acropora cervicornis* followed Michaelis–Menten kinetics and was inhibited by dinitrophenol, indirectly concluded that Ca^{2+} was actively transported to the site of skeletogenesis. By showing stable Sr^{2+} to be a competitive inhibitor of $^{45}Ca^{2+}$ deposition, Chalker (1981) further concluded that Ca^{2+} and Sr^{2+} depositions in corals involved similar biochemical mechanisms. Goreau (1977) postulated that Sr^{2+} was transported by the same biochemical ion pumps as calcium in corals. He arrived at this conclusion not by experimentation, but from the observation that mitochondrial uptake of Sr^{2+} is not discriminated from calcium uptake. Ip and Krishnaveni (1991), however, showed that stable Sr^{2+} exhibited mixed, instead of purely competitive,

Key words: *Galaxea fascicularis*, coral, calcium, strontium, transport, calcification, skeletogenesis.

inhibition of $^{45}Ca^{2+}$ deposition in *Galaxea fascicularis*. They also proved the kinetics of $^{90}Sr^{2+}$ and $^{45}Ca^{2+}$ depositions in *G. fascicularis* to be dissimilar and proposed that different transport mechanisms might be involved.

It is well established that a membrane-bound Ca^{2+}-stimulated adenosine triphosphatase (ATPase) is associated with the active transport of Ca^{2+} across cell membranes (Schatzmann, 1975; Larsen *et al.* 1978). The most direct study on the Ca^{2+} transport mechanism in corals was by Isa *et al.* (1980), who demonstrated the presence of ATPase activities, activated by either Ca^{2+} or Mg^{2+}, in the crude extract of the animal fraction of the hermatypic coral *Acropora hebes*. However, no attempt was made to elucidate the subcellular origin of the Ca^{2+}-ATPase studied or to verify the presence of a high-affinity component of the enzyme activity, which is needed to compensate for the low Ca^{2+} concentration within the cell. It is also unknown whether Sr^{2+} can replace Ca^{2+} in activating this ATPase. In view of the importance of biogenic carbonates to geochemical and geological problems, the present investigation was undertaken to elucidate whether Ca^{2+} and Sr^{2+} are transported by the same mechanism in *G. fascicularis*.

Galaxea fascicularis (Linnaeus) were obtained from coral reefs off Singapore (103°45′, 1°13′). Twenty to thirty polyps were trimmed off from the colony and pounded 35–40 times with a pestle in a 50 ml mortar. Approximately 250–300 *G. fascicularis* polyps were used for the extraction of Ca^{2+}-ATPase. The crushed polyps were transferred to a beaker containing approximately 1 ml per polyp of ice-cold homogenizing buffer (HB) consisting of 0.25 mol l^{-1} sucrose, 5.0 mmol l^{-1} disodium EDTA and 10 mmol l^{-1} imidazole (pH 7.8). All subsequent isolation steps were performed at 0–4 °C. Coral tissues were dislodged from the skeletal fragments, by rubbing them gently between fingers, stirred and decanted. The decanted sample was then homogenized once by an Ikawerk Staufen Ultra-Turrax (Janke and Kunkel Co.) homogenizer at minimum speed for 15 s to release the zooxanthellae from the coral tissues. A 0.5 ml sample of the homogenate was removed for total chlorophyll *a* determination following the method of Jeffrey and Humphrey (1975). The homogenate was centrifuged at 600 *g* for 15 min to precipitate the zooxanthellae and unbroken cells. The resulting supernatant fluid was decanted through two layers of cheesecloth. Chlorophyll *a* analyses were performed on samples of the supernatant fluid and the resuspended precipitate. Only supernatant fluid containing less than 5 % of the total chlorophyll *a* was used for the Ca^{2+}-ATPase extraction. The supernatant fluid was further homogenized with 15 strokes in an all-glass 15 ml Wheaton tissue grinder and centrifuged at 25 000 *g* for 30 min. The supernatant fluid obtained was further centrifuged at 35 000 *g* for 1 h to obtain the heavy microsomal fraction containing plasma membranes. This precipitate was resuspended in HB by homogenizing with two strokes in a 15 ml Wheaton Teflon pestle homogenizer and centrifuged at 35 000 *g* for 1 h. The washing procedure in HB was repeated once more. The resulting precipitate was next suspended by homogenizing with 2–3 strokes in the Teflon pestle homogenizer in a suspension buffer (SB) containing 0.5 mmol l^{-1} imidazole and 0.6 mmol l^{-1} Tris–HCl (pH 7.8) and centrifuged at 35 000 *g* for 1 h. The pellet

was resuspended in the same buffer and centrifuged twice at 100000 *g* for 1 h to remove the EDTA. The final pellet was resuspended in 12 ml of SB, homogenized till homogeneous and stored at −80 °C. This preparation of coral Ca^{2+}-ATPase was used within 3 days from the time of extraction. Preliminary studies using alkaline phosphatase, assayed according to Bergmeyer *et al.* (1974), as the marker for plasma membranes indicated that this enzyme was enriched 3.1-fold in the heavy microsomal fraction; recovery was 30 %.

To determine the various ATPase activities, the reaction mixture contained, in a final volume of 2 ml, 0.1 ml of sample (0.08–0.09 mg of protein), 40 mmol l^{-1} Tris–HCl (pH 7.0), various concentrations of specified ions and 3 mmol l^{-1} ATP (pH 7.0) (unless stated otherwise). The reaction medium without ATP was preincubated at 27 °C for 10 min and the reaction was initiated by the addition of 0.1 ml of ATP. After 5 min of incubation at 27 °C, the reaction was terminated by the addition of 0.5 ml of 7.2 % tungstosilicic acid in 5 mol l^{-1} sulphuric acid. Inorganic phosphate (P_i) released from ATP hydrolysis was determined according to the method of Martin and Doty (1949). The specified ATPase activity, expressed as μmol P_i released mg^{-1} protein min^{-1}, was obtained as the difference in activities assayed in the presence and absence of the specified ion(s), unless stated otherwise.

Standard Ca^{2+}-ATPase activity was determined in the presence of either 0.005 mmol l^{-1} (high-affinity) or 0.15 mmol l^{-1} $CaCl_2$ (low-affinity). For the studies on the effect of Ca^{2+} concentration on the Ca^{2+}-ATPase activity, a Ca^{2+} concentration range of 0.5 μmol l^{-1} to 10 mmol l^{-1} was used. ATP (0.1 ml) was added at a final concentration of 0.75 mmol l^{-1} to start the reaction. Sr^{2+}-ATPase activity was assayed in the presence of either 0.2 or 1.0 mmol l^{-1} $SrCl_2$. For Mg^{2+}-ATPase and Mn^{2+}-ATPase assays, 5 mmol l^{-1} $MgCl_2$ and 5 mmol l^{-1} $MnCl_2$ were included in the reaction mixture, respectively. Na^+/K^+-ATPase activity was determined in the presence of 100 mmol l^{-1} NaCl, 10 mmol l^{-1} KCl and 5 mmol l^{-1} $MgCl_2$. The Na^+/K^+-ATPase activity was obtained as the difference in enzyme activities assayed in the presence and absence of 1 mmol l^{-1} ouabain.

Protein content of the sample was determined by the method of Lowry *et al.* (1951) with bovine serum albumin (Sigma Chemical Company) as the standard for comparison.

Results were presented as means ± S.E. Student's *t*-test was used to evaluate the difference between means. Differences with $P<0.05$ were taken as significant. Linear regression analyses were based on the least-squares method.

Ca^{2+}-ATPase activity in the plasma-membrane-enriched heavy microsomal fraction isolated from *G. fascicularis* exhibited saturation kinetics. Woolf–Augustinnson–Hofstee transformation of data (Hofstee *et al.* 1959) revealed, for the first time, a high-affinity component of Ca^{2+}-ATPase together with a low-affinity component in the heavy microsomal fraction of a coral (Fig. 1). The K_m and V_{max} values for the high-affinity component were 0.002 mmol l^{-1} and 0.048 μmol P_i mg^{-1} protein min^{-1}, respectively. The respective values for the low-affinity component were 0.16 mmol l^{-1} and 0.75 μmol P_i mg^{-1} protein min^{-1}. Mg^{2+}

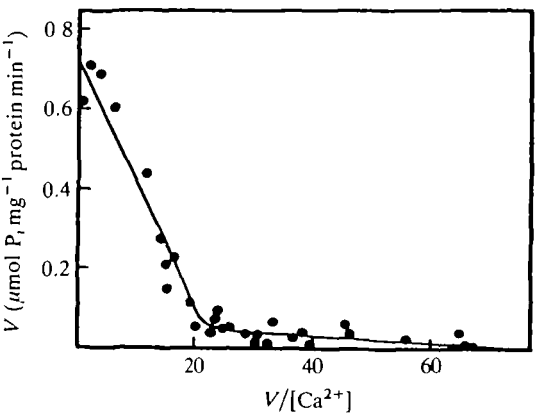

Fig. 1. Woolf–Augustinsson–Hofstee plot of the effect of substrate (Ca^{2+}) concentration on the specific activity (V; μmol P_i mg^{-1} protein min^{-1}) of Ca^{2+}-ATPase in the heavy microsomal fraction isolated from *Galaxea fascicularis*.

is generally regarded as a necessary component for the assay of the plasma membrane Ca^{2+}-ATPase. It is possible that, like the plasma membrane fractions isolated from the corpus luteum (Verma and Penniston, 1981) and adipocytes (Pershadsingh and McDonald, 1980), a minute quantity of membrane-endogenous Mg^{2+} was retained in this preparation. Ruthenium red (0.02 mmol l^{-1}), a potent inhibitor of mitochondrial calcium uptake (Reed and Bygrave, 1974) and the calcium pump of red blood cell ghosts (Sarkadi *et al.* 1980), completely inhibited the activity of the high-affinity Ca^{2+}-ATPase from *G. fascicularis* and significantly inhibited the activity of the low-affinity component by 56.1±6.2 % (N=3). Hence, it is probable that the Ca^{2+}-ATPase assayed in this plasma-membrane-enriched microsomal fraction is the enzyme responsible for the transport of Ca^{2+} in *G. fascicularis*.

Activities of Mg^{2+}-ATPase (1.66±0.12 μmol P_i mg^{-1} protein min^{-1}, N=4), Mn^{2+}-ATPase (1.63±0.03 μmol P_i mg^{-1} protein min^{-1}, N=5) and Na^+/K^+-ATPase (0.23±0.05 μmol P_i mg^{-1} protein min^{-1}, N=4) were also detected in this microsomal fraction extracted from *G. fascicularis*. However, when tested at concentrations of 0.2 and 1 mmol l^{-1}, Sr^{2+} was unable to activate the ATPase activity in the same fraction. Hence, it can be deduced that Sr^{2+} cannot substitute for Ca^{2+} in activating the Ca^{2+}-ATPase in *G. fascicularis*. No Sr^{2+}-ATPase activity was detected in any other subcellular fractions isolated from this coral. Instead, Sr^{2+} competitively inhibited Ca^{2+}-ATPase activities in the heavy microsomal fraction of *G. fascicularis*, with K_i values of 0.0085 mmol l^{-1} for the high-affinity and 2.06 mmol l^{-1} for the low-affinity component (Fig. 2). It is generally accepted that the selectivity of the plasma membrane Ca^{2+} pump for Ca^{2+} is not high, since other divalent metal ions can replace Ca^{2+} in the transport cycle. In resealed red blood cell ghosts, Sr^{2+} is transported in the same way as Ca^{2+} (Schatzmann and Vincenzi, 1969). Hydrolysis of ATP by a purified preparation of the enzyme from human red blood cell membranes is stimulated by alkaline metal earth cations in

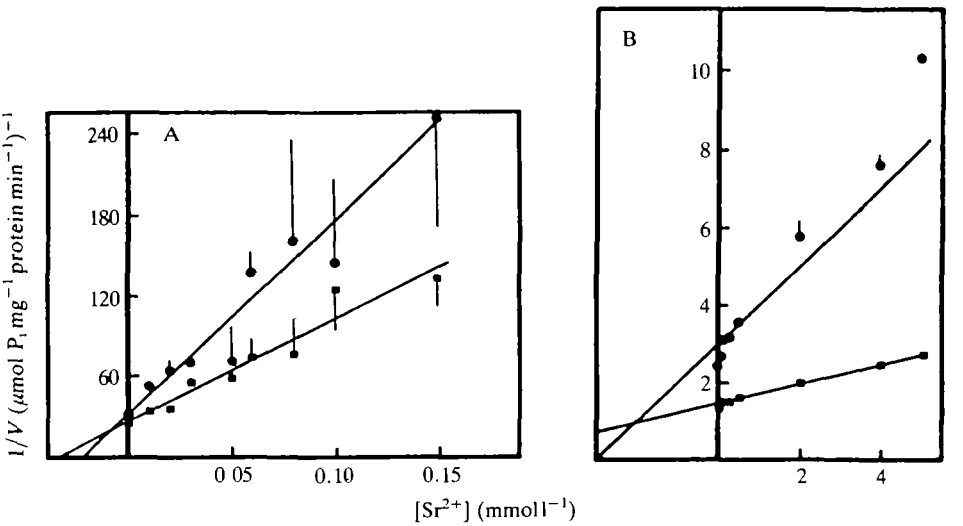

Fig. 2. (A) Dixon plot of the effect of Sr^{2+} concentration on the specific activity (V; μmol P_i mg^{-1} protein min^{-1} ± S.E., N=3) of the high-affinity component of the Ca^{2+}-ATPase, measured at Ca^{2+} concentrations of either 0.006 (●) or 0.01 mmol l^{-1} (■), in the heavy microsomal fraction isolated from *Galaxea fascicularis*. (B) Dixon plot of the effect of Sr^{2+} concentration on the specific activity of the low-affinity component of the Ca^{2+}-ATPase, measured at Ca^{2+} concentrations of either 0.15 (■) or 1.0 mmol l^{-1} (●), in the heavy microsomal fraction isolated from *G. fascicularis*.

the order $Ca^{2+} > Sr^{2+} \gg Ba^{2+}$. Sr^{2+} is about as effective as Ca^{2+} but its apparent affinity is lower. In this regard, the Ca^{2+}-ATPase of *G. fascicularis* is different from those of other organisms reported in the literature.

Contrary to previous hypotheses (Goreau, 1977; Chalker, 1981), the results indicate that Ca^{2+} and Sr^{2+} are transported by different mechanisms in *G. fascicularis*. Although calcification processes in reef corals involve only a negligible change in the molar Sr^{2+}/Ca^{2+} ratio during passage from sea water to the calcification site (Gunatilaka, 1981), the Ca^{2+}-ATPase involved can effectively discriminate between Ca^{2+} and Sr^{2+}. Whether Sr^{2+} transport in *G. fascicularis* involves a mediated or vesicular process without any direct requirement for ATP is uncertain, although Ip and Krishnaveni (1991) have shown that Sr^{2+} deposition in *G. fascicularis* is linearly dependent on Sr^{2+} concentration (up to 20 mmol l^{-1}) and unaffected by the presence of 1 mmol l^{-1} KCN. It is also possible that Sr^{2+} transport in *G. fascicularis* takes a paracellular route, as has been suggested for the epiphyseal growth plate (Krefting *et al.* 1988).

From these results, we can understand why the deposition of Sr^{2+}, unlike that of Ca^{2+}, in *G. fascicularis* is unaffected by light and refractory to 3-(3,4-dichlorophenyl)-1,1-dimethylurea, an inhibitor of photosynthesis (Ip and Krishnaveni, 1991). Reef-building scleractinians are described as hermatypic and contain within their cells large populations of endosymbiotic zooxanthellae. Several hypotheses have been proposed to explain the light stimulation of calcification in corals. It is

possible that photosynthesis acts by providing an energy source for the active transport of Ca^{2+}, either in the form of carbohydrate or by leakage of a high-energy phosphate compound (Chapman, 1974). The observation that no Sr^{2+}-stimulated ATPase activity was detected in *G. fascicularis* would necessarily lead to the conclusion that the ratio of Sr^{2+} to Ca^{2+} depositions will be smaller in the presence of light than in total darkness. Such a conclusion supports the hypothesis that differences in K^{A}_{Sr} in hermatypic and ahermatypic corals are attributable to marked differences in growth rates as a result of the presence of algal endosymbionts in the hermatypes (Thompson and Livingston, 1970). It would also explain previous reports on reef corals that lower skeletal Sr^{2+} contents are associated with higher rates of calcification (Weber, 1973). Hence, the Sr^{2+}/Ca^{2+} ratio of reef coral aragonite may not necessarily reflect the chemical composition of the sea water.

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