Strontium Transport System of Brown Alga *Cystoseira barbata*: Reconstitution on Bilayer Lipid Membranes

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Abstract. A water-soluble protein factor was isolated from the Sr^{2+}-accumulating alga *Cystoseira barbata,* permitting the reconstitution of the Sr^{2+}-transport system of this alga on a bilayer lipid membrane. It was found that the model system studied can distinguish between Sr^{2+} and Ca^{2+} with a discrimination coefficient of 7. The Sr^{2+}/Ca^{2+} discrimination coeficient observed in our model system was considerably higher than the equivalent coefficients for bivalent cations in any hitherto known natural ionophores.

Key words: Strontium — Membrane — Transport — Ion selectivity

Introduction

Among the great variety of sea organisms there are some unique species capable of high affinity Sr^{2+} binding. The acantharia-plankton protozoan the skeleton of which is composed of pure $SrSO_4$ crystals may be given as an example. In these organisms, strontium ions play probably the same role as calcium ions, known to be unique regulators of different cell functions. They appear to have specific binding properties and a Sr^{2+} transport system analogous to that of Ca^{2+}. The brown alga *Cystoseira barbata* that accumulates strontium with the Sr^{2+}/Ca^{2+} selectivity ratio of only 4/1 (Polikarpov 1964) was studied; however, this alga is very useful as it is easily accessible for studies.

Material and Methods

A homogenate was prepared by pounding 10 g of fresh alga in an agate mortar in 100 ml of aqueous medium (AM), containing sucrose 300 mmol/l, Tris HCl 10 mmol/l, pH = 7.5. The homogenate was deproteinized by adding 90 ml of cold acetone to 10 ml of homogenate. The precipitate was dried and pounded. The water-soluble proteins were obtained by extraction of 1.5 g of the powder with 20 ml of deionized water containing 12.3 mg of dithiotreitol; 0.02 ml of ethanolic solution of ionol (100 mg/l) at constant stirring at 4°C overnight. The mixture was then centrifuged at 8000 g for 30 min. The supernatant was used to modify bilayer lipid membranes (BLM). The BLM were formed according to Mueller and Rudin (1963) from lipid extract of ox brain white matter, obtained by the Folch method

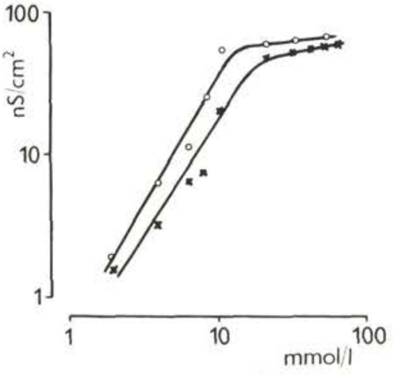

Fig. 1. The effect of $Sr(NO_3)_2$ (o) and $Ca(NO_3)_2$ (x) concentrations on BLM conductance induced by the alga factor at a concentration of 150 mg/l. The aqueous medium was 5 mmol/l acetate buffer, pH = 7.5

(Folch et al. 1957). The membrane active protein factor was added to both sides of BLM. Biionic potentials were measured as they developed across the bilayer due to the presence of 10 mmol/l of Sr^{2+} ions on one side of BLM, and 10 mmol/l of other divalent cations on the other side. The data presented are average values of six experiments.

Results

As it has been shown by our experiments the alga factor studied yields more than a 20-fold increase in BLM conductance when used in a concentration of 150 mg/l in the presence of 10 mmol/l Sr^{2+}. In the case of an ideal cation selectivity the membrane potential is known to change by -28 mV at a 10-fold gradient of bivalent ions. In our experiments the membrane potential was changed by -27 ± 2 mV, and -22 ± 2 mV at 10-fold gradients of Sr^{2+} and Ca^{2+} ions, respectively. Transfer numbers calculated from these membrane potential values were 0.96 and 0.83 for Sr^{2+} and Ca^{2+}, respectively. It was found that a half-maximum increase in BLM conductance induced by this factor occurred at the Sr^{2+} concentration of 9 mmol/l; the respective value for Ca^{2+} ions was 15 mmol/l (Fig. 1).

The following selectivity for bivalent cations could be established by measuring biionic potentials: $Sr^{2+}:Ca^{2+}:Mg^{2+}:Ba^{2+} = 1:0.14:0.17:0.21$.

The study of the influence of pH on the transfer number values and BLM conductance has shown that these parameters were maximal at pH = 7 (Fig. 2). This pH value seems to be optimal for Sr^{2+} complex formation with carboxyl groups of protein molecules participating in the induction of Sr^{2+} conductance. Figure 3 shows the dependence of BLM conductance on the protein concentration in the presence of 10 mmol/l of Sr^{2+} ions. With the increasing protein concentra-

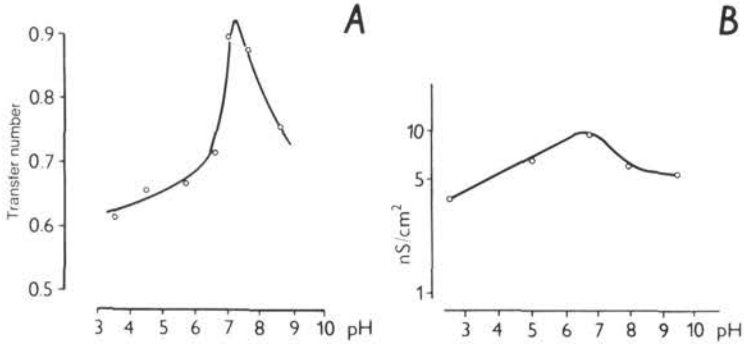

Fig. 2. Effect of pH on the ionic transfer numbers (ordinate) (*A*) and the BLM conductance (*B*), modified by the alga factor. The aqueous medium contained 5 mmol/l of acetate buffer, alga protein factor 150 mg/l, and 10 mmol/l of $Sr(NO_3)_2$.

tion the order of complex formation becomes quadratic at a stoichiometric ratio of transfer molecules per cation of 2:1.

Discussion

Strontium is similar to calcium as for its chemical properties. That is why under experimental conditions Sr^{2+} can substitute for Ca^{2+} in many functional processes. Nevertheless, the cell structures of ordinary „calcium" organisms are able to discriminate between Ca^{2+} and Sr^{2+}. Thus under identical experimental conditions Ca^{2+} accumulates in mitochondria 2 times faster than Sr^{2+} (Tashmukhamedov et al. 1973). The conductance of the sarcoplasmic reticulum transport system reconsittu-

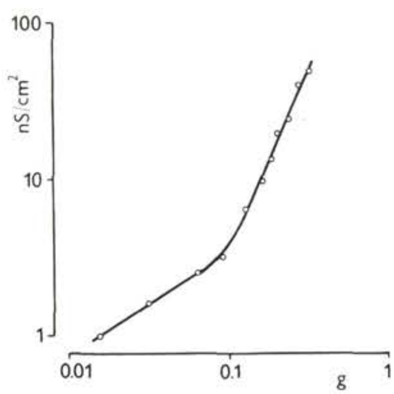

Fig. 3. Effect of the alga protein factor concentration on the BLM conductance. The aqueous medium contained 5 mmol/l of acetate buffer, pH = 7.5, and 10 mmol/l of $Sr(NO_3)_2$.

ted on BLM is 1.5 times higher for Ca^{2+} than for Sr^{2+} (Shamoo and Goldstein 1977). As it was found earlier, *Cystoseira barbata* accumulates strontium with a Sr^{2+}/Ca^{2+} ratio as high as 4/1 (Polikarpov 1964) in the sea water where the Sr^{2+}/Ca^{2+} ratio is 0.08 (Chalker 1981). Under experimental conditions at the Sr^{2+} and Ca^{2+} concentrations being identical, this alga can accumulate bivalent cations with a Sr^{2+}/Ca^{2+} discrimination coefficient of 10 (Polikarpov 1964). In our experiments, the transport system of this alga reconstituted on BLM had a Sr^{2+}/Ca^{2+} discrimination coefficient of 7. This was considerably higher than the discrimination coefficients for bivalent cations in any hitherto known natural ionophores. Strontium ions of this sea organism may function as calcium ions in relation to some bivalent cation-dependent metabolic processes. Acantharia is considered as the most interesting object for the study of Sr^{2+}-dependent processes. The origin of Sr^{2+} accumulating organisms is, in itself, an exciting issue from an evolutionary viewpoint.

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