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ATP-DEPENDENT CALCIUM TRANSPORT IN MEMBRANE VESICLES OF THE CYANOBACTERIUM, *ANABAENA VARIABILIS*

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Transport of Ca^{2+} in membrane vesicles of the cyanobacterium *Anabaena variabilis* has been investigated. The light membranes previously shown to carry a Mg^{2+}-dependent, Ca^{2+}-stimulated ATPase (Lockau, W. and Pfeffer, S. (1982) Z. Naturforsch. 37C, 658–664) accumulate Ca^{2+} upon addition of ATP, whereas the (heavier) thylakoids do not. A stoichiometry of 0.3 Ca^{2+} taken up per ATP hydrolyzed has been determined from initial rates, which is considered to be an underestimation of the true stoichiometry of the pump. Calcium transport and Ca^{2+}-stimulated ATPase activity are both sensitive to Na_3VO_4 (an inhibitor of ATPases forming a phosphorylated intermediate), show the same pH optimum and a comparable dependence on ATP concentration. Calcium transport is also supported by nucleoside triphosphates other than ATP, although at lower rates. Accumulation of calcium is abolished by an ionophore of divalent cations, ionophore A23187, but is resistant to ionophores of monovalent cations and to the inhibitor of F_1-F_0-type ATPases, *N*,*N'*-dicyclohexylcarbodiimide. It is concluded that the ATPase is a primary calcium pump.

Introduction

Two routes of calcium transport have to be considered in bacteria – import and export (review: Ref. 1). Import of calcium, via a uniporter or a leak, is driven by the membrane potential across the plasma membrane. Export of calcium occurs against the membrane potential and often also against a concentration gradient, since the intracellular concentration of free Ca^{2+} seems to be low in general. Calcium is usually exported from a bacterium by a secondary antiport mechanism in exchange for H^+ or, only known for *Halobacterium halobium* [2] and an alkalophilic *Bacillus* [3], in exchange for Na^+. ATP-dependent primary active transport (i.e., a calcium pump) is well-known for animal (see Refs. 4,5) and also for plant cells [6,7]. It has been found so far in only one prokaryote, *Streptococcus faecalis*, where it seems to be involved in calcium export [8]. As reported briefly [9], calcium accumulation by membrane vesicles of *Streptococcus* is sensitive to vanadate and can be reconstituted into liposomes from detergent-solubilized membranes, suggesting the presence of a Ca^{2+}-ATPase similar to eukaryotic ones. There appear to be difficulties, however, in demonstrating a Ca^{2+}-stimulated ATPase activity in this bacterium [8–10].

We have previously shown [11] that the cyanobacterium *Anabaena variabilis* contains a Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase. The enzyme is apparently bound to the plasma membrane (as opposed to the intracytoplasmically located thylakoids) of the bacterium and is inhibited by

Abbreviations: DCCD, *N*,*N'*-dicyclohexylcarbodiimide; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N'*-tetraacetic acid; FCCP, carbonylcyanide-*p*-trifluoromethoxy-phenylhydrazone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Tricine, *N*-tris(hydroxymethyl)methylglycine.

ortho-vanadate. Here we present evidence that the ATPase is a primary calcium pump.

Materials and Methods

Anabaena variabilis Kütz. (ATCC 29413) was grown [12] and membranes isolated therefrom [11] as described. The method includes mechanical disruption of washed spheroplasts followed by sucrose density gradient centrifugation. The fractions of sucrose gradients containing either the thylakoids or the lighter membranes carrying a Ca^{2+}-stimulated ATPase (fractions A and B, respectively, of the previous communication [11]) were pooled, diluted 4-fold with 30 mM Tris-HCl (pH 8.1) and centrifuged for 1 h at $100000 \times g_{max}$. The pellets were suspended in the same buffer to about 1 mg protein/ml.

Uptake of calcium was in most cases assayed with $^{45}CaCl_2$ at room temperature (about 22°C). The reaction mixtures (described in the legends) contained membranes with 0.1 to 0.3 mg protein/ml. At different times, aliquots of 0.05 to 0.15 ml were pipetted into 2 ml of ice-cold assay buffer (lacking $CaCl_2$ and ATP) on a cellulose nitrate filter (0.2 μm pore size, Sartorius, Göttingen, F.R.G.). The solution was sucked off under vacuum and the filter washed with 3.5 ml of the above buffer. Filtration and washing took about 30 s. Radioactivity retained on the filter was counted in 5 ml of a dioxane-based scintillation cocktail. Use of filters of 0.1 μm pore size instead of 0.2 μm did not increase recovery of radioactivity. Calcium uptake was also assayed with the membrane-impermeant optical indicator of Ca^{2+}, murexide. Assay conditions (see Fig. 7) were similar to those recommended [13]. Absorption changes of murexide were followed at 22°C with a temperature-controlled Aminco DW-2 spectrophotometer in the dual wavelength mode (540 minus 507 nm, optical bandpass 4 nm).

ATPase activity was determined at room temperature either by P_i liberation from [γ-^{32}P]ATP according to Ref. 14 or with a coupled spectrophotometric assay (see Ref. 15). In the latter case, the absorption change was followed at 340 minus 400 nm (bandpass 4 nm) at 22°C in the Aminco spectrophotometer. Further details are given in the legends.

Redox difference spectra were recorded as before [16]. Chlorophyll *a* was quantified in methanolic extracts according to Mackinney [17], protein by a modified Lowry procedure [18].

Radiochemicals were obtained from New England Nuclear (Dreieich, F.R.G.). Murexide and EGTA were purchased from Sigma (München, F.R.G.), nucleoside phosphates, phospho*enol*-pyruvate, NADH, hexokinase, lactate dehydrogenase and pyruvate kinase from Boehringer (Mannheim, F.R.G.). All other chemicals were of the highest purity commercially available.

Results

ATP-dependent accumulation of calcium by membrane vesicles from Anabaena

The experiments were performed with membranes from *A. variabilis* separated by sucrose density gradient centrifugation [11]. The procedure

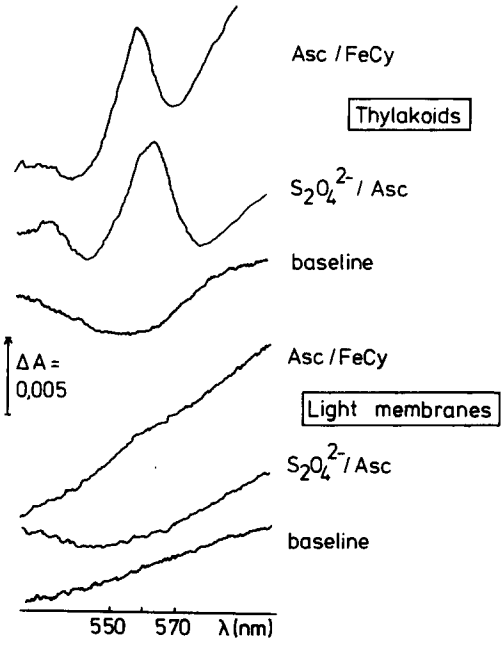


Fig. 1. Redox difference spectra of the thylakoids and the light membranes from *A. variabilis* separated by sucrose density gradient centrifugation. The cuvettes either contained thylakoids with 120 μg protein/ml or light membranes with 280 μg protein/ml in 30 mM Tricine/NaOH (pH 8.0). Asc/FeCy, sodium ascorbate minus potassium ferricyanide; $S_2O_4^{2-}$/Asc, $Na_2S_2O_4$ minus sodium ascorbate; baseline, potassium ferricyanide minus potassium ferricyanide. The protein : chlorophyll ratio of the thylakoids was 5, that of the light membranes was about 300.

separates the chlorophyll-containing thylakoids from lighter, yellowish-brown membranes which carry a Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase as the dominating phosphohydrolase. This latter membrane is thought to be the plasma membrane of the cyanobacterium.

In contrast to the thylakoids, the light membranes do not contain detectable amounts of *b*- or *c*-type cytochromes (redox difference spectra of Fig. 1). They should, therefore, not possess an electron-transport chain or any Ca^{2+} transport linked to electron transport, as found in mitochondria and certain bacteria (see Refs. 1,19). Calcium is taken up by the light membranes upon addition of ATP, Mg^{2+} being already present in the reaction mixture (Fig. 2A). At a concentration of 10 μM, the potent protonophore FCCP does not inhibit calcium uptake (see also Fig. 8). Sequestered calcium appears to be accumulated inside the vesicles since uptake is rapidly reversed by ionophore A23187, an ionophore of divalent cations. Addition of ionophore A23187 before ATP prevents any calcium accumulation (Fig. 7). The initial rate of calcium uptake is half-maximal at a concentration of Ca^{2+} slightly below 10 μM and is saturated at a concentration of about 30 μM (Fig. 2B). In contrast to the light membranes, the thylakoids do not show significant ATP-dependent calcium uptake (Fig. 2A).

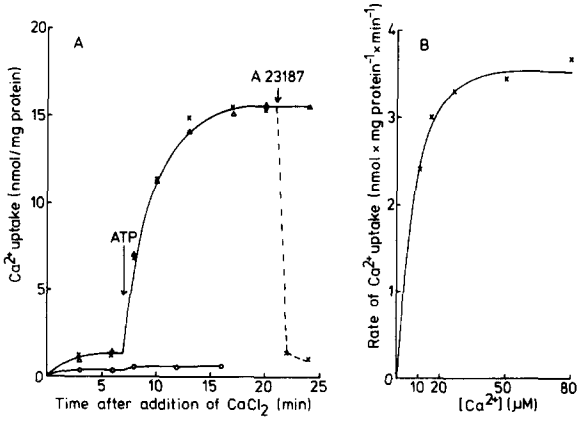


Fig. 2. ATP-dependent uptake of calcium by membranes from *Anabaena variabilis* separated by sucrose density gradient centrifugation. (A) Symbols: Δ and $\times$, light membranes with (Δ) and without ($\times$) 10 μM FCCP; O, thylakoids. At zero time, $^{45}CaCl_2$ was added to a final concentration of 50 μM, at 7 min ATP to a final concentration of 3 mM. Reaction mixture: 50 mM Tricine/NaOH (pH 8.0), 50 mM KCl, 5 mM $MgCl_2$, 50 mM sucrose and either the light membranes (0.1 mg protein/ml) or the thylakoids (0.17 mg protein/ml). Where indicated, ionophore A23187 (1 mg/ml of ethanol) was added to one of the samples to a final concentration of 5 μg/ml ($\times$). FCCP (2 mM in ethanol) was added to the other sample 5 min before addition of $CaCl_2$. (B) Dependence of the initial rate of calcium uptake on the concentration of Ca^{2+}. The reaction mixtures contained 50 mM Hepes/NaOH (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 100 mM sucrose and the light membranes with 0.09 mg protein/ml. The high concentration of $MgCl_2$ was used to minimize binding of Ca^{2+} [13]. 10 min after addition of $^{45}CaCl_2$, ATP was added to 0.8 mM and calcium accumulation determined 1 min thereafter. The values are corrected for radioactivity recovered on the filters in the absence of ATP. Ca^{2+} contaminating the reaction mixture (9 μM) was determined by titration of the absorption change (540 minus 507 nm) of 60 μM murexide with EGTA and was taken into account. Absorption changes of murexide were calibrated with $CaCl_2$ [13]. The specific radioactivity in the individual reaction mixtures range from 53000 to 122000 cpm per nmol Ca^{2+}.

Back-flux of accumulated calcium is observed when external calcium is chelated with EGTA (Fig. 3A). EGTA simultanously inhibits the rate of ATP hydrolysis by about 80% (insert of Fig. 3A, compare Ref. 11). Fig. 3A also shows that the rate of ATP hydrolysis by the membrane preparation is independent of the extent of Ca^{2+} accumulation (the concentration of external Ca^{2+} decreases by about 15% during the experiment; compare the murexide experiment described below). Back-flux of calcium also occurs when ATP is trapped with hexokinase/glucose (Fig. 3B). The experiment shows that Ca^{2+} efflux does not require ATP, at variance to the findings with *Streptococcus* membranes [8]. In the steady state of accumulation, Ca^{2+} turns over rapidly, as demonstrated by $^{45}Ca^{2+}$ uptake into vesicles preloaded with $^{40}Ca^{2+}$ (Fig. 3C). The initial rate of calcium exchange is somewhat higher than the initial rate of net calcium uptake, presumably because of the initially higher specific radioactivity of external calcium in the exchange experiment. Since the rate of ATP hydrolysis does not change, the extent of calcium accumulation by the vesicles appears to be determined by the apparently constant rate of uptake and by the permeability of the membranes to calcium in back-flux. It is unknown whether back-flux occurs via a specific carrier or reflects unspecific leakiness of the membranes.

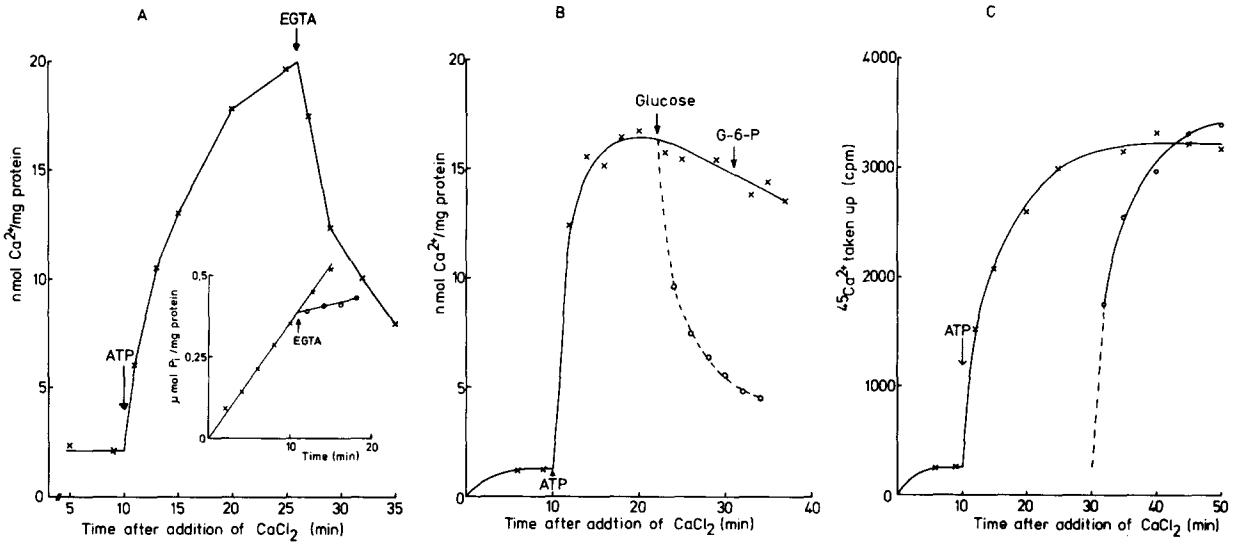
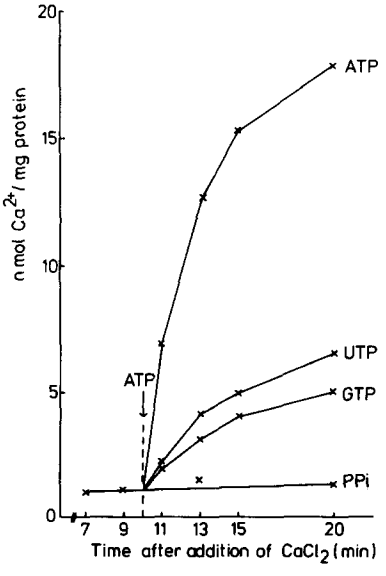


Fig. 3. Evidence for calcium back-flux. (A) Back-flux after complexation of external Ca^{2+} with EGTA. Where indicated, 250 mM EGTA (adjusted to pH 7.5 with NaOH) was added to a final concentration of 0.5 mM. The insert shows the kinetics of ATP hydrolysis measured with the same membrane preparation in a parallel experiment (radiochemical method). After 10 min, 0.5 mM EGTA was added to an aliquot of the reaction mixture (O——O). Assay conditions: 50 mM Hepes/NaOH (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 50 mM sucrose and membranes with 0.12 mg protein/ml. 50 μM $CaCl_2$ (labelled with 14500 cpm $^{45}Ca^{2+}$/nmol in the uptake experiment) was added 10 min before 1 mM ATP (labelled with 1320 cpm [γ-^{32}P]ATP/nmol in the ATPase experiment). (B) Back-flux of calcium after trapping of ATP. The reaction mixture contained 50 mM Tricine/NaOH (pH 8.0), 5 mM KCl, 5 mM $MgCl_2$, 100 mM sucrose, 25 μM $^{45}CaCl_2$ (26800 cpm/nmol), 5 IU hexokinase/ml and membranes with 0.155 mg protein/ml. Uptake was initiated by 0.5 mM ATP. Where indicated, glucose (10 mM final concentration) was added to one part of the reaction mixture (O——O) and glucose 6-phosphate (1 mM final concentration) to another (×——×). (C) Uptake of $^{45}Ca^{2+}$ in the steady state of calcium accumulation. At zero time, one sample (×——×) received 25 μM unlabelled $CaCl_2$ plus tracer, the second one only unlabelled $CaCl_2$. After 10 min, 3 mM ATP was added to both samples. At 30 min, an appropriate amount of tracer was added to the second sample (O——O). The radioactivity of aliquots of 50 μl was assayed by the filtration method. Other conditions were as described under (B).



Nucleotide specificity of calcium accumulation

In addition to ATP also UTP and GTP support calcium uptake (Fig. 4). ITP and CTP supported uptake with kinetics identical to GTP (omitted from Fig. 4). These triphosphates are hydrolyzed by the membranes at lower rates than is ATP [11]. Significant accumulation of calcium was not observed with ADP and *p*-nitrophenylphosphate (data not shown).

Fig. 4. Substrate specificity of calcium uptake. At the arrow, substrates were added to a final concentration of 3 mM. The reaction mixture contained 50 mM Tricine/NaOH (pH 8.0), 5 mM $MgCl_2$, 100 mM sucrose, 50 μM $^{45}CaCl_2$ (19700 cpm/nmol) and membranes with 0.24 mg protein/ml. PP_i stands for sodium pyrophosphate.

TABLE I

INHIBITION OF ATPase ACTIVITY AND OF CALCIUM ACCUMULATION BY *ORTHO*-VANADATE

For measurement of ATPase activity (hydrolysis of [γ-^{32}P]ATP), the reaction was terminated 5 min after addition of ATP. The initial rate of calcium accumulation was determined 1 min, the extent of accumulation 15 min after ATP addition by the filtration method. The values are corrected for calcium bound in the absence of ATP. *ortho*-Vanadate was added 20 min and $CaCl_2$ 10 min before ATP. The reaction mixtures contained: 50 mM Tricine/NaOH (pH 8.0) or 50 mM Hepes/NaOH (pH 7.5), 10 mM $MgCl_2$, 50 mM KCl, 50 mM sucrose, 50 μM $CaCl_2$ and membranes with 0.24 mg protein/ml. The final concentration of ATP was 2 mM. Specific radioactivities of [γ-^{32}P]ATP and of $^{45}CaCl_2$ were as in Fig. 3A.

	A. ATPase activity (nmol/mg protein per min)		B. Calcium accumulation			
			I. Initial rate (nmol/mg protein per min)		II. Extent (nmol/mg protein)	
	–	+40 μM Na_3VO_4	–	+40 μM Na_3VO_4	–	+40 μM Na_3VO_4
pH 8.0	56 (100%)	22 (39%)	5.8 (100%)	2.4 (41%)	18.2 (100%)	9.0 (49%)
pH 7.5	46 (100%)	3 (6.5%)	4.1 (100%)	0.1 (2.5%)	15.8 (100%)	0.4 (2.5%)

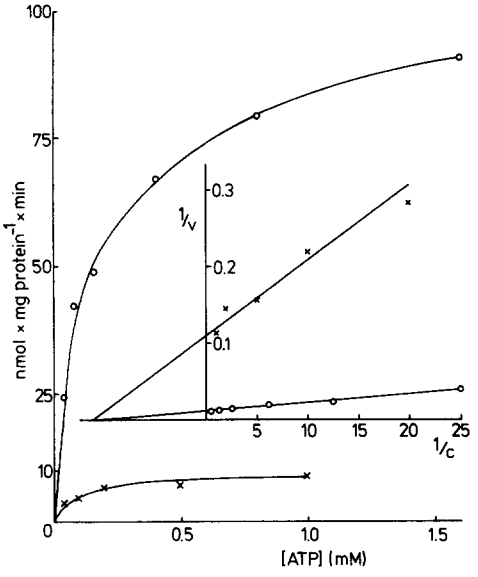


Fig. 5. Dependence of ATPase activity (O——O) and of calcium uptake (×——×) on the concentration of ATP. The reaction mixtures contained 50 mM Tricine/NaOH (pH 8.0), 50 mM KCl, 5 mM $MgCl_2$, 100 mM sucrose, 0.5 mM phospho*enol*pyruvate, 0.2 mM NADH, 4 IU/ml of pyruvate kinase, 2 IU/ml of lactate dehydrogenase, 50 μM $CaCl_2$ (labelled with 22500 cpm $^{45}Ca^{2+}$/nmol in the uptake experiment) and membranes. Calcium accumulation was assayed 1 min after addition of ATP to reaction mixtures containing 0.09 mg protein/ml. The values are corrected for $^{45}Ca^{2+}$ bound in the absence of ATP. The ATPase experiments (spectrophotometric assay) were started by addition of membranes to a concentration of 0.045 mg protein/ml, since the ATP solution contained a trace of ADP.

Correlation of calcium uptake with the ATPase activity

The rather strong inhibition of ATPase activity by EGTA (Fig. 3A), similar in extent to the inhibition found with sarcoplasmic reticulum [20], already suggests that the phosphohydrolase activity of the cyanobacterial membrane is primarily due to a Ca^{2+}-stimulated ATPase acting as the pump.

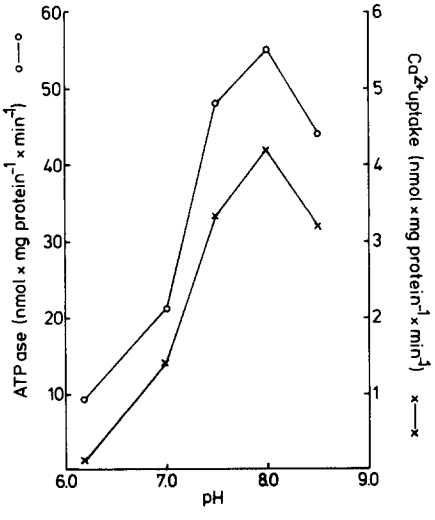


Fig. 6. Dependence of ATPase activity (O——O) and of calcium uptake (×——×) on pH. The assay conditions were as in Fig. 5, except that the following buffers were used at a concentration of 50 mM: 2-(*N*-morpholino)ethanesulphonic acid/NaOH (pH 6.2); Hepes/NaOH (pH 7.0, 7.5); Tricine/NaOH (pH 8.0, 8.5). ATP was added to 2 mM.

Three lines of evidence further strengthen this conclusion. Firstly, ATPase activity and initial rate of calcium uptake have a comparable dependence on ATP concentration (Fig. 5), half-maximal rates occurring at about 90 μM ATP. Secondly, ATPase activity and initial rate of calcium uptake have a similar pH profile (Fig. 6). Thirdly, both activities are inhibited by *ortho*-vanadate (Table I). The only other known cyanobacterial ATPase, the coupling factor, is insensitive to this inhibitor [11]. With 40 μM vanadate, calcium uptake is only partially inhibited at pH 8.0 (the pH optimum of ATPase activity, Fig. 6), but nearly completely at pH 7.5, which corresponds to the inhibition of ATPase activity at the respective pH values (Table I). Increased affinity to vanadate at lower pH has been observed before for yeast and animal ATPases [21,22].

Stoichiometry

Comparison of the initial rate of calcium uptake as determined by the filtration method (3.5 nmol/mg protein per min, Fig. 3A) with the rate of ATP hydrolysis (36 nmol/mg protein per min) gives a stoichiometry of 0.1 Ca^{2+} transported per ATP hydrolyzed, assuming that the Ca^{2+}-stimulated ATPase is the only phosphohydrolase present in the membranes (see above). A similar value can be calculated from the data of Figs. 5 and 6 and Table I. Continuous measurement of calcium uptake by monitoring external Ca^{2+} with the metallochromic indicator murexide [13,23] under conditions identical to those of the experiment shown in Fig. 3A (except for the presence of murexide) gave an approx. 3-fold higher initial rate of calcium uptake (12 nmol/mg protein per min) and of the extent of its accumulation (52 nmol/mg protein, Fig. 7). The properties of calcium transport measured by the murexide method are otherwise comparable to those found with the filtration method. A control experiment showed that murexide has no influence on the ATPase activity. Using the value for initial uptake, a stoichiometry of 0.33 Ca^{2+} transported per ATP hydrolyzed is obtained. Because of the high permeability of the membrane vesicles to Ca^{2+} (Fig.

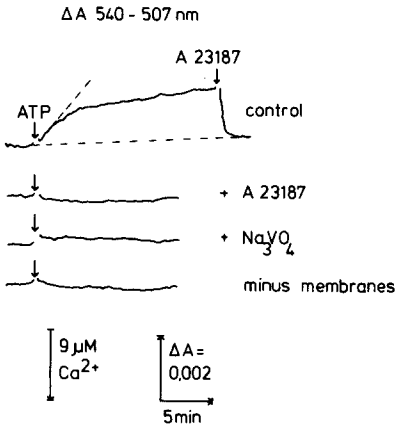


Fig. 7. Measurement of calcium uptake with an optical indicator, murexide. The assay is described under Methods. Na_3VO_4 (40 μM) and ionophore A23187 (4 μg/ml) were added to the reaction mixture containing membranes 10 min before addition of $CaCl_2$. Assay conditions were as in Fig. 3A, except that the solutions contained 60 μM murexide and membranes with 0.12 mg protein/ml. At the arrows, ATP was added to 0.8 mM. Absorption changes of murexide were calibrated with $CaCl_2$ and EGTA [13].

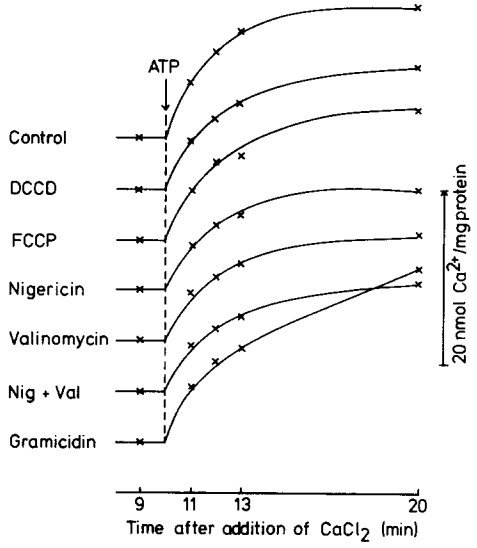


Fig. 8. Effects of *N,N'*-dicyclohexylcarbodiimide and of ionophores on ATP-dependent calcium uptake. The inhibitor and the ionophores were added to the reaction mixture containing membranes (0.12 mg protein/ml) 10 min before addition of $^{45}CaCl_2$ (25 μM, 19000 cpm/nmol) as ethanolic solutions. All assays contained 0.4% (v/v) ethanol. The final concentrations were: *N,N'*-dicyclohexylcarbodiimide (DCCD), 0.4 mM; carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), 4 μM; nigericin (Nig), 4 μg/ml; valinomycin (Val), 4 μg/ml; gramicidin D, 4 μg/ml. Other assay conditions: 50 mM Hepes/NaOH (pH 7.5), 5 mM KCl, 5 mM $MgCl_2$, 100 mM sucrose. At the arrow ATP was added to 2 mM.

3) and the possible existence of unsealed vesicles, the observed stoichiometries are probably understimations of the true stoichiometry of the calcium pump.

Effects of DCCD and of ionophores on calcium accumulation

To obtain information on the nature of the transport system, the effects of DCCD and of ionophores of monovalent cations were studied. DCCD is an inhibitor of proton-translocating ATPases of the F_1-F_0 type such as the cyanobacterial coupling factor [24—26], but inhibits the cyanobacterial Ca^{2+}-stimulated ATPase only marginally [11]. A summary of the ion-translocating properties of the ionophores used, which include a protonophore (FCCP), an ionophore primarily of potassium (valinomycin) and a channel-former with rather broad specificity (gramicidin D), can be found in Ref. 27. Neither DCCD nor the ionophores, all used at high concentration, abolish calcium accumulation by the vesicles (Figs. 2 and 8). On the basis of these experiments, it is concluded that Ca^{2+} is not taken up in response to an ATP-induced gradient of protons or other cations via an antiporter. In this case, uptake should be strongly inhibited by either DCCD or at least one of the ionophores employed. Calcium transport into the vesicles is thus in all probability a primary active process – a calcium pump.

Discussion

The plasma membrane of cyanobacteria has not been isolated and biochemically characterized before. As previously discussed [11], the Ca^{2+}-stimulated ATPase of *Anabaena* is most probably located on this membrane, the principal argument being that, starting from spheroplasts, the ATPase activity can be largely recovered on membranes separated from the heavier thylakoids by sucrose density gradient centrifugation. Since the plasma membrane is the site of calcium transport in other bacteria (see Ref. 1), the observed calcium accumulation by the light membranes from *Anabaena* supports our previous conclusion. Alternative possibilities would be that cyanobacteria contain a so-far unknown calcium-sequestering organelle within their cytoplasm or that, during the isolation procedure, their thylakoids specifically split into chlorophyll-plus cytochrome-containing vesicles and into vesicles deficient in chlorophyll and cytochromes which carry the calcium pump (compare Figs. 1 and 2 and Ref. 11). These last-mentioned possibilities may be considered as remote. The described calcium pump may thus serve as a first simple marker enzyme for further studies on the plasma membrane of *Anabaena*. In analogy to the conclusion of Kobayashi et al. [8] for *Streptococcus*, it is suggested that the physiological role of the pump is calcium excretion from the cyanobacterial cell, calcium being accumulated (Fig. 2A) by everted vesicles formed during the mechanical disruption of spheroplasts (cf. Ref. 28).

Ion transport ATPases other than the F_1-F_0-type ATPase have been detected in bacteria only recently. Epstein and co-workers [29] and Wieczorek and Altendorf [30] described a K^+-stimulated ATPase involved in K^+ transport of *Escherichia coli*, Heefner and Harold [31] a Na^+-stimulated ATPase involved in Na^+ transport of *S. faecalis*. The observation of ATP-dependent primary active transport of Ca^{2+} [8] and of K^+ [32] by *Streptococcus* provides evidence for the presence of corresponding ATPases in that bacterium, although an ATPase activity stimulated by the transported ion has not been found [8] or has not been sought [32]. It may be masked by the high ATPase activity of the F_1-F_0-type ATPase present in the bacterium. Hydrolysis of ATP by the cyanobacterial F_1-F_0-type ATPase (their coupling factor) is slow in cell-free extracts unless the enzyme is activated by certain treatments [24–26,33]. This may be the chief reason why the Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase of *Anabaena* is easily detected and is the dominant ATP hydrolase on the light membranes (Fig. 3A, Ref. 11).

Resistance of calcium accumulation by the cyanobacterial membrane vesicles to a variety of ionophores of monovalent cations and to *N*,*N*′-dicyclohexylcarbodiimide (Figs. 2A and 8) and its sensitivity to *ortho*-vanadate (Table I) strongly suggest it to be primary active transport not requiring a chemiosmotic force. A stimulatory effect of gramicidin D on long-term accumulation was noted (Fig. 8), which may reflect a secondary effect of the channel-former on the membrane properties not related to its ionophoric properties.

On the other hand, none of the ionophores increased the initial rate of calcium uptake by the membrane vesicles. This raises the question of whether the transport is electrogenic or not. In pH jump experiments employing the indicator for a pH gradient, 9-aminoacridine, under conditions similar to those used before for determination of the pH gradient of thylakoids of *Anabaena* [34] it was found that the calcium-accumulating vesicles are permeant to protons (unpublished experiments). This property of the vesicles used may explain why a stimulatory effect of ionophores on calcium transport was not observed: it makes it impossible, however, to exclude an electrogenic nature of the transport.

Comparison of the cyanobacterial primary active calcium transport described here with that of *Streptococcus*, its only known counterpart in prokaryotes, reveals similarities and differences in details. Both pumps are inhibited by *ortho*-vanadate (Table I; [9]). The streptococcal one, however, has been reported [8] to be specific for ATP, whereas the cyanobacterial one is not, but shows a preference for ATP (Fig. 4). A further difference is its greater affinity for Ca^{2+}, the half-maximal rate of accumulation requiring less than 10 μM Ca^{2+} (Fig. 2B) as compared to 150 μM for *Streptococcus* membranes [8].

As suggested by Heefner and Harold [31,35], the reason why *Streptococcus* does contain several primary active transport systems may be that it is a glycolyzing bacterium generating only a relatively small electrochemical gradient of protons across its plasma membrane, which may be insufficient for maintaining the proper ion gradients via secondary active transport systems. However, cyanobacteria usually do not glycolyse but generate ATP exclusively by electron transport-linked phosphorylation [36]. The occurrence of a primary active, ATP-dependent calcium transport system in *Anabaena* nevertheless fits into the suggestion of Heefner and Harold. Only a low [37] or no (Fig. 1, [11]) electron transport capacity is attributed to the cyanobacterial plasma membrane, the principal site of electron flow being the intracytoplasmically located thylakoids. A correspondingly low electrochemical gradient of protons across the plasma membrane of *Anabaena* has been reported [38].

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