Mg^{2+} REGULATES THE ATPase ACTIVITY OF A CALCIUM TRANSPORT PROTEIN BY INTERACTING WITH BOUND PHOSPHATIDIC ACID

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1. Introduction

The liquid-crystalline to gel phase transition of saturated lecithins has a profound effect on the ATPase activity of the calcium transport proteins from sarcoplasmic reticulum of rabbit skeletal muscle: when the associated lipid is in the crystalline phase, the transport protein exhibits no ATPase activity [1,2]. Träuble and Eibl [3] have recently shown that the phase transition temperature of phosphatidic acid bilayers is increased when the divalent cation concentration is raised. This prompted the interesting speculation that physiological effectors such as Mg^{2+} might regulate the function of some membrane proteins indirectly by interacting with negatively-changed lipids in the membrane.

Here we have used a procedure in which the lipids associated with the pure calcium transport protein are replaced by titrating with defined exogenous lipid in cholate. We use this technique to prepare a complex of pure calcium transport protein with dioleoyl phosphatidic acid (DOPA), which demonstrates the feasibility of this novel cellular control mechanism. Mg^{2+} was found to cause a marked inhibition of the ATPase activity of this complex, which we attribute to the interaction of the Mg^{2+} with the negatively-charged phosphatidic acid headgroup. On the other hand, divalent cations interact very weakly with the zwitterionic lecithins, and Mg^{2+} was found to have little effect on the activity of the protein complexed with dioleoyl lecithin (DOL).

2. Materials and methods

In the lipid titration procedure, the pure calcium transport protein (ATPase) and associated defined lipid [4,5] is suspended in cholate together with a large excess of exogenous lipid until equilibration of the lipid pools has been reached. The ATPase activity is then determined by diluting a small aliquot of the mixture 400-fold into the assay medium; this dilution serves to remove nearly all of the cholate associated with the lipid-protein complex. The ATPase activity of this diluted sample then reflects the interaction between the exogenous lipid and the calcium transport protein.

The experiments discussed below involve a comparison between ATPase which has been titrated with either DOL (DOL–ATPase) or DOPA (DOPA–ATPase). The enzymes were prepared by incubating 0.37 mg DOL–ATPase [5] together with 2.0 mg lipid (DOL or DOPA) and 1.0 mg cholate in a total volume of 100 μl of 250 mM sucrose, 50 mM potassium phosphate buffer pH 8.0, 1 M KCl and 5 mM MgATP. The DOL titration mixture contained more than 99.5% DOL; the DOPA titration mixture contained 96% DOPA and 3.5% DOL. After equilibration of the lipid

pools 5 μl samples were diluted in 2.0 ml of 100 mM triethanolamine hydrochloride–KOH buffer pH 7.2, 1 mM ATP, 50 μM EGTA, 100 μM Ca^{2+}, 0.5 mM phosphoenol–pyruvate and 0.15 mM NADH containing 50 μg pyruvate kinase and 100 μg lactate dehydrogenase. The reaction was started by the addition of Mg^{2+} and the rate of NADH oxidation reached 99% of the steady state rate of ATP hydrolysis within 20 sec at all assay temperatures [6]. In all assays > 98% of the ATPase activity was inhibited by 1 mM EGTA.

Valid results can only be obtained using this lipid titration technique when certain criteria have been met which are discussed in detail elsewhere [2]. Here we show that the DOPA– and DOL–ATPase complexes satisfy these criteria.

(i) The protein must not be irreversibly denatured by the exogenous lipid. As shown in fig.1, the ATPase activity is lowered when DOPA replaces DOL as the lipid which is directly associated with the calcium transport protein. Once equilibration of the lipid pools has been reached, the ATPase activity remains constant. The original activity may then be recovered by adding an excess of DOL. Further addition of DOPA lowers the activity again, demonstrating that lipid titrations using DOPA and DOL have no adverse effects on the integrity of the calcium transport protein.

(ii) Cholate must not affect the interaction between the calcium transport protein and the added exogenous lipid. Although only 1% of the cholate in the original titration mixture remains bound to the ATPase after dilution into the assay medium, this represents about 10 moles cholate/mole ATPase. It is therefore important to show that the effect of Mg^{2+} is not a result of its interaction with the negatively charged cholate in the complexes rather than the phosphatidic acid. The equilibrated titration complexes were washed by centrifugation and dialyzed against XAD-2 until the level of cholate had fallen below one mole/mole ATPase. The effect of Mg^{2+} on DOPA-ATPase activity described below, was in no way diminished by this procedure.

(iii) The lipid directly interacting with the protein must reflect the composition of the entire lipid pool. There are about 850 moles of lipid/mole ATPase in the titration mixtures compared with about 30 moles of lipid that are directly bound to one mole of ATPase and are necessary to maintain its activity [1,2]; we must therefore show that these 30 molecules of lipid reflect the composition of the much larger lipid pool in the titration mixture. By preparing ATPase associated with this minimal number of lipid molecules from the equilibrated titration complexes [4,5], the composition of the lipid directly associated with the protein was shown to be that expected for complete equilibration of the lipid pools in the original titration mixtures. However, the pure DOPA–ATPase prepared in this fashion was very inactive and the experiments described below were therefore carried out using titration complexes of DOPA–ATPase. The residual activity of the cholate-free transport protein associated with about 30 molecules of DOPA was inhibited by Mg^{2+} in an identical fashion to the titration complex of the DOPA–ATPase; this further validates the titration technique as a means of investigating the interaction of Mg^{2+} with DOPA–ATPase.

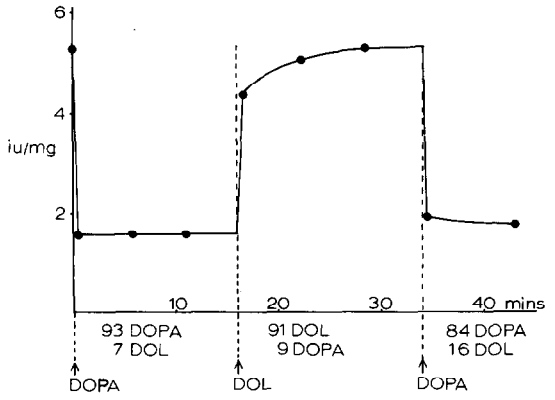

Fig.1. Reversibility of the lipid titration procedure. DOL–ATPase in cholate was treated sequentially with DOPA, DOL and finally DOPA such that the percentage of either lipid in the incubation mixture was that indicated under the abscissa. ATPase activity was assayed at 25°C.

3. Results and discussion

At any given assay temperature the activity of DOPA–ATPase titration complex is inhibited by increasing concentrations of Mg^{2+} in the assay medium: in contrast, the activity of DOL–ATPase is little affected by similar changes in the concentration of Mg^{2+} (fig.2). This suggests that the inhibitory effect of Mg^{2+} on DOPA–ATPase is a result of its interaction with the phosphatidic acid headgroup. The inhibition by Mg^{2+}

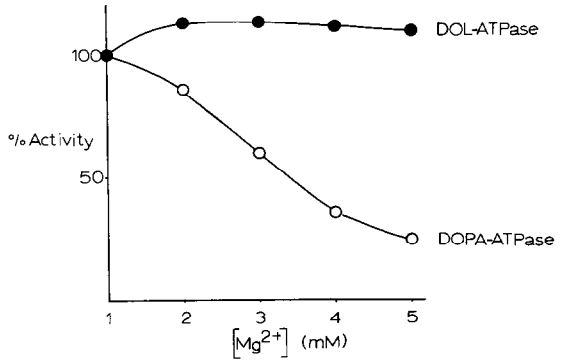

Fig.2. Inhibition of DOPA-ATPase by increasing concentrations of Mg^{2+} in the assay medium. ATPase activity was assayed at 25°C and the 100% activity of DOL–ATPase was 2.46 IU/mg and DOPA–ATPase was 0.69 IU/mg.

can also be observed during the course of any single assay by the sequential addition of small aliquots of Mg^{2+} (fig.3a). This inhibition is relieved by addition of

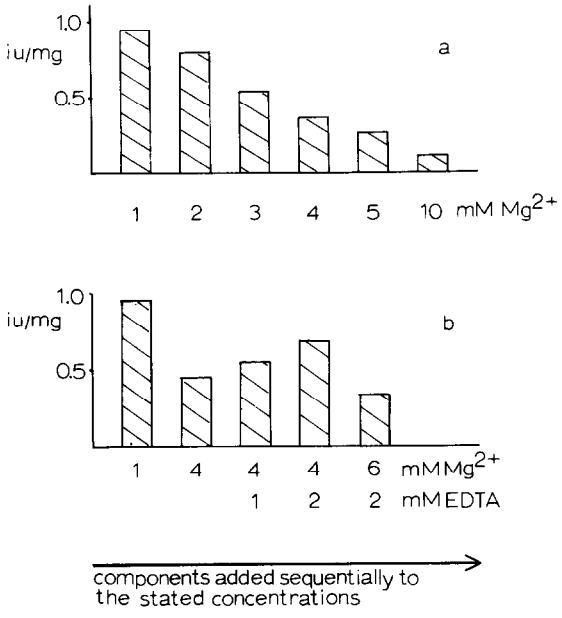

Fig.3a. Inhibition of DOPA–ATPase by increasing concentrations of Mg^{2+} added sequentially during the course of a single ATPase assay at 25°C.

Fig.3b. Relieving the inhibition of DOPA–ATPase by Mg^{2+} by adding EDTA. Addition of Mg^{2+} or EDTA was performed sequentially during the course of a single ATPase assay at 25°C.

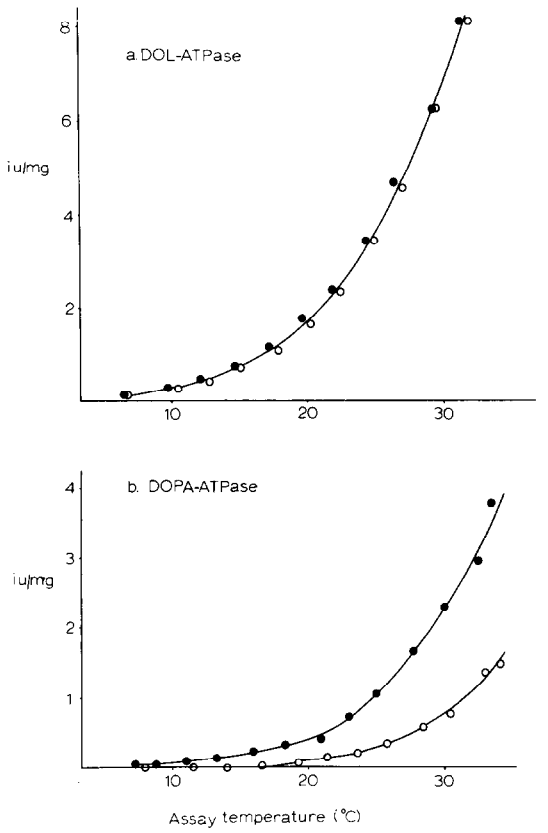

Fig.4. The effect of Mg^{2+} on the temperature-activity profiles for a. DOL–ATPase and b. DOPA–ATPase. The two fixed concentrations of Mg^{2+} were 1.5 mM (•) and 6 mM (○) and the pH of the assay cocktail was maintained at 7.2 at all temperatures.

EDTA which may be overcome by further addition of Mg^{2+} (fig.3b). To analyse this further, detailed temperature-activity plots were constructed at two Mg^{2+} concentrations for both DOL– and DOPA–ATPase. As shown in fig.4a, changes in the level of Mg^{2+} have little effect on the temperature profile for DOL–ATPase activity but have a large effect on DOPA–ATPase activity at all assay temperatures.

Inhibition by Mg^{2+} may be attributable to two related components. Träuble and Eibl [3] have clearly shown that Mg^{2+} raises the phase transition temperature for phosphatidic acid bilayers and we have recently shown [1,2] that the calcium transport protein exhibits no ATPase activity when the associated lipid is in

the crystalline phase. At 1.5 mM Mg^{2+}, DOPA–ATPase is active at all assay temperatures but is only active above about 17°C in the presence of 6 mM Mg^{2+} (fig. 4b). This observation is consistent with Mg^{2+} raising the phase transition temperature of DOPA. DOPA–ATPase is also less active at all assay temperatures when the Mg^{2+} concentration is raised from 1.5 to 6 mM (fig.4b). This suggests that Mg^{2+} bound to the phosphatidic acid headgroup of DOPA may be more inhibitory than the free headgroup.

The overall effect of Mg^{2+} on DOPA–ATPase is a marked inhibition, which results from the interaction of Mg^{2+} with DOPA associated with the calcium transport protein. Although this is a highly simplified system, which is composed almost entirely of defined components, it serves as a model for the regulation of membrane proteins through such changes in their lipid interactions caused by physiological effectors.

Acknowledgements

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