Crystallization of Ca\textsuperscript{2+} ATPase in sarcoplasmic reticulum vesicles by phospholipase treatment

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Ca\textsuperscript{2+} ATPase molecules in sarcoplasmic reticulum, isolated from rabbit skeletal muscle, have been induced to crystallize into two-dimensional arrays by incubating the vesicles with phospholipase A\textsubscript{2} and dialysing against dilute Tris/HCl buffer. These crystals differ in shape and size from those produced by treatment of the sarcoplasmic reticulum vesicles with Na\textsubscript{3}VO\textsubscript{4}. However, the unit-cell dimensions of both types of crystals are similar. The differences in shape and size are presumably due to differences in the mechanisms of crystal formation induced by treatment with phospholipase and Na\textsubscript{3}VO\textsubscript{4}.

Sarcoplasmic reticulum (SR) of the skeletal muscle constitutes a specialized membrane system which regulates intracellular distribution of calcium ions with great speed and efficiency. Ca\textsuperscript{2+} ATPase forms the major membrane protein (about 80\%) of the SR vesicles (Martonosi, 1982). While sequestering of the Ca\textsuperscript{2+} ions by the SR occurs through the Ca\textsuperscript{2+} ATPase, involving enzymatic mechanism of hydrolysis by ATPase (DeMeis & Vianna, 1979), the mechanism of Ca\textsuperscript{2+} release (which occurs at a rate 10\textsuperscript{2}-10\textsuperscript{3} times faster than the rate of Ca\textsuperscript{2+} sequestering) is not understood. However, high rates of Ca\textsuperscript{2+} release have led to the suggestion of a Ca\textsuperscript{2+} release channel in the Ca\textsuperscript{2+} ATPase (MacLennan et al., 1982). A better understanding of the mechanism of Ca\textsuperscript{2+} transport would be facilitated by increasing our current knowledge of the structure of the Ca\textsuperscript{2+} ATPase. The formation of the two-dimensional crystals of a membrane protein offers a unique opportunity to study its structure using the techniques of low-dose electron-diffraction and electron imaging (Unwin & Henderson, 1975).

Ca\textsuperscript{2+} ATPase in SR vesicles has been earlier crystallized by Dux and Martonosi (1983) with the use of Na\textsubscript{3}VO\textsubscript{4} and its structure studied in projection (Taylor et al., 1984). Both SR vesicles and purified Ca\textsuperscript{2+} ATPase show similar crystals after treatment with sodium orthovanadate (Na\textsubscript{3}VO\textsubscript{4}). We have explored alternative treatments for inducing crystallization of Ca\textsuperscript{2+} ATPase which should yield crystals suitable for high resolution structural studies. We report here the crystallization of the Ca\textsuperscript{2+} ATPase in the SR with the use of a mild
detergent (bee venom phospholipase A$_2$). Since the yield of the purified enzyme is low, only SR vesicles were subjected to phospholipase treatment for crystallization. A detailed study of the Ca$^{2+}$ ATPase structure is expected to provide information not only about the structure-function relationship of this enzyme but also about the mechanisms of crystallization by the two methods (phospholipase- and Na$_3$VO$_4$-induced crystallization).

Materials and Methods

Isolation and purification

SR vesicles were isolated from white skeletal and back muscle of albino rabbits, and Ca$^{2+}$ ATPase was purified from these vesicles according to the procedure described by MacLennan (1970). Protein determination was done by Lowry's method (Lowry et al., 1951). The SR vesicles and purified Ca$^{2+}$ ATPase were subjected to SDS polyacrylamide gel electrophoresis (Laemmli, 1970), and the gels were stained with silver nitrate (Wray et al., 1981).

Crystallization with phospholipase A$_2$

Freshly prepared SR vesicles were diluted by varying amounts for crystallization purposes. However, in general, microsomal preparations at a concentration of 5-10 µg/ml were used for crystallization. Bee venom phospholipase A$_2$ was obtained from Sigma Chemical Co. Crystallization was performed by, for example, incubating 5 ml of microsomal fraction at a concentration of 10 µg/ml with 0.5-1.0 unit phospholipase A$_2$/µg of microsomal protein and dialysing against 1 mM Tris/HCl buffer (pH 7.4) at 4°C for 3 to 4 d (Manella, 1984). As a control, microsomal membrane fraction was dialysed against buffer without the addition of phospholipase. Although total microsomal protein was kept at about 50 µg in all the trial experiments, we noticed that better crystals were obtained when the total volume of the starting microsomal fraction was 5 ml at 10 µg/ml protein concentration than 50 ml at 1 µg/ml protein concentration. Dialysed membranes were centrifuged in a Beckmann ultracentrifuge at 60,000 g for 90 min and taken up in double-distilled water at a concentration of about 1 mg/ml.

Crystallization with Na$_3$VO$_4$

The isolated SR vesicles and also purified Ca$^{2+}$ ATPase were crystallized according to Dux and Martonosi (1983) by treatment of 1 mg/ml protein suspensions at 2°C for 3 to 4 d with a solution containing 5 mM Na$_3$VO$_4$, 0.1 M KCl, 10 mM imidazole, 5 mM MgCl$_2$ and 0.5 mM EGTA (pH 7.4).

Electron microscopy

Specimens for electron microscopy were prepared in a similar manner, for both phospholipase- and Na$_3$VO$_4$-induced crystals, in a cold room (4°C), by depositing a 12 µl aliquot of crystalline suspension on a freshly carbon-coated 400 mesh copper grid. The suspension was blotted and stained with 2% uranyl acetate (pH 4.3). Grids were examined in a Philips 400T electron microscope and micrographs