TIME-RESOLVED STRUCTURAL STUDIES OF THE SARCOPLASMIC RETICULUM MEMBRANE

J. K. BLASIE, D. PASCOLINI, L. HERBETTE, D. PIERCE, F. ITSHAK, V. SKITA, AND A. SCARPA
Departments of Chemistry and Biochemistry-Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19014

We have used x-ray and neutron diffraction, coupled with the deuteration of selected membrane molecular components, to determine the time-averaged electron density profiles, to ~10 Å resolution, of the phospholipid bilayer and of the Ca^{2+} ATPase molecule within the total profile of fully functional, isolated sarcoplasmic reticulum membranes (1). Synchrotron x-ray diffraction studies of these membranes with a time resolution of 0.2–0.5 s, coupled with the flash photolysis of caged ATP to initiate synchronously the calcium transport processes of the Ca^{2+}-ATPase molecules in oriented membrane multilayers, have been used to determine the profile structure of the Ca^{2+}-ATPase predominantly in the first phosphorylated intermediate state of the enzyme in the calcium transport process under higher temperature conditions of enzyme turnover (2).

We have recently used lower-temperature conditions to trap the first-phosphorylated intermediate state of the enzyme in the calcium transport process for much longer time periods of several seconds (3). Synchrotron x-ray diffraction studies analogous to those described in reference (2) with a time-resolution of only 2–5 s have now provided the profile structure of the Ca^{2+} ATPase as transiently trapped in the first phosphorylated intermediate state of the enzyme, with no turnover of the enzyme ensemble detectable on a time-scale of <10–20 s.

RESULTS

In Fig. 1, we show the low-resolution, difference electron density profile for the isolated sarcoplasmic reticulum

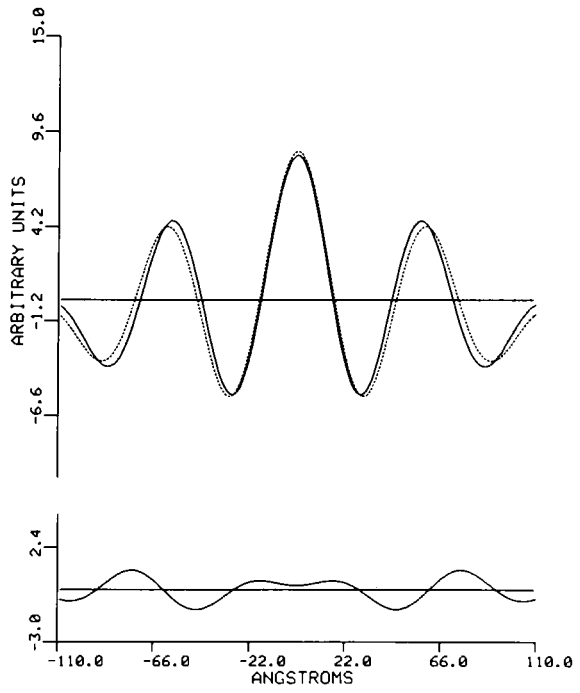

FIGURE 1 *Top*: Unit cell electron density profiles at low resolution (~40 Å) for the isolated sarcoplasmic reticulum membrane under higher-temperature conditions (7°C). The profiles contain two apposed single-membrane profiles, each contained within 0 Å and ±110 Å. The solid line corresponds to the SR membrane profile immediately before the UV flash-photolysis of caged-ATP; the dashed line corresponds to the membrane profile immediately after the UV flash-photolysis using 0.2–0.5 s time-resolution. *Bottom*: Difference electron density profile obtained from the profiles above. This is the electron density profile for which the Ca^{2+} ATPase is in its first phosphorylated intermediate state minus the profile before ATP-initiated calcium transport.

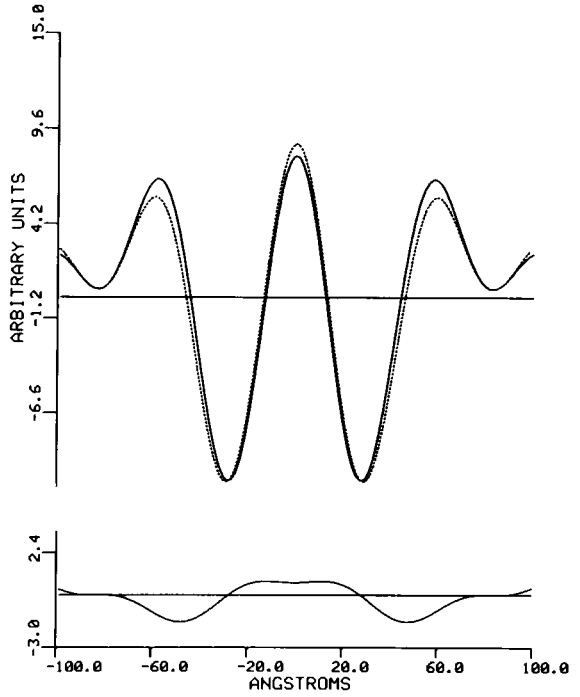

FIGURE 2 *Top*: Unit cell electron density profiles at low-resolution (~40 Å) for the isolated sarcoplasmic reticulum membrane under lower-temperature conditions (0°C). The profiles contain two apposed single membrane profiles, each contained within 0 Å and ±100 Å. The solid line corresponds to the SR membrane profile immediately before the UV flash-photolysis of caged-ATP, the dashed line to the membrane profile immediately after the UV flash-photolysis using 2–5 s time-resolution. *Bottom*: Difference electron density profile obtained from the profiles above, the electron density profile for which the Ca^{2+} ATPase is transiently trapped in its first phosphorylated state minus the profile before the ATP-initiated phosphorylation.

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membrane for which the Ca^{2+} ATPase is predominately in the first phosphorylated intermediate state of the enzyme, minus the profile before ATP-initiated calcium transport, as adopted from reference (2).

In Fig. 2, we show the low-resolution, difference electron density profile for the isolated sarcoplasmic reticulum membrane for which the Ca^{2+} ATPase is transiently trapped in the first phosphorylated state of the enzyme minus the profile before ATP–initiated phosphorylation of the enzyme.

Simple inspection of the two difference profiles reveals a close similarity between them, and verifies the earlier detection of the profile structure of the first phosphorylated intermediate state of the enzyme under conditions of enzyme turnover (2). The smaller differences between the two difference profiles are currently being subjected to a model refinement analysis to provide the Ca^{2+} ATPase profile structure for the transiently trapped, first-phosphorylated intermediate state of the enzyme.

CONCLUSIONS

These time-resolved x-ray diffraction studies using intense synchrotron radiation sources appear to be fully capable of detecting and characterizing structurally distinct intermediate states within the ATP-driven calcium transport process in sarcoplasmic reticulum membranes. The ultimate time resolution achievable depends primarily upon the incident x-ray flux and the kinetics of the flash-photolysis reactions producing enzyme substrate, and possibly on radiation damage to the membrane multilayer.

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USE OF VIBRATIONAL SPECTROSCOPY IN DEFINING THE ROLE OF CLATHRIN IN COATED VESICLE FORMATION

IRA W. LEVIN,* JAMES S. VINCENT,* ROBERT BLUMENTHAL,‡ AND CLIFFORD J. STEER‡
**Laboratory of Chemical Physics, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases; and ‡Laboratory of Theoretical Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205*

Clathrin, the major structural protein characteristic of the fibrous networks associated with the coat material of membrane pits and vesicles, has been implicated in the dynamics of a variety of endocytotic processes and intracellular transfer mechanisms (1). The basic unit of the membrane coat, the triskelion, occurs as a M_r = 650,000 protein trimer of clathrin subunits (M_r = 180,000) in combination with three associated proteins (M_r = 30,000–36,000). Under defined conditions, dissociated clathrin triskelions reassemble in the absence of the vesicle membrane into cagelike structures resembling the surface lattice of coated vesicle systems.

The sensitive, noninvasive molecular probes provided by infrared and Raman spectroscopy for examining lipid bilayer disturbances on the vibration time scale ($\sim 10^{-13}$ s) allow an assessment of the individual contributions of membrane bilayer components to the conformational, dynamical and functional properties of biological membrane assemblies. We have applied infrared spectroscopic techniques to clarify the pH-dependent conformational reorganizations paralleling the evolution of triskelions, isolated from bovine brain tissue, into the assembled high-molecular weight, latticelike species. Infrared spectral parameters were also used to determine the extent of the perturbation of the clathrin coat protein on the lipid matrix of the isolated coated vesicle membrane. In a complementary Raman spectroscopic study, we investigated the effects of clathrin on the lipid bilayer intrachain and intermolecular order/disorder characteristics of model systems comprised of unilamellar dipalmitoyl phosphatidylcholine (DPPC) dispersions.

MATERIALS AND METHODS

Coated vesicles were isolated from bovine brain by the method of Nandi et al. (2). The clathrin trimers were isolated and reassembled as described previously (3). The model membrane unilamellar systems were formed