Zoological Studies 34, Supplement I (1995)

Zoological Studies

Images of a Membrane-bound Detoxification Enzyme at 4 Å Resolution Obtained by Electron Cryomicroscopy

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INTRODUCTION

The significance of electron microscopy as a tool in structural molecular biology is directly related to the resolution that can be reached. In general, the goal should be to obtain information at a level which allows an accurate spatial localization of all the building blocks of a biological macromolecule such as the amino acids of a protein. Recently, this has been achieved for two membrane proteins (Henderson et al. 1990, Kühlbrandt et al. 1994). A requirement has been that protein molecules be arranged on a lattice forming a two-dimensional crystal. If such an array is sufficiently large and well ordered, averaging can produce significant high resolution information. Thus, it is possible to circumvent one of the fundamental problems in high resolution biological electron microscopy: the conflict between using a sufficient number of electrons to produce significant contrast and protecting the specimen from the dose dependent beam damage.

Electron crystallography is a method which is of particular interest to the study of membrane proteins. Formation of two-dimensional crystals can be induced in a natural membrane environment with phospholipids, and such thin specimens are ideal objects for electron microscopy. In the present work, we have obtained large and well ordered two-dimensional crystals of a membrane-bound detoxification enzyme - microsomal glutathione transferase (molecular weight of the monomer 17.3 kDa). Image data obtained through electron cryomicroscopy and subsequent image processing including compensation for lattice disorder and phase contrast reconstruction has resulted in a projection structure at 4 Å resolution that allows interpretation in terms of secondary structure elements.

MATERIALS AND METHODS

Pure microsomal glutathione transferase was solubilized in 1 % Triton X-100 and mixed with bovine liver lecithin at a lipid to protein molar ratio of approximately three. Slow removal of the detergent was obtained through dialysis. The frequency, size and order of crystalline domains were monitored following negative staining. Image acquisition and on-line Fourier transformation were performed with a Tietz slow scan CCD system attached to a Philips CM120 electron microscope. High resolution images were collected on film at 200 kV with a Jeol 2000EX. The specimens were stabilized in tannin and cooled by liquid nitrogen (Wang and Kühlbrandt 1991). Some of the images were subjected to image processing which included compensation for lattice disorder and phase contrast reconstruction (Henderson et al. 1986). A weight function was applied to the final image amplitude data.

RESULTS AND DISCUSSION

Removal of the detergent resulted in a spontaneous formation of vesicles and membrane sheets. About 20 % of them contained microsomal glutathione transferase arranged on a lattice (Fig. 1a). These 2D crystals often measured about 1 μm in cross section and consisted of a single layer of protein molecules. An R-value test determined the projection symmetry to be pgg and

the unit cell dimensions were a=b=90.5 Å. The scanned areas (4400*4400 pixels at a pixel size on the specimen level of 1.4 Å) contained about 4000 unit cells. The lattice distortions were relatively small as measured by deviations from a perfect least-squares adapted lattice. The Fourier transforms of the images contained significant information extending to 4 Å. The phase residual was typically 20 to 25 degrees for 320 observed reflections.

The unit cell contains four tightly packed protein domains forming a staggered pattern (Fig. 1b). A local three-fold axis relates three molecules into a trimeric structure. The presence of a trimer is consistent with indications from other experiments (Morgenstern et al. 1982, Boyer et al. 1986, Lundqvist et al. 1992). Thus, we conclude that microsomal glutathione transferase is a trimer in the membrane-bound state. The projection structure of each trimer can be described as consisting of two concentric rings (Fig. 1c). The innermost of these has a triangular shape and is subdivided into six strong densities that have a center to center distance of 10 Å. In addition, the Fourier transform has strong reflections at the corresponding spatial frequency. Thus, we interpret the inner core as consisting of six parallel α helices with the helical axes running perpendicular to the plane of the membrane. These helices delineate a central featureless region. The outer ring consists of three elongated domains that do not form a completely closed structure. Since CD spectroscopy measurements have indicated the presence of β structure in microsomal glutathione transferase, this may correspond to β strands running more or less perpendicular to the plane of the membrane as in bacterial porins (Weiss et al. 1991, Cowan et al. 1992).

Interestingly, soluble glutathione utilizing enzymes have a common structural motif consisting of two parallel α helices and four β strands (Dirr et al. 1994). The projection of this conserved domain along the helical axes presents a structure that fits the projection of microsomal glutathione transferase as determined in the present work. This indicates that a similar glutathione binding domain could be present also in this membrane-bound form. In addition, this would mean that the microsomal glutathione transferase consists of both α helices and β structure. This has not been observed in other membrane proteins that have had their structures determined at high resolution (Cowan and Rosenbusch 1994). An alternative interpretation that can be made from the projection data alone is that the elongated outer domain contains one or two slightly tilted helices. The sequence of microsomal glutathione transferase predicts at least three membrane inserted domains. One of these is known to pass through the lipid bilayer (Andersson et al. 1994).

In the present work we have demonstrated the formation of large, well ordered 2D crystals of membrane-bound microsomal glutathione transferase. From electron crystallographic analysis it can be concluded that the protein forms trimers. Each of these consists of two parallel α helices and an elongated domain that could arise from a β sheet or tilted α helices. The projection structure resembles the glutathione binding domain found in soluble proteins with a similar catalytic function. Obtaining a more detailed model of the membrane topology will require the recording of three-dimensional data. This work is now in progress and it is hoped that this will allow localization of all the 154 amino acid residues of the protein

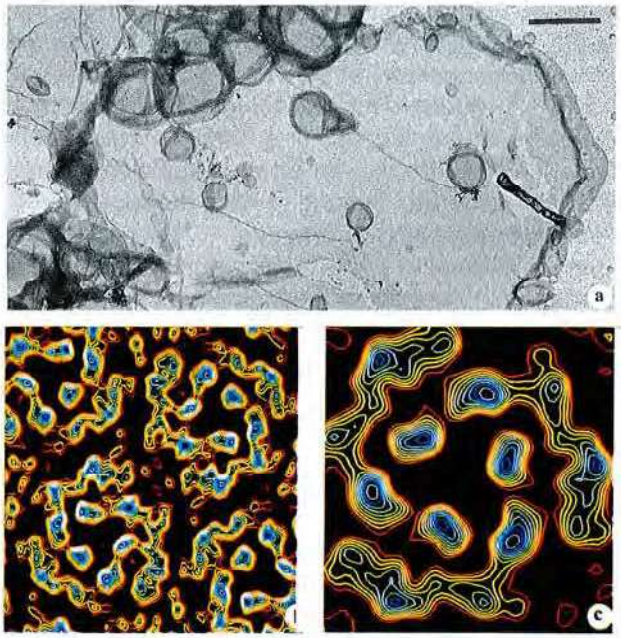

Fig. 1. (a) Electron micrograph of membrane sheets containing two-dimensional crystals of glutathione transferase. The specimen was stained with uranyl acetate. Scale bar 1 μm. (b) Projection map of the unit cell of the microsomal glutathione transferase two-dimensional crystals after image processing including data to 4 Å resolution. The unit cell dimensions are a=90.5, b=90.5 Å and one asymmetric unit contains one trimer of the protein arranged about a local three-fold symmetry axis. (c) The microsomal glutathione transferse trimer after imposing three-fold symmetry. The inner core consists of six parallel transmembrane α helices oriented with their helical axes perpendicular to the plane of the membrane.

monomer. Such a result is of interest, not only for understanding the functional properties of this protein, but also as contributing to the knowledge of membrane protein structure. Since this large, important yet elusive group of proteins is difficult to study with other structural methods, electron crystallography may provide a tool which can be applied more generally. It would be advantageous if the requirement to form large and well ordered cystals of the proteins could be relaxed to some extent since this could still be a limiting factor. Instrumental aspects play a key role in such a development.

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