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Crystallization of microsomal triglyceride transfer protein from bovine liver

SHARI L. OHRINGER, HARIS JAMIL, CHING-HSUEN CHU, LYDIA TABERNERO, HOWARD M. EINSPAHR, JOHN R. WETTERAU AND JOHN S. SACK, *Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, New Jersey 08543, USA*

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Abstract

The microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein required for the assembly of plasma very low density lipoproteins in the liver and chylomicrons in the intestine. Bovine MTP was purified by a modification of a previously published procedure and crystals of MTP were grown reproducibly with polyethylene glycol as a precipitant at pH 7.0. MTP crystals, which diffract to Bragg spacings of better than 3.2 Å, have the symmetry of space group $P2_12_12_1$ with refined lattice constants of $a = 88.7$, $b = 100.9$ and $c = 201.1$ Å, with one heterodimer per asymmetric unit.

1. Introduction

Plasma very low density lipoproteins (VLDL) in the liver and chylomicrons in the intestine transport triglycerides from sites of synthesis to peripheral tissues where the triglycerides are hydrolyzed to free fatty acids, which are taken up by cells and utilized as a source of energy (Havel & Kane, 1989). The remnant lipoproteins from this process (low-density lipoproteins and chylomicron remnants) are cholesterol-rich particles that are important risk factors for the development of arteriolosclerotic plaques and premature coronary heart disease. Although the metabolism of VLDL and chylomicrons has been studied extensively, little is known about the means by which these lipoproteins are assembled. Within the endoplasmic reticulum (ER), the primary structural protein of VLDL and chylomicrons, apolipoprotein B, associates with phospholipid, cholesterol, and triglycerides to form the mature lipoprotein particle. The molecular details of this process remain to be determined. Several studies indicate that the microsomal triglyceride transfer protein (MTP) is a key component of the assembly apparatus for VLDL and chylomicrons (Wetterau *et al.*, 1992; Sharp *et al.*, 1993; Shoulders *et al.*, 1993; Gordon *et al.*, 1994).

MTP catalyzes the transport of neutral lipids and phospholipids between membranes (Wetterau & Zilversmit, 1984; Jamil *et al.*, 1995). It is found within the lumen of microsomes isolated from the liver and intestine (Wetterau & Zilversmit, 1986). It is unique among a class of proteins referred to as lipid-transfer proteins in that it is a protein complex containing two different subunits with relative molecular weights of 55 000 and 97 000 (Wetterau & Zilversmit, 1985; Wetterau, Combs, Spinner & Joiner, 1990; Wetterau, Aggerbeck, Laplaud & McLean, 1991, Sharp *et al.*, 1993). Other lipid-transfer proteins are single polypeptide chains (Helmkemp, 1986; Wirtz, 1991). MTP is also unusual among lipid-transfer proteins in that it is one of only two proteins that catalyze the transfer of neutral lipids, like triglycerides and cholesteryl esters, between phospholipid surfaces.

The small subunit of MTP has been identified as protein disulfide isomerase (PDI) (E.C. 5.3.4.1) (Wetterau *et al.*, 1990). PDI is a soluble protein and its retention in the ER is mediated through the peptide sequence KDEL at the C-terminus. Within the ER, PDI has several different functions. In addition to being a component of MTP, it catalyzes the proper folding of newly synthesized proteins by promoting the proper pairing of cysteine residues during the formation of disulfide bonds (Bulleid & Freedman, 1988); it is the beta subunit of the tetrameric enzyme, prolyl 4-hydroxylase (Koivu *et al.*, 1987); and it has dehydroascorbate reductase activity (Wells, Xu, Yang & Rocque, 1990). The precise roles of the two subunits of MTP in the lipid transport process are not clear. Minimally, PDI is required to maintain the transfer protein in an active form, however, a direct role in the transfer process has not been excluded (Wetterau, Combs, McLean, Spinner & Aggerbeck, 1991). Isolated PDI has no lipid-transfer activity (Wetterau *et al.*, 1990).

Determining the detailed structure of MTP by X-ray crystallography will help to elucidate further the molecular details of lipoprotein assembly. In this study, we report the crystallization of MTP as an initial step toward obtaining a detailed structure of the transfer protein complex. This structure will also provide additional insights into the mechanism by which neutral lipids are transported between membranes and will further our understanding of the roles of the two subunits of MTP in the lipid transport process.

2. Purification

Bovine liver MTP was purified by a modification of a previously published isolation procedure that included DEAE–Sephacel, DE52 cellulose, Sephacryl S-300, hydroxylapatite gel chromatography, and Mono Q fast protein liquid chromatography (FPLC) steps (Wetterau & Zilversmit, 1985; Wetterau *et al.*, 1990; Wetterau, Aggerbeck, Laplaud & McLean, 1991). Purified MTP showed only two bands of apparent molecular weights 58 and 88 kDa on sodium dodecyl sulfate polyacrylamide-gel electrophoresis (SDS–PAGE) (Laemmli & Favre, 1973). The protein was dialyzed against 15 m*M* Tris–HCl pH 7.0 containing 75 m*M* NaCl and concentrated to 6–8 mg ml^{-1} prior to crystallization.

3. Crystallization

Crystallization of MTP was carried out at 293 K by the hanging-drop vapour-diffusion method in 24-well tissue-culture plates (Flow laboratories). The best crystals were obtained from drops containing 5 µl protein mixed with an equal volume of a reservoir solution of 18%(*w*/*v*) polyethylene glycol (average $M_r = 4000$, Fluka Chemical) without further purification, 10 m*M* magnesium chloride, 40 m*M* Tris–maleate buffer, pH 7.0. Crystals may also be obtained in a range of pH from 5.5 to 7.0 and in several different types of polyethylene glycol.

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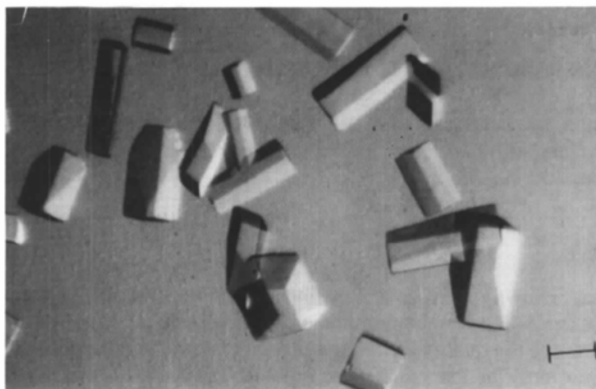

Fig. 1. Crystals of microsomal triglyceride transfer protein. The scale bar is 0.2 mm long.

This crystallization procedure produced small, rod-shaped crystals within 10 d. To improve crystals for X-ray analysis, seed crystals are transferred to fresh drops composed of equal volumes of the protein and reservoir solutions. Crystals grow to dimensions of up to 1.0 × 0.3 × 0.15 mm (Fig. 1) within one week. The identity of the crystalline material was determined by SDS–PAGE, where the presence of two protein bands of apparent molecular weights 58 and 88 kDa confirms the crystallization of the components of MTP.

4. X-ray analysis

For X-ray analysis, the crystals were mounted and sealed in thin-walled glass capillary tubes. Native data were collected with a Siemens X-1000 area-detector system mounted on a Rigaku RU-200 rotating anode, equipped with mirror optics, producing Cu $K\alpha$ radiation. The detector was placed at 200 mm from the crystals. A total of 360 frames each with 0.25° oscillation were collected. The crystals diffracted to Bragg spacings of better than 3.5 Å.

Analysis of the diffraction data was done with the computer programs *Xengen* (Howard, Gilliland, Finzel & Poulos, 1987) and *XDS* (Kabsch, 1988*a*,*b*). The crystals have the symmetry of space group $P2_12_12_1$ with refined lattice constants of $a = 88.7$, $b = 100.9$ and $c = 201.1$ Å. Based upon the deduced amino-acid sequences of bovine PDI and the large subunit of MTP (Sharp *et al.*, 1993), the molecular weights of the two components of MTP are 55 and 97 kDa. Assuming one heterodimer of molecular weight 152 000 per asymmetric unit, the average crystal volume per unit mass (V_M) is 2.96 $Å^3$ Da^{-1} in good agreement with values for other proteins (Matthews, 1968). The data collection resulted in a nearly complete (89.7%) data set to Bragg spacings of 3.5 Å with an overall R_{symm} on intensity of 9.1%.

X-ray diffraction data have also been collected on flash-frozen crystals of MTP. Prior to freezing, a crystal was transferred from the hanging drop into a solution containing the mother liquor and 10% glycerol. Three subsequent transfers were performed into solutions containing increasing concentrations of glycerol for a period of 1 h, with the final solution containing 25% glycerol. From the final solution, the crystal was transferred to a nylon loop and flash cooled in a stream of nitrogen gas to 100 K. Data from frozen crystals extends to at least 3.2 Å resolution.

A search for heavy-atom derivatives is currently underway for phase determination by isomorphous-replacement methods.

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