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Crystallization and preliminary X-ray crystallographic studies on apolipoprotein H (β2-glycoprotein-I) from human plasma

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Abstract

Apolipoprotein-H (Apo-H, $M_w \simeq 50$ kDa) is a carbohydrate-rich human-plasma protein which exists in blood serum in the free form as well as distributed between several classes of lipoproteins. Single crystals of apo-H have been obtained and crystallographic data sets have been collected. The crystals belong to the orthorhombic space group $C222_1$, with cell dimensions $a = 158.47$, $b = 169.25$, $c = 113.28$ Å (at 100 K). The data indicate that the crystallographic asymmetric unit contains one tetramer of the protein.

1. Introduction

Apolipoprotein-H (apo-H), also known as β2-glycoprotein-I, has been found to attach to several classes of lipoproteins to varying degrees. It has been implicated in a variety of physiological pathways, including lipoprotein metabolism (Kamboh & Ferrell, 1991), blood coagulation (Roubey, 1994) and the production of antiphospholipid antibodies (aPA) (Schousboe, 1985). The mechanism of action of apo-H is not known with certainty, but it seems to bind to various kinds of negatively charged substances, *i.e.* phospholipids, dextran sulfate (Shousboe & Rasmussen, 1988; Shousboe, 1988) and lipoproteins (Polz & Kostner, 1979). Recent studies have shown that one specific part, the fifth domain of apo-H, forms the lipid-binding region and is important for lipid–protein interaction (Sanghera *et al.*, 1997). The high-resolution structure of apo-H is of great importance not only to obtain further insight into the mode of lipid–protein interaction, but also to understand the possible cause for apo-H acting as an autoantigen (Schousboe, 1985).

Apo-H is a single-chain glycoprotein of 326 amino-acid residues (molecular mass 50 kDa) determined from purified protein (Lozier *et al.*, 1984) and subsequently confirmed by cDNA cloning and sequencing (Kristensen *et al.*, 1991; Mehdi *et al.*, 1991; Steinkasserer *et al.*, 1991). It has a high carbohydrate content of about 16% by weight (Finlayson & Mushinsky, 1967). Apo-H shows extensive internal homology, with five consecutive domains of 60 amino-acid residues each, referred to as the GP-I domain (Davie *et al.*, 1986), sushi domain (Ichinose *et al.*, 1990) and complement-control protein (CCP) repeats (Kristensen *et al.*, 1987) (Fig. 1). Such domains are observed in a number of complement and non-complement proteins (Davie *et al.*, 1986). The three-dimensional structure analyses of various apolipoproteins (Wilson *et al.*, 1991; Breiter *et al.*, 1991; Borhani *et al.*, 1997) suggest that elongated amphiphatic helical bundles are common structural motifs for apolipoproteins. The primary and secondary structures of apo-H are entirely different from those of other lipoproteins, as there is a complete absence of amphiphatic α-helical structure. Because of its remarkable characteristics compared to other apolipoproteins, we undertook the crystallization of this protein and the analysis of its three-dimensional structure by X-ray crystallography.

2. Experimental procedures

2.1. *Purification and crystallization*

Apo-H was purified from citrated plasma by treatment with 1.4%(*v*/*v*) $HClO_4$ followed by affinity chromatography over heparin-Sepharose as described previously (Gries *et al.*, 1989). The purity of the protein was judged by SDS–PAGE and immunochemistry.

Crystals were grown according to Haupt & Heide (1966) with some modifications in the crystallization procedure. The best crystals, suitable for X-ray diffraction studies, were obtained by the sitting-drop vapour-diffusion technique at 277 K using ammonium sulfate as precipitant. Drops containing 5 µl of 10 mg ml^{-1} protein solution and 5 µl precipitant buffer were equilibrated against 1 ml precipitant buffer.

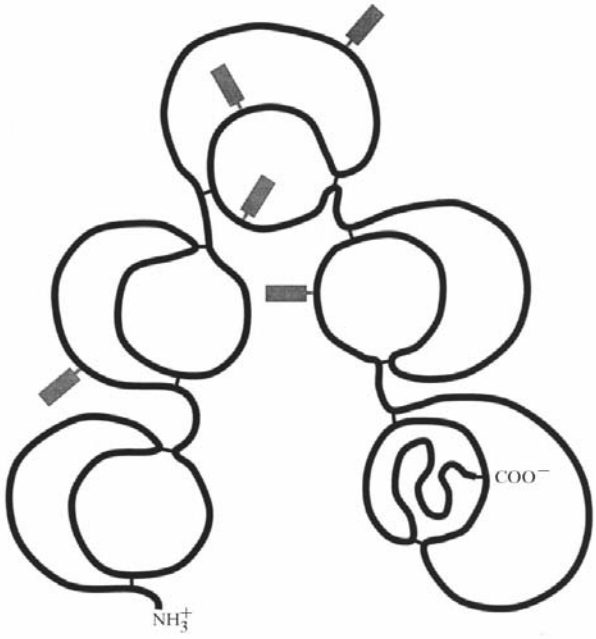

Fig. 1. Schematic view of apo-H, based upon the primary structure, from Kato & Enjyoji (1991) (simplified).

The precipitant buffer contained 1.6–2.0 *M* ammonium sulfate buffered with 0.2% sodium carbonate at pH 7.2. Crystals were obtained after 3–4 days. Two different crystal forms have been observed during crystallization: plates and rhombs, with dimensions of 0.9 × 0.7 × 0.2 mm, which often form tight clusters of twinned crystals with irregular shape.

2.2. *X-ray data collection and processing*

X-ray diffraction experiments were performed using the cryotemperature data-collection technique, as crystals deteriorated in the X-ray beam at room temperature. Crystals of apo-H were cryo-preserved by soaking in the solution used for their growth and 25%(*v*/*v*) glycerol. We have collected intensity data up to 3.0 Å resolution at liquid-nitrogen temperature (100 K) using the synchrotron radiation wiggler beamline BW7B (λ = 0.837 Å) of the EMBL outstation at DESY, Hamburg and an imaging-plate scanner (MAR Research, Germany). For data collection, 0.5° oscillation per image was used and the crystals were rotated about the *c* axis through a net rotation of 90°. Typical exposure time was 3 min per image, and a crystal-to-detector distance of 280 mm was used. The data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski, 1993).

3. Results and discussion

Human plasma apo-H has been crystallized using ammonium sulfate as precipitant at pH 7.2 (Fig. 2). Under cryogenic conditions (100 K), the rhombic shaped crystals diffracted to better than 3.0 Å resolution with synchroton radiation and remained stable during collection of the complete data set. A representative experimental data frame is shown in Fig. 3.

The observed systematic absences are $h + k = 2n + 1$ for hkl reflections and $00l = 2n + 1$ for $00l$ reflections, indicating the *C*-centred orthorhombic space group $C222_1$. The cell dimensions at 100 K are $a = 158.47$, $b = 169.25$, $c = 113.28$ Å with a unit-cell volume of 3.04×10^6 Å^3. The standard merging residual (on intensities) was 6.8%. The intensity-data collection and reduction statistics are given in Table 1. The measured value of 1.35 g cm^3 for the crystal density indicates four molecules per asymmetric unit (V_m = 1.9 Å^3 Da^{-1}) with a solvent content (V_{sc}) of 36.0%. This value is in the expected V_m

Table 1. *Data-collection statistics and completeness of the data sets*

Number of measured reflections	443123
Number of unique reflections	24160
Average $I/\sigma(I)$	13.3
R_{sym} (%)†	6.8
R_{sym} in last resolution shell (%)	23.0

	Completeness (%)	
Resolution (Å)	$I > -3\sigma(I)$	$I > 2\sigma(I)$
99.0–6.41	65.8	62.3
6.41–5.08	75.9	68.2
5.08–4.44	77.5	69.1
4.44–4.04	77.5	66.5
4.04–3.75	79.1	65.5
3.75–3.53	79.1	60.0
3.53–3.35	79.1	55.8
3.35–3.20	80.3	46.6
3.20–3.08	80.1	39.9
3.08–2.97	68.7	30.4

† $R_{sym} = \sum_h \sum_i |I_{ih} - \langle I_h \rangle| \sum_h \sum_i \langle I_h \rangle$, where $\langle I_h \rangle$ is the mean intensity of the i observation of reflection h.

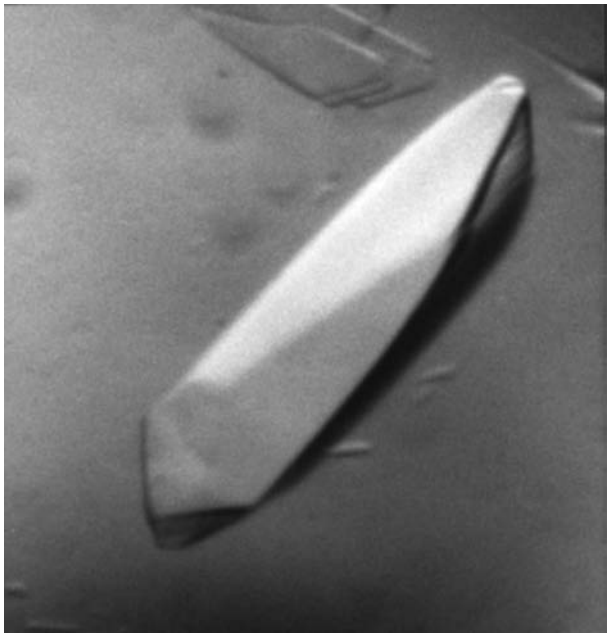

Fig. 2. Single crystals of human apo-H grown from 1.6–2.0 *M* ammonium sulfate as a precipitant, buffered with 0.2% sodium carbonate at pH 7.2.

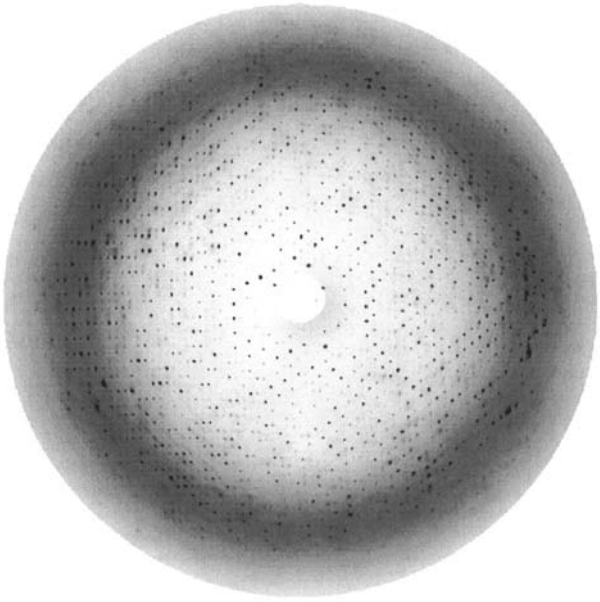

Fig. 3. 0.5° rotation image-plate pattern of a crystal of apo-H from human plasma. The pattern was obtained under cryogenic condition (100 K) with synchrotron radiation at beamline BW7B of EMBL at DESY (Hamburg). The crystal-to-film distance was 280 mm, the diameter of the collimator was 0.3 mm, the wavelength was 0.837 Å, the exposure time was 3 min and the resolution was 3.0 Å.

range of 1.6–3.5 $Å^3$ Da^{-1} for most protein crystals (Matthews, 1968).

We are pursuing the conventional multiple-isomorphous-replacement technique (MIR) and screening of various heavy-atom compounds for suitable isomorphous derivatives in order to analyse the phases.

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