Crystallization and Preliminary Analysis of Crystals of Apolipophorin III Isolated from Locusta migratoria*

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Crystals of apolipophorin III, isolated from the locust Locusta migratoria, have been reproducibly grown from ammonium sulfate solutions and are well suited for an x-ray crystallographic analysis. Locust apolipophorin III is a glycosylated protein with a molecular weight of 19,100 and interacts with lipophorin, the major lipoprotein complex in insects. The crystals belong to the space group P6_22 or P6_22 with unit cell dimensions of a = b = 67.5 Å, c = 155.6 Å and diffract to a nominal resolution of 2.5 Å. They are physically robust and are stable in the x-ray beam for over a week. A complete native x-ray data set has been collected and processed to 3.0-Å resolution.

In some insect orders such as Lepidoptera and Orthoptera β-oxidation of fatty acids serves as the source of fuel for prolonged flight (Beenakkers et al., 1985). During flight triacylglycerols normally stored in the fat body cells are converted to diacylglycerols and released into the hemolymph and have molecular weights of approximately 2,000 and 80,000, respectively (Ryan et al., 1986). In some insects there exists another apolipoprotein designated as apoLp-III. This apolipoprotein has a molecular weight of approximately 20,000 and exists as a soluble monomer in the hemolymph. During flight, however, when lipophorin is loaded with diacylglycerol, apoLp-III associates with the lipophorin, the major lipoprotein complex in insects. The crystals described in this report were produced from Locust apoLp-III and have a molecular weight of approximately 250,000 and 80,000, respectively (Ryan et al., 1986). In recent years the protein from the sphinx moth has been the most extensively characterized (Kawooya et al., 1984; Wells et al., 1985). It has been suggested that this apoLp-III is a prolate ellipsoid with an axial ratio of approximately $c/a = 1.1$, that this apoLp-III is glycosylated with a total sugar content of approximately 12%. A cDNA coding for locust apoLp-III has recently been cloned, and the amino acid sequence has been determined based on the cDNA sequence.

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Crystallographic data and structural analysis of apoLp-III is of considerable interest since it will not only provide a better understanding of the role of apolipoprotein in lipid transport systems. The three-dimensional structure determination of this protein is of considerable interest since it will not only provide a better understanding of the role of apolipoprotein in lipid transport systems.

EXPERIMENTAL PROCEDURES

Locust apoLp-III was purified according to the method of Kanost et al. (1987). This purification procedure takes advantage of the exceptional thermal stability of apoLp-III. Nearly all of the hemolymph proteins, with the exception of apoLp-III, precipitate when the hemolymph is heated to 100 °C. Minor contaminants are then removed by gel filtration and reversed phase high performance liquid chromatography. ApoLp-III is stored as a lyophilized powder.

For crystallization experiments the protein was dissolved in a buffer solution of 10 mM Hepes, 5 mM sodium azide, pH 7.0, to a concentration of 30 mg/ml. A survey for potential crystallization conditions was conducted at room temperature using the hanging drop method of vapor diffusion. Once promising conditions were identified, the sitting drop method was employed in order to grow large single crystals of the protein isolated from the locust L. migratoria crystallized readily in a form suitable for a high resolution structural analysis.

In this report we describe the crystallization and preliminary analysis of crystals of locust apoLp-III. The three-dimensional structure determination of this protein is of considerable interest since it will not only provide a better understanding of the role of apolipoprotein in lipid transport systems.
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larger crystals (for a review of crystallization techniques, see McPherson, 1982).

X-ray diffraction photographs were recorded using nickel-filtered copper Kα radiation from an Elliot GX20 rotating anode x-ray generator operated at 35 kV and 40 mA with a 200-μm focal cup. Because the apoLp-III crystals tended to slip in the quartz capillary tubes, a small volume of a 0.2% solution of poly(vinylformal) in 1,2-dichloroethane was drawn over each crystal and allowed to dry. This formed a very thin plastic film around the crystal and was exceedingly helpful in preventing such problematic slippage (Rayment et al., 1977).

Precession photographs were typically recorded using a precession angle of 10°, a crystal-to-film distance of 100 mm, and exposure times of about 17–20 h. The three-dimensional native data set was collected by the method of oscillation photography using an Arndt-Wonacott camera. An oscillation angle of 2.0°/film pack was used with an exposure time of 6 h and a crystal-to-film distance of 100 mm. The crystal was rotated about the c axis through a net rotation of 30°.

The x-ray films were digitized with an Optronics P1000 film scanner and subsequently processed using a set of programs originally developed by Dr. Michael Rossmann and modified by Dr. Brian Matthew’s laboratory (Rossmann, 1979; Schmid et al., 1981).

RESULTS AND DISCUSSION

Crystals of apoLp-III were first obtained using 3.0 M ammonium sulfate as the precipitant and a protein concentration of 30 mg/ml. The ammonium sulfate solution was buffered with 50 mM potassium/sodium phosphate, pH 7.4. The crystals grew as a cluster of small needles. Subsequently, single hexagonal rods with dimensions of 0.2 × 0.2 × 1.0 mm were produced in about 2 weeks by lowering the protein concentration to 10 mg/ml and the ammonium sulfate concentration to 2.8 M. There was, however, some difficulty in growing large crystals reproducibly. Indeed, many experiments failed to produce crystals at all.

In an effort to overcome this nucleation problem we used the technique of "macro-seeding" which proved extremely successful in other crystallization studies (Thaller et al., 1981; Thaller et al., 1985). For such experiments, small apoLp-III crystals, grown from 2.8 M ammonium sulfate, were first washed in 2.4 M ammonium sulfate, 50 mM phosphate, pH 7.4, for approximately 15 min and then transferred to 50-μl droplets containing 12 mg/ml protein and 2.4 M ammonium sulfate, 50 mM phosphate, pH 7.4. The droplets were then equilibrated by vapor diffusion against 2.8 M ammonium sulfate, 50 mM phosphate, pH 7.4. The seed crystals increased in size over a period of 2 weeks with some achieving dimensions of 0.4 × 0.4 × 4.0 mm. With this technique large single crystals can be obtained from at least 80% of the crystallization experiments. A photomicrograph of a typical crystal is shown in Fig. 1.

Fig. 1. Crystal of locust apoLp-III. The crystal shown was grown from 2.8 M ammonium sulfate, 50 mM phosphate, pH 7.4. The thick bar corresponds to a length of 0.5 mm. By using the technique of "macro-seeding" crystals with a length of 4.0 mm can be readily grown.

Fig. 2. 10° precession photograph. The precession photograph shown is of the h0l zone and was recorded using a crystal-to-film distance of 100 mm and an exposure time of 19 h at 35 kV and 40 mA. Diffraction maxima at the edge of the precession circle correspond to a resolution of 4.4 Å.

Fig. 3. Typical oscillation photograph. The photograph shown here was recorded using an oscillation angle of 2.0° and an exposure time of 6 h. The crystal-to-film distance was 100 mm. Diffraction maxima at the edge of the film correspond to a resolution of 3.0 Å.
ApoLP-III crystals belong to the hexagonal space group P6\(_{1}\)2\(_2\) or P6\(_{2}\)2\(_1\) with unit cell dimensions of \(a = b = 67.5 \text{ Å}\) and \(c = 155.6 \text{ Å}\). A typical 10\(^{\circ}\) precession photograph is shown in Fig. 2. Assuming one molecule/asymmetric unit and a molecular weight of 19,100, the volume/unit molecular weight in the unit cell or \(V_m\) is 2.7 \(\text{Å}^3/\text{dalton}\). Most globular proteins lie within the range of 1.68–3.53 \(\text{Å}^3/\text{dalton}\) (Matthews, 1974).

The crystals are easily manipulated and diffraction strongly to 3.0-Å resolution using synchrotron radiation. At present, a three-dimensional native x-ray data set has been collected and processed to 3.0-Å resolution. A typical oscillation photograph is shown in Fig. 3, and relevant data processing statistics are given in Table I. Since the crystals are long rods it was possible to collect the entire data set from one crystal by making appropriate translations along its length. Data collection and processing took 1 week. A search for heavy atom derivatives is in progress.

In recent years there have been many investigations directed toward understanding those structural elements of the apolipoproteins that allow them to interact with lipid surfaces. It has been suggested that amphiphilic helices may play a key role in such protein:lipid interactions (Segrest et al., 1974). These helices are thought to contain polar and nonpolar amino acid residues distributed in such a manner that the nonpolar residues penetrate into the lipid milieu while the charged and polar residues interact with the aqueous environment. Evidence for the amphiphilic helix comes from a variety of studies including protein sequence comparisons, peptide syntheses, and numerous physicochemical investigations (for a review see Gotto et al., 1986). Furthermore, Eisenberg et al. (1982, 1984) have formulated a mathematical model to quantify the amphiphilicity of a helical region, and this theoretical approach has been recently applied to various mammalian apolipoproteins (De Loof et al., 1987). Analysis of the amino acid sequence of locust apoLP-III also suggests the presence of amphiphilic regions. To date, however, there is a lack of three-dimensional structural information concerning apolipoproteins and the putative amphiphilic helices. The crystals of apoLP-III described here present a unique opportunity to examine, on a structural basis, the current hypotheses concerning the interactions between apolipoproteins and lipid surfaces.

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REFERENCES