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CRYSTALLIZATION OF α_1-ACID GLYCOPROTEIN

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Crystals of α_1-acid glycoprotein have been grown reproducibly from delipidated protein in the presence of chlorpromazine. The crystals are large hexagonal prisms of space group either P622 or $P6_222$ and the unit cell dimensions are a = b = 101 Å and C = 201 Å. The unit cell is very highly hydrated and is nearly 80% solvent. It contains one molecule of protein per asymmetric unit. The crystals diffract only to low resolution, presumably reflecting the extensive hydration and accompanying disorder. © 1984 Academic Press, Inc.

Orosomucoid (α_1-acid glycoprotein; OMD) is one of the most abundant and widely studied of the serum proteins. It is characterized by possessing a high carbohydrate content (42%), 28% of which is N-acetyl neuraminic acid (NANA) (1). These two features render the molecule both extremely soluble and acidic. In the total molecular weight of 41,000 reside a single polypeptide chain and five complex carbohydrate side chains linked to asparagine. An acute phase protein, orosomucoid, is one of the handful of serum proteins with no known physiological function. A number of biological activities have been described for it however, ascribable to either or both the carbohydrate and polypeptide portions of the molecule. These activities include promotion of collagen fibril formation (2), inhibition of platelet aggregation (3), inhibition of the heparin accelerated antithrombin III mediated inactivation of thrombin and factor Xa (4), binding of Δ_4 ketosteroids (5), sequestration of a glycosaminoglycan cofactor in the lipoprotein lipase reaction (6) and the preferential binding of more than 60 cationic therapeutic drugs (7-9).

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It has become apparent that this latter activity can be of considerable clinical significance in drug therapy, particularly in those syndromes where the orosomucoid levels are elevated (10). Both physical (11) and chemical (12) studies have been directed toward evaluating the topography of, and the amino acid residues contained in, the drug binding site; however, X-ray crystallography remains as the definitive technique for gathering structural information. We report here the results of some encouraging crystallization trials and the properties of one crystal form grown from delipidated protein in the presence of the phenothiazine tranquilizer, chlorpromazine.

MATERIALS AND METHODS

Preparation of Oroscomucoid. Successful protein crystallization is frequently dependent upon subtle differences between preparations which are in all other respects identical. For this reason orosomucoids were prepared from a number of sources, and also from the same source on different occasions. With the exception of two preparations from pooled human serum, generously provided by the American Red Cross National Fractionation Centre, and Dr. Karl Schmid, all orosomucoids were isolated from the urine of individual patients suffering from nephrotic syndrome, essentially as reported previously (13). All preparations were delipidated, according to Westphal (14) or Chen (15), and characterized by the chemical and physical criteria used earlier (13), and, in addition, a binding assay using spin-labeled propranolol (11).

Crystallization. After numerous trials involving both native and delipidated protein in the presence of numerous potential ligands, we found that crystals could be grown by vapor diffusion of 20 μl droplets of 30 mg/ml protein solution, also containing 10 mM chlorpromazine·HCl and 35% saturated with ammonium sulfate, against 63 to 70% saturated ammonium sulfate. The protein solutions were not buffered. Vapor diffusion was successful using both hanging drops or depression plates for equilibration (16). Large, perfect crystals were initially grown by equilibration against 64% ammonium sulfate after three months time but appeared about one week after the ambient temperature was reduced from 25°C to 18°C. Subsequently we found that crystals of equal size and quality could be induced to grow in less than a weeks time by such a temperature drop and we are certain that this is an important component in the crystallization procedure. The crystals have also been grown in the presence of 1 to 4 mM Pb^{+++}, Cu^{++}, Mg^{++}, Mn^{++}, Cd^{++}, Zn^{++}, Ni^{++}, Co^{++} and Ca^{++} but appear no different than those grown in the absence of the transition metals. Chlorpromazine is perhaps not unique in the ability to enhance crystallization, but an equal number of crystallization attempts using other ligands such as dipyridamate have not proven successful. Similarly, we have had virtually no success with any but delipidated protein.

We have frequently observed crystals to grow and then partially or completely disappear in a matter of a few days, suggesting some inherent crystal instability. Those maintained at 18°C or below, however, appear to be stable and not subject to change. The crystals show good birefringence and extinction and tend to hang suspended in the mother liquor unless attached to a glass surface.

Six different crystals were mounted in quartz capillaries along with a small amount of mother liquor and examined by X-ray photography using a Buerger

precession camera. The X-ray source was an Elliott rotating anode generator operated at 40 kV and 40 mA with a focal spot size of 200 μ^2. Exposure times ranged from one to six hours at precession angles of one to four degrees. The ambient temperature for photography was 4°C.

RESULTS

Crystals of the delipidated α_1-acid glycoprotein grown from ammonium sulfate are shown in Figure 1. The crystals are variations on the hexagonal prismatic habit and frequently have a diameter similar to their length. The crystals are quite large and often reach lengths greater than a millimeter with well defined faces and clean edges. They are mechanically sturdy. The crystals grown here are completely different than the crystals previously reported grown from PEG 4000 by McPherson et al. (17) but those were essentially only microcrystals. These crystals do, however, appear to be morphologically identical to those grown by Schmidt in 1952 (1) from ethanol in the presence of lead acetate.

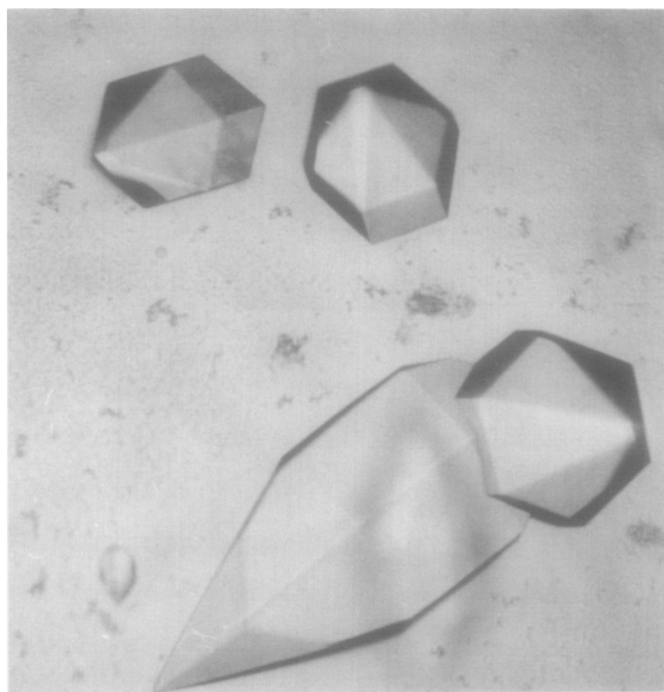

Figure 1. Low power light microscope photograph of hexagonal prismatic form of delipidated α_2-acid glycoprotein crystals grown from 64% saturated ammonium sulfate in the presence of chlorpromazine at 18°C. Crystals are very highly hydrated and produce diffraction intensities only at low Bragg angles.

Unfortunately, in spite of the large size of the crystals, they diffracted very poorly, yielding diffraction intensities to no more than about 12 Å resolution Bragg spacings. In spite of this, and aided somewhat by the apparent absence of decay with time over several days of exposure, we were able to determine the unit cell parameters and the symmetry of the reciprocal lattice. The diffraction distribution falls on a hexagonal net with symmetry 6 mm corresponding to real cell dimensions of a = b = 101 ± 1 Å and c = 201 ± 2 Å. Because the resolution of the photographs is so low, it is difficult to state with certainty the space group. We do, however, observe a strong 002 reflection which would require the probable space group, therefore, to be either P622 or $P6_222$ (or the enantiomorph $P6_422$).

The volume of the crystallographic unit cell is 2.12×10^6 $Å^3$. Since the possible symmetry groups require 12 asymmetric units per unit cell, the volume per asymmetric unit is 1.76×10^5 $Å^3$. Assumption of one molecule of protein (M.W. 41,000) per asymmetric unit yields a volume to mass ratio of $V_m = 4.3$ $Å^3$/dalton while assumption of two molecules implies $V_m = 2.15$ $Å^3$/dalton. A value of 2.15 falls within the range observed for common proteins (18) but does so near the lower end of the range and implies, in general, reasonably well packed protein in a well ordered lattice and is usually associated with crystals that produce high resolution diffraction patterns. α_1-Acid glycoprotein is not a common protein, it has an axial ratio of 5:1, contains 49% carbohydrate by weight and is known to be extremely hydrated. In addition, the crystals described here have an obvious lack of order in the lattice and yield only very low resolution diffraction patterns. Thus we would conclude that one molecule of protein per asymmetric unit and a $V_m = 4.3$ $Å^3$/ dalton is most probably the case. This implies that the crystal contains on the order of 80% solvent and that the protein molecules are forming little more than a gel structure. This is not unprecedented, certain tRNA crystals (19) and the protein tropomyosin (20) have also demonstrated this behavior. We presume the very extensive carbohydrate component is responsible in this case and the character of the crystal simply reflects that of the protein in solution.

We have also observed a second crystal form of α_1-acid glycoprotein in several samples containing the same components and exposed to the same conditions as those described above. They are needle clusters, and the individual crystals can be seen under the light microscope to have a rectangular cross section. These have as yet not grown to sufficient size for an X-ray examination but are clearly a different crystal form than those described above. Polymorphism in crystals is not uncommon among macromolecules that exhibit highly hydrated lattices, tRNA again being a good case in point (19).

We are at present continuing to survey a broad range of additives, other precipitants and potential modifiers, to obtain crystals yielding improved resolution. We are also treating the hexagonal crystals with a variety of reagents with the objective of increasing the order of the molecules in the lattice. We believe that with some additional effort the crystals described here, or perhaps a different modification of the delipidated protein may permit us to deduce at least a low resolution image of α_1-acid glycoprotein.

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