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Authors
Koszelak, S
McPherson, A
Bouillon, R
et al.

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SHORT COMMUNICATION

CRYSTALLIZATION AND PRELIMINARY X-RAY ANALYSIS OF THE VITAMIN D-BINDING PROTEIN FROM HUMAN SERUM

STANLEY KOSZELAK,* ALEXANDER MCPHERSON,*† ROGER BOUILLON‡ and HUGO VAN BAELEN‡
*Department of Biochemistry, University of California, Riverside, CA 92521, U.S.A. and ‡Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Campus Gasthuisberg, 3030 Leuven, Belgium

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Summary—The vitamin D-binding protein from human serum has been crystallized from polyethylene glycol as a complex with 25-hydroxyvitamin D_3 and examined by X-ray diffraction photography. The space group of the crystals is C2 with $a = 203.0$ Å, $b = 75.8$ Å, $c = 90.9$ Å and $\beta = 109.5°$. There are two molecules of 56,000 dalton in the asymmetric unit. The crystals diffract to about 3.0 Å resolution but the patterns exhibit a substantial level of diffuse scatter.

INTRODUCTION

In humans, vitamin D and its metabolites are transported in the blood serum complexed with a specific protein known as the vitamin D-binding protein (DBP) and previously described as group-specific component (Gc-globulin). This protein has been isolated and characterized in many laboratories and can now be purified in substantial amounts by affinity chromatography using immobilized anti-DBP antibodies [1] The estimated molecular weight of protein is 56,000 dalton and its amino acid composition is known. The protein exists mainly as a monomeric species and it is known to contain about 1% covalently linked carbohydrates of which sialic acid residues contribute to a significant level of microheterogeneity.

The protein binds 1 mol of 25-hydroxyvitamin D_3/mol of protein with an affinity of the order of 10^8 M^{-1}. DBP is thermally stable especially in the presence of an excess of 25-hydroxyvitamin D_3 and maintains its activity over long periods of time. Because it is in most ways representative of the broad class of steroid-transporting proteins in the blood, it presents an attractive system for physical–chemical and structural studies. With the intention of ultimately determining the three-dimensional structure of this protein, we have endeavored to obtain crystalline samples of DBP suitable for X-ray diffraction analysis. We have had some initial success in this regard that we would like to describe here.

EXPERIMENTAL

Human DBP was purified from pooled serum by affinity chromatography on immobilized anti-DBP antibodies as described earlier [1] followed by a final chromatofocusing step. This preparation was devoid of endogenous vitamin D metabolites and contained the three common DBP components (Gc-1 Fast, Gc-1 Slow and Gc-2) [2] in almost equimolar concentrations.

Attempts were made to grow crystals of the protein from both salt solutions and from solutions of polyethylene glycol 4000 both in the presence and absence of added 25-hydroxyvitamin D_3. Trials were conducted at both 4 and 25°C principally using the vapor diffusion method in glass plates or hanging drops [3]. Variables investigated in the trials, in addition to precipitant concentrations, were pH, protein concentration, ligand concentration and time.

Two crystal forms of DBP have been obtained and these have been observed in several cases to coexist within the same crystallization sample. While the needle form is essentially useless for crystallographic analyses, the second form seen in Fig. 1 diffracts reasonably well and has been characterized by us.

The crystals seen in Fig. 1 have been grown reproducibly using vapor diffusion in glass plates with PEG 4000 as the precipitating agent [4]. The concentration of PEG 4000 in the 25 ml reservoirs was 12 to 15% w/v and the reservoirs were unbuffered. The sample volumes were 15 μl and contained initially 10 μl of protein dissolved in water at a concentration of 11 mg/ml plus 6 μl of 25-hydroxyvitamin D_3 in ethanol at a concentration of 0.25 mg/ml, plus 5 μl of the reservoir solution containing 12–15% w/v PEG 4000. The sample was made by first adding the 25-hydroxyvitamin D_3 to the glass depressions and allowing the ethanol to completely evaporate before addition of the other components. This was to avoid placing the protein in direct contact with ethanol which was observed to precipitate the DBP.

At 25°C, crystals like those in Fig. 1 appeared in from 5 days to 3 weeks. Once nucleation occurred they appeared to grow rapidly to terminal size over a few days time. The whisker-like crystals appeared much later, after from 3 to 6 months and often formed as neighbors to the other form. No crystals could be obtained in the absence of added 25-hydroxyvitamin D_3.

Determination of the crystal parameters and characteristics of the specimens seen in Fig. 1 was carried out using Buerger precession cameras with a crystal to film distance of 75 mm. The X-ray source was a GX21 Elliott rotating anode generator producing CuK_α radiation. The source was operated at 40 kV and 40 mA with a focal spot size of 200 μm^2.

RESULTS AND DISCUSSION

Photographs from several crystals as well as photographs of multiple orientations from independent crystals were

†To whom correspondence should be addressed.

Fig. 1. Light microscope photograph at low magnification of crystals of human vitamin D-binding protein. The monoclinic crystals, many of them appearing twinned, were grown from polyethylene glycol, and were analyzed by X-ray diffraction. Maximum dimensions are about 1.0 mm.

consistent with a monoclinic net of reciprocal lattice points. The photographs could be indexed such that reflections $h + k \neq 2n$ were systematically absent. Thus the space group of the crystals is C2. The unit-cell dimensions of the crystals were measured from the photographs to be $a = 203.0$ Å, $b = 75.8$ Å, and $c = 90.9$ Å with $\beta = 109.5°$. The calculated volume of the unit cell is therefore 1.32×10^6 Å^3 and it must, by space-group symmetry, contain four asymmetric units. Assumption of two molecules of 56,000 dalton in the asymmetric unit would imply a volume to mass ratio of 2.9 Å^3/dalton. This is consistent with similar ratios for most other crystalline proteins studied [5], while values calculated from assumption of three or more protein molecules per asymmetric unit are not. The value of 2.9 Å^3/dalton is somewhat high and implies a solvent content of about 60%, suggesting the protein to be quite hydrated in the crystal. The value does not, however, appear to be inconsistent with those found for some other glycoproteins [6], and this probably reflects the influence of the carbohydrate moiety.

The crystals reported here can be obtained reproducibly and are mechanically sturdy. Their properties are, however, far from ideal at this time, and we are continuing efforts to improve their quality and diffraction characteristics. These crystals are still somewhat small in size and diffraction intensities have been observed to extend only to about 3.0 Å resolution in most photographs. In addition, many of the crystals show a strong tendency towards twinning and most diffraction photographs appear to contain an unusually high level of diffuse scatter. This suggests that the crystal lattice is less well-ordered than we would like and this may be an unavoidable consequence of the glycoprotein character of the molecule. It should be noted that the DBP preparation used here is heterogeneous in the carbohydrate moiety since the pooled serum used as starting material contains all common phenotypes of DBP. The observation that crystals can be obtained only in the presence of 25-hydroxyvitamin D_3 is probably a result of a more stable conformation being induced in DBP upon binding its ligand. In any case, these crystals provide a starting point for the analysis of the three-dimensional structure of this interesting and physiologically significant molecule.

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