A Preliminary Crystallographic Investigation of Avidin

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Avidin was crystallized in various forms from 3M-ammonium sulphate or sodium phosphate at pH 5.2. X-ray-crystallographic analysis of crystals from phosphate showed them to be orthorhombic, space group $C222$, containing two molecules per unit cell and with a single subunit as the asymmetric unit. The formation of the complex with biotin did not affect the morphology of the crystals.

The preceding paper (Green & Toms, 1970) describes the purification and crystallization of avidin and its analytical characterization. We give here an account of some of the properties of avidin crystals. The first crystals of avidin were observed in samples that had been left in 3M-ammonium sulphate at various pH values for several months in the refrigerator. Although the isoelectric point of avidin is above pH 10 the crystals were initially obtained only at pH 5-6, and all subsequent work has been carried out in this pH region, either in ammonium sulphate or in concentrated sodium

phosphate buffers. Attempts were made to grow larger crystals in both these media for crystallographic work by slowly raising the salt concentration of small (0.5 ml) volumes of solution (10-20 mg/ml) in Perspex cells fitted with a dialysis membrane. These cells facilitated precise control of salt concentration of small volumes of protein solution and permitted observation of the crystals through a low-power microscope. The crystals grown from ammonium sulphate solutions, at room temperature were usually thin, almost square, plates (Fig. 1a). Specimens up to 0.4 mm square could be obtained.

Fig. 1. Crystals of avidin. (a) Thin plates from 3M-ammonium sulphate, pH 5.1 (magnification $\times 25$). (b) Prisms from 3.1M-sodium phosphate, pH 5.2; stained with 4-hydroxyazobenzene-2'-carboxylate (magnification $\times 40$). The sodium phosphate solutions were prepared from 5.5M- NaH_2PO_4 , whose pH, measured after dilution to 2.0M, was adjusted to 5.2 with 70% (w/v) NaOH. This stock solution (final phosphate concn. 5.2M) was used to bring the avidin solution to the point of crystallization. (c) Needles from 3.1M-sodium phosphate, pH 5.2 (magnification $\times 15$).

Occasionally needles and rather thicker plates were observed. Crystals grown from sodium phosphate solutions, pH 5.2, also at room temperature, appeared when the buffer concentration was raised to about 3.0M. They were either prisms with a maximum dimension of 0.2 mm (Fig. 1b) or needles (Fig. 1c), with a similar, rhombic cross section. The interfacial angles were 60° and 120° .

The avidin-biotin complex gave crystals that were macroscopically isomorphous with those of avidin, confirming the suggestions from physico-chemical evidence (Green, 1963) that there was no major conformational change accompanying the binding of biotin. The binding of biotin by crystals of avidin could be followed under the microscope by treating them first with a solution of the dye 4-hydroxyazobenzene-2'-carboxylic acid (Green, 1965), which occupied the binding sites and stained the crystals red (Fig. 1b). Subsequent addition of biotin led to a slow displacement of the dye with eventual decolorization of the crystals. The shape of the crystals was unchanged by this treatment. After fixation in 1% glutaraldehyde at pH 6 for 30 min the crystals from sodium phosphate became insoluble in water or dilute buffers. Their ability to bind dye and biotin was qualitatively unimpaired.

Crystallographic studies were carried out on crystals from sodium phosphate, which were in general needle-shaped with long dimensions up to 0.5 mm and short dimensions up to 0.05 mm (i.e. of the same form but rather more chunky than those shown in Fig. 1c). Zero and upper-layer precession photographs established that the crystals were orthorhombic and belonged to space group $C222$ with cell dimensions $a = 62 \text{ \AA}$, $b = 107 \text{ \AA}$, $c = 43 \text{ \AA}$. The diffraction pattern (Plate 1) extended to $2.5 \text{ \AA}$ and the crystals did not appear to be particularly sensitive to prolonged exposure.

In space group $C222$ there are eight asymmetric units in the unit cell, so that the volume of crystal/asymmetric unit is $35700 \text{ \AA}^3$.

If V_M is defined by $V_M = \text{volume of asymmetric unit/mol.wt. of protein per asymmetric unit}$ and if the molecular weight of a subunit is taken to be 16000, then V_M would be $2.23 \text{ \AA}^3 \text{ dalton}^{-1}$ if there were one subunit/asymmetric unit and $1.11 \text{ \AA}^3 \text{ dalton}^{-1}$ if there were two subunits/asymmetric unit. Matthews (1968) has shown that V_M normally lies within the range 1.68–3.53 for proteins with molecular weights up to 70000, so that the asymmetric unit most probably consists of a single subunit.

If the partial specific volume of the avidin molecule is taken to be $0.72 \text{ cm}^3 \text{ g}^{-1}$ (Green, 1964), the proportion of solvent in the crystals is 50% by volume (for one subunit/asymmetric unit).

The size of the asymmetric unit confirms the presence of four identical subunits in the avidin

Fig. 2. Diagram of the unit cell of orthorhombic crystals of avidin. Neighbouring subunits are indicated by + and - and are related by dyad axes in the plane of the paper.

molecule, which was suggested by sedimentation and osmotic-pressure measurements (Green, 1963, 1964) and by the results of amino acid analysis (Green & Toms, 1970). The space group $C222$ contains three mutually perpendicular dyad axes of symmetry. This shows that the four asymmetric units in each molecule of avidin are related to each other by three dyad axes, which must be parallel to the crystallographic axes. This is consistent with the results of electron-microscopic examination of avidin and the polymers that it gave with bisbiotinyl-polymethylenediamines (Green, 1967, and unpublished work). These showed that the four subunits of avidin were arranged with 222 symmetry and that the dimensions of the molecule were approx. $60 \text{ \AA} \times 55 \text{ \AA} \times 40 \text{ \AA}$. Such molecules could readily be assembled into the orthorhombic space group described with the shortest dimension in the c direction. The pattern of molecules in the a - b plane would be as shown in Fig. 2.

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EXPLANATION OF PLATE I

Precession photograph (9°) of the $hk0$ zone of avidin.