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Crystallization of the Fv Fragment of Mouse Myeloma Protein M315

Rudolf ASCHAFFENBURG,* David C. PHILLIPS,* David R. ROSE,*

Brian J. SUTTON,* Steven K. DOWER† and Raymond A. DWEK†

*Laboratory of Molecular Biophysics, Department of Zoology, University of Oxford,
South Parks Road, Oxford OX1 3PS, U.K., and

†Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

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The Fv fragment of mouse myeloma protein M315 was crystallized from poly(ethylene glycol) solution in the form of monoclinic crystals, space group *C*2 and unit cell dimensions $a = 5.96\text{ nm}$ ($59.6\text{ \AA}$), $b = 5.66\text{ nm}$ ($56.6\text{ \AA}$), $c = 13.79\text{ nm}$ ($137.9\text{ \AA}$) and $\beta = 99.7^\circ$. Some unusual effects of poly(ethylene glycol) on protein crystals were noted and are discussed.

The Balb/c mouse myeloma (immunoglobulin A, λ 2) protein M315, first isolated by Eisen *et al.* (1968), has been of particular interest in structural studies of immunoglobulins because of two unusual features: first, its light chain, designated λ 2, differs in the amino acid sequence of its constant region from that of the prototype λ 1 chain (Dugan *et al.*, 1973; Tonegawa *et al.*, 1978); secondly, the protein is susceptible to an unusual cleavage by pepsin that allows its Fab (antigen-binding) fragment to be further digested into a smaller fragment, called Fv, which comprises the variable regions of the light chain and heavy chain and shows hapten-binding behaviour identical with that of the intact immunoglobulin A molecule (Inbar *et al.*, 1972).

Hochman *et al.* (1973) have shown by isoelectric focusing that the reaction yields a homogeneous product with a molecular weight, by sedimentation equilibrium, of 25250 ± 1200 . By comparing the amino acid composition of the light-chain variable region with the sequence determined by Dugan *et al.* (1973), they deduced that the primary point of cleavage was between residues 114 and 117 of the light chain. Similar reasoning applied to the heavy chain, which has since been sequenced by Francis *et al.* (1974), suggested that here the main cleavage point was probably between residues 114 and 116.

The Fv fragment has been shown to combine with various haptens containing 2,4-dinitrophenyl and 2,4,6-trinitrophenyl groups, and a model of the combining site was built by using structural information from other Fab fragments (Padlan *et al.*, 1976). This model was modified and refined by using data from n.m.r., e.s.r. and chemical modification studies (Dwek *et al.*, 1977; Sutton *et al.*, 1977), but some details are still being called into question (Kumar *et al.*, 1978).

In the structural studies of immunoglobulins, X-ray crystallography has clearly been an important tool (for recent reviews see Poljak, 1978; Kabat,

1978), but a previous attempt to obtain suitable crystals of the Fab fragment of myeloma protein M315 was unsuccessful (Inbar *et al.*, 1971). We now report the crystallization of the Fv fragment of myeloma protein M315.

Materials and Methods

Preparation

Reduced and alkylated myeloma protein M315 immunoglobulin A monomer was extracted from mouse serum by using an ϵ -N-dinitrophenyl-lysine-Sepharose column, as in the previously published procedure (Goetzl & Metzger, 1970). The immunoglobulin A was eluted with 0.1 M-ammonia, according to a slight modification suggested by D. Givol (personal communication). The pH of the fractions was lowered immediately to 8.2 when necessary, and the fractions with $A_{280} > 0.05$ were pooled and dialysed overnight against 10 litres of 0.05 M-sodium acetate buffer containing 0.15 M-NaCl at pH 4.7. The protein solution was then concentrated to 10 mg/ml by ultrafiltration (Amicon PM 30), its pH lowered to 4.5 with acetic acid, and sufficient pepsin added to give a concentration of 0.2 mg/ml. After incubation at 37°C for 3 h to release the Fab fragment, the solution was further acidified to pH 3.5 with acetic acid and again incubated for 3 h to release the Fv fragment (Inbar *et al.*, 1972). The digest was neutralized with 2 M-Tris, dialysed overnight against 10 vol. of 0.05 M-Tris buffer containing 0.15 M-NaCl at pH 7.2, and centrifuged at 15 000 g for 20 min. The Fv fragment was then isolated by applying the concentrated (Amicon PM 10) supernatant to a Sephadex G-75 column (80 cm $\times$ 3 cm), and elution with the Tris-buffered NaCl at pH 7.2. The fractions containing Fv fragment (elution volume approx. 200 ml) were pooled, dialysed overnight against 5 litres of 0.05 M-NH₄HCO₃, pH 7.0, and freeze-dried.

After reduction and alkylation, the product gave two merging bands on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, corresponding to the variable domains of the light chain and heavy chain.

Crystallization

Single crystals suitable for X-ray analysis were prepared by using poly(ethylene glycol) (mol.wt. 6000) (BDH Chemicals, Poole, Dorset, U.K.). Freeze-dried Fv fragment was dissolved in 0.1 M-imidazole/HCl buffer, pH 7.0, to a clear solution containing 1 mg/150 μ l of buffer. Samples (100 μ l) were pipetted into narrow glass tubes, and 35 μ l of poly(ethylene glycol) stock solution was added. The stock solution consisted of equal weights of poly(ethylene glycol) and water; the rather viscous mixture was stirred before each use to enhance its fluidity. On addition of the poly(ethylene glycol), the protein solution goes distinctly turbid, and on standing fine floccules form on the walls of the tubes. Changes occur, usually after 1–2 weeks, when isolated crystals are seen to develop between the floccules. The size and number of crystals tend to increase subsequently over a period of several weeks. Large crystals are usually distinctly yellow, probably owing to traces of the dinitrophenyl hapten used in the isolation procedure.

X-ray studies

Precession and oscillation X-ray photographs were taken by using nickel-filtered Cu K_α radiation, generated at 40 kV and 40 mA.

Results

The crystals are prisms with rectangular cross-section, elongated along the z -axis, the best developed form being {110}. The space group is $C2$, with unit cell dimensions $a = 5.96 \pm 0.01$ nm (59.6 ± 0.1 Å), $b = 5.66 \pm 0.01$ nm (56.6 ± 0.1 Å), $c = 13.79 \pm 0.02$ nm (137.9 ± 0.2 Å) and $\beta = 99.7 \pm 0.2^\circ$.

The diffraction pattern extends to a resolution of better than 0.28 nm (2.8 Å), and crystals mounted in sealed glass capillary tubes at room temperature sustain over 100 h of exposure to X-rays before showing noticeable radiation damage.

In a survey of 116 different crystal forms of globular proteins, Matthews (1968) observed that the fraction of the crystal volume occupied by solvent is most commonly near 43% and always between 27 and 65%. Only if two molecules of Fv fragment of molecular weight 25250 lie in the asymmetric unit (8 molecules in the unit cell) is the calculated fractional solvent content, 47%, within this range. However, the density of the crystals was measured on xylene/

bromobenzene density gradients (Low & Richards, 1952) and found to be 1.27 ± 0.03 g/ml. Despite this unexpectedly high value (see the Discussion section), packing considerations and the extremely low fractional solvent content of 22% resulting from the analogous calculation lead us to believe it unlikely that there are three molecules in the asymmetric unit.

Discussion

The use of poly(ethylene glycol) as a crystallizing agent (McPherson, 1976) has become more common in recent years, though its mode of action is not clearly understood. In examining crystals of Fv fragment and others grown from poly(ethylene glycol) in this laboratory, we have found that the measured density of the crystals is greater than expected. One would expect, for essentially salt-free crystals, that the volume of the unit cell not occupied by protein would contain solvent of density close to 1.0 g/ml. If it can be assumed that the partial specific volume can be used to estimate the volume occupied by a certain mass of crystalline protein, the solvent densities of hen's-egg-white lysozyme (Imoto *et al.*, 1972) and horse methaemoglobin (Perutz, 1946) are calculated to be 1.025 and 1.006 g/ml respectively. However, the corresponding calculation for the Fv fragment, using a partial specific volume of 0.723 ml/g (Hochman *et al.*, 1973), gives a solvent density of 1.15 ± 0.05 g/ml.

One explanation might be that polymers of ethylene glycol very much smaller than the average molecular weight of 6000 can enter the crystal lattice and perhaps cross-link the protein molecules. Alternatively, if one assumes that no poly(ethylene glycol) can enter the crystals and that the solvent within the crystal has a density close to 1.0 g/ml, the high overall density could be explained by a coating of poly(ethylene glycol) around the crystal. The thickness of solid poly(ethylene glycol) covering an average-sized crystal required by this explanation would be of the order of 10^{-3} mm. Either of these alternatives could explain the observation that, after a period of weeks, the crystals did not dissolve in poly(ethylene glycol)-free buffer solutions, or even in pure water.

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References

- Dugan, E. S., Bradshaw, R. A., Simms, E. S. & Eisen, H. N. (1973) *Biochemistry* **12**, 5400–5416

- Dwek, R. A., Wain-Hobson, S., Dower, S., Gettins, P., Sutton, B., Perkins, S. J. & Givol, D. (1977) *Nature (London)* **266**, 31–37
- Eisen, H. N., Simms, E. S. & Potter, M. (1968) *Biochemistry* **7**, 4126–4134
- Francis, S. H., Leslie, R. G. Q., Hood, L. & Eisen, H. N. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1123–1127
- Goetzl, E. J. & Metzger, H. (1970) *Biochemistry* **9**, 1267–1278
- Hochman, J., Inbar, D. & Givol, D. (1973) *Biochemistry* **12**, 1130–1135
- Imoto, T., Johnson, L. N., North, A. C. T., Phillips, D. C. & Rupley, J. A. (1972) *Enzymes 3rd Ed.* **7**, 665–870
- Inbar, D., Rotman, M. & Givol, D. (1971) *J. Biol. Chem.* **246**, 6272–6275
- Inbar, D., Hochman, J. & Givol, D. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2659–2662
- Kabat, E. A. (1978) *Adv. Protein Chem.* **32**, 1–75
- Kumar, K., Phelps, D. J., Carey, P. R. & Young, N. M. (1978) *Biochem. J.* **175**, 727–735
- Low, B. W. & Richards, F. M. (1952) *J. Am. Chem. Soc.* **74**, 1660–1666
- Matthews, B. W. (1968) *J. Mol. Biol.* **33**, 491–497
- McPherson, A. (1976) *J. Biol. Chem.* **251**, 6300–6303
- Padlan, E. A., Davies, D. R., Pecht, I., Givol, D. & Wright, C. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41**, 627–637
- Perutz, M. F. (1946) *Trans. Faraday Soc.* **42B**, 187–195
- Poljak, R. J. (1978) *CRC Crit. Rev. Biochem.* **5**, 45–84
- Sutton, B. J., Gettins, P., Givol, D., Marsh, D., Wain-Hobson, S., Willan, K. J. & Dwek, R. A. (1977) *Biochem. J.* **165**, 177–197
- Tonegawa, S., Maxam, A. M., Tizard, R., Bernard, O. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1485–1489