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Preliminary X-ray Study of Crystals of Human C-reactive Protein

Two different crystal forms of human C-reactive protein have been grown from solutions of 2-methyl-2,4-pentanediol. Both crystal forms are tetragonal, the space group for form I is $P4_122$ (or $P4_322$), and that for form II is $P4_222$. The unit cell parameters for form I are $a = b = 103{\cdot}0(5)$ Å, $c = 308{\cdot}5(7)$ Å and for form II are $a = b = 103{\cdot}1(2)$ Å, $c = 312{\cdot}7(6)$ Å. The crystals of form II diffract to at least 3·0 Å resolution, and are suitable for detailed structural studies.

In mammals, infection or tissue injury uniformly leads to a complex systemic response, including an increase in the concentration of certain plasma proteins, termed acute-phase proteins (Kushner, 1982). In humans, C-reactive protein (CRP) is the most characteristic acute-phase protein, since its plasma concentration rises by as much as 1000-fold within 24 to 48 hours from tissue injury. These high levels persist for the duration of the injury, returning to normal with restoration of tissue structure and function.

CRP is composed of five identical subunits in a planar pentameric arrangement (Osmand *et al.*, 1977). The primary structure of the subunit has been determined at the protein (Oliveira *et al.*, 1979) and the nucleotide level from cDNA and genomic clones (Lei *et al.*, 1985; Woo *et al.*, 1985). It consists of 206 amino acid residues with a combined molecular weight of 23,017. A molecular weight of 118,000 was calculated for the native pentamer from sedimentation equilibrium studies (Volanakis *et al.*, 1978). Crystallization of CRP from 75% saturated solutions of sodium sulfate was reported 40 years ago by McCarty (1947), but the crystals described as "rhomboid plates" were not characterized.

CRP is a calcium-binding protein displaying one or two calcium-binding sites per subunit (Gotschlich & Edelman, 1967). Circular dichroism (Young & Williams, 1978) and immunochemical (Kilpatrick *et al.*, 1982) data indicate a calcium-induced conformational change of CRP essential for the binding of its main ligand phosphocholine (Volanakis & Kaplan, 1971). Numerous additional specificities have been described, including monophosphates (Gotschlich & Edelman, 1967), polycations (DiCamelli *et al.*, 1980), chromatins (Robey *et al.*, 1984), and perhaps galactans.

On the basis of its pentameric structure and its calcium-dependent binding specificities, CRP is classified as a pentraxin (Osmand *et al.*, 1977). Pentraxins represent a phylogenetically ancient family of proteins with members found in the hemolymph of the invertebrate *Limulus polyphemus*, the blood of marine teleosts and the blood of mammals examined (Pepys *et al.*, 1978). Not all pentraxins are acute-phase proteins but the stable conservation of structure and binding specificities through an extremely long evolutionary time suggests a biologically important function. With regards to CRP, its exact biological function is unknown. However, participation of CRP in host defense has been suggested by its ability to activate the complement system (Kaplan & Volanakis, 1974) and to enhance phagocytosis (Kilpatrick & Volanakis, 1985).

Human CRP was isolated using a previously described procedure (Volanakis *et al.*, 1978). The purified protein solution was dialyzed in 0·025 M-Tris·HCl (pH 8·2), 0·1 M-NaCl, and concentrated by vacuum dialysis against the same solution to a protein concentration of 5 mg ml^{-1}. Crystals of each form were obtained by vapor diffusion in depression plates, using 30 µl of protein solution with 5% (v/v) 2-methyl-2,4-pentanediol. This solution was equilibrated against a reservoir of 0·25 M-Tris·HCl, 30% 2-methyl-2,4-pentanediol. The solutions, stored at 22°C, produced small crystals (<0·1 mm × 0·05 mm × 0·05 mm) of both crystal forms within 24 hours. Initially, multiple seedings were necessary to obtain crystals 0·6 mm × 0·4 mm × 0·4 mm in size. However, a specially constructed vapor diffusion apparatus that dynamically controls the equilibration rate by pumping reservoir solution into the crystallization chamber will produce crystals of similar size and of higher quality. Quality X-ray precession photographs are particularly difficult to obtain due to the limited lifetime of crystals in the X-ray beam and the weak diffraction patterns shown by both forms. Small-angle precession photographs confirm two crystal forms, often twinned together, both with the Laue group 4/mmm. The space groups $P4_122$ (or $P4_322$) for form I and $P4_222$ for form II are specified by the absence of reflections $00l$ with $l = 4n$ for form I and $l = 2n$ for form II. These results are confirmed

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by diffractometer studies and extensive synchrotron radiation data collection using oscillation photography. Resolution limits of 3·5 Å for crystal form I and 2·9 Å for crystal form II were observed using the synchrotron radiation source.

The unit cell parameters for form I are $a = b = 103{\cdot}0(5)$ Å, $c = 308{\cdot}5(7)$ Å derived from a $1/\sigma^2$ weighted average from least-squares refinements of reflections from still photographs obtained from a total of five crystals. Each crystal contributed around 50 reflections to the least-squares procedure. The parameters for form II, derived from 12 crystals, are $a = b = 103{\cdot}1(2)$ Å, $c = 312{\cdot}7(6)$ Å.

Assuming a pentameric structure with $M_r = 115{,}085$, the V_m value (Matthews, 1968) based on the cell parameters and one pentamer per asymmetric unit is 3·6 Å^3 dalton^{-1}, corresponding to a solvent volume fraction of 66%. While these figures are at the upper end of the normal range (Matthews, 1968), there is a tendency for higher molecular weight proteins to form crystals containing a relatively higher fractional volume of solvent. In addition, the crystals scatter weakly and are relatively unstable. A decameric structure suggests $V_m = 1{\cdot}8$ Å^3 dalton^{-1}, and a solvent content of only 33%, very much towards the lower end of the normal range.

The density of the CRP crystals, as measured by Ficoll gradient techniques (Matthews, 1968) is 1·217 g cm^{-3} for form I and 1·167 g cm^{-3} for form II. It should be noted, however, that crystals exposed to Ficoll gradient for density determination did not show any diffraction pattern on subsequent exposure to X-rays. The calculated density based on a partial specific volume of 0·735 cm^3 g^{-1} is 1·12 g cm^{-3} for a pentameric and 1·24 g cm^{-3} for a decameric molecule (Colman & Matthews, 1971). Whilst it is our opinion that the measured densities are not sufficiently reliable to allow conclusions to be drawn regarding the number of subunits per molecule, the possibility that the crystalline CRP forms I and II might contain decamers and pentamers, respectively, can not be discounted. Rotation function studies underway should help to clarify the subunit arrangements in these two crystal forms.

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