CRYSTALLIZATION OF FRAGMENT Fab OF HUMAN IgG MYELOMA PROTEINS[1]

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The availability of crystalline antibodies should permit the eventual determination of their three-dimensional structure by X-ray crystallography. Northrop (1942) crystallized a preparation of trypsin-treated horse diphtheria antitoxin. Human macroglobulins have been crystallized by Kratochvil and Deutsch (1956) and by Caputo and Appella (1960). Crystallization of a rabbit γG-globulin (IgG) antihapten antibody, specifically purified from the serum of a rabbit producing an unusually large amount of the antibody, was recently reported from this laboratory (Nisonoff et al., 1967). Deutsch (1967) has crystallized light chains derived from Waldenstrom macroglobulins. Fragment Fc of IgG of several species crystallizes readily (Porter, 1958; Kern, Helmreich and Eisen, 1961; Hershgold et al., 1963), and X-ray crystallographic studies have been initiated on Fragment Fc of rabbit IgG and of a human IgG myeloma protein (Poljak et al., 1967). Fragment Fc does not possess the antigen-binding site of the molecule but has other interesting biological properties. X-ray crystallographic studies have not been carried out with the other crystallized preparations, presumably because of irregularity or small size of the crystals, or insufficient material. Despite strong evidence for chemical homogeneity, the crystals of rabbit antihapten antibody mentioned above gave a poor diffraction pattern. Except for Fragment Fc and the light chains of

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macroglobulins, none of the immunoglobulins or their subunits has been routinely crystallized, and each of the few reports represents an isolated case.

We describe here the crystallization of Fragment Fab of 3 myeloma proteins of the IgG type, one of which, designated "Hil", was a cryoglobulin. Since we have investigated only 6 myeloma proteins so far, it seems likely that Fragment Fab of many myeloma proteins will prove to be crystallizable. These fragments are of particular interest since a number of myeloma proteins have been found to possess antibody activity, and because the Fab fragment contains the active site of the molecule.

Each of the 3 myeloma serums contained more than 40 mg/ml of IgG, as estimated by ultracentrifugation of the serum diluted with 9 volumes of neutral buffer. Each myeloma protein belonged to the same subgroup, IgG1, and the allotype of each was Gm (1+ 3- 4- 5-). The subgroup of protein "Hil" was not determined directly, but was deduced from its Gm type. The light chain subtype of proteins "New" and "Hil" was lambda; that of protein "Gor" was kappa.

The method used for isolation and crystallization of Fragment Fab of myeloma protein "New" will be described in detail. A globulin fraction was prepared from the serum by precipitation with sodium sulfate at a final concentration of 18%. It was chromatographed on DEAE-cellulose equilibrated with 0.0125 M sodium phosphate buffer, pH 6.9; the size of the column was 1 cm^3 for each 10 mg of protein. The fraction that was not retarded on the column (approximately 85% of the protein applied) was collected, dialyzed against 0.01 M sodium phosphate buffer, pH 6.5, and chromatographed on carboxymethyl cellulose equilibrated with the same buffer. The following buffers, each at pH 6.5, were passed through the column sequentially: 0.01 M Na phosphate, 0.03 M NaCl; 0.01 M phosphate, 0.04 M NaCl; 0.01 M phosphate, 1.0 M NaCl. The major fraction of the protein was eluted with the buffer containing 0.04 M NaCl. The overall yield was 23 mg per ml of serum. This protein, as well as "Gor" and "Hil", was identified as IgG by immunoelectrophoresis and ultracentrifugation. It was concentrated by ultrafiltration, dialyzed against 0.1 M phosphate buffer, pH 7.5, and treated with 0.5% of its own weight of papain

in the presence of 0.002 M EDTA and 0.01 M cysteine (Porter, 1959). After digestion for 2 hours at 37° the mixture was dialyzed against 0.01 M phosphate buffer, pH 8.0.

To separate Fragment Fab the digest was filtered through a DEAE-cellulose column equilibrated with 0.01 M phosphate buffer, pH 8.0 (Spiegelberg and Weigle, 1967). The column volume was approximately 1 cm^3 for each 4 mg of digest. The protein that was not retarded contained Fragment Fab and a small amount of undigested IgG, as shown by immunoelectrophoresis. Fragment Fc was collected by elution with 0.3 M phosphate buffer, pH 8.0. The IgG contaminant was removed from Fragment Fab by gel filtration on Sephadex G-100. The major product was identified as Fragment Fab by its sedimentation coefficient, 3.6 S; immunoelectrophoresis (the band was cathodal to that of Fc); reaction with antiserum specific for lambda chains; and the absence of carbohydrate. Also, after reduction and alkylation two major bands formed upon starch gel electrophoresis in 8 molar urea-formate buffer, pH 3, one of which corresponded in mobility to that of light chains isolated from the myeloma IgG and electrophoresed at the same time. Fragment Fab was concentrated to approximately 20 mg/ml by ultrafiltration and dialyzed at 5°, in tubing that had been extracted with boiling water, against ammonium sulfate, pH 5.6, at a concentration equal to 40% of a saturated solution made at 5° C (40% SAS). The protein was then dialyzed at 5° against 42% SAS and finally against 45% SAS. At this concentration clusters of very small needles settled out of solution (Fig. 1A). A second portion of the protein was dialyzed against 35% SAS and seeded with the needles. Crystals formed which were much larger than those first obtained, and which appeared as elongated rectangular prisms. The largest crystals of Fragment Fab of protein "New" that we have been able to obtain are shown in Fig. 1B. These were prepared by seeding a solution at 35% SAS and allowing the mixture to stand for several weeks at 5°. The crystal form of the Fab fragment of protein "New" was the same in each of 4 complete procedures that have been carried out. Each of these crystallized proteins, and also those described below, was birefringent as shown by viewing under crossed Nicol prisms.

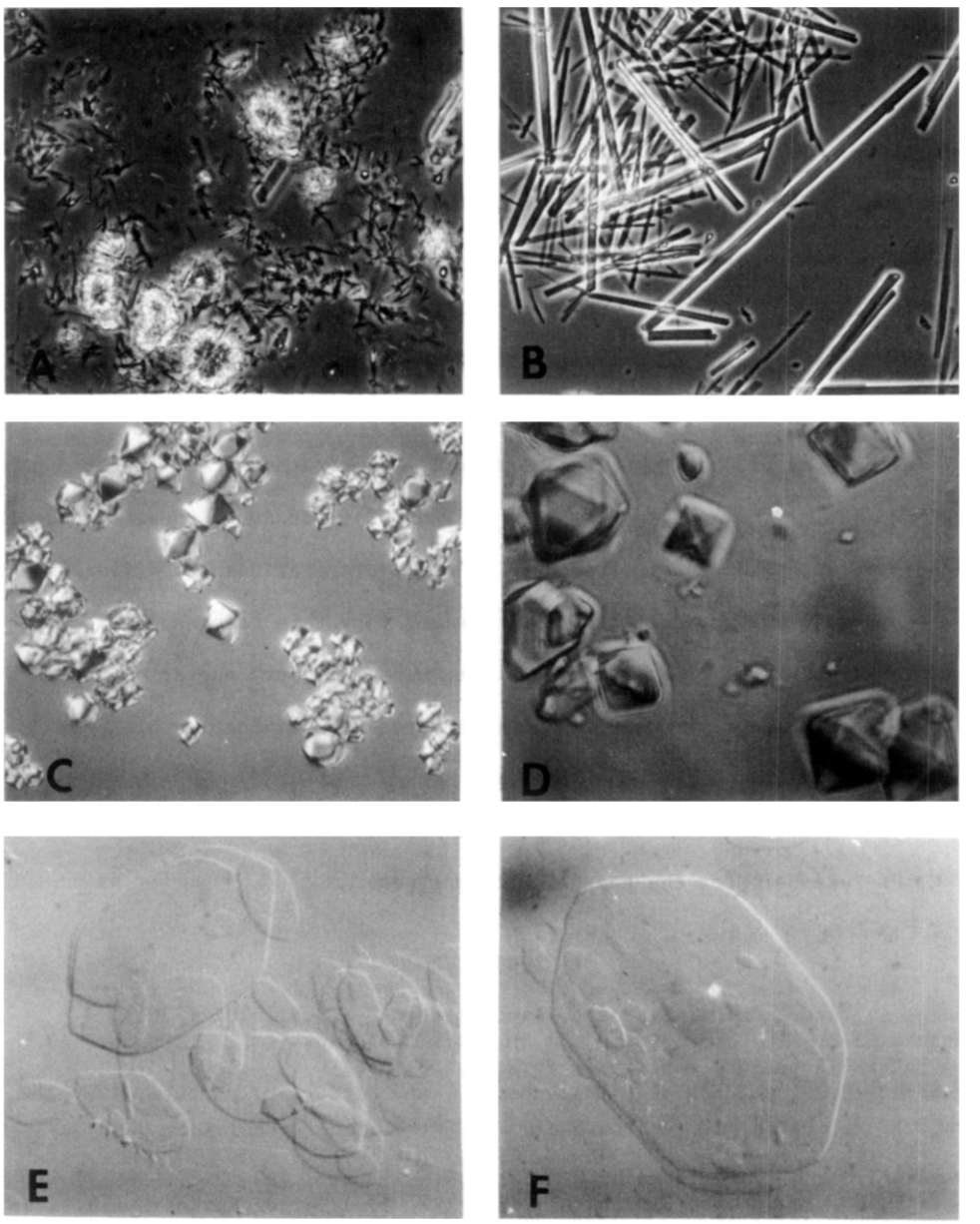

Figure 1. Crystals of Fragments Fab of myeloma proteins "New" (A and B), "Gor" (C and D) and "Hil" (E and F). Crystals in B were obtained by using those shown in A as seeds. Magnifications: A,400x, B,250x; C,800x; D,2000x; E,800x; F,2000x.

The method used for preparation of crystals of Fragment Fab of protein "Gor" was similar except that the bulk of the IgG was eluted from carboxymethyl cellulose with 0.01 M phosphate buffer, pH 6.5, containing 0.025 M rather than 0.04 M NaCl. Crystallization occurred in 48% SAS at pH 6.3.

Isolation of the cryoglobulin ("Hil") was based on its insolubility at low temperature. It was precipitated and centrifuged at 0^{o} to 5^{o} and the supernatant was removed. The protein was redissolved in saline-borate buffer, pH 8, at 37^{o} and precipitated again at low temperature. This procedure was repeated 3 times. Fragment Fab was isolated from a papain digest and crystallized at 5^{o} from 25% SAS, pH 7.7. The solubility of Fragment Fab at 5^{o} was much greater than that of the parent cryoglobulin.

Photographs of the crystals of Fragments Fab obtained from myeloma proteins "Gor" and "Hil" are shown in Fig. 1(C, D, E and F). It is of interest that the morphologies of the crystals from each of the 3 preparations are different. Those from "Gor" and "Hil" appear as octahedrons and very thin hexagonal plates, respectively. Although the crystals of proteins "New" and "Gor" appear very regular they are small, and larger crystals would be required for examination by X-ray diffraction.

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