Properties of Crystalline IgG_3 Globulin[1]

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Summary: The first crystallizable gamma-G globulin of the IgG_3 subclass is described. Crystals were spontaneously obtained upon cooling the serum and in neutral salt solutions. Crystal growth was achieved in 33% of saturated ammonium sulfate. Reduction and alkylation did not abolish the crystalline nature of the protein. Dialysis of the reduced and alkylated protein against 1M acetic acid, and pepsin digestion of the protein abolished the crystalline properties. The preliminary data suggest that the Fc region of the protein is the molecular segment primarily involved in conferring the crystalline and self-associating properties to this protein.

A requirement for determining the three dimensional structure of intact human gamma-G globulin is the availability of readily crystallizable protein. The information derived by X-ray crystallography at a high order of resolution could be combined with primary amino acid sequence data to help elucidate structural differences present on the Fc fragment of the intact molecule which account for the variations in biologic and biochemical function between the various subclasses of human gamma-G globulin.

A preliminary, three-dimensional model of a crystallized IgG_1 myeloma protein has been obtained at 6Å of resolution by X-ray crystallography (1). A crystallizable IgG of another subclass which could provide similar information has not been previously available. Further, previously crystallized gamma-G globulins have not had the characteristic of

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spontaneous crystallizability, hence, localization of the molecular regions primarily involved in the precise self-association necessary for crystallization was not possible. Preliminary chemical studies of the first IgG_3 globulin which preferentially exists in a crystalline rather than a precipitated form are described.

A spontaneously crystallizable IgG_3 globulin, whose subclass was determined by specific antisera using a modification of the radio-precipitin inhibition assay of Fahey and Lawrence (2), was obtained from the serum of a patient with a unique clinical syndrome. Seven to ten micron long fusiform crystals were obtained within 10 minutes of cooling the patient's serum to 4° C (Fig. 1a). These also formed at 23° C at a slower rate. The crystals were collected by centrifugation at 4° C and were purified by multiple washes in cool (5° C) phosphate buffered 0.154M NaCl pH 7.35.

Larger crystals were obtained by dialyzing solutions against 33% of saturated ammonium sulfate at protein concentrations of 1, 2.5, 5 and 10 mgm/ml of solution. The preparations were first warmed to 37° C to achieve solubilization of the crystalline solutions and gradually cooled to 25° C. Using this procedure more orderly, fragile, straw-like crystals, 0.02 to 0.2 mm long were obtained in all preparations within one week (Fig. 1b). These were weakly birefringent in polarized light.

Attempts were made to localize the region(s) of, and structures on the molecule primarily responsible for the spontaneous crystalline nature and self-associating properties of this protein. Solutions of protein at a concentration of 10 mgm/ml in 0.2M Tris-HCl buffer, pH 8.3, were warmed at 37° C to dissolve the crystals. Reduction was performed for 90 minutes by making the solution 0.03M in Dithiothreitol. Alkylation was performed for 60 minutes by adding iodoacetamide to a concentration of 0.06M. During the procedure the solution gradually regained its original, silky cloudiness. This cloudiness remained during dialysis for 12 hours at 4° C

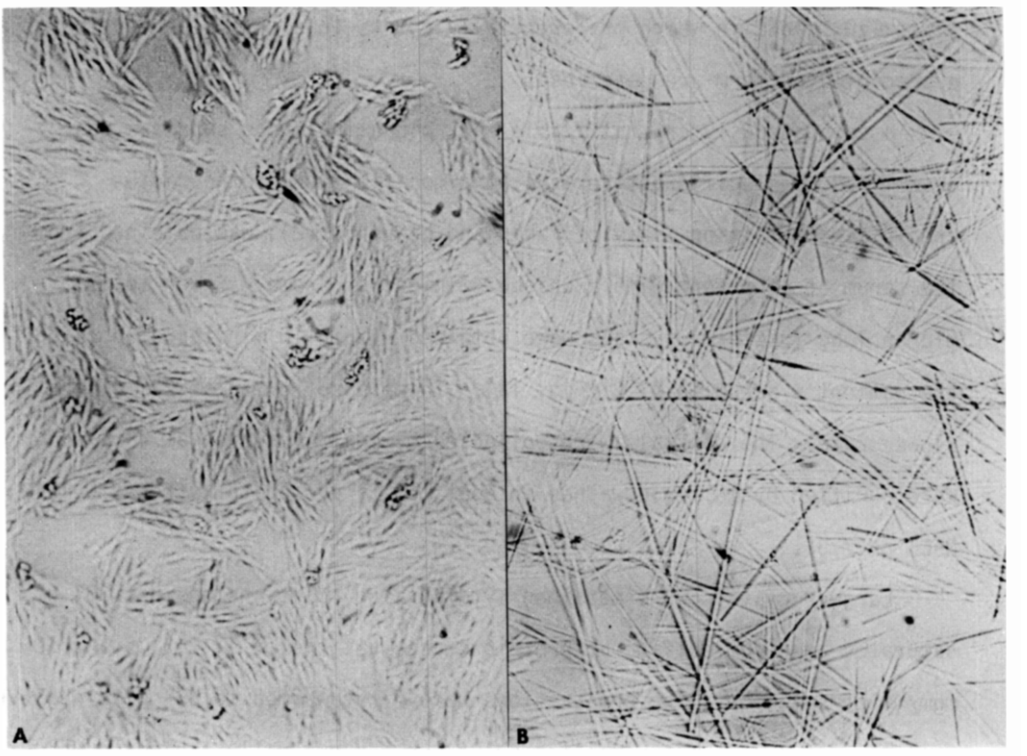

Fig. 1a: Oil immersion photograph at 1000x of 7-10 micron long serum crystals.

Fig. 1b: 165x photograph of crystals after 1 week of cultivation in 33% of saturated, pH 7.0 $(NH_4)_2$ SO_4. Size range is 0.02 to 0.2 mm in length. Birefringence under polarized light was clearly noted at crossover points of the crystals.

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against pH 7.35 phosphate buffered saline to remove excess alkylating agent, and the fusiform micro-crystals were again visible in the solution

by light microscopy. Subsequent dialysis against 1M acetic acid totally abolished the turbidity.

One portion of the acidified sample was subjected to Sephadex G-100 column chromatography in 1M acetic acid. This yielded quantitative proportions of H and L chains which indicated that total reduction of the interchain disulfide bonds had occurred. The H and L chain peaks were pooled and dialyzed against distilled water and phosphate-buffered saline pH 7.35. The L chain preparation was concentrated to 5 mgm/ml and remained completely soluble. The pool of H chains formed a precipitate during dialysis which was not able to be solubilized by warming to 40° C. No crystals were seen by microscopy. The other portion of the acidified, reduced and alkylated protein was redialyzed against pH 7.35 phosphate-buffered 0.154M saline to achieve reassociation of the H and L chains. A white, non-crystalline, amorphous precipitate was obtained which was solubilized by warming the solution to 37° C. When the solution was gradually recooled to 23° C the precipitate reformed and no crystals were seen in the preparation.

This protein is similar to other IgG_3 proteins in its sensitivity to papain digestion and the initial attempts to isolate the Fc fragment in quantity by selective papain digestion have been unsuccessful.

Pepsin digestions were performed exactly as described by Nisonoff (3) for periods of 4 and 8 hours. Complete digestion was determined by analytical ultracentrifugation of the samples which revealed a single 5.1S peak in both samples. It was not possible to crystallize the $(Fab)_2$ portions of the molecule obtained using the previously described conditions. Control protein in the pH 4.5 0.1M sodium acetate buffer but without the pepsin or reducing agent (cysteine) added, spontaneously recrystallized when dialyzed against pH 7.35 phosphate-buffered saline at 4° C.

These data provided indirect evidence that the major structures responsible for the crystalline nature and the self-associating properties

are present on the Fc portion of this IgG_3 molecule. Further, in view of the previously reported hyperaggregability of some IgG_3 myeloma proteins (4) it is quite probable that other crystalline IgG_3 globulins will be found. These should be routinely searched for in any instance in which a cryoprecipitating gamma-G globulin occurs.

References

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