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Characterization of a Human Cryoglobulin Complex: A Crystalline Adduct of a Monoclonal Immunoglobulin G and Albumin†

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ABSTRACT: An unusual human cryoglobulin complex was characterized as a two-component system containing monoclonal immunoglobulin G (IgG) and serum albumin in a 1:2 mole ratio. This complex appeared to be an antibody–antigen complex, since the mole ratio was appropriate and the isolated Fab of the IgG associated with the albumin. The cryoglobulin apparently arose as a result of specific association and/or aggregation of the IgG albumin adduct, since the cryoglobulin complex formed a crystalline precipitate. The IgG and albumin were separated and characterized with respect to immunological cross-reactivities, sedimentation velocities, isoelectric properties, and amino acid composition. The extent of precipitation of the cryoglobulin complex was maximal at pH 7.8, was decreased by added ions including citrate, ethylenediaminetetraacetic acid, NaCl, and $CaCl_2$, and was decreased by increasing temperature. Both the nature of the cold-precipitable complex and the solubility properties differed from those described for other cryoglobulins.

Cryoglobulins comprise those immunoglobulins which precipitate reversibly in the cold. In humans, the following three classes or types have been recognized: type I, monoclonal immunoglobulins with reduced cold solubility; type II, mixed immunoglobulins with a monoclonal component possessing antibody activity toward polyclonal immunoglobulins; and type III, mixed polyclonal immunoglobulins which may also contain nonimmunoglobulin molecules (Brouet et al., 1974). This paper describes the Tu cryoglobulin complex, a cryoglobulin which does not readily fit any of the above categories. The characterization of the Tu cryoglobulin as a cold-insoluble, crystalline 1:2 complex of monoclonal immunoglobulin G (IgG) and serum albumin is described in this report.

The mechanism of cryoprecipitation has been investigated for a number of human cryoglobulins of all three types. No unifying mechanism has been reported (Middaugh et al., 1978); rather, a variety of different mechanisms have been

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shown to exist for cryoprecipitation, including precipitation of antibody–antigen complexes, altered protein–water interactions (Saha et al., 1968), temperature-induced conformational changes (Klein et al., 1977; Saluk & Clem, 1975), and aggregation caused by electrostatic and dispersion forces (Middaugh & Litman, 1977; Middaugh et al., 1980).

Experimental Procedures

Materials. Antiidiotypic antisera to Tu IgG and anti-λ, anti-κ, and anti-Fc antisera were prepared in rabbits after the method of Bernier (1973). Antiserum against human serum albumin was obtained from Meloy. Immunoelectrophoreses (Wieme, 1959) were performed with monospecific antisera (Bernier & Hines, 1967). Horse anti-human serum was purchased from the Transfusion Service of the Netherlands Red Cross. Chemicals and chromatography supplies were analytical grade or higher.

Isolation of Tu Cryoglobulin. Plasma was obtained via therapeutic plasmapheresis of a patient identified as Tu and stored frozen at −20 °C. The Tu plasma was clotted by addition of 25 mM calcium and incubation at 37 °C for 1 h. The serum was incubated overnight at 4 °C and the resulting cryoprecipitate isolated by centrifugation in an International PR-2 at 7000 rpm for 30 min at 4 °C. The cold-precipitable fraction was further purified by several cycles of (1) centrifugation, (2) washing with cold 50 mM Tris-HCl at pH 8.0, (3) dissolving the precipitate at 37 °C, and (4) reprecipitation by overnight incubation at 4 °C. Agar gel electrophoresis (Wieme, 1959) and sodium dodecyl sulfate ($NaDodSO_4$)[1] gel electrophoresis (Laemmli, 1970) and immunoelectrophoresis using anti-whole human serum of the resulting cryoglobulin complex showed bands or arcs consistent with an electrophoretically homogeneous IgG and albumin. Soluble cryoglobulin cross-reacted only with anti-human IgG and anti-human albumin. The cryoprecipitate was composed of small cubic crystals ca. 1–5 μm in diameter. The regular arrangement of components in the crystals is evident in the electron micrograph at 17 250 × magnification shown in Figure 1 (top plate).

Separation of IgG and Albumin. The cryoglobulin complex was solubilized in 18 mM citrate, pH 7, and subjected to DEAE-Sephadex A-50 chromatography in the same buffer (Brettman & Bernier, 1976). IgG was not retained on the column under these conditions, while 1 M NaCl was required to elute the albumin. Alternatively, the cryoglobulin complex could be solubilized in 50 mM Hepes and 0.2 M NaCl, pH 8.0, and added to a column of Blue Sepharose CL-6B (Pharmacia). Under these conditions, up to 35 mg of albumin was absorbed per g of column material (N. Jentoft, J. Sikela, and D. G. Dearborn, personal communication), while homogeneous Tu IgG washed through. Tu albumin was eluted with 1.4 M NaCl in the column buffer (Travis & Pannell, 1973). Isolated Tu IgG was soluble at 4 °C at concentrations up to 100 mg/mL. However, a cryoprecipitate appeared when Tu IgG was added to isolated Tu albumin, human serum, dialyzed human serum, or bovine serum albumin.

Preparation of Fc and Fab Fragments. Fab and Fc fragments were obtained from Tu IgG by a modification of the method of Porter (1959), using 0.1 mg/mL mercuripapain (12 units/mg, Worthington), 0.01 M cysteine, 2 mM EDTA, and 10 mg/mL IgG in 0.1 M phosphate buffer, pH 7.5. Fab and Fc were separated on a DEAE-cellulose column (Whatman),

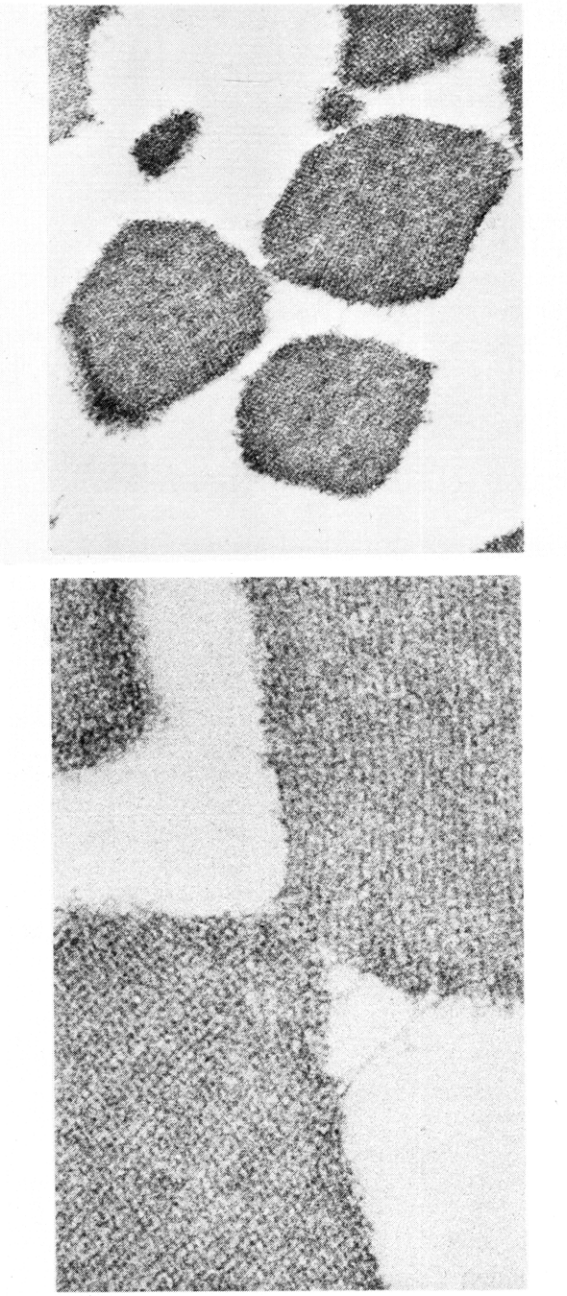

FIGURE 1: Electron micrographs of cryoglobulin crystals fixed with OsO_4 and treated with tannic acid. (Top plate) Sections of several cryoglobulin crystals, magnified 17 250 ×. (Bottom plate) Details of cryoglobulin crystals, enlarged 38 550 ×. At least two distinct packing patterns are displayed in these crystals. These electron micrographs were obtained by Dr. B. Tandler.

following which each gave a single band on agar gel electrophoresis (Wieme, 1959). Ouchterlony double diffusion was performed with antiidiotypic anti-Tu IgG antiserum in the center well, and the Fc and Fab preparations in the side wells. The Fab preparation gave a single precipitin line while no cross-reaction was observed for the Fc preparation. In similar experiments, the Fab preparation gave a single precipitin line against anti-λ antiserum but did not cross-react with anti-κ or anti-Fc antisera.

Electron Microscopy. Pellets of cryoglobulin were fixed for 2 h at room temperature in half-strength Karnovsky's fixative (1965) buffered with phosphate (pH 7.3). They were rinsed in a phosphate-buffered sucrose solution and then postfixed for 2 h in phosphate-buffered 2% osmium tetraoxide (Millonig). After the pellets were rinsed in distilled water, they were

[1] Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

Table I: Amino Acid Analysis of Tu IgG, Fab, and Fc As Compared to Eu IgG1[a]

amino acid	Tu IgG, 1334 residues assumed	Eu IgG (residues/ 150 000 g)	Tu Fc, 224 residues assumed	Fc from Eu IgG1,[b] 224 residues	Tu Fab, 447 residues assumed
Asp	86.3	98.9	15.9	16	25.3
Thr	106.6	112	16.1	15	47.2
Ser	178.5	166	24.4	20	70.6
Glu	112.7	146	21.7	21	31.5
Pro	93.7	103	18.4	21	24.7
Gly	112.1	95.5	11.7	11	45.6
Ala	66.9	72.9	7.1	6	26.6
Val	116.2	129	26.6	16	35.5
Met	9.7	17.7	2.6	3	2.4
Ile	38.1	33.2	5.4	4	13.2
Leu	93.9	94.2	18.3	17	33.7
Tyr	58.0	53.5	8.6	8	16.0
Phe	37.7	46.9	6.6	6	10.0
His	30.5	23.6	6.4	5	9.0
Lys	95.2	92.0	18.6	17	26.6
Arg	47.8	34.3	6.2	7	15.6
CM-Cys[c]	22.7	33.9	4.9	7	6.0
Trp[d]	27.3	22.4	4.4	4	7.4

[a] Edelman et al. (1968). [b] From the sequence of the Fc region of human IgG1 (Edelman, et al., 1969). [c] Carboxymethylcysteine. [d] Estimated spectrophotometrically (Beaven & Holiday, 1952).

treated with tannic acid in a minor modification of Takahashi's (1978) method. This involved soaking the specimens in phosphate-buffered 4% tannic acid for 2 h, rinsing in buffered sucrose, and refixing in 2% osmium tetraoxide for 30 min. Rinsing in distilled water was followed by dehydration in ascending concentrations of ethanol, passage through propylene oxide, and embedment in Epon-Maraglas (Tandler & Walter, 1977). Thin sections were mounted on bare copper grids and examined without further staining in a Siemens Elmiskop 1a electron microscope.

Physical Methods. Isoelectric focusing was performed in 7.5% acrylamide gels containing 2% v/v of the appropriate ampholines. Gels were run for at least 4 h at 120 V. Samples for electrophoresis were applied in pairs; after being focused, one sample was stained for protein by using procedure B of Vesterberg et al. (1977), while the second gel was sliced into 0.5-cm sections for measurement of the pH gradient. The pH of focusing was estimated from the R_f of the protein band.

Amino acid analysis was performed on a Beckman Model 119CL amino acid analyzer by using the single-column method of Fauconnet & Rochemont (1978) for resolving amino acids from hexosamines. Samples were S-carboxymethylated (Hirs, 1967) prior to hydrolysis in vacuo in 6 N HCl at 117 °C for 24, 47, or 71 h. Ultracentrifuge experiments were performed in a Beckman Model E analytical ultracentrifuge, UV measurements were made with a Gilford-modified Beckman DU or a Model 635 Varian Techtron UV–visible spectrophotometer, and pH measurements were made with a Radiometer PHM 64 Research pH meter.

The concentration of Tu albumin was calculated by using an $E^{1\%,1cm}_{280nm}$ value of 5.3 (Putnam, 1977). An $E^{1\%,1cm}_{280nm}$ of 13 was calculated for Tu IgG from the amino acid analysis. An $E^{1\%,1cm}_{280nm}$ value of 9.3 was estimated for the cryoglobulin complex, assuming a 1:2 mole ratio of IgG and albumin and using a weighted average of the respective absorbance values.

Solubility Studies. Solutions for solubility experiments were prepared by mixing aliquots of stock solutions of known concentrations of Tu cryoglobulin complex, buffer, and perturbants in plastic test tubes. Water was added to bring the total volume of the solution to 1 mL. Solutions were incubated at 37 °C for 30 min, followed by incubation at the temperature of the experiments. After 24 h, an aliquot of the supernatant of the cryoprecipitated samples was obtained and the absorption at 280 nm measured. For each experiment, the absorption of a series of dilutions of the stock solution of Tu cryoglobulin was also measured and used to determine the initial concentration of Tu cryoglobulin in each sample. The data are expressed as the percent precipitated cryoglobulin. Solubility data were reproducible to approximately 5% provided fresh cryoglobulin was used to prepare each sample.

Results and Discussion

Determination of the IgG/Albumin Ratio. Purified Tu cryoglobulin was quantitatively separated into IgG and albumin by chromatography on Blue Sepharose columns, and the respective fractions were pooled to determine constituent quantities by the absorbance at 280 nm. The fractions were free of cross-contamination as indicated by Ouchterlony double diffusion against appropriate antisera. Two such analyses, for which 100% of the applied material was recovered, resulted in a molar ratio of 2.0 for albumin/IgG, as calculated by using the appropriate extinction coefficients. The mole ratio is consistent with the bivalent nature of IgG, suggesting that the basic unit of this cryoglobulin is an antigen–antibody complex (a suggestion further substantiated below).

Characterization of Tu IgG. The amino acid analyses of Tu IgG and derivative papain fragments are given in Table I. The composition of another IgG1 and that of a Fc fragment from an IgG1 are reported for purposes of comparison. The amino acid composition is comparable to that of the IgG1 used as reference, particularly for the Fc fragment although the Tu Fc has significantly more valine residues than the reference. The whole IgG molecule appears to have fewer acidic and more basic residues, as would be suggested by the rather high p*I* of Tu IgG.

The isoelectric points (p*I*) of the components of the cryoglobulin and the Tu Fc and Fab fragments were determined. Albumin from the cryoglobulin complex had a normal p*I*, 5.4, and the isolated Tu IgG had a p*I* of 8.4, on the high end of the normal range for IgG1 molecules (Howard & Virella, 1969). The p*I* of the Tu Fc was normal, with several bands between 6.0 and 6.6, but the p*I* of the Fab fragment was very high, ≥8.6. Some heterogeneity of the isoelectric focusing bands existed for all of the Tu IgG species, consistent with the reported progressive loss of the labile amides of asparagine and glutamine from IgG in the serum (Awdeh et al., 1970).

Table II: Sedimentation Velocity Data for Tu Cryoglobulin Components

component(s)[a]	concn (mg/mL)	$s_{20,w}$ (S)
Tu IgG	3.2	6.45 ± 0.03
Tu albumin	2.3	4.17 ± 0.02
Tu Fab	2.5	3.90 ± 0.08
Tu cryoglobulin complex	4.1	11.73 ± 0.06, 4.12 ± 0.14
Tu IgG mixed with Tu albumin	1.5 (IgG), 1.1 (albumin)	10.67 ± 0.08, 4.10 ± 0.03
Tu Fab mixed with Tu albumin	2.5 (Fab), 1.1 (albumin)	6.01 ± 0.02, (3.0 ± 0.2)[b]

[a] All solutions contained 50 mM Tris at pH 8.0. The sedimentation velocity runs were performed at 20 ± 1 °C, in 12-mm double-sector cells at a speed of 60 000 rpm. [b] The slowly moving component was not well-defined, and the $s_{20,w}$ value is highly uncertain.

Subtyping of the isolated Tu IgG was kindly performed by Dr. A. G. Steinberg. The monoclonal protein expressed the Gm determinant 3, a genetic factor associated with the IgG1 subclass. The light chain expressed no Km determinants.

Sedimentation velocity experiments performed on Tu cryoglobulin components are described in Table II. The $s_{20,w}$ values for the isolated IgG and albumin were in good agreement with the published values of 6.6 S (Putnam, 1977) and 4 S (Oncley et al., 1947), respectively. The $s_{20,w}$ values for the Tu cryoglobulin, the reconstituted cryoglobulin, and the Fab–albumin mixture indicate that the albumin associated with the Fab portion of the Tu IgG to form an adduct in equilibrium with the constituent components. The peak at 11.7 S, which was asymmetric, presumably arose from overlapping peaks of uncomplexed IgG and the adduct. At higher concentrations of the Tu cryoglobulin complex, components which sedimented more rapidly were suggested, although these species could not be quantitated (data not shown).

Solubility Properties of the Tu Cryoglobulin Complex. The percentage of the Tu cryoglobulin complex which precipitated at 4 °C increased with increasing amounts of total cryoglobulin, as shown in Figure 2A. A maximum of about 90% precipitate occurred in the presence of 30 mM Tris at pH 8.0, while under more physiological conditions, i.e., in the presence of buffer plus 0.16 M NaCl, the plateau value dropped to about 75% precipitate. At 22 °C, the percent precipitate also decreased, although the shape of the precipitation curve was similar (data not shown). The concentrations of the Tu cryoglobulin complex required for a given percent precipitation were much greater than those reported for cryoglobulins composed only of monoclonal IgG (Middaugh et al., 1978).

The sensitivity of the percentage precipitation to pH is shown at 4 and 22 °C in Figure 2B. The pH for maximum precipitation was in the region of physiological pH values. The Tu cryoglobulin has a very abrupt pH optimum for precipitation relative to that reported for the monoclonal cryoglobulins reviewed by Middaugh et al. (1978) where a broad maximum of precipitation was observed over the pH range of 5–9.

The effect of added ions on the percent precipitation of Tu cryoglobulin (8.1 mg/mL) is shown in Figure 3. The effects of NaCl and $CaCl_2$ were qualitatively similar since both caused an initial increase in precipitation at lower concentrations and a decrease in precipitation at higher concentrations. The effect of $CaCl_2$ was much more abrupt, with enhanced precipitation at physiological concentrations (6 mM), while physiological concentrations of NaCl (0.16 M) tended to decrease precipitation. Increasing the $CaCl_2$ concentration above 10 mM caused a rapid decrease in the extent of precipitation; a similar pattern involving the initial enhancement of precipitation

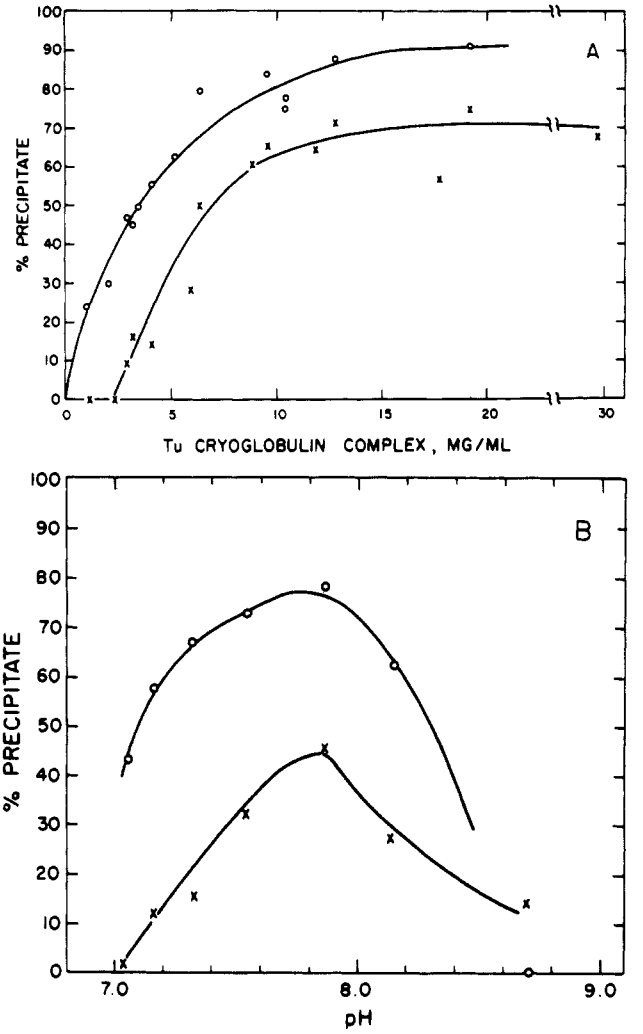

FIGURE 2: (A) Solubility of the Tu cryoglobulin complex as a function of increasing cryoglobulin concentration at 4 °C and pH 8. The data represented by (O) were obtained from the Tu cryoglobulin complex in 30 mM Tris-HCl while the data represented by (×) were obtained from the complex in 30 mM Tris-HCl containing 0.16 M NaCl. The solid lines through the data have no theoretical significance. (B) Solubility of the Tu cryoglobulin complex as a function of pH at 4 °C (O) and at 22 °C (×). The solutions contained 8.7 mg/mL Tu cryoglobulin complex in 30 mM Tris–acetate buffer. The solid lines through the data have no theoretical significance.

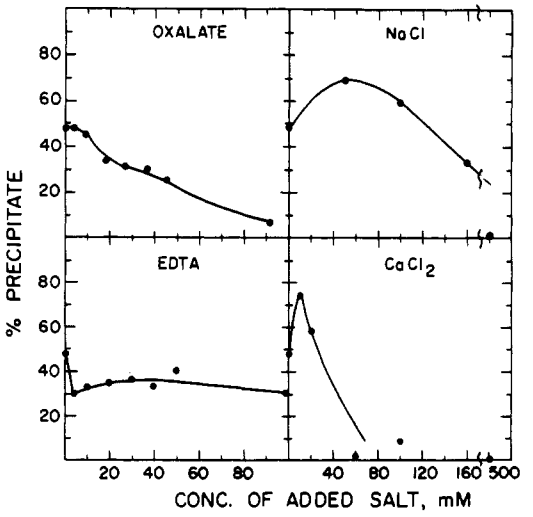

FIGURE 3: Solubility of the Tu cryoglobulin complex at 4 °C as a function of the concentration of added salts. The solutions contained 8.7 mg/mL Tu cryoglobulin complex in 30 mM Tris buffer at pH 8.0.

followed by an increase in solubility with further increases in the $CaCl_2$ concentration has been reported (Middaugh & Litman, 1977; Middaugh et al., 1978) and has been attributed to nonspecific Debye–Huckle charge shielding interactions. However, the effect of $CaCl_2$ occurred over a relatively narrow concentration range in the case of the Tu cryoglobulin complex. Three additional factors may have influenced the effect of calcium on cryoprecipitation. The first is that $CaCl_2$ is known to have a chaotropic effect on local water structure; this is predicted to increase the solubility of exposed nonpolar groups and thus to increase the solubility of the cryoprecipitate at some concentration. Second, there may be binding sites for calcium ions on the IgG which would affect the charge–charge interactions with albumin. The prediction of calcium binding sites on IgG is derived from the observation that Gd^{3+} binds tightly to IgG (Dower et al., 1975) which in turn is related to recent studies in which lanthanides have been used as probes of calcium binding sites on proteins (Matthews & Weaver, 1974; Sperling et al., 1978). Third, the antigenic determinant on albumin may only be recognized in the presence of calcium ions. Frankel & Liberti (1980) have reported the presence of such determinants in human serum albumin. These possibilities are being investigated.

The inhibition of precipitation by the small, highly charged anions citrate, oxalate, and EDTA is also complex (see Figure 3). Citrate under identical conditions reduced precipitation to a limit of 15% precipitate at a concentration of only 4 mM. All three ions may have chelated residual bound calcium from the IgG, albumin, or both, or these ions may have interacted with positively charged sites on the IgG or in some other way interfered with the IgG–albumin interaction.

The precipitation of a sample of 50% soluble Tu cryoglobulin at 4 °C was nearly doubled in the presence of 49 mM ethanol while the same concentration of urea completely solubilized the complex. Both glucose and sucrose increased the solubility of the cryoglobulin, but sucrose was about 3 times as effective at solubilizing the complex when both sugars were present at a concentration of 0.5 M. These results were quantitatively similar to the results obtained with the McE IgM cryoglobulin described by Middaugh & Litman (1977). Some involvement of hydrogen bonds in the interaction leading to cryoprecipitation was suggested to explain these results. This is particularly reasonable because of the low concentrations of urea and ethanol required to affect cryoprecipitation substantially.

Conclusions

The association of Tu IgG and albumin to form a cryoprecipitable species appears to be governed by some of the same forces which have been implicated in the simpler aggregation of monoclonal IgG cryoglobulins, particularly charge–charge interactions and intramolecular hydrogen bonding. An antibody–antigen-type interaction probably explains the initial association of the IgG and albumin, but the aggregation and subsequent precipitation of the complex in a crystalline form are clearly not a typical antibody–antigen effect for a monoclonal antibody. The solubility properties described for the Tu cryoglobulin complex are quantitatively different from those described for other cryoglobulins. For example, the solubility of the Tu cryoglobulin is significantly higher than that described for many cryoglobulins (Brouet et al., 1974; Middaugh & Litman, 1977), the pH dependence of solubility is much more abrupt than that reported for other cryoglobulins (Middaugh & Litman, 1977), and the effect of added ions upon the solubility is different (Middaugh & Litman, 1977; Klein et al., 1977; Middaugh et al., 1978).

The Tu cryoglobulin possessed two highly unusual characteristics: (1) cryoprecipitation required aggregation of an apparent antibody–antigen complex, and (2) the precipitate was crystalline. Spontaneous crystallization has been described for cryoglobulins (Brouet et al., 1974) although the nature of the crystalline complexes has not been characterized in detail. A crystalline precipitate of the type described in this paper involving albumin and monoclonal IgG has not been previously reported. Obviously, very strong forces exist between the individual IgG–albumin adducts to cause them to crystallize spontaneously at low temperatures. However, the affinity of the two proteins for each other is of such magnitude that were the Tu monoclonal IgG present at microgram per milliliter concentrations, similar to those of most normal IgG's produced by single clones, no evidence of a cryoprecipitate would have been observed. The Tu cryoglobulin complex precipitated in vivo because both proteins were present in relatively high concentrations in the serum.

The evidence that the cryoglobulin complex was the result of an antibody–antigen interaction is not complete. The fact that the Fab fragment of Tu combined with albumin and the fact that a 1:2 ratio existed between the IgG and albumin in the complex are both consistent with this possibility. If this is the case, the monoclonal IgG would be expected to combine with a single determinant on the albumin molecule, and there would be no possibility for cross-linking to occur. Hence, the crystal would be built from ordered arrays of IgG(albumin)$_2$ units.

We plan to study this system as a model antigen–antibody complex. Since the cryoglobulin is crystalline, the possibility of an X-ray crystallographic investigation will be explored.

Acknowledgments

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Biochemical Characterization of the Sixth Component (C6) of Human Complement†

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ABSTRACT: The sixth component (C6) of complement was purified from human serum in fully hemolytically active form by anti-C6 and anti-impurities immunoadsorbent column chromatography. When 3-4 L of pooled normal human serum containing 10 mM EDTA as starting material was employed, the final C6 preparations exhibited yields ranging from 40% to 56%, with 1780-1940-fold purification factors based on recovery of specific hemolytic activity. Highly purified C6 was found to be a relatively stable serum glycoprotein, containing 11.3% carbohydrate, that retained 80-100% functional hemolytic activity upon incubation under the following conditions: (1) 6 M guanidine hydrochloride or 6 M urea at 37 °C for 3 h, (2) 4 M potassium thiocyanate at 4 °C for 18 h, (3) 56 °C for 90 min, or (4) pH 5-11 at 37 °C for 2 h. C6 exhibited 4.7 *p*-(chloromercuri)benzoate (pCMB) and 6.3 DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] binding sites per molecule; the C6 hemolytic activity was completely inhibited by 6 mM mercuric chloride and 10 mM pCMB. C6 was highly susceptible to inactivation by trypsin or thermolysin, with a 50% reduction in hemolytic activity occurring after 30 min at 37 °C with 0.2% and 0.4% w/w, respectively. The C6 functional activity was also inhibited by diisopropyl fluorophosphate (DIFP), *p*-tosyl-L-lysyl chloromethyl ketone (TLCK), phenylmethanesulfonyl fluoride (PMSF), and dansyl fluoride over a concentration range of 10^{-2}–10^{-3} M; (*p*-amidinophenyl)methanesulfonyl fluoride (pAPMSF) was inhibitory over a concentration range of (0.5–2) × 10^{-4} M. The synthetic substrate acetylglycyl-L-lysine methyl ester was able to completely protect C6 from inactivation by pAPMSF but was unable to protect C6 from inactivation by mercuric chloride or pCMB. These results strongly suggest that C6 is a serine active site protease and that expression of the C6 enzymatic activity is essential for complement membrane attack complex (MC5b-9) membranolytic function.

Complement (C) is a sequential, multimolecular system of plasma proteins which can be activated by a variety of immunological as well as nonimmunological stimuli (Müller-Eberhard, 1975). C activation, which can proceed via either the classical or alternative pathway, is mediated through a series of cascading reaction steps which are dependent upon the conversion of serum zymogens to active serine esterase enzymes. The classical C pathway is activated by IgG and IgM containing immune complexes and is composed of 11 plasma proteins which are identified numerically as C1–C9 (Müller-Eberhard, 1969). The first component (C1) is a calcium-dependent complex of three plasma proteins, C1q, C1r, and C1s (Lepow et al., 1963). Activated C1r, C1s, and C2 represent the three proteolytic enzymes of the classical pathway which have been described previously (Haines & Lepow, 1964; Naff & Ratnoff, 1968; Porter & Reid, 1978). The alternative C pathway is activated by plant, fungal, and bacterial polysaccharides and lipopolysaccharides in particulate form and is composed of 11 plasma proteins identified as factor D, factor B, properdin, the modulating proteins β_1H and C3b INA, and C3–C9 (Müller-Eberhard & Schreiber, 1980). The C3–C9 components are thus common to both C pathways. Factor D, C3b INA, and activated factor B represent the three proteolytic enzymes of the alternative C pathway which have been described previously (Müller-Eberhard & Götze, 1972; Götze, 1975; Pangburn et al., 1977).

Activation of either C pathway results in the expression of multiple biological activities which include (1) deposition of C3b molecules on the surface under C attack, resulting in particle opsonization and clearance by C3 receptor positive neutrophils, monocytes, and macrophages (Lay & Nussenz-

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