Interconversion of Conformational Isomers of Light Chains in the Mcg Immunoglobulins†

J. R. Firca,‡ K. R. Ely,§ P. Kremser, F. A. Westholm, K. J. Dorrington,¶ and A. B. Edmundson*,§

ABSTRACT: Previous crystallographic studies in this laboratory demonstrated that immunoglobulin light chains with the same amino acid sequence can have at least two and probably three or more conformations, depending on whether the second member of an interacting pair is a light or heavy chain. If a heavy chain is not available in the assembly medium, a second light chain plays the structural role of the heavy chain in the formation of a dimer. In the present work, the λ-type light chains were dissociated from the heavy chains of a serum IgG1 immunoglobulin from the patient Mcg and reassembled noncovalently into a dimer. The reassembly process was completed by allowing the penultimate half-cystine residues to form an interchain disulfide bond. The covalently linked dimer was compared with the Mcg urinary Bence-Jones dimer, for which an atomic model has been fitted to a 2.3-Å electron density map. The assembled dimer and the native Bence-Jones protein were indistinguishable in their chromatographic and electrophoretic properties, as well as in their activity in the binding of bis(dinitrophenyl)lysine. These results indicate that the light chains can be converted into the two types of Bence-Jones conformational isomers. The procedure was also reversed: the two Bence-Jones isomers were dissociated and reassembled as the single type of isomer associating with each of two heavy chains in the IgG1 protein. The change in activity occurring when a light chain associates with a heavy chain instead of a second light chain is illustrated by the fact that the Mcg IgG1 immunoglobulin does not bind bis(dinitrophenyl)lysine in measurable amounts.

IgG1 immunoglobulins consist of two light (mol wt = 23 000) and two heavy chains (mol wt = 50 000), held together by noncovalent interactions and by covalent interchain disulfide bonds (Edelman and Poulik, 1961; Fleischman et al., 1962). The light (L) chains are composed of two homology units ("domains") of about 110 amino acid residues, while the heavy (H) chains are divided into four domains (Edelman et al., 1969). The amino-terminal domains (V_L and V_H) of the L and H chains vary (V) in sequence in different immunoglobulins (Hilschmann and Craig, 1965; Titani et al., 1965). The remaining domains (C_L, C_H1, C_H2, and C_H3) are relatively constant (C) in sequence in immunoglobulins of the same antigenic class and species.

In collaboration with H. F. Deutsch, we have isolated and crystallized a monoclonal serum IgG1 protein and a urinary λ-type light chain dimer (Bence-Jones protein) from a patient (Mcg) with multiple myeloma and amyloidosis (Deutsch, 1971; Deutsch and Suzuki, 1971; Schiffer et al., 1970; Edmundson et al., 1970, 1971; for review of properties of Bence-Jones proteins and light chains, see Solomon, 1976). The crystallographic structure of the IgG1 immunoglobulin is currently being determined, and Watson-Kendrew skeletal models have been fitted to an electron density map of the Bence-Jones dimer at 2.3-Å resolution (Edmundson et al., 1974a, 1975; see also Schiffer et al., 1973). The amino acid sequence determined by Fett and Deutsch (1974, 1975, 1976) was essential for construction and interpretation of the atomic model.

The light chain in the Mcg serum immunoglobulin is combined with an unusual heavy chain, which has a deletion of 15 residues in the "hinge" region between the C_H1 and C_H2 domains (Deutsch and Suzuki, 1971; Fett et al., 1973). The hinge region is responsible for segmental flexibility between the Fab (V_H, V_L, C_H1, and C_L domains) and Fc regions (C_H2 and C_H3 domains) in other immunoglobulin molecules (Noelken et al., 1965). Moreover, the half-cystine residues participating in the interchain disulfide bonds between heavy chains (H-H) and between heavy and light chains (H-L) are located in the hinge region in normal IgG1 proteins. Because of the deletion in the hinge region, H-H and H-L disulfide bonds are absent in the Mcg immunoglobulin. However, the penultimate half-cystine residue is present in the Mcg light chain, and L-L interchain bridges are found in the IgG1 molecule, as well as in the Bence-Jones dimer (Deutsch, 1971).

In the Mcg and Dob IgG1 molecules (Edmundson et al., 1970; Sarma et al., 1971), the light chains in the two Fab regions have identical conformations, as indicated by the presence of a crystallographic twofold axis of rotation between halves of the molecules. However, Mcg light chains, with the same amino acid sequence as those in the IgG1 molecule, behave as conformational isomers when present in the Bence-Jones dimer (Schiffer et al., 1973; Edmundson et al., 1974a,b). The dimer is so closely similar to Fab fragments in size, structure and binding properties that we proposed it be considered as a model for a primitive antibody (Edmundson et al., 1974b). In one monomer the spatial relations between the V and C domains are similar to those of the heavy chain component of an Fab fragment, while the second isomer resembles the light chain component (compare references above with those for Fab regions: Poljak et al., 1974; Poljak, 1975; Amzel et al., 1974; Segal et al., 1974; Davies et al., 1975; Padlan and Davies, 1975; Padlan et al., 1976; Colman et al., 1976). In the absence of conformational isomerism, binding sites would not be formed as we see them in the Bence-Jones dimer.

† From the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, and the Departments of Biochemistry and Biology, University of Utah, Salt Lake City, Utah 84112. *Received May 13, 1977.* This work was supported by the U.S. Energy Research and Development Administration, and by U.S. Public Health Service Grant CA-19616.

‡ Present address: Abbott Laboratories, North Chicago, Illinois 60064.

§ Present address: Department of Biochemistry, University of Utah, Salt Lake City, Utah 84112.

¶ Present address: Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A1.

0006-2960/78/0417-0148$01.00/0

Light chains from immunoglobulins of known specificities have previously been dissociated from their complementary heavy chains, allowed to renature and dimerize, and checked for binding specificity (Goodman and Donch, 1965; Mangalo et al., 1966; Mangalo and Raynaud, 1967; Yoo et al., 1967; Painter et al., 1972a,b; Nakamura et al., 1973; Stevenson, 1973). Since the light chains in the Mcg IgG1 protein have the same sequence as the Bence-Jones monomers, it should be possible to convert them into the Bence-Jones types of conformational isomers (see Anfinsen, 1973). The binding of bis(Dnp)lysine[1] can be used to monitor the conversion. The reverse process of replacing the light chains in the IgG1 protein with Bence-Jones monomers should also be possible. The steps in the interconversions of light chain isomers will be described in this article. We used studies of the dissociation and reassembly of the Bence-Jones dimer as guides for the interconversion experiments. The chemical procedures outlined in the present report were supplemented by crystallographic studies, which will be discussed in the accompanying article (Ely et al., 1978).

Materials and Methods

Preparation and Purification of the Proteins. Samples of serum and urine were obtained from the patient Mcg at the University of Wisconsin by Drs. H. F. Deutsch and D. Cripps. The IgG1 and Bence-Jones proteins were isolated and purified at Argonne National Laboratory.

Solid ammonium sulfate was added to 37% saturation (1.6 M) to serum containing about 2.5 g of IgG1 protein per 100 mL (Deutsch and Suzuki, 1971). The precipitate was dissolved in 0.02 M sodium phosphate buffer, pH 7.4, and dialyzed against this buffer at 4 °C. Aliquots containing 4–8 g of protein (~40 mg/mL) were placed on a 7 × 20 cm column of DEAE[1]-cellulose, equilibrated with the 0.02 M sodium phosphate buffer. The protein was eluted with this buffer and subsequently dialyzed at 4 °C against deionized water to initiate crystallization. The crystalline fraction was removed by centrifugation and stored at 4 °C as an aqueous suspension. The supernatant solution was treated with ammonium sulfate (to 1.9 M), and the precipitated protein was stored as a filter paste at −10 °C. After prolonged storage (2–3 years), both the aqueous suspension and the filter paste were found to contain aggregates and smaller fragments of the IgG1 protein. These were separated from the principal component by gel filtration at 4 °C on 5 × 88 cm columns of Sephadex G-200, equilibrated at pH 8.4 with 0.05 M Bicine–0.15 M NaCl and operated in the ascending mode.

The Bence-Jones protein was precipitated from urine with solid ammonium sulfate (to 90% saturation). After dialysis of the precipitate against 0.1 M Tris-HCl, pH 8.0, the protein (40–100 mg/mL) was crystallized by dialysis against deionized water. Further purification was achieved by recrystallization under the same conditions. The protein was stored as an aqueous suspension at 4 °C.

General Procedure for the Dissociation and Reassembly of the Mcg Immunoglobulins. For the dissociation and reassembly of the Mcg Bence-Jones and IgG1 proteins, we followed procedures developed for typical immunoglobulins (e.g., Fleischman et al., 1962; Metzger and Singer, 1963; Olins and Edelman, 1964; Roholt et al., 1964; Nisonoff and Palmer, 1964; Grey and Mannik, 1965; Porter and Weir, 1966; Haber and Richards, 1966; Stevenson and Dorrington, 1970; Björk and Tanford, 1971a–c; Cathou and Dorrington, 1975). Since crystallization of each reassembled protein was one of our prime objectives, all procedures were carried out as rapidly and gently as possible.

After cleavage of interchain disulfide bonds, the light–light or light–heavy chain complex was dissociated in acid, separated into its components, and reassembled. In the reassembly process, the chains were first mixed under conditions favorable for noncovalent association, after which the interchain disulfide bonds were reestablished ("covalent reassembly"). Most immunoglobulins, including the IgG series, did not reassemble if the added light chains were covalently linked by a disulfide bond (Stevenson and Dorrington, 1970). The only known exceptions to this generalization were the molecules of the $Am_2(+)$IgA2 subclass (Jerry and Kunkel, 1972).

Reversible Thiol Interchange Reactions with the Bence-Jones Protein. Reversible thiol interchange reactions (Smithies, 1965) were used to cleave the interchain bond and produce mixed disulfides $(BJ\text{-}S\text{-}S\text{-}R)_2$ of the Bence-Jones dimer with either neutral or cationic or anionic alkyl groups. After dialysis against borate-EDTA buffer, pH 8.7 (0.1 M boric acid, 0.04 N sodium hydroxide, and 0.004 M EDTA), samples of the Bence-Jones dimer (10 mg/mL) were reacted at 37 °C for 2 h with the neutral reducing agent, 0.004 M 2-mercaptoethanol (ME) and a 0.1 M solution of its disulfide, hydroxyethyl disulfide. Positively charged groups were introduced by reaction with 0.004 M 2-mercaptoethylamine (MEA) and 0.1 M cystamine dihydrochloride. Negative charges were added by reactions with 0.004 M 2-mercaptoacetic acid (MAA) and 0.1 M dithiodiglycolic acid.

Dissociation and Reassembly of the Mixed Disulfides of the Bence-Jones Dimer. The mixed disulfides $(BJ\text{-}S\text{-}S\text{-}R)_2$ were dissociated into monomers (BJ-S-S-R) by passage through a 2.5 × 77 cm column of Sephadex G-100, equilibrated at 4 °C with 0.4 M propionic acid. For noncovalent reassembly the monomers (<0.5 mg/mL) were dialyzed at 4 °C against 0.004 M, pH 5.4, sodium acetate (Stevenson and Dorrington, 1970).

Covalent reassembly was performed at pH 8.7. The proteins were dialyzed at 4 °C against pH 8.7 borate-EDTA buffer and were concentrated to 10–12 mg/mL by ultrafiltration. In preliminary experiments the concentrations were varied from 2 to 50 mg/mL. The alkyl groups were removed by reaction with 0.01 M ME, MEA, or MAA for 24 h at 22 °C. More dilute solutions (0.004 M) of reducing agent were also tested. Covalently reassembled dimers were separated from residual mixed disulfides by chromatography at 4 °C on a 1.5 × 30 cm column of CM-Sephadex C-50. The two molecular species were crystallized in ammonium sulfate (Ely et al., 1978).

Dissociation and Reassembly of the Light Chains from the IgG1 Protein. Because of the absence of light–heavy interchain disulfide bonds in the Mcg IgG1 protein, the covalent light chains can be dissociated from the heavy chains by treatment with acid. A sample of IgG1 protein (10 mg/mL) in pH 8.7 borate-EDTA buffer was placed on a 2.5 × 85 cm column of Sephadex G-100, equilibrated at 4 °C with 0.2 M acetic acid–0.025 M NaCl. Fractions containing covalent light chain dimers were dialyzed successively against the acetate and borate buffers used in reassociation experiments (see preceding sections). The sample was concentrated to 23 mg/mL and treated with ammonium sulfate. This protein did not crystallize.

The procedure was then modified to cleave the interchain bond before dissociation. After formation of mixed disulfides

[1] Abbreviations used are: Dnp, 2,4-dinitrophenyl; EDTA, (ethylenedinitrilo)tetraacetic acid, disodium salt; ME, 2-mercaptoethanol; MEA, 2-mercaptoethylamine; MAA, 2-mercaptoacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; DEAE, diethylaminoethyl; Bicine, *N,N*-bis(2-hydroxyethyl)glycine.

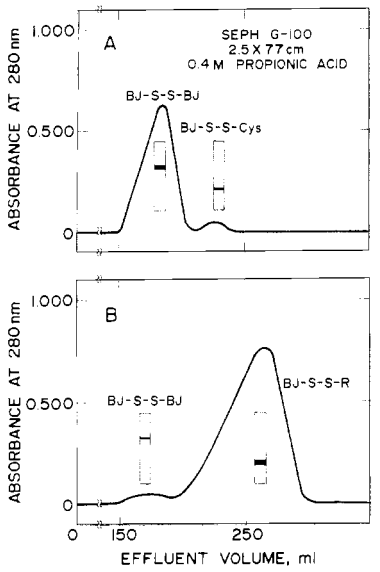

FIGURE 1: (A) Behavior of Bence-Jones starting material on a 2.5 × 77 cm column of Sephadex G-100, equilibrated at 4 °C with 0.4 M propionic acid. Fractions of 3 mL were collected. The covalent dimer is designated BJ-S-S-BJ and the monomer is marked BJ-S-S-Cys. In the monomer the second member contributing to the interchain disulfide linkage is a half-cystine (Cys) residue. Electrophoretic patterns at pH 2.5 are shown near the appropriate peaks. Covalent dimers emerging from this column were dialyzed against the buffers used for renaturation and subsequently were crystallized in ammonium sulfate. These molecules formed needles rather than the trigonal crystals characteristic of the native Bence-Jones dimer (see text for details). (B) Dissociation of noncovalent dimers (mixed disulfides) into monomers (BJ-S-S-R, where R is MEA) in 0.4 M propionic acid, and separation of the monomers from unreacted covalent dimers (BJ-S-S-BJ) on the column of Sephadex G-100.

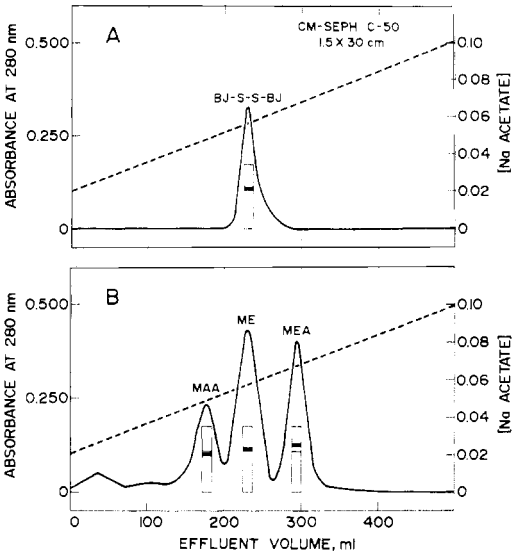

FIGURE 2: Separation of three types of mixed disulfides (MAA, ME, and MEA) of the Meg Bence-Jones dimer by ion-exchange chromatography on a 1.5 × 30 cm column of CM-Sephadex C-50, equilibrated at 4 °C with sodium acetate. The chromatographic behavior of the native covalent dimer (BJ-S-S-BJ) is shown in panel A. The proteins were eluted with a sodium acetate gradient, increasing from 0.02 M (pH 6.0) to 0.1 M (pH 8.0); see dashed lines on effluent curves. Fractions of 3 mL were collected. Electrophoretic patterns at pH 9.5 are shown under the appropriate peaks. Note that the ME derivative is eluted in the same position as the native protein.

with MEA, light chain monomers were separated from heavy chain dimers and higher aggregates by gel filtration in 0.2 M acetic acid–0.025 M NaCl. The MEA monomers were noncovalently reassembled into dimers by the procedure used for the Bence-Jones protein. Removal of the MEA groups was carried out by the method described above, with the exception that the preferred protein concentration was only 3–4 mg/mL (concentrations up to 15 mg/mL were also used). The covalent dimers were separated from the residual mixed disulfides by chromatography on CM-Sephadex C-50. Both types of dimers were crystallized with ammonium sulfate (Ely et al., 1978).

Covalent dimers (20 mg/mL) were also placed in microdiffusion tubes (Zeppezauer et al., 1968) and crystallized by dialysis against water at 20 °C.

Association of Heavy Chains with Monomers from the Bence-Jones Protein to Form IgG1 Molecules. The heavy chains in 0.2 M acetic acid–0.025 M NaCl were mixed at 4 °C with MEA Bence-Jones monomers in 0.4 M propionic acid in a molar ratio of 1.0 to 1.5 (heavy to light). The mixture was exhaustively dialyzed at 4 °C against 0.004 M sodium acetate, pH 5.4 (Stevenson and Dorrington, 1970). MEA groups were removed as before, with a protein concentration of 7–8 mg/mL. The reaction mixture was separated into its components on CM-Sephadex C-50. Reconstituted IgG1 protein was dialyzed at 4 °C against 0.05 M Bicine–0.15 M NaCl, pH 8.4, and concentrated to ~20 mg/mL by ultrafiltration. The protein was crystallized at 4 °C by slow dialysis in microdiffusion tubes against 0.01 M sodium phosphate, pH 6.2 (Ely et al., 1978).

Heavy chains not participating in the reassembly process precipitated on the column of CM-Sephadex C-50. Excess Bence-Jones proteins were recovered as covalent dimers or as mixed disulfides. The covalent dimers were crystallized in ammonium sulfate.

Analysis of Proteins by Disc Electrophoresis on Polyacrylamide Gels. Reactions were monitored by disc gel electrophoresis in alkaline and acidic buffers and in solutions of sodium dodecyl sulfate ($NaDodSO_4$). At alkaline pH, 7% acrylamide gels were used with Tris-glycine buffer (resolving pH of 9.5; Davis, 1974). Electrophoresis was performed at 8 mA per gel until 0.005% (w/v) bromphenol blue tracking dye reached the anode. In acidic solutions the following two systems were employed: (1) 7.5% gels in β-alanine-acetate, with a resolving pH of 3.8 (Maurer, 1974); and (2) 7% gels in propionic acid, with a resolving pH of 2.5 (Choules and Zimm, 1965). Electrophoresis was continued at 8 mA per gel until the 0.05% (w/v) methylene blue tracking dye reached the cathode. Experiments with $NaDodSO_4$ gels (Weber and Osborn, 1969) were performed in the absence of 2-mercaptoethanol.

Gels were stained with a 0.1% (w/v) solution of amido black in a 4:4:1 (v/v/v) mixture of methanol:water:acetic acid or with a 0.25% solution of Coomassie brilliant blue in a 5:5:1 mixture of the same three solvents. The gels were destained with 7% (v/v) acetic acid in a Canalco parallel plate instrument.

The relative quantities of proteins in the bands were estimated with a Gilford gel scanner (Model 2400).

Equilibrium Dialysis with Bis(Dnp)lysine. As one of the criteria for restoration of the conformations of the isomers in the Bence-Jones dimer, the binding of bis(Dnp)lysine by the reassembled Bence-Jones or light chain dimer was studied in solution by the equilibrium dialysis method (Karush, 1962; Voss and Eisen, 1968). The results were compared with those for the native Bence-Jones dimer and IgG1 protein. Dialysis was performed at 4 °C in microchambers with 50 μL of protein or bis(Dnp)lysine per side. Concentrations of proteins ranged from 1.5 to 3.4 mg/mL for dimers and up to 9.0 mg/mL for IgG1 samples. The quantities of bound ligand were determined

spectrophotometrically at 365 nm.

Circular Dichroism. Spectra were obtained at 25 °C with an ORD/CD-15 spectropolarimeter (Japan Spectroscopic Co.) equipped with the SS-20 CD modification (Sproul Scientific Inst.), as described previously by Dorrington and Smith (1972). Between 250 and 320 nm, protein concentrations of 0.8 to 1.2 mg/mL were used in cells of 1.00-cm pathlength. Below 250 nm, protein concentrations were 0.1 to 0.3 mg/mL in 0.10-cm cells. Spectra were recorded using a time constant of 16 s and scanning speeds between 1.1 and 3.6 nm/min. The results were presented as mean residue ellipticity (deg cm^2/dmol) at each wavelength (Dorrington and Smith, 1972). Protein concentrations were determined spectrophotometrically at 280 nm, using a value of 12.5 for $A_{1cm}^{1\%}$.

Results

Purity of the Starting Materials. Crystalline samples of the IgG1 protein (mol wt = 145 000) were monodisperse in the ultracentrifuge and migrated as a single band on gel columns (Edmundson et al., 1970; Deutsch and Suzuki, 1971). After storage for 5 years, however, the samples contained both aggregates and degradation products. The additional components were removed by gel filtration. The contamination level in the purified protein was estimated by gel electrophoresis to be lower than 5%.

Previous analyses of the Mcg Bence-Jones protein in crystals used for diffraction showed only the presence of covalent dimers (Schiffer et al., 1970; Edmundson et al., 1971). However, the supernatant solutions over the crystals contained smaller molecules. Samples employed in the present studies also consisted mainly (~98%) of covalent dimers (mol wt = 46 000). The only minor component was assumed to be a light chain monomer (see Figure 1A).

Thiol Interchange Reactions with the Bence-Jones Dimer. Reactions of the Bence-Jones dimer with MAA, ME, or MEA resulted in mixed disulfides with −2, 0, and +2 net charge differences from the unreacted dimer. The separation of the three disulfides on a column of CM-Sephadex C-50 is illustrated in Figure 2, with the elution profile for the native covalent dimer included for comparison. Electrophoretic patterns are shown with the appropriate peaks. The MEA and MAA derivatives were chromatographically separated from the covalent dimer in milligram quantities, but the ME derivative emerged from the column in the same position as the native protein. All three derivatives were distinguished from the covalent dimer by electrophoresis. The combined chromatographic and electrophoretic analyses indicate that the thiol interchange reactions were 90–98% complete. The elution profile for the fractionation of the products in the MEA reaction mixture was consistent with this view (see Figure 1B). The unreacted covalent dimers are represented by the first small peak in Figure 1B. Monomers formed by dissociation of the noncovalent MEA dimers are indicated by the large second peak. These monomers were used in reassembly procedures.

Reassembly of the Bence-Jones Dimer. The separation of covalent and noncovalent dimers after reassembly is presented in Figure 3. The eluted proteins were electrophoretically homogeneous and present in sufficient quantities (5–10 mg) for crystallization attempts. Because of the ease with which the products could be fractionated, the MEA and MAA derivatives were chosen in preference to the ME derivative for most of the reassembly experiments.

Removal of the alkyl groups and reoxidation of the interchain disulfide bond at pH 8.7 were sensitive to the concentrations of the reactants. When MEA Bence-Jones dimers in concentrations of 40 mg/mL were mixed with 0.004 M MEA,

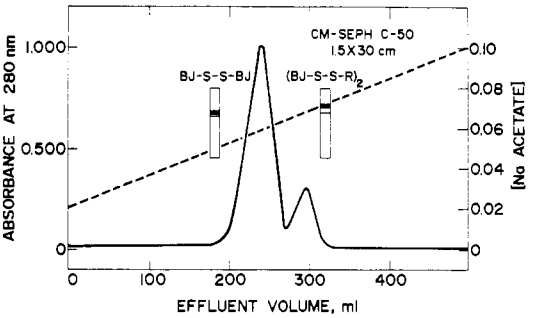

FIGURE 3: Separation of reassembled covalent Bence-Jones dimers (BJ-S-S-BJ) from residual noncovalent dimers $(BJ\text{-}S\text{-}S\text{-}R)_2$, where R is MEA. Operating conditions for the column are similar to those described in Figure 2. Estimation of the areas under the peaks indicates that the conversion of noncovalent to covalent dimers was about 85% complete. The concentrations of protein and thiol (2-mercaptoethylamine) in the reaction mixture were 12 mg/mL and 0.01 M, respectively.

only about 30% of the molecules were covalently reassembled, as estimated by gel electrophoresis. This percentage could be increased to ~85% if the protein concentration was lowered to 10–12 mg/mL and the thiol concentration was increased to 0.01 M (see Figure 3).

Crystal morphology was found to be sensitive to changes in the molecular structures during the dissociation and reassembly procedures. The native covalent dimer only crystallized in one form (trigonal) in ammonium sulfate. The presence of needles in the crystallization mixtures was taken as evidence for conformational changes, but not substantial disordering of the molecules. More serious structural alterations were indicated by the failure of the proteins to crystallize.

Reconstituted Bence-Jones dimers crystallized predominantly in the trigonal form, but needles appeared in small quantities after 3–4 months. Mixed disulfides also crystallized in both forms, but with a greater proportion of needles than the covalent dimers. After treatment of covalent dimers with acid, needles were the only crystals produced. Extended exposure to acid, exhaustive dialysis against acetate buffers, and even ion-exchange chromatography predisposed noncovalent dimers to needle formation. The needle habit was stable in the sense that dissolved crystals always reappeared in the same form during recrystallization experiments.

Dissociation and Reassembly of Light Chains from the IgG1 Protein. Covalently linked light chains could be dissociated from the IgG1 protein with 0.2 M acetic acid–0.025 M NaCl and separated from heavy chains on Sephadex G-100 (see Figure 4A). Separation was possible because the heavy chains were dimers (mol wt 100 000) or higher aggregates in the acetic acid.

The covalent light chain dimers failed to crystallize in ammonium sulfate even after dialysis under conditions favoring reversal of the denaturing effects of the acid. When the interchain disulfide bond was cleaved before dissociation of the light chains from the IgG1 protein, however, the results of the reassembly experiments were similar to those for the Bence-Jones protein (see Figure 4B and preceding section).

The ratio of needles to trigonal crystals in samples of reassembled light chain dimers was usually higher than that in reconstituted Bence-Jones protein. The relative yields of trigonal crystals produced by light chain dimers were greatest after the MEA groups were removed at protein and thiol concentrations of 2–3 mg/mL and 0.01 M, respectively.

Comparison of the CD Spectra of the Native Bence-Jones Dimer and the Proteins Crystallizing as Needles. CD spectra of various samples of Bence-Jones and light chain dimers are

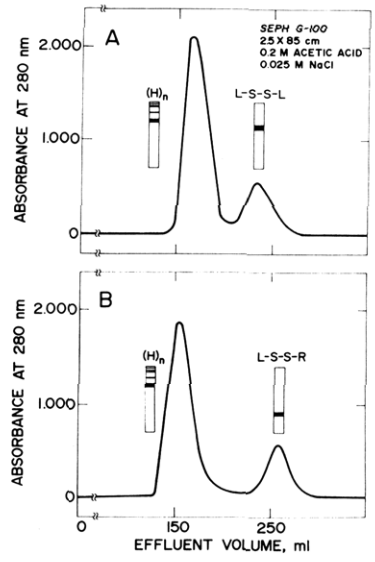

FIGURE 4: Dissociation of heavy (H) and light (L) chains from the IgG1 protein in 0.2 M acetic acid–0.025 M NaCl, and separation of these components at 4 °C on a 2.5 × 85 cm column of Sephadex G-100. The fraction size was 3 mL and the resolving pH for gel electrophoresis was 2.5. (A) Separation when the interchain disulfide bond between the light chains (L-S-S-L) was intact. (B) Fractionation after formation of mixed disulfides of light chains (L-S-S-R, where R is MEA). The heavy chains formed dimers and higher aggregates, $(H)_n$. These H chains recombined with monomers of mixed disulfides (BJ-S-S-R) of the Bence-Jones protein to produce IgG1 molecules (see Figure 10). The H chain aggregates would not recombine with BJ-S-S-R molecules, before or after treatment with 8 M urea in acid to dissociate the aggregates.

presented in Figure 5. The native Bence-Jones dimer (panel a) shows a series of well-resolved CD bands, some of which can be assigned to particular species of chromophores. The bands at 291^-, 286^+, and 282^- nm almost certainly arise from the indole chromophore of tryptophan (Strickland et al., 1969). The positive band centered near 276 nm probably represents a transition in the phenolic ring of tyrosine. Assignments are difficult below 270 nm because tyrosine, tryptophan, and phenylalanine side chains all have transitions in this region. There are also alternative explanations for the broad, skewed band with an apparent center near 298 nm. For example, tryptophan has a long wavelength transition which is occasionally observed in protein spectra (for lysozyme spectra, see Halper et al., 1971). Alternatively, the band may represent the long wavelength end of the broad transition associated with particular values of the dihedral angle in a disulfide chromophore (Sears and Beychok, 1973).

The CD spectrum of the trigonal form of the MEA Bence-Jones dimer shows minor quantitative differences from that of the parent covalent dimer, but the overall shape is unchanged (see Figure 5a). The slight changes in intensity of the spectrum may be accounted for by the loss of optical activity associated with the interchain disulfide bond in the covalent dimer.

Conversion of covalent Bence-Jones or light chain dimers into the needle form is accompanied by profound changes in side-chain optical activity (see Figure 5b). The weak positive ellipticity above 300 nm observed for the trigonal form is replaced by an intense negative band centered near 308 nm. A tryptophanyl transition is apparent at 291 nm and a broad, complex envelope of positive optical activity is apparent between 255 and 285 nm. Although less well-resolved, the spectrum of the needle form appears to retain some features of the "native" spectrum since there is evidence of transitions at 280 to 285 (shoulder), 276, 270, and 263 nm. The spectrum for the needle form of the MEA derivative closely resembles that of the needle form of the covalent dimer.

In contrast to the aromatic absorption region, no significant differences in optical activity associated with the peptide chromophore were observed for any of the samples regardless of the crystal habit they adopted (Figure 5c). This strongly suggests that the secondary folding remains relatively unchanged in the conversion of the trigonal to the needle form. The simplest interpretation of the CD changes involves localized conformational changes affecting the environment of aromatic chromophores. The qualitative features of the aromatic CD in the needle form indicate dynamic coupling between two transitions: one centered near 310 nm and the other around 275 nm. While it is difficult to assign either of these unambiguously, the most likely candidate for the long wavelength band is the indole ring of tryptophan, and tyrosine seems

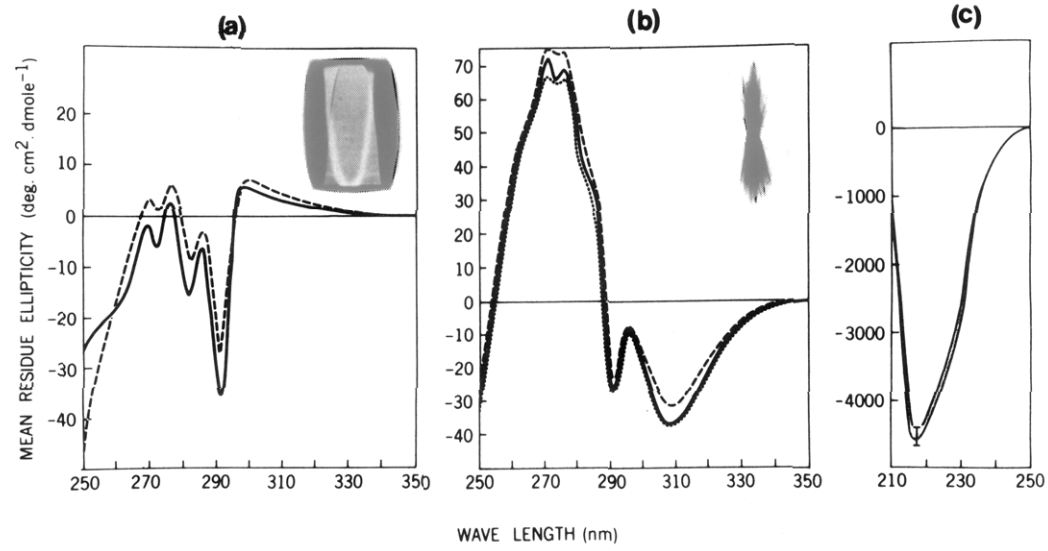

FIGURE 5: (a) CD spectra, between 350 and 250 nm, of the native covalent Bence-Jones dimer (—) and the same protein reduced and alkylated with mercaptoethylamine (- - -). Both samples crystallized in the trigonal form, an example of which is shown above the spectra. (b) CD spectra, again between 350 and 250 nm, of the dissolved needle forms of the Bence-Jones covalent dimer (—), the light chain dimer isolated from the Mcg IgG1 protein (···), and the Bence-Jones dimer reduced and alkylated with mercaptoethylamine (- - -). A photograph of some needles is presented over the spectra. (c) CD spectra below 250 nm. The shaded area represents the spectra for the samples listed in panels a and b.

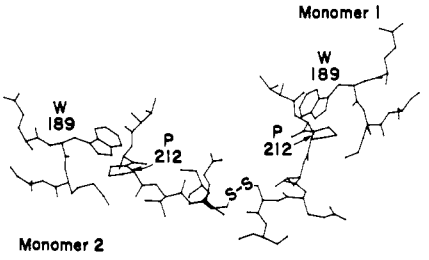

FIGURE 6: Conformations of COOH-terminal segments leading to the interchain disulfide bond (S-S), with the relative location of the helical segment containing tryptophan-189 also shown for each monomer. Note that the chain makes a right-angled turn at proline-212 in monomer 2, but not in monomer 1.

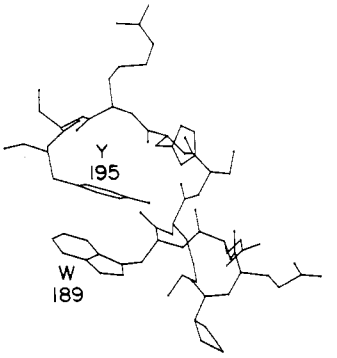

FIGURE 7: Relative orientations of the side chains of tryptophan-189 and tyrosine-195 of monomer 1 in the native Bence-Jones dimer. Segments containing these residues are situated near the interchain disulfide bond (see Figure 6) and are likely participants in the set of conformational changes in molecules crystallizing as needles. These conformational changes are reflected in the striking differences in the aromatic regions of the CD spectra of the trigonal and needle forms (see Figure 5).

a reasonable choice for the 275 nm component.

Inspection of the atomic model of the native Bence-Jones dimer (Edmundson et al., 1975) shows that only one (no. 189) of the three tryptophan residues (nos. 37, 152, and 189) in each monomer is likely to be associated with the CD spectral differences. One tryptophan residue in each domain (no. 37 in the V and 152 in the C domain; Fett and Deutsch, 1974; Schiffer et al., 1973) is buried in the hydrophobic interior and therefore not accessible to solvent. The third tryptophan residue (no. 189) is present in a helix at the end of the C domain near the interchain disulfide bond (see Figure 6). The indole ring of tryptophan-189 is in close proximity to the phenolic ring of tyrosine-195, as shown in Figure 7. While directed toward the interior of the C domain in the native protein, the side chains of these aromatic residues could readily be moved by perturbation of the structures around the interchain disulfide bond. Such changes in the interactions between these two chromophores result in coupling of transitions which are unable to mix in the native conformation.

The spectral changes observed with the Mcg λ-type light chain are not unique to this protein. Klein and Dorrington (to be published) have observed comparable effects when a number of different λ chains were subjected to chemical manipulations similar to those described here.

Reversal of Conformational Changes in Proteins Crystallizing as Needles. The conformational changes involved in needle production in samples of noncovalent dimers could be reversed by careful application of the procedure for covalent reassembly. A simple procedure was also developed to reverse the conformational changes in the needle form of covalent dimers. When dissolved needles were treated with reducing agent to cleave the interchain disulfide bonds and then covalently reassembled, trigonal crystals were again produced in ammonium sulfate (Ely et al., 1978). Together with the CD spectra, these results suggest that the principal conformational differences between dimers in the trigonal and needle forms are found in the vicinity of the interchain bonds.

Conversion of Light Chains to the Bence-Jones Types of Conformational Isomers. The interconversion of conformational isomers can be considered in terms of the known three-dimensional structures of the Mcg Bence-Jones dimer and related immunoglobulin components (see Figure 8). Domains in the Mcg proteins, as well as those in all other light and heavy chains thus far examined, have a common immunoglobulin "fold" (Schiffer et al., 1973; Edmundson et al., 1975; Poljak et al., 1974; Poljak, 1975; Segal et al., 1974; Davies et al., 1975; Padlan and Davies, 1975; Epp et al., 1974; Fehlhammer et al., 1975; Colman et al., 1976; Deisenhofer et al., 1976; Huber et al., 1976; Wang and Sax, 1974). The fold is a bilayered structure with three antiparallel strands of β-pleated sheet in one layer and four antiparallel segments in the second layer (see Figures 8a and 8b). During evolution the domains have rotated in such a way that homologous pleated sheets perform different functions in the V and C domains (see rotation axes in Figures 8a and 8b). In the Mcg light chains the angle of rotation is about 165° between V and C domains, which can be considered rotational allomers (molecules with different amino acid sequences, but similar three-dimensional structures which are related by rotation). The V and C domains of heavy chains and other light chains are also rotational allomers, although the angles of rotation vary from the above value by a few degrees.

The three-stranded layers of the V domains face each other to form the binding regions in the Bence-Jones dimer and in Fab fragments (see Figures 8a, 8b, and 8d). These layers also supply the side chains that interact to stabilize the dimer of V domains. In the C domains the three-stranded layers provide the outer surfaces of the dimer.

If one of the domains is rotated, as in the case of the C domain, a different surface is exposed to the second component of the binary complex. After rotation the four-stranded layers face each other to stabilize the C domain dimer. In the V domains the four-chain layers supply the external surfaces of the dimeric module.

In the dissociation of light chains from their heavy chain companions, the interactions to be broken involve the three-stranded layers of the V domains and the four-chain layers of the C domains. Similar interactions have to be later reestablished between pairs of light chains during the reassembly procedure.

Conformational changes occurring during the conversion to Bence-Jones types of isomers can be surmised by examining models of the native dimer (see Figures 8c and 8d). Since the conformation of monomer 2 is similar to that of the light chain in an Fab fragment, we assume that the major changes occur in the transition to monomer 1, the heavy chain analogue. The most striking differences are observed in the spatial relations between the V and C domains, as illustrated in Figure 8c (see Schiffer et al., 1973; Edmundson et al., 1975). These changes are permissible because the switch regions are capable of large conformational shifts, particularly around glycine-111.

The steps in the conversion of light chains to the Bence-Jones types of isomers are summarized in Figure 9. The extensive changes necessary to achieve the conversion are difficult to envision if the light chains remain covalently anchored by the interchain disulfide bond. Therefore, it is not surprising that light chains dissociated from the IgG1 protein as covalent di-

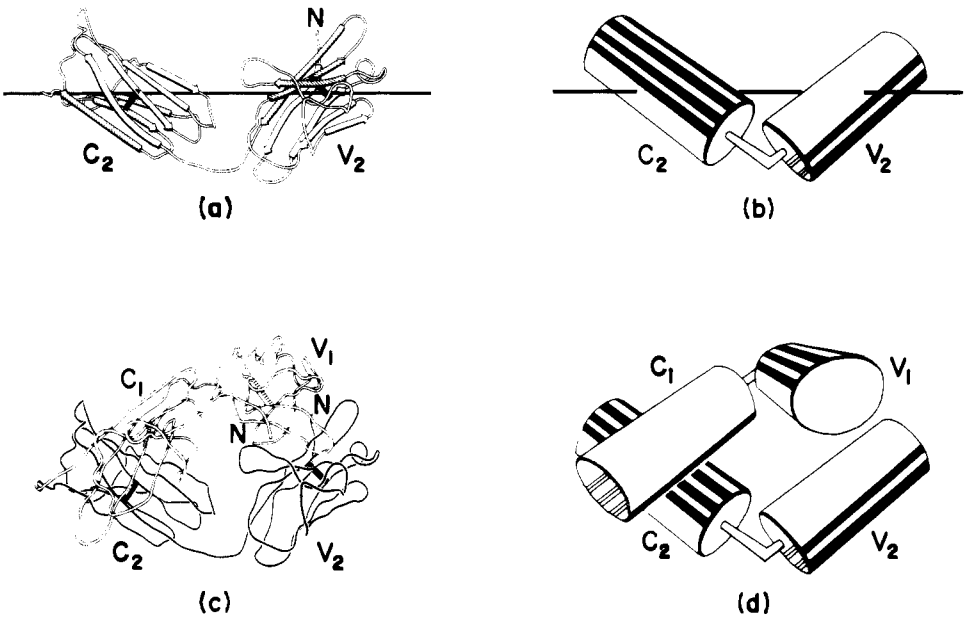

FIGURE 8: Use of models of the Bence-Jones dimer and its monomer 2 component to illustrate rotational allomerism and conformational isomerism. (a) Tracing of the course of the polypeptide chain of monomer 2 with directional arrows superimposed on chain segments. Each domain is a bilayered structure, consisting of three-stranded (striated arrows) and four-stranded (white arrows) β-pleated sheets. The two sheets are connected by an intrachain disulfide bond (black bar) near the center of each domain. The black line through the V and C domains represents a rotation axis relating the two domains. Note that the pleated sheets in the V domain face different directions than their counterparts in the C domain. The two domains can be superimposed if the V domain is rotated about 165° around the axis and translated about 44 Å. We therefore refer to the V and C domains as rotational allomers (molecules related by rotation, with similar three-dimensional structures but different amino acid sequences). (b) Monomer 2, with the V and C domains represented by cylinders. The four-stranded β-pleated sheets are indicated by stripes, and the three-chain layers by blank regions on the cylinders. The rotation axis is shown as a black line. (c) Tracings of the polypeptide chains in the Bence-Jones dimer. Monomer 1 is represented by the white chain. The binding region is located on the far right and the interchain disulfide on the far left. The N terminus of each chain is marked N. Note the striking differences in the spatial relations between the V and C domains in the two monomers, which are conformational isomers. Monomer 1 plays the structural role of the heavy chain component in an Fab fragment, and monomer 2 resembles the light chain. (d) Bence-Jones dimer represented by cylinders. As a result of rotational allomerism, the three-stranded layers of the V domains face each other to form the binding region (between the two V domains at far right), while the four-stranded layers of the C domains face each other across a solvent-free zone to stabilize the dimeric module. The conformational isomerism can be visualized by comparing the relative orientations of the V and C domains in the two monomers.

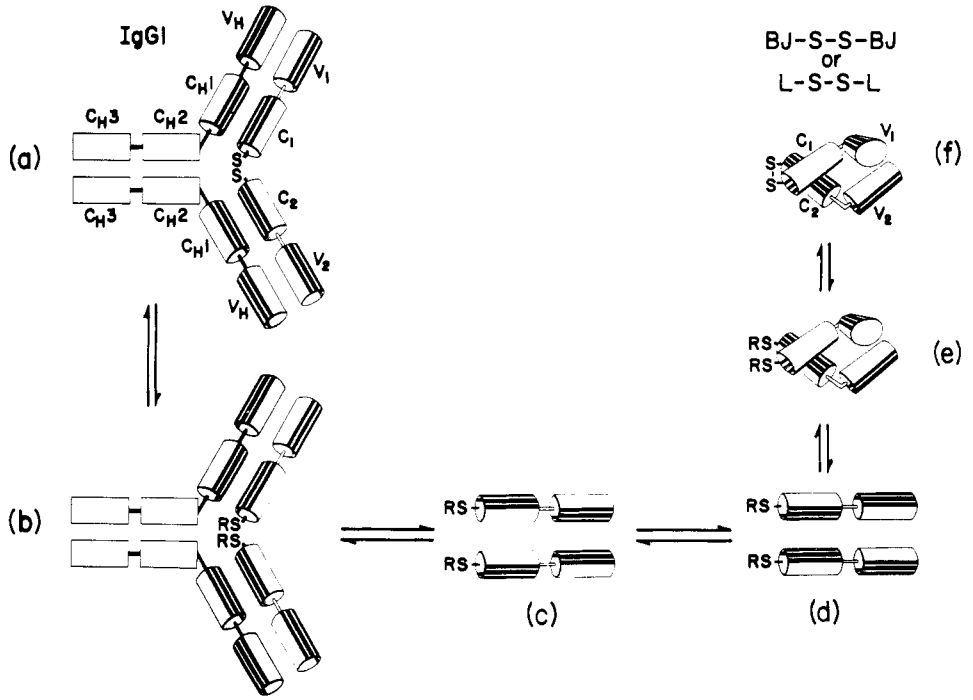

FIGURE 9: Interconversion of conformational isomers of Mcg light chains. (a) The serum IgG1 immunoglobulin with the light and heavy chain components of the Fab regions represented by cylinders. The four-stranded layers are indicated by stripes as in Figure 8. The C_1 and C_2 domains of the light chain are connected by an interchain disulfide bond (SS). (b) The IgG1 protein with the interchain disulfide bond cleaved and converted to mixed disulfides (RS). (c) Dissociation and separation of the light chains from the heavy chains in acid. (d and e) Noncovalent reassembly of light chains into a dimer with the Bence-Jones types of conformational isomers. (f) Covalent reassembly into a disulfide-linked dimer (L-S-S-L). The reactions are reversible, and the native Bence-Jones dimer (BJ-S-S-BJ) can be converted to the light chain type of isomer and incorporated into the IgG1 protein.

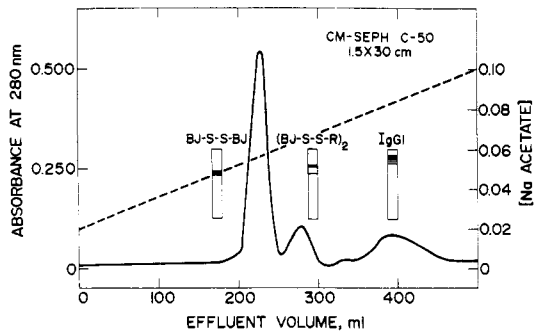

FIGURE 10: Isolation of IgG1 protein, reassembled from heavy chains (see Figure 4) and monomers of the mixed disulfides of the Bence-Jones protein (BJ-S-S-R, where R is MEA). Chromatography and electrophoresis were carried out as in Figure 2. Aggregated heavy chains not combining with Bence-Jones proteins precipitated on the column. Excess Bence-Jones protein formed covalent (BJ-S-S-BJ) and noncovalent $(BJ\text{-}S\text{-}S\text{-}R)_2$ dimers during the reassembly procedure. Light and heavy chains were mixed in a molar ratio of 1.5 to 1.0. The yield of IgG1 molecules was about 10%.

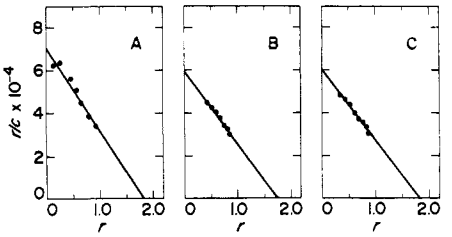

FIGURE 11: Results of equilibrium dialysis of Bence-Jones protein with bis(Dnp)lysine. Data are shown for the following samples: (A) native covalent Bence-Jones dimers (BJ-S-S-BJ); (B) reassembled covalent dimers; and (C) noncovalent dimers (mixed disulfides, $(BJ\text{-}S\text{-}S\text{-}R)_2$, where R is MEA). The concentration of each protein was 5.0×10^{-5} M (2.3 mg/mL). Similar plots were obtained for reassembled covalent and noncovalent dimers of light chains from the IgG1 protein. In the plots r is the number of moles of ligand bound per mole of dimer, and c is the concentration of ligand. The lines were drawn by the method of least squares.

TABLE I: Summary of Results of Equilibrium Dialysis with Bis(Dnp)lysine as Ligand.

Protein	Concn ($M \times 10^{-5}$)	t (°C)	n^a	$K_0{}^b$ ($M^{-1} \times 10^4$)
BJ-S-S-BJ	5.0	4	1.8	3.9
Recombinant BJ-S-S-BJ	5.0	4	1.8	3.4
$(BJ\text{-}S\text{-}S\text{-}R)_2$	5.0	4	1.8	3.3
Recombinant BJ-S-S-BJ	3.3	4	1.9	4.0
L-S-S-L	3.3	4	1.7	3.9
$(L\text{-}S\text{-}S\text{-}R)_2$	3.3	4	1.7	3.1

[a] n is the intercept on ordinate in Figure 11; the number of binding sites in each dimer. [b] K_0 is the average association constant. The abbreviations for the Bence-Jones and light chain dimers are the same as those in Figures 1-4.

mers fail to produce crystals of any kind. If the bond is cleaved, the light chains are free to assume conformations similar to those of Bence-Jones monomers. Such monomers can then be reassembled into a binary complex crystallizing in the trigonal form or as needles which can be converted into the trigonal form.

Conversion of Bence-Jones Conformational Isomers to Light Chains in IgG1 Molecules. All of the conformational changes described in the preceding section are reversible and the atypical Mcg IgG1 protein can be reconstituted from Bence-Jones monomers and heavy chains in quantities adequate for crystallization (see Figure 9 and Ely et al., 1978). Interactions between the three-strand layers of the V domains and the four-chain layers of the C domains in the noncovalent Bence-Jones dimer (mixed disulfide) are first interrupted in acid, and later reestablished between each monomer and a heavy chain. The differences in association constants favor the formation of light-heavy over light-light complexes (Cathou and Dorrington, 1975). In the reassembly of IgG1 immunoglobulins, the reactive species is the heavy chain dimer, and intermediates of light-heavy-heavy complexes and light-heavy half-molecules are produced (Stevenson and Dorrington, 1970). The reassembly is accompanied by conformational changes in both the light and heavy chains (Steiner and Lowey, 1966; Dorrington et al., 1967; Stevenson and Dorrington, 1970; Björk and Tanford, 1971c; Painter et al., 1972a; Dorrington and Smith, 1972; Jirgensons, 1973; Azuma et al., 1975).

The separation of reconstituted IgG1 molecules from unreacted heavy chains and Bence-Jones proteins is illustrated in Figure 10. By $NaDodSO_4$ gel electrophoresis in the absence of reducing agent, the isolated IgG1 proteins were found to be composed exclusively of heavy chains and covalent light chain dimers. The reconstituted proteins were recovered in low yields (~10%), which were attributable to the irreversible formation of heavy chain aggregates higher than dimers (see Figure 4). These aggregates remained soluble for at least 6 months at pH 7.0 and 4 °C in 0.01 M Bicine-0.07 M NaCl, but failed to combine with MEA derivatives of Bence-Jones monomers even after treatment with 8 M urea. The ease of oligomer formation may explain the tendency of the IgG1 protein to aggregate during the "aging" processes.

The presence of oligomers apparently does not have an adverse effect on the excess Bence-Jones monomers, which covalently reassemble during the procedure for reconstituting the IgG1 protein. The reassembled Bence-Jones proteins behave like native dimers in subsequent chromatography and crystallization experiments (see Ely et al., 1978).

The necessity for sequential disruption and reestablishment of interactions during the changing of partners helps to explain why covalent light chain dimers do not recombine with heavy chains in IgG proteins (Stevenson and Dorrington, 1970; Jerry and Kunkel, 1972; Stevenson, 1973). After noncovalent reassembly, however, the two Fab arms in the Mcg immunoglobulin are sufficiently close to permit the formation of an interchain disulfide bond between the light chains.

Binding of Bis(Dnp)lysine by Native and Reconstituted Dimers. The results of equilibrium dialysis are summarized in Figure 11 and Table I. These results indicate that reconstituted Bence-Jones covalent dimers, reassembled light chain covalent dimers, and MEA derivatives of the Bence-Jones and light chain dimers bind bis(Dnp)lysine to approximately the same extent as the native Bence-Jones protein (i.e., two molecules of ligand with average association constants of $3\text{-}4 \times 10^4\ M^{-1}$). Alterations resulting in the formation of needles had no apparent effect on the binding properties of the dimers.

The murine MOPC-315 IgA myeloma protein also has anti-Dnp specificity, with an association constant of $5.4 \times 10^6\ M^{-1}$ for Dnp-lysine (Schechter et al., 1976). The λ-type light chains isolated from the parent IgA molecule bind two Dnp ligands, but the association constant decreases by about three orders of magnitude. Reassembled heavy chains do not bind the Dnp hapten. Thus, while the primary contact residues for the Dnp groups are located on the light chain, the optimal fitting of the hapten is dependent on the geometry provided by interactions of the light and heavy chains.

There are other examples of hapten binding by light chains

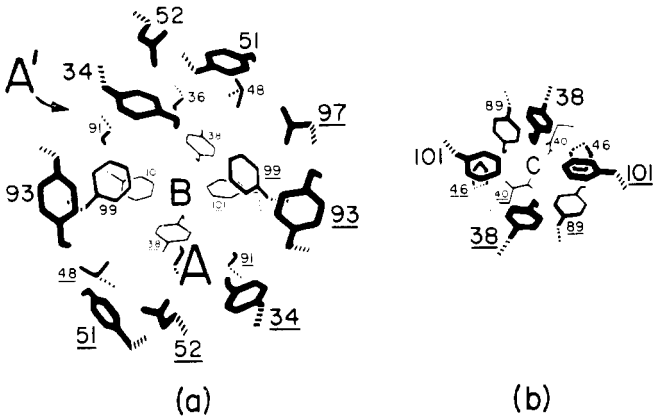

FIGURE 12: Perspective drawings of the binding regions of the Bence-Jones dimer. (a) Side chains of residues lining the main binding cavity between the V domains. Constituents of monomer 2 are underlined. The α-β carbon bonds are designated by dashed lines. In crystals, sites A and B are bridged by bis(Dnp)lysine, with one Dnp ring in each site. Accessibility to site A′ is blocked by interactions with another dimer in the crystal lattice. In solution, A′ is available for binding, and two molecules of bis(Dnp)lysine are accommodated in the cavity. (b) Deep pocket at the base of the main cavity, containing site C.

alone (Goodman and Donch, 1965; Mangalo et al., 1966; Mangalo and Raynaud, 1967; Yoo et al., 1967; Painter et al., 1972a,b; Nakamura et al., 1973; Stevenson, 1973). As in the case of the MOPC-315 protein, the activity is generally low when compared with that of the specific antibody from which the light chains are obtained.

The association constants (see Table I) for the binding of bis(Dnp)lysine by covalent and noncovalent Mcg dimers are approximately the same as those ($\sim 10^5$ M^{-1}) for binding of ligands by the New and McPC 603 Fab fragments (Amzel et al., 1974; Segal et al., 1974). The combined evidence indicates that the Mcg dimers look and act like Fab fragments.

Comparison of the Binding of Bis(Dnp)lysine in Solution and in Crystals. It is important to compare the binding studies in solution with those in the crystals, in which access to the sites is dependent on the packing of protein molecules in the crystal lattice (see Ely et al., 1978). The locations of binding sites A, B, and C in the crystals are shown in Figure 12 (Edmundson et al., 1974b). A potential binding site (A′) opposite site A is blocked by interactions with another protein molecule in the lattice. The solvent channel containing sites A, A′, and B is 15 Å wide at the entrance and 17 Å deep. The space available for binding is significantly more extensive than that in the human New Fab′ fragment (Amzel et al., 1974; Poljak et al., 1974) or the murine McPC 603 Fab fragment (Segal et al., 1974). In particular the interactions between the light and heavy chains in the Fab fragments seal the binding regions at levels corresponding to roughly the entry to site B in the Mcg dimers. Below the floor of the main cavity of the latter there is an additional pocket of solvent housing site C.

The lining of site A is associated with residues of monomer 2, particularly tyrosines-34 and -93. The phenolic ring of tyrosine-34 is the primary contact region for the Dnp group, and for other ligands like 1,10-phenanthroline, ϵ-dansyllysine, methadone, and merthiolate in the crystal. In solution tyrosines-34 and -93 of monomer 1 (site A′) should be accessible for binding similar ligands. Sites B and C are lined by constituents of both monomers 1 and 2, with the principal contact residues being hydrophobic in character (e.g., both monomers contribute tyrosine-38 and phenylalanines-99 and -101 to site B; pairs of proline-46 and tyrosine-89 residues line site C). Site B accommodates Dnp ligands, merthiolate, or purine derivatives like caffeine and theophylline in the crystal. Bis(Dnp)-lysine bridges A and B, with one Dnp group in each site. Colchicine also spans sites A and B. Compounds like menadione, *p*-nitrophenylphosphorylcholine, phenylmercuric groups, and pyrimidine derivatives lodge in site C (for discussion of the binding of menadione and Dnp ligands in antibodies, see Rosenstein and Richards, 1976).

The binding of compounds larger than the available solvent space caused local conformational changes and expansion of the sites to accommodate the ligands (e.g., colchicine in the main cavity and menadione in the pocket). Binding of bis(Dnp)lysine was accompanied by changes in site A, including displacement of the tyrosine-34 side chain. The conformational changes in the binding regions progressively became more pronounced: site C was occupied by the oversized ligand and eventually the crystal was destroyed. However, there was no evidence in the difference Fourier maps for the binding of bis(Dnp)lysine in any other region of the dimer.

We have assumed that the two molecules of bis(Dnp)lysine bound by the dimer in solution are located in the main cavity in sites A, A′, and B. Direct crystallographic evidence for this assumption can be provided only by binding studies with a crystal form in which site A′ is not blocked. However, we have performed experiments pertinent to the problem. When solutions of dimer were treated with 1-fluoro-2,4-dinitrobenzene at the pH (6.2) used for noncovalent binding studies, histidine-192 was the only residue outside the main cavity forming a covalent Dnp derivative (Edmundson et al., 1974b). The two histidine-192 residues are accessible in the crystal lattice, but do not bind Dnp ligands. When crystals were treated with an iodinated derivative of 1-fluoro-2,4-dinitrobenzene, covalent Dnp derivatives were formed in the main cavity with tyrosines-34 and -38 of monomer 2, and no other site of reaction was detected in the difference Fourier map.

Differences in the Binding Properties of the Mcg Dimer and the IgG1 Immunoglobulin. While the Mcg dimers bind a wide variety of compounds, the parent IgG1 protein has no measurable affinity for bis(Dnp)lysine in solution. Characterization of binding properties of the IgG1 protein in the crystal must await the determination of its three-dimensional structure.

We have emphasized that the available space in the binding regions is expected to be smaller in the Fab arms of the immunoglobulin. This assumption is supported by properties of anti-Dnp antibodies. Rabbit IgG anti-Dnp molecules have capacities for only one Dnp group per binding region, while chicken IgG antibodies can bind two molecules of mono-Dnp-substituted haptens, or one molecule of bis(Dnp)lysine in each Fab region (Hoffmeister and Voss, 1975).

The differences in the binding properties of the IgG proteins and the Mcg dimer are mainly attributable to the fact that such a large proportion of the interface between the V domains of the dimer is available for binding. The dimer therefore appears to bind a broader range of compounds and shows less specificity than modern antibodies and their Fab fragments.

References

Amzel, L. M., Poljak, R. J., Saul, F., Varga, J. M., and Richards, F. F. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71*, 1427.

Anfinsen, C. B. (1973), *Science 181*, 223.

Azuma, T., Isobe, T., and Hamaguchi, K. (1975), *J. Biochem. (Tokyo) 77*, 473.

Björk, I., and Tanford, C. (1971a), *Biochemistry 10*, 1271.

Björk, I., and Tanford, C. (1971b), *Biochemistry 10*, 1280.

Björk, I., and Tanford, C. (1971c), *Biochemistry 10*, 1289.

Cathou, R. E., and Dorrington, K. J. (1975), in Biological Macromolecules, Subunits in Biological Systems, Part C, Vol. 7, Fasman, G. D., and Timasheff, S. N., Ed., New York, N.Y., Marcel Dekker, p 91.
Choules, G. L., and Zimm, B. H. (1965), *Anal. Biochem. 13,* 336.
Colman, P. M., Deisenhofer, J., Huber, R., and Palm, W. (1976), *J. Mol. Biol. 100,* 257.
Davies, D. R., Padlan, E. A., and Segal, D. M. (1975), *Annu. Rev. Biochem. 44,* 639.
Davis, B. J., as quoted by Nees, S. (1974), in Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel, Allen, R. C., and Maurer, H. R., Ed., New York, N.Y., Walter de Gruyter, p 189.
Deisenhofer, J., Colman, P. M., Epp, O., and Huber, R. (1976), *Hoppe-Seyler's Z. Physiol. Chem. 357,* 1421.
Deutsch, H. F. (1971), *Seibutsu Butsuri Kagaku 16,* 73.
Deutsch, H. F., and Suzuki, T. (1971), *Ann. N.Y. Acad. Sci. 190,* 472.
Dorrington, K. J., and Smith, B. R. (1972), *Biochim. Biophys. Acta 263,* 70.
Dorrington, K. J., Zarlengo, M. H., and Tanford, C. (1967), *Proc. Natl. Acad. Sci. U.S.A. 58,* 996.
Edelman, G. M., and Poulik, M. D. (1961), *J. Exp. Med. 113,* 861.
Edelman, G. M., Cunningham, B. A., Gall, W. E., Gottlieb, P. D., Rutishauser, U., and Waxdal, M. J. (1969), *Proc. Natl. Acad. Sci. U.S.A. 63,* 78.
Edmundson, A. B., Wood, M. K., Schiffer, M., Hardman, K. D., Ainsworth, C. F., Ely, K. R., and Deutsch, H. F. (1970), *J. Biol. Chem. 245,* 2763.
Edmundson, A. B., Schiffer, M., Wood, M. K., Hardman, K. D., Ely, K. R., and Ainsworth, C. F. (1971), *Cold Spring Harbor Symp. Quant. Biol. 36,* 427.
Edmundson, A. B., Ely, K. R., Girling, R. L., Abola, E. E., Schiffer, M., and Westholm, F. A. (1974a), *Prog. Immunol. Proc. Int. Congr. Immunol., 2nd 1,* 103.
Edmundson, A. B., Ely, K. R., Girling, R. L., Abola, E. E., Schiffer, M., Westholm, F. A., Fausch, M. D., and Deutsch, H. F. (1974b), *Biochemistry 13,* 3816.
Edmundson, A. B., Ely, K. R., Abola, E. E., Schiffer, M., and Panagiotopoulos, N. (1975), *Biochemistry 14,* 3953.
Ely, K. R., Firca, J. R., Williams, K. J., Abola, E. E., Fenton, J. M., Schiffer, M., Panagiotopoulos, N. C., and Edmundson, A. B. (1978), *Biochemistry 17* (following paper in this issue).
Epp, O., Colman, P., Fehlhammer, H., Bode, W., Schiffer, M., Huber, R., and Palm, W. (1974), *Eur. J. Biochem. 45,* 513.
Fehlhammer, H., Schiffer, M., Epp, O., Colman, P. M., Lattman, E. E., Schwager, P., Steigemann, W., and Schramm, H. J. (1975), *Biophys. Struct. Mech. 1,* 139.
Fett, J. W., and Deutsch, H. F. (1974), *Biochemistry 13,* 4102.
Fett, J. W., and Deutsch, H. F. (1975), *Immunochemistry 12,* 643.
Fett, J. W., and Deutsch, H. F. (1976), *Immunochemistry 13,* 149.
Fett, J. W., Deutsch, H. F., and Smithies, O. (1973), *Immunochemistry 10,* 115.
Fleischman, J. B., Pain, R. H., and Porter, R. R. (1962), *Arch. Biochem. Biophys. 98, Suppl. 1,* 174.
Goodman, J. W., and Donch, J. J. (1965), *Immunochemistry 2,* 351.
Grey, H. M., and Mannik, M. (1965), *J. Exp. Med. 122,* 619.
Haber, E., and Richards, F. F. (1966), *Proc. R. Soc. London, Ser. B 166,* 176.
Halper, J. P., Latovitzki, N., Bernstein, H., and Beychok, S. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 517.
Hilschmann, N., and Craig, L. C. (1965), *Proc. Natl. Acad. Sci. U.S.A. 53,* 1403.
Hoffmeister, M. J., and Voss, E. W., Jr. (1975), *Immunochemistry 12,* 745.
Huber, R., Deisenhofer, J., Colman, P. M., Matsushima, M., and Palm, W. (1976), *Nature (London) 264,* 415.
Jerry, L. M., and Kunkel, H. G. (1972), *J. Immunol. 109,* 982.
Jirgensons, B. (1973), *Biochim. Biophys. Acta 317,* 131.
Karush, F. (1962), *Adv. Immunol. 2,* 1.
Mangalo, R., and Raynaud, M. (1967), *Ann. Inst. Pasteur, Paris 113,* 549.
Mangalo, R., Iscaki, S., and Raynaud, M. (1966), *C. R. Acad. Sci., Ser. D 263,* 204.
Maurer, H. R. (1974), in Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel, Allen, R. C., and Maurer, H. R., Ed., New York, N.Y., Walter de Gruyter, p 23.
Metzger, H., and Singer, S. J. (1963), *Science 142,* 674.
Nakamura, H., Grossberg, A. L., and Pressman, D. (1973), *Immunochemistry 10,* 485.
Nisonoff, A., and Palmer, J. L. (1964), *Science 143,* 376.
Noelken, M. E., Nelson, C. A., Buckley, C. E., III, and Tanford, C. (1965), *J. Biol. Chem. 240,* 218.
Olins, D. E., and Edelman, G. M. (1964), *J. Exp. Med. 119,* 789.
Padlan, E. A., and Davies, D. R. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 819.
Padlan, E. A., Davies, D. R., Rudikoff, S., and Potter, M. (1976), *Immunochemistry 13,* 945.
Painter, R. G., Sage, H. J., and Tanford, C. (1972a), *Biochemistry 11,* 1327.
Painter, R. G., Sage, H. J., and Tanford, C. (1972b), *Biochemistry 11,* 1338.
Poljak, R. J. (1975), *Nature (London) 256,* 373.
Poljak, R. J., Amzel, L. M., Chen, B. L., Phizackerley, R. P., and Saul, F. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3440.
Porter, R. R., and Weir, R. C. (1966), *J. Cell. Physiol., Suppl. 1, 67,* 51.
Roholt, O., Onoue, K., and Pressman, D. (1964), *Proc. Natl. Acad. Sci. U.S.A. 51,* 173.
Rosenstein, R. W., and Richards, F. F. (1976), *Immunochemistry 13,* 939.
Sarma, V. R., Silverton, E. W., David, D. R., and Terry, W. D. (1971), *J. Biol. Chem. 247,* 3753.
Schechter, I., Ziv, E., and Licht, A. (1976), *Biochemistry 15,* 2785.
Schiffer, M., Hardman, K. D., Wood, M. K., Edmundson, A. B., Hook, M. E., Ely, K. R., and Deutsch, H. F. (1970), *J. Biol. Chem. 245,* 728.
Schiffer, M., Girling, R. L., Ely, K. R., and Edmundson, A. B. (1973), *Biochemistry 12,* 4620.
Sears, D. W., and Beychok, S. (1973), in Physical Principles and Techniques of Protein Chemistry, Part C, Leach, S. J., Ed., New York, N.Y., Academic Press, p 445.
Segal, D. M., Padlan, E. A., Cohen, G. H., Rudikoff, S., Potter, M., and Davies, D. R. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 4298.
Smithies, O. (1965), *Science 150,* 1595.
Solomon, A. (1976), *N. Engl. J. Med. 294,* 17.
Steiner, L. A., and Lowey, S. (1966), *J. Biol. Chem. 241,* 231.

Stevenson, G. T. (1973), *Biochem. J. 133*, 827.
Stevenson, G. T., and Dorrington, K. J. (1970), *Biochem. J. 118*, 703.
Strickland, E. H., Horwitz, J., and Billups, C. (1969), *Biochemistry 8*, 3205.
Titani, K., Whitley, E., Jr., and Putnam, F. W. (1966), *Science 152*, 1513.
Voss, E. W., Jr., and Eisen, H. N. (1968), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 27*, 684.
Wang, B. C., and Sax, M. (1974), *J. Mol. Biol. 87*, 505.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244*, 4406.
Yoo, T. J., Roholt, O. A., and Pressman, D. (1967), *Science 157*, 707.
Zeppezauer, M., Eklund, H., and Zeppezauer, E. S. (1968), *Arch. Biochem. Biophys. 126*, 564.

Crystal Properties as Indicators of Conformational Changes during Ligand Binding or Interconversion of Mcg Light Chain Isomers†

K. R. Ely,‡ J. R. Firca,§ K. J. Williams, E. E. Abola,‡ J. M. Fenton, M. Schiffer, N. C. Panagiotopoulos, and A. B. Edmundson*,‡

ABSTRACT: Crystals were prepared from samples of noncovalent and covalently linked dimers previously isolated as intermediates and end products in the interconversion of conformational isomers of immunoglobulin light chains from the patient Mcg. In ammonium sulfate these dimers crystallized in the trigonal form characteristic of the native Bence-Jones protein and in an abnormal needle form associated with conformational changes in the vicinity of the interchain disulfide bond. Trigonal forms were compared with the native covalent dimer by difference Fourier analysis at 3.5-Å resolution. Criteria were established for recognizing and cutting twinned trigonal crystals into fragments useful for diffraction experiments. The packing of molecules in the trigonal crystal lattice was examined in detail to determine the steric limitations governing chemical modifications, chemical modifications within crystals or in solutions slated for crystallization. When the modifications involved only the cleavage and alkylation of the interchain disulfide bond, the difference Fourier maps indicated only local conformational changes mainly in the COOH-terminal pentapeptide segment of monomer 2 of the dimer. When light chains were dissociated from heavy chains and reassembled into a dimer, there were changes in segments which interact to stabilize the V domain dimer (i.e., in the lining of the deep binding pocket in the V interface). These changes, as well as the more extensive changes in the needle forms, could be reversed by dissolving the crystals, cleaving the interchain disulfide bond, and allowing it to reoxidize. The resulting proteins crystallized as trigonal forms indistinguishable from those of the native dimer. After the binding of two molecules of bis(dinitrophenyl)lysine and subsequent removal of the ligands by dialysis, the dimer crystallized only as needles. The needles could be converted into trigonal forms as before. These results suggest that the binding of ligands by the V domains can lead to conformational changes in the most distal regions of the C domain dimer.

In the interconversions of conformational isomers of the Mcg immunoglobulin light chains (see Firca et al., 1978), we initially considered crystallization of the products to be just a routine step in the correlation of chemical and diffraction results. Instead, the unexpected appearance of different crystal forms and variations in the properties of the expected crystal forms directed us into a more enlightened study of conformational changes in the constituent molecules. By using the crystal properties in conjunction with chemical modifications and CD spectroscopy (see Firca et al., 1978), we were able to identify regions involved in conformational changes during the interconversion experiments. The morphology and pertinent properties of these crystals will be described in the present article. In cases in which the crystal morphology was similar to that of the trigonal form of the native Bence-Jones dimer (Edmundson et al., 1971), subtle conformational changes were studied in detail by difference Fourier analysis. The crystal packing in the trigonal form was examined to determine what changes were possible without destruction of the lattice.

The presence of other crystal forms was taken as evidence of more substantial conformational differences, as shown in solution by the comparisons of the CD spectra of trigonal and needle forms. Information on the nature of the latter differences was obtained from the procedures required to convert the altered molecules into species crystallizing like native dimers.

In contrast to the results for the New and McPC 603 Fab fragments (Amzel et al., 1974; Poljak et al., 1974; Segal et al., 1974; Davies et al., 1975; Padlan et al., 1976), local conformational changes can be induced by the binding of some hapten-like molecules in crystals of the Bence-Jones dimer (Edmundson et al., 1974). We now consider whether such induced changes in the combining sites may also lead to alterations in distal parts of the molecules (for discussions of this subject in functional antibodies, see Schur and Christian, 1964; Warner and Schumaker, 1970; Hyslop et al., 1970; Holowka

† From the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, and the Departments of Biochemistry and Biology, University of Utah, Salt Lake City, Utah 84112. *Received May 13, 1977.* This work was supported by the U.S. Energy Research and Development Administration and by U.S. Public Health Service Grant No. CA-19616.

‡ Present address: Department of Biochemistry, University of Utah, Salt Lake City, Utah 84112.
§ Present address: Abbott Laboratories, North Chicago, Illinois 60064.

0006-2960/78/0417-0158$01.00/0