Conformation and Association of the Light Chain from a Homogeneous Human Immunoglobulin†

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ABSTRACT: The light chain from a human myeloma immunoglobulin (Wes) is a monomer–dimer system between pH 5 and 8 having a pH-dependent association constant of 10^4–10^5 l./mol. A shift in pK of an imidazole group and a carboxyl group upon dimerization can account for the pH dependence of the association constant. The monomer and dimer structures have different conformations which appear to be independent of pH and ionic strength. Acid denaturation occurs below pH 5 leading to an acid-denatured state at pH 1.5 where conformation and association are ionic strength dependent. The association at pH 1.5 does not appear to involve a conformational change.

Structural studies of a homogeneous immunoglobulin light chain, while adding to our general understanding of protein structure, can also provide information which is applicable to other immunoglobulin subunits and their proteolytic fragments. Sequence homologies of immunoglobulin subunits and fragments suggest that their three-dimensional structures are similar (Dayhoff, 1972). Conformational similarities have been demonstrated by physical measurements (Dorrington and Tanford, 1970; Björk and Tanford, 1971a,b).

Light chains are of particular interest since they exist as independent proteins in immunoglobulin synthesizing cells (Laskov *et al.*, 1971). Knowledge of light-chain structure is needed to understand the function of the light chain in assembly, transport, and cellular excretion of immunoglobulins. Recently, the structure of rabbit light chain was examined by hydrodynamic and optical methods (Björk and Tanford, 1971b). Similar studies were done with human light chain (Jirgensons *et al.*, 1966; Stevenson and Dorrington, 1970; Takahashi *et al.*, 1970; Azuma *et al.*, 1972). Although studies of a heterogeneous population of light chains do reveal general structural properties, some results, due to the heterogeneity, may lead to concepts which do not describe the biological system. Several analogous studies of different homogeneous light chains would better define the general structural features.

For some time it has been known that human light chains dimerize (Edelman and Gally, 1962). However, the structural details, the energies involved, and the effects of environment on dimerization have not been investigated. In this study, the native and acid-denatured states of a human homogeneous light chain were established by optical rotation. The self-associating properties of the light chain were then studied by sedimentation equilibrium.

Materials and Methods

A human myeloma immunoglobulin (Wes), classified as Gl, κ, Gm(F), was mildly reduced with 0.2 M mercaptoethanol and then treated with iodoacetamide at pH 8.5. The light chain, having a blocked interchain sulfhydryl group, was isolated from the heavy chain by gel filtration through Sephadex G-100 in 0.01 M HCl. The light chain was judged homogeneous by immunoelectrophoresis against goat anti-human serum, by polyacrylamide gel electrophoresis in 6 M urea at pH 3.2 (Panyim and Chalkley, 1969), and by sedimentation velocity in a Spinco Model E analytical ultracentrifuge.

Specific volume, 0.72 ml/g, and mean residue weight, 109 g/mol, were calculated from amino acid analysis. The extinction coefficient, determined by dry weight, is 1.07 ml/mg per cm at 278 mμ in water and in 0.1 M sodium chloride at pH 6. Below pH 3, the extinction coefficient, calculated from difference spectra, is 1.05 ml/mg per cm at 277 mμ. Light scattering corrections were made by applying Rayleigh's law after measuring apparent absorbance at 333 mμ. These corrections never exceeded 3% of the absorbance in the 280-mμ region and were usually much less.

Optical Rotation. Optical rotation was measured at 25° with a Cary 60 recording spectropolarimeter (Cary Instruments, Monrovia, Calif.). Cells of 0.05–5.0-cm path lengths were used to keep the absorbance always below 1.5. The following relationship was used to calculate reduced mean residue rotation (eq 1) where n is the index of refraction of the solvent,

$$[m'] = [3/(n^2 + 2)](M_0/100)[\alpha] \quad (1)$$

M_0 is the mean residue weight, and $[\alpha]$ is the specific rotation. Since the solvents were dilute salt solutions, refractive indices of water were used for these calculations.

The low pH conformational transition was measured by optical rotation at 240 mμ where the change in $[m']$ is relatively large and where the signal-to-noise ratio is favorable due to the low extinction coefficient at this wavelength. The pH was adjusted by adding 3 M HCl or 3 M NaOH to a protein solution while rapidly stirring at ice temperature. The effect of ionic strength on optical rotation was measured after adding increments of 3.5 M NaCl to protein solutions. Measurements of optical rotation, pH, and protein concentration were made when solutions showed no further change in rotation. pH was measured at 25° with a Radiometer type PHM22q pH meter equipped with a Radiometer GK2302C combined electrode.

Sedimentation Equilibrium. A Spinco Model E analytical ultracentrifuge (Spinco Division, Beckman Instruments Inc., Palo Alto, Calif.) was used for sedimentation equilibrium

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experiments. Rotor speeds, calculated from the odometer readings, were maintained by a mechanical gear box speed control. The average deviation of the rotor speed for 13 experiments near 22,000 rpm was 0.1 rpm. Temperature was controlled at 25.0 ± 0.03° by the standard RTIC unit. Data, recorded on Kodak II-G spectroscopic plates, were obtained using the interference optical system with a Wratten 77A filter.

A 12-mm double sector synthetic boundary cell with sapphire windows was used for all experiments. Fluorocarbon, FC-43 (20 μl), was used as a bottom oil in each sector of the centerpiece. The cell was then loaded with 400 μl of dialysate and 60 μl of protein solution having a concentration of 0.6 mg/ml. When the accelerating rotor reached approximately 5000 rpm, the dialysate layered onto the protein solution. This layering procedure (Chervenka, 1970) has the advantage of providing more data in a shorter time at lower rotor speeds, compared to shorter column experiments where dialysate is not layered onto protein solution. The rotor speeds used in these experiments were between 20,000 and 26,000 rpm. Experiments were terminated when no change in fringe displacement occurred over a 3-hr interval. Water blank measurements at the end of the experiments were used to correct for window distortion. The displacement of three white or three black fringes was measured with a Gaertner microcomparitor (Gaertner Scientific Corporation, Chicago, Ill.). Near the bottom of the solution column, where fringe displacements were large, readings were made at plate radial intervals of 10 or 20 μ. All photographic plates could be read to the fluorocarbon interface.

Generally, plots of the natural logarithm of the fringe displacement, $\ln f$, *vs.* the square of the radial distance, r^2, showed upward curvature indicating an associating system. Adjacent values of fringe displacement and radial distance were used to calculate weight average molecular weight at different radial distances by the equation

$$M_w = \frac{2RT}{(1 - \bar{v}\rho)\omega^2} \frac{\Delta \ln f}{\Delta r^2} \quad (2)$$

where $\bar{v}$ is the protein specific volume, ρ is the solvent density, and ω is the angular velocity. The protein concentration, c, at each radial distance was calculated from the fringe displacement by the experimentally determined conversion factor of 0.250 mg/ml per fringe (Richards *et al.*, 1968; Babul and Stellwagen, 1969).

Sets of four successive values of M_w and c from each experiment were averaged and plotted. The smooth curve drawn through these data points was used to obtain weight average molecular weights at different protein concentrations. Four experiments between pH 4.5 and 5.7 were used to confirm that the associating system above pH 4.5 is a monomer–dimer equilibrium. The method used (Chun and Kim, 1970) requires that the weight fraction of monomer, c_1/c, be plotted against M_w/M_1, where c_1 is the monomer concentration and M_1 is the monomer weight. The smooth curves of M_w *vs.* c were used to evaluate c_1/c by eq 3 which is applicable to self-associating

$$c_1/c = \exp \int_{c=0}^{c} [(M_1/M_w) - 1](dc/c) \quad (3)$$

systems where nonideality is negligible (Steiner, 1952; Fugita, 1962). Values of c_1/c at different protein concentrations were obtained by numerical integration using the trapezoid rule.

For a monomer–dimer system, the following linear relationship can be used to calculate the association constant, k, in units of milliliters per milligram (eq 4). To avoid inaccuracy

$$c/c_1 = 1 + kc_1 \quad (4)$$

from curve fitting to plots of M_w *vs.* c, the integration of eq 3 was attempted using the original fringe displacement and radial distance data. However, this procedure resulted in excessive scatter of the integrand in eq 3 *vs.* c at concentrations below 0.2 mg/ml. Consequently, the following form of this equation was used (eq 5) where A is the exponential of

$$c_1/c = A \exp \int_{c=0.2}^{c} [(M_1/M_w) - 1](dc/c) \quad (5)$$

the integration from 0 to 0.2 mg/ml. For each experiment A was varied until the plot of c/c_1 *vs.* c_1, by the least-squares method, had an ordinate intercept of 1, as required by eq 4. The slope of this line is the association constant. A programmable electronic calculator was used for these calculations.

Results

Monomer Molecular Weight. The minimum molecular weight of Wes light chain was obtained when the protein was in 0.1 M NaCl between pH 2.7 and 3.5. A plot of the natural logarithm of the fringe displacement, $\ln f$, *vs.* the square of the radial distance, r^2, is linear at pH 3.5, as is predicted for a homogeneous and nonassociating protein. The calculated mol wt, 23,300 g/mol, agrees closely with the molecular weights of other light chains, calculated from amino acid sequences (Dayhoff, 1972). The plots of $\ln f$ *vs.* r^2 for Wes light chain in 0.1 M NaCl at pH 2.7 and 2.9 showed slight downward curvature for fringe displacements above 800 μ, indicating nonideality. Consequently, pH 3.5 was judged the minimum pH in 0.1 M NaCl where the second virial coefficient could be neglected.

Molecular weight measurements of Wes light chain in 0–3 M guanidinium chloride at pH 7, using the meniscus depletion method of Yphantis (1964), indicated that complete dissociation occurs when the guanidinium chloride concentration is above 2 M. Under such conditions, mol wt values of 23,000–24,000 were obtained using a specific volume of 0.71 ml/g (Hade and Tanford, 1967). In calculations of association constants, 23,300 g/mol was used for the monomer molecular weight.

A plot of $\ln f$ *vs.* r^2 for Wes light chain in 0.1 M NaCl at pH 5.1 is shown in Figure 1. The upward curvature away from the dashed line indicates an associating system. The dashed line represents a calculated plot of $\ln f$ *vs.* r^2 for the monomer.

Molecular Weight vs. pH and Ionic Strength. Figure 2 shows plots of weight average molecular weight at two protein concentrations (0.1 and 0.6 mg/ml) and in two ionic strength conditions (0.1 and 0.2 M NaCl) between pH 2 and 7. These results were read from the curves fitted to plots of weight average molecular weight *vs.* protein concentration. The associating system above pH 4 is not significantly ionic strength (NaCl) dependent between 0.1 and 0.2 M NaCl. Near pH 3.5 in 0.1 M NaCl, Wes light chain is completely dissociated. However, below pH 3 association occurs which is strongly ionic strength (NaCl) dependent. Nonideality, if present and corrected for, would enhance this effect.

Conformational Transitions. To clarify the results of Figure

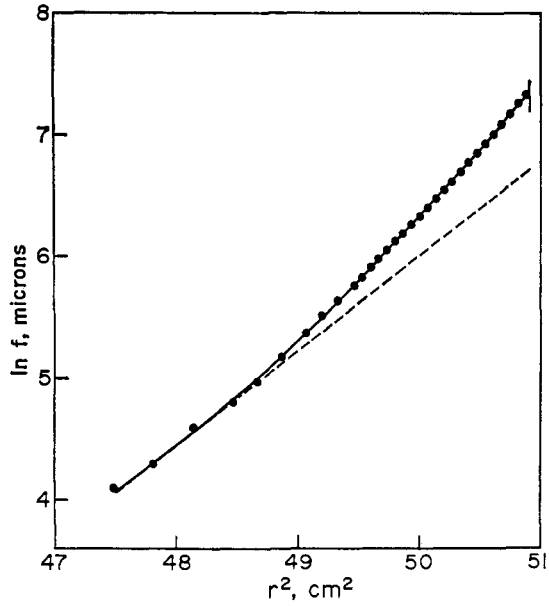

FIGURE 1: Sedimentation equilibrium of Wes light chain at 23,000 rpm, 25°, 0.1 M NaCl, pH 5.1. Natural logarithm of the fringe displacement, ln f, *vs.* the square of the radial distance, r^2. The bottom of the solution column is indicated by a vertical line. The dashed line was calculated for the monomer.

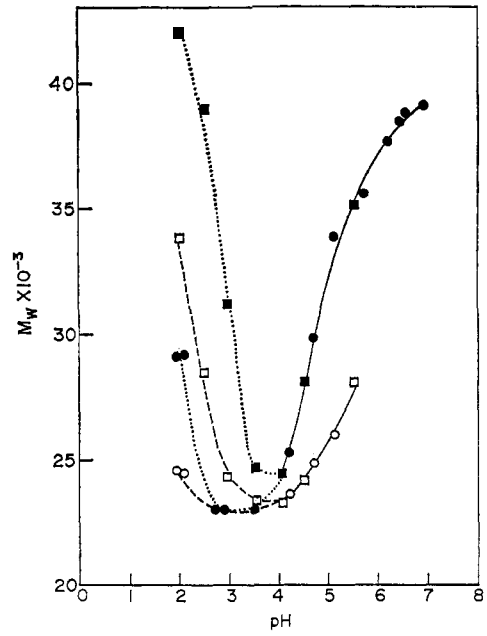

FIGURE 2: Weight average molecular weight, M_w, *vs.* pH of Wes light chain at two protein concentrations and two ionic strength conditions: 0.10 mg/ml in 0.10 M NaCl (○); 0.10 mg/ml in 0.20 M NaCl (□); 0.60 mg/ml in 0.10 M NaCl (●); 0.60 mg/ml in 0.20 M NaCl (■). Sedimentation equilibrium was done beteeen 21,000 and 26,000 rpm at 25°.

2, where associating systems are indicated above and below pH 4, conformational transitions of Wes light chain in the acid region were measured. The wavelength of 240 mμ was chosen for observing optical rotation, because the protein extinction coefficient is small at this wavelength, resulting in a good signal-to-noise ratio, and because the change in rotation is relatively large at 240 mμ. Figure 3 shows the conformational transitions of Wes light chain in the acid region for the protein in water and in 0.1 M NaCl. Reduced mean residue rotation at 240 mμ is plotted against pH. Open symbols represent protein solutions made from a pH 7 stock solution, while closed symbols represent solutions made from a pH 2 stock solution. The results shown in Figure 3 demonstrate that the conformational transitions are reversible. Equilibrium, measured by optical rotation, was reached immediately upon adjusting protein solutions to pH 2. Rates of equilibration for other pH perturbations were not measured.

To describe the effect of ionic strength on the conformation of Wes light chain in the acid pH region, the results of Figures 3 and 4 should be compared. Data were obtained with the protein at 0.6 mg/ml except for one set of data in Figure 3 where the protein was 0.1 mg/ml in 0.1 M NaCl. Between pH 5 and 8, the reduced mean residue rotation, [m'], increases slightly. Figure 4 shows that within this pH range, namely at pH 6.2, ionic strength (NaCl) has virtually no effect on [m']. Measurements were made up to 0.4 M NaCl. The invariance of [m'] suggests that Wes light chain has a stable conformation between pH 5 and 8.

Below pH 5, where Wes light chain undergoes acid denaturation, the conformational transition curves of Figure 3 diverge, indicating that Wes light chain in water is more stable to acid denaturation than when sodium chloride is present. The decrease in [m'] by an increase in sodium chloride concentration at pH 3, shown in Figure 4, indicates that the inflection point of the conformational transitions shifts to higher pH values as the salt concentration is increased, confirming the observation that acid denaturation is facilitated by the presence of sodium chloride.

The denatured state at pH 1.5, shown in Figure 3, is ionic strength dependent. Figure 4 shows that at pH 1.5 a stable denatured conformation is reached at approximately 0.2 M NaCl, since at higher sodium chloride concentrations little change in [m'] occurs.

The conformational transitions of Wes light chain, shown in Figures 3 and 4, can be compared with the association behavior of Wes light chain shown in Figure 2. Above pH 4, a pH-dependent association of native light chain occurs. Acid denaturation below pH 4 results in a different associating system which is ionic strength dependent. The coincidence of the inflection point in acid denaturation with the minimum molecular weight of Wes light chain indicates that the mono-

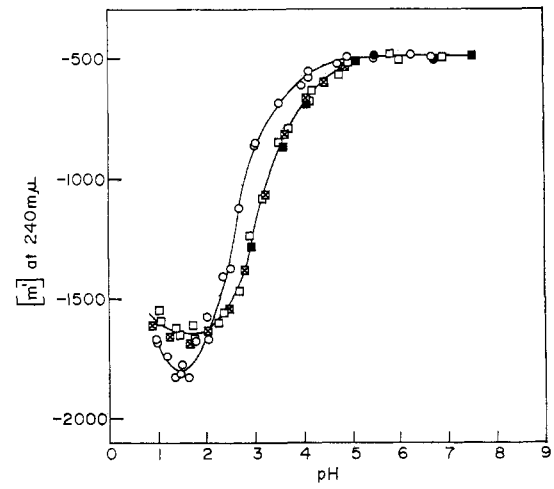

FIGURE 3: Reduced mean residue rotation at 240 mμ *vs.* pH at 25°: 0.60 mg/ml in water, pH 7-1 (○), pH 2-7 (●); 0.60 mg/ml in 0.10 M NaCl, pH 7-1 (□), pH 2-7 (■); 0.10 mg/ml in 0.10 M NaCl, pH 5-1 (⊠).

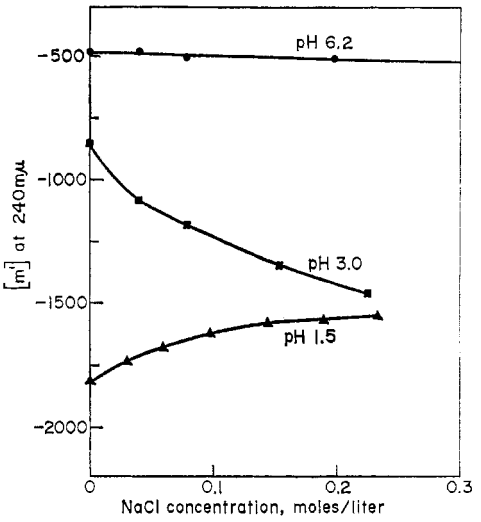

FIGURE 4: Reduced mean residue rotation at 240 mμ *vs.* sodium chloride concentration at 25°.

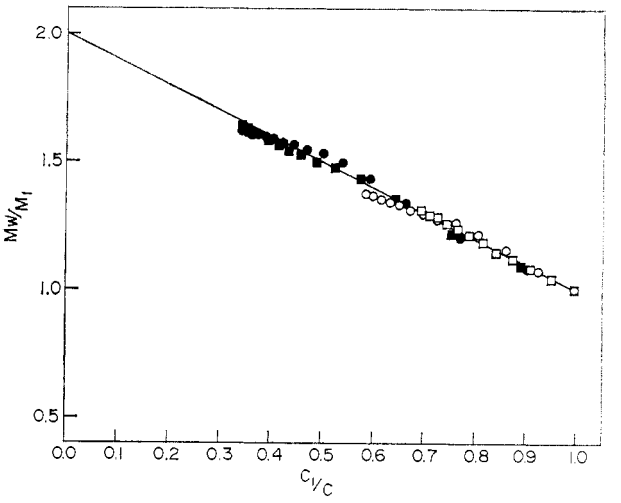

FIGURE 5: Weight average molecular weight divided by monomer molecular weight, M_w/M_1, *vs.* weight fraction of monomer, c_1/c, at 25°: pH 4.5 (□); pH 4.7 (○); pH 5.5 (■); pH 5.7 (●).

meric form is involved in the denaturation and renaturation process rather than associated structures.

Monomer–Dimer Equilibrium. The associating system above pH 4 was analyzed in some detail since conformational analyses indicated that native conformation occurs in the pH range between 4 and 8. To analyze a self-associating system in terms of association constants, the components that are in equilibrium must be known. It is generally concluded from sedimentation velocity and sedimentation equilibrium data that light chains are monomer–dimer systems having variable association constants. However, a more convincing demonstration of the type of self-association is desirable. The method of Chun and Kim (1970) was used to demonstrate that the association of Wes light chain above pH 4 is a monomer–dimer equilibrium. Curves of M_w *vs.* protein concentration between pH 4.5 and 5.7 were used to calculate the weight fraction of monomer, c_1/c, at different values of M_w/M_1. For these calculations, eq 3 was solved by numerical integration. The coincidence of the plotted values with the straight line shown in Figure 5 is consistent with a monomer–dimer equilibrium. More complicated association would have positive deviation from this line as the protein concentration increases.

The definition of the association constant for a monomer–dimer equilibrium, rearranged as shown in eq 4, was used to calculate association constants. To avoid inaccuracy from curve fitting of M_w *vs.* protein concentration data, the original values of fringe displacement *vs.* radial distance were used. Since plots of $[(M_1/M_w) - 1]/c$, the integrand of eq 3, *vs.* c from the original centrifuge data were somewhat scattered for protein concentrations below 0.2 mg/ml, only the original data above 0.2 mg/ml was iterated by eq 5 until eq 4 was achieved. Typical plots of eq 4 are shown in Figure 6. The slopes of these lines are the equilibrium constants in units of milliliters per milligram.

The pH dependence of the association constant for Wes light chain in the pH 4–8 range is given in Figure 7, where the logarithm of the association constant in units of liters/mole is plotted against pH. These results were obtained for the protein in 0.1 and 0.2 M NaCl. Some solutions were buffered with small amounts of sodium acetate or Tris.

Figure 7 shows that a decrease in pH causes a decrease in association constant. This effect may be explained as a change in charge interactions in the contact region of the dimer as pH is changed. Either a cationic–anionic attractive interaction could be eliminated by protonation of the anionic group or protonation could form an unfavorable electrostatic repulsion. A pH-dependent conformational change may also explain the results of Figure 7 since electrostatic interactions, hydrogen bonding, or hydrophobic interactions in the contact region may be affected by the conformational change.

Optical Rotatory Dispersion Spectra. Figure 8 shows that the optical rotatory dispersion spectrum of Wes light chain at pH 5.5 is concentration dependent for measurements between 0.12 and 3.4 mg/ml. Calculations of the weight ratio of monomer, c_1/c, from the association constant at pH 5.5 resulted in 0.75 and 0.24, respectively, for these two protein concentrations.

The following equation (6) was used to calculate the reduced mean residue rotations for the monomer, $[m']_M$, and the dimer, $[m']_D$, at pH 5.5. These calculated spectra are shown

$$[m'] = (c_1/c)[m']_M + [1 - (c_1/c)][m']_D \quad (6)$$

as dotted and dashed curves in Figure 8. The difference between the monomer and dimer spectra at pH 5.5 is significant and must be taken into consideration when analyzing the pH dependence of the association constant.

The optical rotatory dispersion spectrum of Wes light chain for the denatured state at pH 1.5 in 0.1 M NaCl is also shown in Figure 8. Measurements made at 0.1 and 0.6 mg/ml did not reveal a concentration dependence of optical rotation. This result is supported by the coincidence of the transition curves for the protein at 0.1 and 0.6 mg/ml in 0.1 M NaCl as shown in Figure 3. The components involved in the associating system below pH 4 apparently have the same conformation. This conclusion is based on a relatively small difference in protein concentration. However, precision was good because of the favorable signal-to-noise ratio for measurements of optical rotation at low pH.

The optical rotatory dispersion spectrum of Wes light chain in 0.1 M NaCl at pH 1.5 has a small shift of its minimum from 224 mμ in the native state to 222 mμ in the denatured state. The shape of the spectrum indicates that some structure exists at pH 1.5 and that Wes light chain is not a random coil in this low pH state (Tanford *et al.*, 1967).

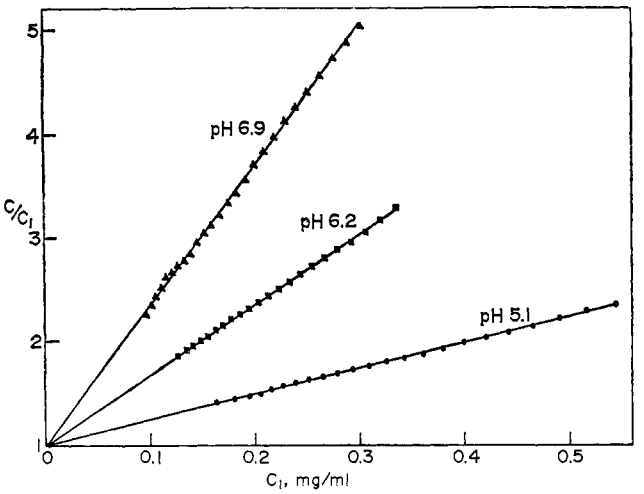

FIGURE 6: Reciprocal of weight fraction of monomer, c/c_1, *vs.* monomer concentration, c_1, at 25°.

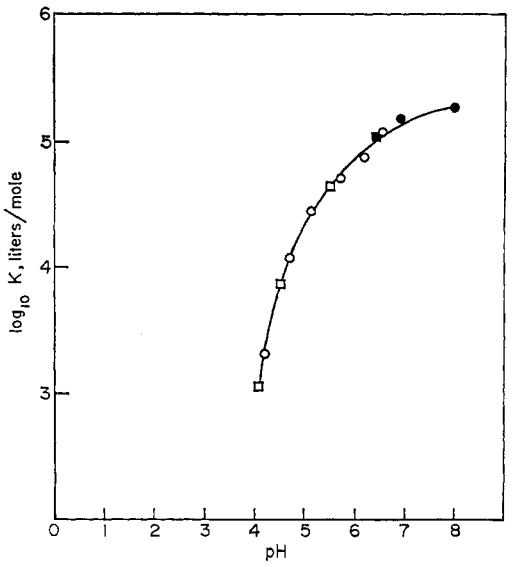

FIGURE 7: Logarithm of the association constant (log K) *vs.* pH at 25°: 0.10 M NaCl (○); 0.20 M NaCl (□); 0.10 M NaCl and 0.005 M Tris (●); 0.10 M NaCl and 0.005 M sodium acetate (■).

Discussion

The light chain from Wes myeloma immunoglobulin is a self-association system above pH 4 involving monomers and dimers. The monomer mol wt, calculated from sedimentation equilibrium data, is 23,300, which agrees closely with molecular weights calculated from sequence determinations of other homogeneous human light chains (Dayhoff, 1972).

At pH 5.5, the calculated optical rotatory dispersion spectra for monomer and dimer are significantly different, indicating that a change in conformation occurs upon dimerization. The nature of this structural change and the free energy associated with it are not known.

Figure 3 shows that Wes light chain increases slightly in reduced mean residue rotation, [m'], between pH 5 and 8. The corresponding variation in weight fraction of monomer, c_1/c, is 0.60–0.27, calculated from eq 4 and the association constants. Assuming that the optical rotatory dispersion spectra for monomer and dimer shown in Figure 8 are invariant between pH 5 and 8, eq 6 can be used to calculate [m']. The calculated [m'] values, presented in Table I, show a positive trend with increasing pH and are close to the experimental results read from Figure 3. This close agreement suggests that the monomer and dimer conformations are not significantly pH dependent above pH 5. Figure 4 shows that within this pH range, namely at pH 6.2, NaCl concentrations up to 0.4 M have very little effect on [m'], indicating that the monomer and dimer conformations are independent of ionic strength. Because of the pH and ionic strength stability of light chain above pH 5, measured properties of the protein, such as dimerization equilibrium, are expected to exist in the native environment of the protein within the immunoglobulin synthesizing cell.

TABLE I: Native Reduced Mean Residue Rotation[a] at 240 mμ.

pH	Calcd [m']	Obsd [m']
5	−512	−500
6	−483	−489
7	−470	−486
8	−466	−483

[a] The protein concentration is 0.6 mg/ml.

The association constants of Wes light chain are in the range of 10^4–10^5 l./mol above pH 5. Such association is not strong, and consequently a relatively large amount of monomer is always present in equilibrium with dimer. It is unlikely that the two- to threefold excess synthesis of light chain found in cells that make immunoglobulins (Laskov *et al.*, 1971) is necessary to provide an adequate supply of light-chain monomers for rapid assembly of immunoglobulin if dissociation of dimer is rapid. The rates of association and dissociation of light chain are of interest since they may affect the rate of assembly of the immunoglobulin. The conformational difference between the monomer and dimer forms of Wes light

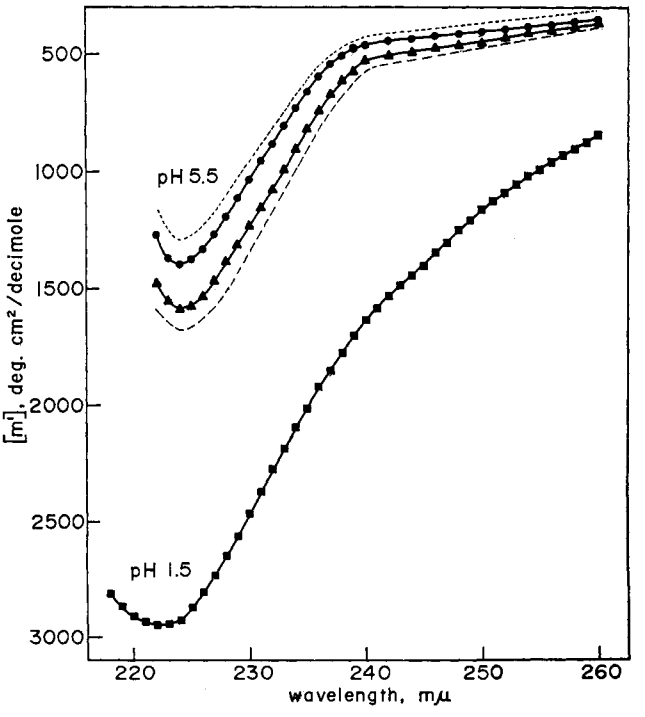

FIGURE 8: Reduced mean residue rotation *vs.* wavelength at 25° in 0.10 M NaCl; 0.12 mg/ml at pH 5.5 (●); 3.4 mg/ml at pH 5.5 (▲); 0.11 mg/ml at pH 1.5 (■); calculated monomer spectrum (···); calculated dimer spectrum (- - -).

chain may affect the rate constants for the monomer–dimer system.

At constant temperature and pressure, the relationship between monomer–dimer equilibrium and the perturbing ligand, the hydrogen ion, can be expressed as an extension of Wyman's derivation (Wyman, 1964)

$$\frac{\partial \ln K}{\partial \ln a_H} + \frac{\partial \ln K_{conf}}{\partial \ln a_H} = \Delta\bar{\nu}_H \quad (7)$$

where K is the apparent association constant, K_{conf} is the conformation equilibrium constant, a_H is the hydrogen ion activity, and $\Delta\bar{\nu}_H$ is the difference between the number of protons bound to the dimer and the number bound to two monomers. The partial derivatives are taken with the activities of other perturbing ligands kept constant.

The contribution of the acid-denatured state to the equilibria above pH 5 is considered to be negligible. The small increase of $[m']$ above pH 5 is fully accounted for by the conformational change accompanying dimerization. Because of the large difference in $[m']$ between the native and acid-denatured states, as shown in Figures 3 and 8, a larger increase in $[m']$ above pH 5 would be expected if the denatured state were significantly present.

If the free energy of conformational change accompanying the dimerization of Wes light chain above pH 5 is not significantly pH dependent, then the second term of eq 7 is near zero, resulting in eq 8 which requires that the acid dissociation

$$\partial \ln K/\partial \ln a_H = \Delta\bar{\nu}_H \quad (8)$$

constants of some residues in the contact region change upon dimerization.

$\Delta\bar{\nu}_H$ can be expressed as the summation of terms containing the acid dissociation constants of residues in the monomer, $K_{i,M}$, and in the dimer, $K_{i,D}$ (Aune and Timasheff, 1971) (eq 9). Corresponding residues from each monomer acquire the same environment upon dimerization. The factor 2 in eq 9 is necessary because the dimer has a twofold axis of symmetry.

$$\Delta\bar{\nu}_H = 2\Sigma\left[\frac{a_H}{a_H + K_{i,D}} - \frac{a_H}{a_H + K_{i,M}}\right] \quad (9)$$

Equation 9 does not include terms arising from long-range electrostatic interactions. Calculations of these effects would require pH titration data, or preferably residue coordinates from crystal-structure data. In the dimerization study of α-chymotrypsin in 0.1 M NaCl between pH 3 and 6 (Aune and Timasheff, 1971), the calculated effect of long-range electrostatic interactions on pK values in the contact region was small and unimportant for the identification of groups responsible for the pH dependence of dimerization. Neglecting long-range electrostatic effects, eq 8 can be integrated after combining with eq 9 to give

$$\log K = \log K_0 + 2 \log \frac{\Pi[1 + (a_H/K_{i,D})]}{\Pi[1 + (a_H/K_{i,M})]} \quad (10)$$

where K_0 is an integration constant.

Equation 10 was iterated with successive pK values until the curve of Figure 7 was fitted. The simplest fit of eq 10 to the curve of Figure 7 was obtained by allowing pK shifts of two residues from 6.5 to 6.2 and from 4.8 to 2.8 upon dimerization. Considering the pH range where the calculated pK shifts are occurring, an imidazole group and a carboxyl group are implicated as two components of the contact surface whose changes of pK upon dimerization account for the pH dependence of the dimerization constant. In the foregoing analysis, the free-energy change associated with the conformational change accompanying association was assumed to be independent of pH and long-range electrostatic effects were assumed to be negligible.

Below pH 5, acid denaturation of Wes light chain occurs. The information presented in Figures 2 and 3 plus free-energy considerations of the two associating systems above and below pH 4 suggest the following mechanism for the transition from native dimer to denatured and associated light chain: native dimer $\rightleftharpoons$ native monomer $\rightleftharpoons$ denatured monomer $\rightleftharpoons$ denatured aggregate. Denaturation and renaturation occur only to the monomer form.

The acid-denatured state of Wes light chain at pH 1.5 has an optical rotatory dispersion spectrum which indicates that the protein has some structure and is not a random coil. In the absence of sodium chloride at pH 1.5, the high charge density on immunoglobulin light chain[1] is expected to cause electrostatic distortion of the structure. Adding sodium chloride to mask these charges should change the denatured conformation. The ionic strength dependence of $[m']$ at pH 1.5 is consistent with such a model.

Figure 2 shows that self-association at low pH is enhanced by an increase in ionic strength (NaCl). This effect can be explained as a masking of cation–cation repulsion allowing association by hydrophobic interactions. Other forces which might contribute to self-association, such as hydrogen bonding and electrostatic attractions, would not be prominent near pH 1.5. This study suggests that acid denaturation may expose amino acid residues which can participate in interchain hydrophobic interactions resulting in protein aggregation.

Summary

In this study, a homogeneous human immunoglobulin light chain was found to be native above pH 5, where a pH-dependent dimerization occurs. The association constant is between 10^4 and 10^5 l./mol. A shift in pK of an imidazole group and a carboxyl group upon dimerization can account for the pH dependence of the association constant. This association is accompanied by a conformational change. The acid-denatured state was reached at pH 1.5 where self-association occurs, apparently by hydrophobic interactions.

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[1] The isoionic point of human immunoglobulin light chain is ~pH 8.

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Interaction of Histone f2a1 with T7 Deoxyribonucleic Acid. Cooperativity of Histone Binding†

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ABSTRACT: Hydrodynamic studies are performed on histone f2a1 and reconstituted histone f2a1–T7 DNA complexes. The results of cesium chloride banding of intact and sheared nucleohistone complex indicate that histone f2a1 molecules anneal to DNA in a cooperative fashion starting at the two ends of the DNA molecule. Histone f2a1 is modified by the removal of 18 amino acids from its carboxyl-terminal end by cleavage with cyanogen bromide. The CNBr-modified histone f2a1 molecule no longer binds cooperatively to the DNA. Histone f2a1 aggregates in 0.1 M NaCl but not in 0.005 M NaCl. On the other hand, the CNBr-modified histone f2a1 does not aggregate in 0.1 M NaCl. Sedimentation velocity and viscosity studies indicate that histone binding to DNA causes folding of the DNA only under conditions where the histone molecules themselves aggregate. We suggest that the basic halves of the histone molecules bind electrostatically to the DNA and the carboxyl ends aggregate through the interactions of hydrophobic amino acid residues. A "roof-shingling" mechanism is proposed to illustrate the cooperative binding of histone molecules to DNA. Both the cooperativity of histone binding and the folding of the DNA are observed only with the intact histone f2a1 molecules.

Histones may play roles in the maintenance of the structural integrity of the nucleoprotein (Pardon and Wilkins, 1972), the control of chromosome conformational changes (Sadgopal and Bonner, 1970) and as modifiers of template activity in gene regulation (Johns, 1972). An extreme biological restriction on structural variation is demonstrated by the fact that in over one billion years since the divergence of plants and animals, very little change in the amino acid sequences of the histones has occurred (DeLange and Smith, 1972).

We report here that histone f2a1 molecules appear to anneal to DNA in a cooperative fashion starting at the two ends of the DNA molecule. This artificial nucleohistone may be cross-linked with glutaraldehyde and banded in CsCl density gradients.

Histone f2a1 may be modified by the removal of 18 amino acid residues from the carboxyl-terminal end by cleavage with cyanogen bromide. The residual protein which remains after removal of the peptide will also bind to DNA. But it is not stabilized by glutaraldehyde and dissociates from DNA in concentrated CsCl. Furthermore, we find that the interactions induced by 0.1 M NaCl, which occur with this histone both when free in solution and when bound to DNA, are lost when the peptide is removed from the C-terminal end.

These observations may be taken to imply the existence of a "roof-shingle" fit between successive histone molecules, in analogy with shingling a roof where it is necessary to start at the eaves. The hydrophobic end of each histone may overlap the basic portion of the previous molecule, locking it into place where it winds around the DNA groove.

Materials and Methods

Histone f2a, a mixture of f2a1 and f2a2, and histone f2a1 were isolated from calf thymus as previously described (Ziccardi and Schumaker, 1972).

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