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Equilibrium and Kinetics of the Denaturation of a Homogeneous Human Immunoglobulin Light Chain†

Elizabeth S. Rowe‡ and Charles Tanford*,§

ABSTRACT: A study of the denaturation equilibrium and the kinetics of the approach to equilibrium as a function of guanidine hydrochloride (Gdn·HCl) concentration of a homogeneous human κ light (L) chain (Wes) is described. The results of this study are analyzed quantitatively in terms of the present understanding of Gdn·HCl perturbations of denaturation processes. In terms of these principles it is shown that the denaturation of monomeric Wes L chain is quantitatively consistent with a domain model in which the two halves of Wes L chain denature independently, giving rise to stable half-denatured intermediates in the transition. This result confirms the domain hypothesis and demonstrates that the domains are essentially independent in the intact L chain in solution. Analysis of the kinetic and equilibrium data in terms of this model gives the result that the two domains of Wes L chain have similar relatively low intrinsic stabilities of 5.5 kcal/mol in aqueous solution at pH 7.0 and 25°.

Amino acid sequence studies of homogeneous light (L) chains of immunoglobulins[1] have shown that L chains are composed of two homologous regions of sequence, one which is essentially constant within a given class and type and one which contains a large proportion of variable residues (for a review, see Edelman and Gall, 1969). This finding and the similar finding that IgG heavy (H) chains are composed of four such homology regions have led to the hypothesis that each of these homologous regions of sequence is independently folded into discrete globular entities called "domains" which contribute to some particular function of the IgG molecule (Edelman *et al.*, 1969). Circumstantial evidence in favor of this hypothesis for isolated L chains is provided by recent demonstrations (Solomans and McLaughlin, 1969; Karlsson *et al.*, 1969) that isolated L chains can be cleaved by a variety of proteolytic enzymes into compact mol wt 11,000 fragments corresponding to these homology regions which retain their original gross conformation (Björk *et al.*, 1971; Karlsson *et al.*, 1972). Preliminary 6-Å resolution X-ray crystallographic data on an L-chain dimer are consistent with this hypothesis (Edmundson *et al.*, 1972). However, the relationship between these homology regions in the intact L chain in solution has not been established.

Denaturation studies are capable of yielding information about the native state in terms of its cooperativity, intrinsic stability, and the nature of the forces responsible for maintaining its tertiary structure (see Tanford, 1968, 1970). Denaturation studies of model proteins such as lysozyme (Aune and Tanford, 1969a,b) and ribonuclease (Salahuddin and Tanford, 1970) in the presence of Gdn·HCl[2] have provided some general principles of Gdn·HCl induced denaturations which are useful in the analysis of such studies of more complicated proteins of biological importance. Many model proteins undergo a two-state transition from the native state N to the cross-linked random coil state D; that is, during the transition $N \rightleftharpoons D$ no states other than N and D are significantly present. This finding has been interpreted to mean that the native state is highly cooperative, such that disruption of any portion of its noncovalent interactions destabilizes the entire structure. In the case of the hypothetical domain structure of L chains, it would be expected that its denaturation would not be two state; if the two homologous halves of L chain are independently folded into discrete globular entities which do not interact, then stable partially denatured intermediates would be expected to appear during unfolding.

In the present article is reported the results of a study of the Gdn·HCl denaturation equilibrium and the kinetics of the approach to equilibrium of a κ-type L chain derived from the hyman myeloma protein Wes. A manuscript is currently in preparation which describes a similar study of the whole Wes Fab fragment.

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‡ National Institutes of Health Predoctoral Fellow (1968–1971); supported by Grant 5-F01-GM-39,557-03 from the U. S. Public Health Service. This material was submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biochemistry, Duke University. Present address: Department of Chemistry, Georgetown University, Washington, D. C. 20007.

§ Research career awardee, National Institutes of Health, U. S. Public Health Service.

[1] The nomenclature and the abbreviations for the immunoglobulins and their subunits produced by reduction and proteolysis are those recommended by the World Health Organization (1964).

[2] Abbreviation used is: Gdn·HCl, guanidine hydrochloride.

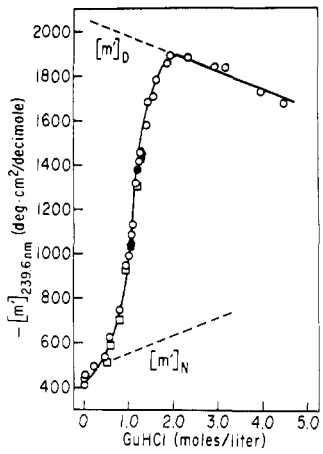

FIGURE 1: Optical rotation of the Wes L chain at 239.6 nm as a function of [Gdn·HCl] and protein concentration: (○) forward solutions; (□) reverse solutions. Protein concentrations are: (○,□) 0.017%; (▲) 0.0017%; (●) 0.034%.

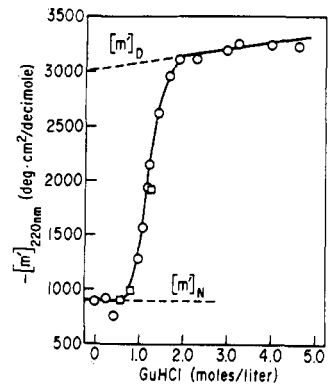

FIGURE 2: Optical rotation of the Wes L chain at 220 nm as a function of [Gdn·HCl]: (○) forward solutions; (□) reverse solutions.

Materials and Methods

Protein. Human serum from myeloma patient Wes was kindly donated by Dr. H. G. Kunkel; it was of the γ_1 subclass of IgG and contained L chains of the κ type. The purification of the protein from the serum was performed according to standard techniques (Fahey, 1967). The separation of the chains of the mildly reduced and S-carboxamidomethylated protein was performed by gel filtration at pH 2.0 (Green, 1973). Wes L chain was found by gel filtration in 6 M Gdn·HCl (Fish *et al.*, 1969) to have a mol wt of 23,000. The concentrations of stock solutions of isolated Wes L chain were determined by optical density measurements using an extinction coefficient for Wes L chain of $E_{278}^{1\%,1\,cm} = 10.8$ (Green, 1973).

Guanidine Hydrochloride. Gdn·HCl was UHP GuHCl Lot 777, purchased from Heico, Inc. Stock solutions were filtered and used without further purification. The concentrations of stock solutions were determined by measuring the index of refraction at 5892 Å and determining the concentration from the data of Kielly and Harrington (1960).

Optical Rotation Measurements. Optical rotation measurements were performed using a Cary 60 recording spectropolarimeter. The indices of refraction of water and Gdn·HCl solutions as a function of wavelength, required for the calculation of mean residue rotation, were obtained according to Hooker (1966).

Equilibrium and Kinetic Experiments. Solutions for equilibrium and kinetic measurements were made gravimetrically from stock solutions of concentrated protein and Gdn·HCl and doubly glass distilled water. The pH of each solution was measured at the end of each experiment and found to be between 6.8 and 7.1, with no systematic variations. For unfolding experiments the Gdn·HCl was added last, and for refolding experiments the Gdn·HCl and protein were combined and allowed to equilibrate and the water was added last. Kinetic measurements were made by continuous observation using the Cary 60 spectropolarimeter, with zero time taken as the time of addition of the last reagent.

Results and Discussion

The isothermal transition of the κ-type Wes L chain from the native state to the cross-linked random coil state as a function of Gdn·HCl concentration was followed by optical rotation at two wavelengths. The transition curves obtained by optical rotation at 239.6 and 220 nm are shown in Figures 1 and 2, respectively. At both wavelengths a single stage transition is observed which is complete at 2.0 M Gdn·HCl. The data of Figures 1 and 2 are plotted together in Figure 3 as the fractional extent of transition *vs.* Gdn·HCl concentration. Fractional extent of reaction is calculated by $f_{obsd} = ([m'] - [m']_N)/([m']_D - [m']_N)$ where $[m']_N$ and $[m']_D$ represent the optical rotation of the states N and D, respectively, and are obtained from the dashed lines in Figures 1 and 2. In the case of the data at 239.6 nm it is difficult to determine the dependence of $[m']_N$ on Gdn·HCl concentration because of the rather steep slope and the fact that the transition begins at very low Gdn·HCl concentration; however, the resulting uncertainty in $f_{obsd,239.6}$ at low values of $f_{obsd,239.6}$ will be seen to have little effect on the conclusions derived from this data.

Reversibility. The thermodynamic reversibility of the L-chain transition is demonstrated in Figures 1 and 2 by the agreement of solutions made by adding Gdn·HCl to solutions of native protein, and by diluting preequilibrated solutions of protein in concentrated Gdn·HCl.

Molecularity. The native state of Wes L chain in the absence of Gdn·HCl has a tendency to dimerize at pH 7.0 (Green, 1973). Therefore, it is necessary to establish whether association is significant during denaturation. If significant dimerization of native material occurs, the observed extent of unfolding under a given solvent condition must depend on the total concentration of protein. As shown in Figure 1 the conformational state of Wes L chain above 1.0 M Gdn·HCl is independent of protein concentration over a 20-fold range, demonstrating that the unfolding of L chain is unimolecular. It must be concluded that dissociation of any L-chain dimers is completed between 0.0 and 1.0 M Gdn·HCl.

Presence of Stable Intermediates. Useful information can be obtained from denaturation studies only if the data obtained can be interpreted in terms of defined thermodynamic states. If the native state N and the denatured state D are the only ones present in significant amounts throughout the transition, the transition is said to be two state, and the denaturation equilibrium constant $K_D = f_D/f_N$ can be obtained directly from the transition curve; in this case $f_{obsd} = f_D$ and the experimental equilibrium constant $K_{ap} = f_{obsd}/(1 - f_{obsd}) = K_D$. On the other hand, if stable intermediates X_i are present in the transition, the fractional extent of transition as observed by parameter y is $f_{obsd,y} = f_D + \Sigma f_i z_{i,y}$ where f_i is the weight fraction of species X_i and $z_{i,y} = (y_i - y_N)/(y_D - y_N)$ where y_i is the value of y that would be observed for a solution of pure X_i. If equilibrium constants $K_i = f_i/f_N$ are defined, then

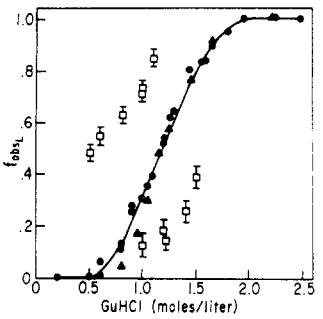

FIGURE 3: Normalized transition curve for the Wes L chain as a function of [Gdn·HCl]. f_{obsd} is calculated from the data of Figure 1 (●) and Figure 2 (▲) as described in the text. Both forward and reverse points are included: (□) extrapolated zero times from kinetic experiments.

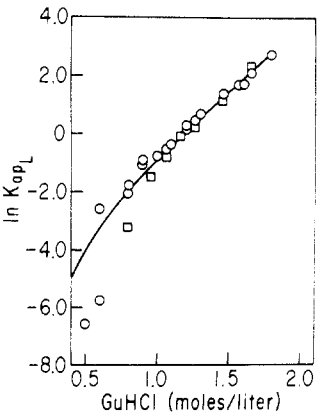

FIGURE 4: Dependence of the apparent denaturation constant on [Gdn·HCl]: (○) data from Figure 1; (□) data from Figure 2.

the apparent equilibrium constant is given by eq 1 (Tanford,

$$K_{ap,y} = K_D\left[\frac{1 + \Sigma z_{i,y}K_i/K_D}{1 + \Sigma(1 - z_{i,y})K_i}\right] \quad (1)$$

1968).

One means of detecting the presence of stable intermediate states from equilibrium data is by comparing properties of the apparent equilibrium constant K_{ap} with predicted properties of the true denaturation constant K_D. Using the procedure described by Tanford (1970) it is possible to approximately predict the dependence of the true denaturation constant K_D on Gdn·HCl concentration, with the aid of model protein and model compound data.

The effect of altering the solvent composition on the equilibrium between states can be expressed by the relation

$$\delta \ln K_D = -\Sigma\Delta\alpha_i\delta g_{tr,i}/RT \quad (2)$$

where $\Delta\alpha_i$ is the average change in exposure to solvent of group i during unfolding and $\delta g_{tr,i}$ is the free energy of transfer of group i from one solvent composition to another. In this treatment the free energy of denaturation at a given solvent composition is given by the relation

$$\Delta G = -RT \ln K_D = \Delta G^\circ + \Sigma\Delta\alpha_i\delta g_{tr,i} \quad (3)$$

where ΔG° is the free energy of the process in aqueous solution. For the transition of globular proteins from N to D, $\Delta\alpha_i$ is estimated from solvent perturbation studies and model protein studies to be 0.30–0.35 for the peptide and hydrophobic groups (Tanford, 1970); the $\delta g_{tr,i}$'s for these groups as a function of Gdn·HCl concentration have been determined from solubility studies of model compounds in various concentrations of Gdn·HCl (Nozaki and Tanford, 1970). Using these parameters, the slope of a plot of ln K_D *vs.* [Gdn·HCl] can be approximately predicted if the amino acid composition of the protein is known. It is clear from eq 1 that if stable intermediates are present the dependence of ln K_{ap} on [Gdn·HCl] would be less than that predicted for ln K_D.

In Figure 4 is shown a plot of ln K_{ap} *vs.* [Gdn·HCl] for the Wes L chain transition data shown in Figure 3. The slope estimated from eq 2 for a plot of ln K_D *vs.* [Gdn·HCl] for this protein, between 1.0 and 2.0 M Gdn·HCl, is 10. It is seen that the slope of the curve of Figure 4 for the experimentally significant data ($0.1 < K_{ap} < 10$) is 4.8, approximately half that predicted if $K_{ap} = K_D$, clearly indicating the presence of stable intermediates in this transition.

If, as suggested by the domain hypothesis, the Wes L chain is composed of two globular half-molecules which can denature independently with equilibrium constants K_1 and K_2, the resulting intermediate species expected to appear are illustrated in Figure 5. In this model $z_A + z_B = 1$, and the apparent equilibrium constant as given by eq 1 is

$$K_{ap} = \frac{K_1K_2 + z_BK_1 + (1 - z_B)K_2}{1 + (1 - z_B)K_1 + z_BK_2} \quad (4)$$

As before eq 2 can be used to estimate the dependence of K_1 and K_2 on Gdn·HCl concentration. This calculation is heavily influenced by the number of peptide groups; since the two homology regions of L chain are of similar size and similar "hydrophobicity" (Welscher, 1969a,b) the predicted dependence of ln K_1 and ln K_2 on [Gdn·HCl] is 5.0. Inspection of eq 4 shows that if $K_1 = K_2$, $K_{ap} = K_1 = K_D^{1/2}$ so that the maximum slope of ln K_{ap} *vs.* [Gdn·HCl] for this model is that predicted for K_1; if K_1 and K_2 are significantly different, the dependence of ln K_{ap} on [Gdn·HCl] would be <5.0.

Our finding that the slope of Figure 4 is no more than 5.0 is consistent with the domain model, though of course the particular half-denatured states indicated in Figure 5 are not the only intermediate states which can account for the lowered cooperativity of the Wes L transition. This interpretation of the equilibrium data is supported by the results of Karlsson *et al.* (1972), in which transition curves, under conditions similar to ours, of a λ L chain and of the isolated C_λ and V_λ fragments are reported. Analyzing their transition curves for the isolated domains in terms of the cooperativity with respect to [Gdn·HCl], we find that each gives a slope of ln K_{ap} *vs.* [Gdn·HCl] of ~4.5. Similar analysis of their curve for the whole L chain gives a slope of ln K_{ap} *vs.* [Gdn·HCl] of ~3.5, consistent with eq 4, and reflecting the fact that the transitions for C_λ and V_λ occur at different Gdn·HCl concentrations, so that at any given Gdn·HCl concentration K_1 and K_2 are significantly different.

Another means of detecting the presence of stable intermediates is to follow the transition by several parameters for which the $z_{i,y}$'s of eq 1 are expected to be different. It is seen in Figure 3 that the data at 239.6 and 220 nm are in good agreement above 1.0 M Gdn·HCl. Below 1.0 M Gdn·HCl the data do not coincide; however, the small disagreement of the two curves at low values of f_{obsd} could be due to the difficulty of determining the solvent effect on the optical rotation of

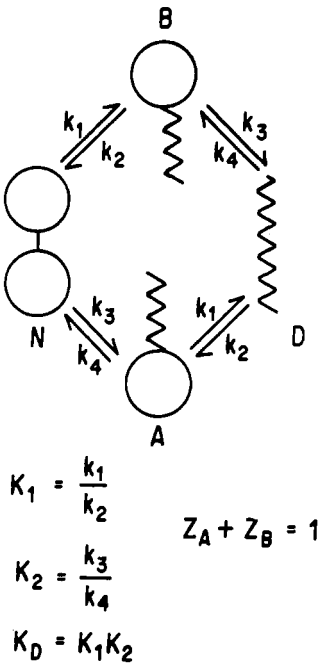

FIGURE 5: Domain model for L-chain denaturation: circles represent native domains; angles represent denatured domains.

native L chain at 239.6 nm or to a small change in optical rotation due to the dissociation to monomer which occurs below 1.0 M Gdn·HCl, as well as to a difference in the z_i for the stable intermediates at these two wavelengths. Although it is quite possible that the z_i's are the same for optical rotation at the two wavelengths examined, it is of interest to note that in the domain model z_B cancels out of eq 4 if $K_1 = K_2$, and the transition becomes independent of the parameter used to follow it.

Our equilibrium data are consistent with the domain denaturation model illustrated in Figure 5 in which the half-molecules denature independently with equilibrium constants K_1 and K_2. The finding that the slope of Figure 4 is nearly 5.0 suggests that K_1 and K_2 are of similar magnitude throughout the transition, since according to eq 4, if K_1 and K_2 are significantly different, the dependence of ln K_{ap} on [Gdn·HCl] would be less than 5.0. It may be noted that any intermediates in addition to those illustrated in Figure 5, for example, intermediates in the unfolding of either domain, would also result in a slope less than 5.0.

Kinetics of the Approach to Equilibrium. The examination of the kinetics of the approach to equilibrium is a rigorous test for the presence of stable intermediates (Tanford, 1968, 1970); if stable intermediates are present, as already demonstrated for the Wes L chain, kinetic studies provide information concerning the identity of intermediate states and the mechanism of folding and unfolding. If a transition is two state, *i.e.*

$$N \underset{k_r}{\overset{k_f}{\rightleftharpoons}} D$$

then solution of the appropriate rate equations gives the result

$$\left|\frac{\alpha_\infty - \alpha_t}{\alpha_\infty - \alpha_0}\right| = e^{-(k_f + k_r)t} \qquad (5)$$

where α is the optical rotation at the subscripted time. If

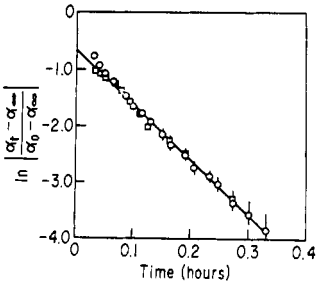

FIGURE 6: Kinetics of folding and unfolding of the Wes L chain at 1.0 M Gdn·HCl: (○) unfolding kinetics; (□) refolding kinetics; followed by optical rotation at 239.6 nm.

stable intermediates are present at equilibrium, solution of the appropriate rate equations takes the form

$$\left|\frac{\alpha_\infty - \alpha_t}{\alpha_\infty - \alpha_0}\right| = \sum_i p_i e^{-\lambda_i t} \qquad (6)$$

where the λ's are functions of the rate constants and the p's are functions of the initial conditions, the rate constants, and the contributions (z_i) of the intermediates to the observed parameter. The exact solution depends on the mechanism, but all reversible mechanisms give solutions of this form. For a two-state process (eq 5) a plot of ln $(\alpha_\infty - \alpha_t)/(\alpha_\infty - \alpha_0)$ *vs.* time gives a linear plot which extrapolates to zero at zero time, whereas if stable intermediates exist (eq 6) such linearity could only occur if several of the rate constants were fortuitously identical.

In order to survey the kinetic properties of the denaturation of the Wes L chain, the approach to equilibrium was followed by monitoring the time dependence of the optical rotation at 239.6 nm. The kinetics were measurable in the narrow range of Gdn·HCl concentration between 0.5 and 1.5 M; outside this range the process was too fast to be measured by this technique in which the first observation is made 125 sec after mixing.

In Figure 6 are shown the first-order plots for an example of a forward and reverse experiment in 1.0 M Gdn·HCl. In these examples, as in all the experiments, the first-order plot is linear but it does not extrapolate to zero at zero time. The extrapolated zero time points for the kinetic experiments are shown in Figure 3 along with the equilibrium curve. It is clear that at least two exponential terms in eq 6 are required to describe the results, confirming the presence of stable intermediates in this transition.

The first portion of the reaction at all concentrations of Gdn·HCl was too fast to be observed. The coefficients P_{2F} and P_{2R} and the apparent rate constant λ_2 for the observable portion of the reaction are summarized in Table I. λ_2 ranges from 10 to 18 hr^{-1}, and good agreement is obtained for forward and reverse experiments under conditions where both were measured. In Table I it is seen that the coefficients P_{2F} for the forward experiments and P_{2R} for the reverse experiments are within experimental error, both approximately 0.5 for all the experiments.

The solutions of the appropriate rate equations for several simple denaturation mechanisms involving one or two intermediates, including a two-intermediate parallel step mechanism such as that illustrated in Figure 5, have been obtained by Ikai (Ikai and Tanford, 1973). Applying their diagnostic criteria for these mechanisms to our data, we find that our data are consistent with the mechanism illustrated in Figure 5

TABLE I: Kinetic Parameters for the Wes L Chain.

[Gdn·HCl] (M)	Direction	λ_2 (hr^{-1}) ±1.0	P_{2F} or P_{2R}
0.50	Reverse	12	0.5 ± 0.1
0.60	Reverse	11	0.5 ± 0.1
0.78	Reverse	10	0.6 ± 0.1
0.97	Reverse	12	0.6 ± 0.1
0.99	Reverse	11	0.5 ± 0.1
1.00	Forward	10	0.5 ± 0.2
1.20	Forward	18	0.7 ± 0.2
1.20	Reverse	19	0.6 ± 0.1
1.26	Forward	20	0.7 ± 0.2
1.46	Forward	17	0.6 ± 0.1
1.50	Forward	18	0.5 ± 0.1

in which the molecule consists of two portions which denature independently. According to these criteria, all sequential mechanisms involving a small number of intermediates are ruled out by the relationships among the coefficients of eq 6 for the forward and reverse experiments. Because the initial portion of the kinetics was too fast to be followed by our technique, additional intermediates on one of the parallel pathways cannot be ruled out by the kinetic data alone; however, the cooperativity of the equilibrium transition curve with respect to Gdn·HCl concentration indicates that no additional intermediates occur.

In the solution of the mechanism shown in Figure 5, eq 6 has two terms; both are significant for experiments in both directions. Above the transition ($K_{ap} \gg 1$) the coefficient $P_{2F} = z_A$ and below the transition ($K_{ap} \ll 1$) P_{2R} also equals z_A. In the transition region where both forward and reverse experiments can be performed, $P_{1F}/P_{1R} = K_1/K_{ap}$ and $P_{2F}/P_{2R} = K_2/K_{ap}$. The finding that P_{2F} and P_{2R} are 0.5 above and below the transition, respectively, is consistent with this mechanism and indicates that the independently denaturing portions of L chain each contribute half of the total change in optical rotation at 239.6 nm during the transition. The finding that P_{2F} and P_{2R} are 0.5 throughout the transition, such that $P_{1F}/P_{1R} = P_{2F}/P_{2R} = 1$, indicates that the equilibrium constants K_1 and K_2 are similar throughout the transition and are therefore approximated by the experimental equilibrium constant K_{ap}. The apparent rate constants are $\lambda_1 = k_1 + k_2$ and $\lambda_2 = k_3 + k_4$ (see Figure 5); the large difference observed between the apparent rate constants indicates that in spite of the similarity between the equilibrium constants for the two independent regions of the Wes L chain, one of them equilibrates more rapidly than the other.

According to the domain hypothesis, L chains are composed of two independently folded half-molecules corresponding to the regions of constant and variable sequence. The kinetics of the approach to equilibrium show that the Wes L chain is composed of two independently unfolding regions; the equilibrium data, in particular the cooperativity of the transition with respect to Gdn·HCl concentration, are consistent with the kinetics and indicate that the independently denaturing portions correspond to half-molecules. Together, the equilibrium and kinetic results provide strong confirmation of the domain hypothesis of L-chain structure and demonstrate that the two domains of L chain are essentially independent of each other in the intact L chain. Our data show that the independent domain denaturation constants K_1 and K_2 are similar as a function of Gdn·HCl concentration, whereas the rate constants governing the equilibration of the two domains are different.

Intrinsic Stability of L-Chain Domains. If the true denaturation constant for a protein can be measured as a function of Gdn·HCl concentration, the value of the equilibrium constant extrapolated to zero Gdn·HCl concentration provides an estimate of the free energy of stabilization of the native structure over the random coil under physiological conditions. Several theoretically based functions have been used for the extrapolation of experimentally measured denaturation constants in the study of model proteins which undergo two-state transitions (see Tanford, 1970). The use of eq 3 in estimating the intrinsic stabilities of globular proteins from denaturation data is not as theoretically sophisticated as some of the other functions which have been developed; however, this relation has been shown to give results in good agreement with the more sophisticated relations and is well within the accuracy of our data.

The transition of the Wes L chain has been found to be a multi-state process; however, the thermodynamic and kinetic results have been analyzed in terms of the true denaturation constants K_1 and K_2 for the two domains. Since it has been shown that $K_1 \simeq K_2 \simeq K_{ap}$ for Wes L domains, the extrapolation of ln K_{ap} to zero Gdn·HCl concentration may be taken to approximate the values of K_1 and K_2 in the absence of Gdn·HCl. This extrapolation, using eq 3, gives the result that $\Delta G^\circ = 5.5$ kcal/mol for the C_L and V_L domains of the Wes L chain at pH 7.0 and 25°. Thus, the globular domains of the Wes L chain are only marginally stable under native conditions, even less so than such small model proteins as lysozyme ($\Delta G^\circ = 12$ kcal/mol) and ribonuclease ($\Delta G^\circ = 10$ kcal/mol).

Comparison of our results with the rather limited data in the literature is instructive. Transition curves for the isolated C_λ and V_λ domains of a λ L chain at 25° and pH 7.3 have midpoints at 1.05 M Gdn·HCl and 1.6 M Gdn·HCl, respectively (Karlsson *et al.*, 1972), as compared with 1.15 M Gdn·HCl for Wes L domains. Transition curves for several κ and λ Bence-Jones proteins at pH 6.0 show two-stage transitions, the first having a midpoint between 1.0 and 1.5 M Gdn·HCl, and the second at a slightly higher concentration (Azuma *et al.*, 1972). These data are difficult to interpret because the possibility of dimerization was not evaluated. Within their limitations, however, these data suggest that the individual domains of L chains are in general of low intrinsic stability, similar to Wes L domains, but that small variations in stability occur due to the differences in amino acid sequences among them. The similarities of intrinsic stability among L domains are consistent with the prevailing concept that the homologous domains have similar tertiary structures which are largely determined by the constant residues in the sequences.

The relatively low intrinsic stability of L domains compared to model proteins such as lysozyme and ribonuclease may be a reflection of their slightly smaller size. Alternatively, however, the low stability of L domains may be a consequence of the multiplicity of evolutionary pressures upon L domain sequences, since L chains exist *in vivo* primarily as a subunit of a complicated molecule having multiple functions. From this point of view it might be expected that the C_L domain would be more stable than the V_L domain, since V_L domain variability has been related to an evolutionary pressure other than intrinsic stability, that of antibody diversity. On the contrary, we have found the V_L and C_L domains of the Wes L chain to be of similar stability, and two investi-

gators have suggested that the C domains are less stable than the V domains for several other L chains and Bence-Jones proteins (Karlsson *et al.*, 1972; Azuma *et al.*, 1972). These considerations, if valid, raise the possibility that the sequence of the C domain has been subject to evolutionary pressures related to an as yet unknown specific function in the intact immunoglobulin. From this point of view a study of the relationships among the domains in an intact Fab fragment will be of interest; a manuscript describing such a study of the Wes Fab fragment is currently in preparation.

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Hydroxystilbamidine. A Nonintercalating Drug as a Probe of Nucleic Acid Conformation†

Bernard Festy‡ and Michel Daune*,§

ABSTRACT: The binding to DNA and polynucleotides of hydroxystilbamidine, a new trypanocidal drug, was followed by spectrophotometric and viscosimetric studies. The purified dye itself displays a peculiar fluorescence spectrum with two emission bands situated around 450 and 600 nm, respectively, and corresponding to the same wavelength of excitation. Both the blue and red parts of the spectrum are observed when the dye is bound to DNA, but only the blue emission is present when bound to RNA or synthetic polyribonucleotides. In the case of DNA, the enhancement of blue fluorescence increases linearly with the square of the percentage of A + T. The fluorescence light is often characteristic of a given nucleic acid and hydroxystilbamidine appears as a new interesting fluorescent probe. No elongation of the molecule can be detected from viscosimetric measurements with sonicated rod-shaped DNA or covalently circular DNA and therefore the dye is not intercalated. Spectral modifications of the dye may only be explained in terms of an outside binding in which the ionization of the phenolic group is very sensitive to the presence of vicinal proton acceptors. Finally, hydroxystilbamidine binding to DNA offers a very simple model for interactions between proteins and nucleic acids.

An incredible number of substances, particularly drugs, have been studied with regard to their interaction with nucleic acids, DNA in particular, and very often in order to prove they are intercalated between the base pairs in relation to their biological properties (Waring, 1970).

The present study of hydroxystilbamidine is not intended to add a new name to the list but rather to pose a problem connected with its biological properties. This drug is not only a good trypanocidal agent but has also antimalarial, antifungal, and carcinostatic properties. A recent *in vivo* study of the action of hydroxystilbamidine on *Trypanosoma cruzi* has given clear evidence of a selective binding of the drug to the kinetoplast, followed by important changes of the organization of the kinetoplastic DNA (Delain *et al.*, 1971). At the same time, the replication of this DNA is modified, leading to molecules of lower density and probably a higher A + T content.

† From the Unité de Biochimie et Enzymologie (directeur Professor C. Paoletti), Institut Gustave Roussy, 75-Villejuif, France, and the Centre de Recherches sur les Macromolécules, 67-Strasbourg, France. *Received January 12, 1973.*

‡ Institut Gustave Roussy. Present address: Laboratoire d'Hygiène de la Ville de Paris, Paris, France.

§ Centre de Recherches sur les Macromolecules. Present address: Groupe de Biophysique, Université Louis Pasteur, 67-Strasbourg, France.