Thermodynamics of Unfolding of the All β-Sheet Protein Interleukin-1β†

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ABSTRACT: The thermal denaturation of interleukin-1β in solution has been studied by differential scanning calorimetry at various pH values. It is shown that the thermal transition of interleukin-1β is completely reversible below pH 2.5, only partly reversible in the pH range 2.5–3.5, and irreversible above pH 3.5. Analysis of the reversible unfolding of interleukin-1β shows that the heat denaturation is well approximated by a two-state transition and is accompanied by a significant increase of heat capacity. The partial heat capacity of denatured interleukin-1β is very close to that expected for the completely unfolded protein. This permitted us to assign the thermodynamic characteristics of interleukin-1β denaturation to its complete unfolding and to correlate them with structural features of the protein. The contributions of hydrogen bonding and hydrophobic interactions to the stability of interleukin-1β are analyzed and compared to those for other globular proteins. It is shown that the Gibbs energy of a hydrogen bond in a β-sheet structure is greater than in α-helices.

Human interleukin-1β is a globular protein of 153 amino acid residues, with a molecular weight of 17 381. The protein does not contain disulfide bonds, and its structure is not stabilized by any cofactors. The three-dimensional structure of the protein has been solved by NMR in solution (Clore *et al.*, 1991) and by X-ray crystallography in the solid state (Finzel *et al.*, 1989; Priestle *et al.*, 1989; Veerapandian *et al.*, 1992), revealing a fold made up of 12 β-strands (Clore & Gronenborn, 1991). The stability of this all β-sheet protein has been studied by circular dichroism and fluorescence and UV absorbance (Craig *et al.*, 1987). The kinetics of folding have been followed by NMR, circular dichroism, and fluorescence spectroscopy (Varley *et al.*, 1993; Gronenborn & Clore, 1994). At neutral pH, unfolding of the protein by guanidine hydrochloride is fully reversible and proceeds without any detectable population of intermediate states. These findings suggest that interleukin-1β is a good object for the study of the relationship between structure and stability. Interest in the thermodynamics of unfolding of this all β-sheet protein is also enhanced by the fact that not many proteins of this class have been studied before.

In this paper, the thermodynamic parameters of interleukin-1β unfolding are characterized by scanning microcalorimetry. A correlation is made between the thermodynamic parameters obtained and the structural features of the protein molecule such as the buried water accessible surface area (ASA), the number of hydrogen bonds in the molecule, their average length, and the amount of secondary structure.

MATERIALS AND METHODS

Human interleukin-1β was expressed in *Escherichia coli* and purified as described earlier by Wingfield *et al.* (1986) and Clore *et al.* (1991). Due to differential processing, the protein product was heterogeneous in sequence, either possessing or lacking the N-terminal methionine residue.

The concentration of interleukin-1β in solution was determined spectrophotometrically using an extinction coefficient of 0.61 cm^{-1} at 280 nm (Wingfield *et al.*, 1986). A correction for light scattering was applied according to Winder and Gent (1971).

The calorimetric experiments were performed on a DASM-4M scanning microcalorimeter (Privalov & Plotnikov, 1989) in the temperature range 5–125 °C, at a heating rate of 1 °C/min, in 10 mM glycine/HCl (for pH range 2.0–3.0) and 10 mM sodium acetate/acetic acid (at pH 4.0). The protein sample at a concentration of 1.5–2.0 mg/mL was extensively dialyzed at 4 °C against the corresponding buffer using Spectrapor 2 dialysis membranes with molecular weight cutoff 3000 Da. Insoluble material was removed by centrifugation for 15–20 min at 5000*g*. The partial heat capacity of protein, $C_p(T)$, was computed from the heat capacity difference, $\Delta C_p^{app}(T)$, between the sample cell, containing protein solution, and the reference cell, containing corresponding solvent, as follows (Privalov & Potekhin, 1986):

$$C_p(T) = C_{p,sol}(T)\frac{V_{pr}(T)}{V_{sol}(T)} - \frac{\Delta C_p^{app}(T)}{m(T)} \quad (1)$$

$V_{sol}(T)$ and $V_{pr}(T)$ are the partial volumes of solvent and protein, respectively, $C_{p,sol}(T)$ is the partial heat capacity of solvent, and $m(T)$ is the amount of protein in the calorimetric cell at a given temperature, T. A partial specific volume of 0.726 $cm^3\ g^{-1}$ for interleukin-1β (Wingfield *et al.*, 1986) was employed for the computation of the partial heat capacity.

The water accessible surface area of different groups was calculated as described previously (Makhatadze *et al.*, 1993). Four structures of interleukin-1β from the Protein Data Bank (Bernstein *et al.*, 1977) were used, the three X-ray structures 1il b (Finzel *et al.*, 1988), 2il b (Priestle *et al.*, 1989), and 4il b (Veerapandian *et al.*, 1992), and the solution NMR structure 6il b (Clore *et al.*, 1991). The results of calculations have been averaged over the four structures.

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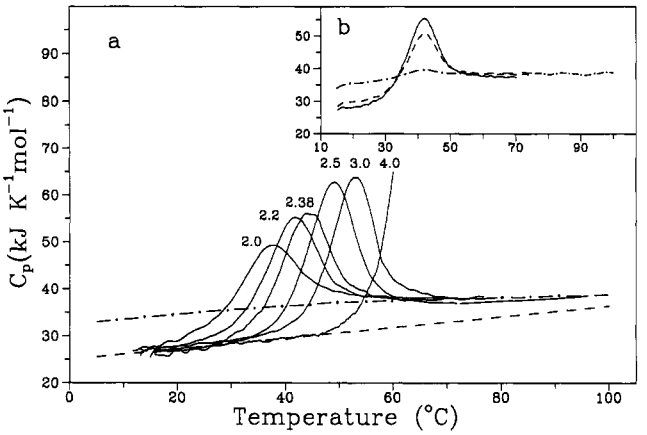

FIGURE 1: (a) Temperature dependence of the partial molar heat capacity of interleukin-1β in solutions with the pH values indicated above the curves. At pH 4.0, the protein provides the heat capacity of the native state over a wider temperature range, although it aggregates in the unfolded state. The dashed line represents the partial molar heat capacity of native interleukin-1β. The dashed-and-dotted line represents the partial molar heat capacity of unfolded interleukin-1β, calculated according to Privalov and Makhatadze (1990). (b) Three successive scans of interleukin-1β at pH 2.2. The first scan (solid line) was stopped at 70 °C and the second (dashed line) at 100 °C. The reversibility of the second scan is estimated to be about 90%, by comparison of the areas under the heat capacity endotherms.

The unfolded state of interleukin-1β was modeled as an extended conformation of the actual sequence using the sequence builder of QUANTA (Molecular Simulations, Inc.). In most prior studies, the ASA of the unfolded state was estimated by calculating the surface area of each amino acid in an extended tripeptide, Gly-X-Gly or Ala-X-Ala, and then summing up these contributions over the amino acid composition of the protein (Lee & Richards, 1971; Shrake & Rupley, 1973; Miller *et al.*, 1987; Spolar *et al.*, 1989; Privalov & Makhatadze, 1990, 1992, 1993; Lesser & Rose, 1990). Since Gly does not have a side chain and Ala has a very small one, these methods give a net water accessible surface of the side chain X which is not screened by the neighboring side chains. It appears that the ASA of the polypeptide in the extended conformation is the best one can currently use as an approximation of the polypeptide in the random coiled state. Accordingly, for the analysis presented in this paper in difference to our previous papers, we used for the unfolded state the ASA determined for the extended conformation. This is by about 20% lower than for the tripeptides.

The ASA exposed upon unfolding was obtained as a difference between surface areas of the native and extended conformations. The following Δ^{U}_{N}ASA values were obtained (Å^2), aliphatic, 6531; aromatic, 1374; polar parts of: Arg, 101; Asn, 29; Asp, 104; Cys, 113; Gln, 364; Glu, 320; His, 29; Lys, 247; Met, 201; Ser, 192; Thr, 99; Trp, 10; Tyr, 101; –CONH–, 2231.

The number of hydrogen bonds in the molecule, N_{HB}, as well as the fraction of protein with helical hydrogen-bonding geometry, f_α, and the average length of the hydrogen bond, L_{HB}, were calculated using the algorithms described by Stickle *et al.* (1992). The following numbers for interleukin-1β have been obtained, N_{HB} = 118; f_α = 0.183; L_{HB} = 2.98 Å.

RESULTS

Figure 1a presents the partial molar heat capacity of interleukin-1β at different pH values. The protein undergoes a transition from the native to the denatured state with increasing temperature. This transition is accompanied by a significant heat absorption, with the midpoint temperature, T_t, depending upon the pH of the solution. With a change of pH from 2 to 2.5, the transition temperature increases by 11 °C. In this pH range, the denaturation transition of interleukin-1β is highly reversible. Three consecutive scans of interleukin-1β at pH 2.2 are presented in Figure 1b, showing that its reversibility depends on the upper temperature of heating. A similar decrease of reversibility with the increase of the upper temperature of heating was observed with many other proteins and was explained by chemical modification of some groups and hydrolysis of the polypeptide chain (Klibanov, 1983). Further increase of pH to 3 leads to an increase in the transition temperature by an extra 6 °C and a decrease in reversibility. Increasing the pH above 3.0 leads to complete irreversibility of the denaturational transition, not only because of thermal degradation of the polypeptide chain but also due to aggregation in the unfolded state.

Table 1: Thermodynamic Characteristics of Interleukin-1β Heat Denaturation

pH	T_t (°C)	ΔH_{cal} (kJ/mol)	ΔH_{vH} (kJ/mol)	$\Delta H_{cal}/\Delta H_{vH}$
2.0	37	227	242	0.94
2.2	41	266	277	0.96
2.38	44	247	235	1.05
2.5	49	330	327	1.01
3.0	53	351	369	0.95

The calorimetrically measured enthalpies of interleukin-1β denaturation are in good correspondence with the van't Hoff enthalpies, ΔH_{vH}, calculated from the sharpness of the calorimetric profile as

$$\Delta H_{vH} = \frac{4RT_t^2 C_{p,max}}{\Delta H_{cal}} \quad (2)$$

where $C_{p,max}$ is the heat capacity at the transition temperature and R is the gas constant (Table 1). As one can see, the ratio $\Delta H_{cal}/\Delta H_{vH}$ is close to 1 which means that the unfolding of interleukin-1β can be closely approximated by a two-state mechanism.

The slope of the dependence of the enthalpies of the interleukin-1β denaturation on transition temperature, obtained from calorimetric experiments, represents the heat capacity change upon interleukin-1β denaturation. Linear regression analysis gives a value of 7.9 kJ K^{-1} mol^{-1} for the ΔC_p. The heat capacity change obtained in this way reflects ΔC_p averaged over the temperature range where the enthalpy of denaturation can be obtained experimentally. This accessible temperature interval is unfortunately rather narrow.

The heat capacity change upon denaturation can also be obtained from the temperature dependencies of the heat capacities of a protein in the native and denatured states. This method for the determination of the heat capacity change is more advantageous, since ΔC_p depends significantly upon temperature (Privalov & Makhatadze, 1990, 1992; Makhatadze *et al.*, 1993). The partial molar heat capacity of the native state of interleukin-1β, C_p^N, can be obtained experimentally up to 50 °C (Figure 1a). In this temperature range, the heat capacity of the native protein appears to be a linear function of temperature. Assuming that this functional dependence is valid for higher temperatures, the heat capacity of the native state can be extrapolated to higher temperatures using a slope of approximately 0.08 kJ K^{-2} mol^{-1} (Figure 1a and Table 2).

Table 2: Temperature Dependencies of the Heat Capacity, Enthalpy, and Entropy for Unfolding of Interleukin-1β

	temperature (°C)					
	5	25	50	75	100	125
	Heat Capacity (kJ mol^{-1} K^{-1})					
C_p^N	26.4	28.1	30.1	32.2	34.3	36.4
C_p^U	33.3	35.6	37.6	38.4	39.2	39.2
$\Delta_N^U C_p$	6.9	7.5	7.5	6.2	4.9	2.8
	Enthalpy (kJ mol^{-1})					
$\Delta_N^U H_{cal}$	7	151	330	501	640	736
$\Delta_N^U H_{alp}^{hyd}$	−1084	−797	−457	−137	170	451
$\Delta_N^U H_{arm}^{hyd}$	−247	−203	−153	−106	−63	−25
$\Delta_N^U H_{pol}^{hyd}$	−5048	−5178	−5320	−5449	−5567	−5675
$\Delta_N^U H^{vdW}$	1081	1065	1041	1003	944	857
$\Delta_N^U H^{HB}$	5305	5264	5219	5190	5156	5128
	Entropy (J mol^{-1} K^{-1})					
$\Delta_N^U S^{exp}$ [a]	−99	401	1006	1516	1903	2155
$\Delta_N^U S_{alp}^{hyd}$	−4768	−3775	−2671	−1718	−875	−144
$\Delta_N^U S_{arm}^{hyd}$	−591	−438	−273	−135	−16	85
$\Delta_N^U S_{pol}^{hyd}$	−2924	−3372	−3827	−4201	−4534	−4809
$\Delta_N^U S^{cnf}$	8182	7981	7774	7579	7308	7009
	Gibbs Energy (kJ mol^{-1})					
$\Delta_N^U G$[a]	34.5	31.5	5.1	−26.6	69.8	−121.7
$\Delta_N^U G_{alp}^{hyd}$	242	327	405	464	490	503
$\Delta_N^U G_{arm}^{hyd}$	−84	−73	−65	−59	−58	−59
$\Delta_N^U G_{pol}^{hyd}$	−4235	−4173	−4084	−3987	−3876	−3761

[a] $\Delta_N^U S^{exp}$ and $\Delta_N^U G$—experimental entropy and Gibbs energy obtained at pH 3.0. Experimental error in the absolute values of the heat capacities of the native and unfolded states is 3–5% and in other experimental quantities is 5–7%; estimated errors in the calculated quantities is 15–25%. C_p^N—the calorimetrically measured and extrapolated values of the partial specific heat capacity of the native protein; C_p^U—the heat capacity of the unfolded polypeptide chain of interleukin-1β; $\Delta_N^U H_{cal}$—the calorimetrically measured enthalpy of protein unfolding at considered temperature; $\Delta_N^U H_{pol}^{hyd}$ and $\Delta_N^U H_{npl}^{hyd}$—the enthalpies of hydration of polar and nonpolar groups upon protein unfolding, respectively; $\Delta_N^U H^{vdW}$—the enthalpy of van der Waals interactions between nonpolar groups in protein; $\Delta_N^U H^{HB}$—the total enthalpy of internal hydrogen bonding in protein; $\Delta_N^U S^{exp}$—the experimentally determined entropy of protein unfolding; $\Delta_N^U S_{pol}^{hyd}$ and $\Delta_N^U S_{npl}^{hyd}$—the entropies of hydration of polar and nonpolar groups upon protein unfolding, respectively; $\Delta_N^U S^{cnf}$—the configurational entropy of unfreezing of the backbone chain and of unpacking of the side chain groups; $\Delta_N^U G_{npl}^{hyd}$ and $\Delta_N^U G_{pol}^{hyd}$—the Gibbs energies of hydration of nonpolar and polar groups upon protein unfolding, respectively.

The heat capacity of denatured interleukin-1β is significantly larger than that of native interleukin-1β. Its values are very close to the heat capacity expected for the fully unfolded interleukin-1β, C_p^U (Figure 1a), calculated using the procedure of Privalov and Makhatadze (1990) and Makhatadze and Privalov (1990), and are listed in Table 2. The correspondence between the heat capacities of denatured and unfolded interleukin-1β implies that these two states are indistinguishable, at least thermodynamically. Thus, we can assign the measured thermodynamic parameters of interleukin-1β denaturation to its complete unfolding.

The temperature dependence of the heat capacity change upon unfolding of interleukin-1β can be obtained as $\Delta_N^U C_p = C_p^U - C_p^N$. The functional dependence of $\Delta_N^U C_p$ on temperature is presented in Table 2. Knowing the temperature dependence of the heat capacity change upon unfolding, one can calculate the enthalpy, $\Delta_N^U H(T)$, and entropy, $\Delta_N^U S(T)$, and Gibbs energy of stabilization, $\Delta_N^U G(T)$, difference between the unfolded and native states in all considered temperature ranges as

$$\Delta_N^U H_{cal}(T) = \Delta_N^U H_{cal}(T_o) + \int_{T_o}^{T} \Delta_N^U C_p(T)\, dT \quad (3)$$

$$\Delta_N^U S(T) = \Delta_N^U S^{exp}(T_o) + \int_{T_o}^{T} \Delta_N^U C_p(T)\, d \ln T \quad (4)$$

$$\Delta_N^U G(T) = \Delta_N^U H(T) - T\Delta_N^U S(T) \quad (5)$$

These functions are presented in Table 2.

DISCUSSION

The contributions of different interactions to the stability of interleukin-1β can be analyzed using information on the structural features of this molecule, such as the number of hydrogen bonds, the fraction of amino acid residues present in regular elements of secondary structure, and the amount of buried water accessible surface area using the formalism described by Makhatadze and Privalov (1993) and Privalov and Makhatadze (1993).

Contributions of Hydration, van der Waals Interactions, and Hydrogen Bonding to the Enthalpy of Interleukin-1β Unfolding. The experimentally measured enthalpy of interleukin-1β unfolding, $\Delta_N^U H_{cal}$, can be represented as the sum of the hydration enthalpies of polar, $\Delta_N^U H_{pol}^{hyd}$, and nonpolar, $\Delta_N^U H_{npl}^{hyd}$, groups upon protein unfolding, the enthalpy of van der Waals interactions between nonpolar groups, $\Delta_N^U H^{vdW}$, and the enthalpy of internal hydrogen bonding between polar groups, $\Delta_N^U H^{HB}$:

$$\Delta_N^U H_{cal} = \Delta_N^U H_{pol}^{hyd} + \Delta_N^U H_{npl}^{hyd} + \Delta_N^U H^{vdW} + \Delta_N^U H^{HB} \quad (6)$$

Each of these contributions is related to structural features of the protein as follows:

$$\Delta_N^U H_{npl}^{hyd}(T) = \sum_i \Delta_N^U ASA_{npl,i} \Delta \hat{H}_{npl,i}^{hyd}(T) \quad (7)$$

$$\Delta_N^U H_{pol}^{hyd}(T) = \sum_k \Delta_N^U ASA_{pol,k} \Delta \hat{H}_{pol,k}^{hyd}(T) \quad (8)$$

$$\Delta_N^U H^{vdW} = \Delta \hat{H}_{arm}^{vdW} \Delta_N^U ASA_{arm} + \Delta \hat{H}_{alp}^{vdW} \Delta_N^U ASA_{alp} \quad (9)$$

$$\Delta_N^U H^{HB} = N_{HB} \Delta h^{HB} \quad (10)$$

where $\Delta \hat{H}_i^{hyd}$ is the reduced enthalpy of hydration of a given type of surface area and $\Delta \hat{H}^{vdW}$ is the reduced enthalpy of van der Waals interaction between aliphatic (alp) or aromatic (arm) residues (Makhatadze & Privalov, 1993); $\Delta_N^U ASA_i$ is the exposure of a given type of surface area upon unfolding; Δh^{HB} is the enthalpy of an internal hydrogen bond in a protein.

In eqs 5–9, the only unknown parameter is the specific enthalpy of the internal hydrogen bond. Thus, the Δh^{HB} can be easily determined by solving these equations. The obtained value can be compared with the enthalpy of hydrogen bonding in other proteins by treating the thermodynamics of their unfolding in the same way as that of interleukin-1β (*e.g.*, eqs 6–10). A comparison is made in Figure 2 with eight other proteins: bovine pancreatic trypsin inhibitor, ubiquitin,

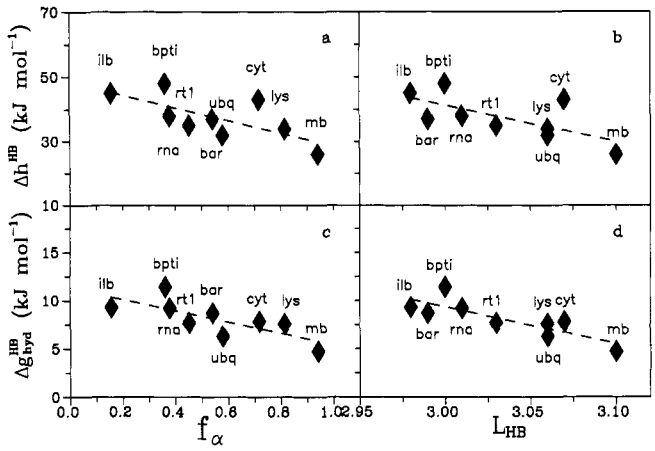

FIGURE 2: Dependence of the enthalpy of one internal hydrogen bond, Δh^{HB}, and the total Gibbs energy of one hydrogen bond, Δg^{HB}_{hyd}, on the fraction of protein in α-helical structure, f_α, or the average hydrogen bond length, L_{HB}. Correlation coefficients are 0.89 (a), 0.80 (b), 0.88 (c), and 0.88 (d). The estimated error is represented by the size of the symbols. The proteins are interleukin-1β (ilb), bovine pancreatic trypsin inhibitor (bpti) (Makhatadze *et al.*, 1993), ubiquitin (ubq) (Wintrode *et al.*, 1994), barnase (bar) (Griko *et al.*, 1994), ribonuclease T1 (rt1) (Yu *et al.*, 1994), myoglobin (mb), cytochrome *c* (cyt), ribonuclease A (rna), and lysozyme (lys) (Makhatadze & Privalov, 1993).

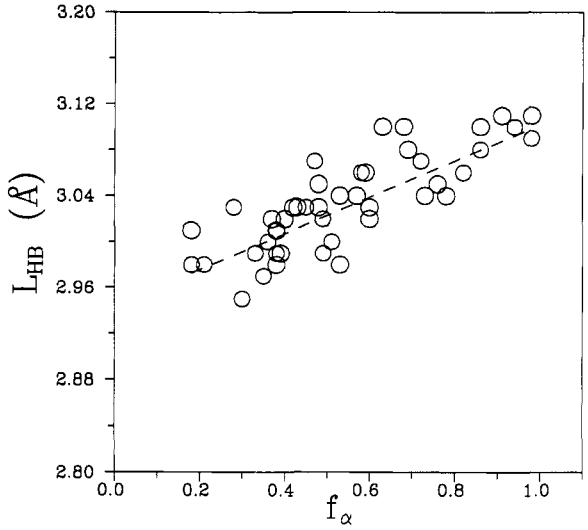

FIGURE 3: Dependence of the average length of a hydrogen bond, L_{HB}, on the fraction of protein in helical structure, f_α, for the proteins listed in Figure 2 as well as for an additional 37 other proteins, used by Stickle *et al.* (1992). Both parameters are calculated according to the algorithm described by Stickle *et al.* (1992). The correlation coefficient r = 0.83.

barnase, ribonuclease T1, ribonuclease A, cytochrome *c*, lysozyme, and myoglobin. As follows from Figure 2, the enthalpy of hydrogen bonding in interleukin-1β is close to that of BPTI. It is, however, almost twice as large as that of the hydrogen bond in myoglobin, a purely α-helical protein of similar molecular weight (Makhatadze & Privalov, 1993). It is most striking that the enthalpy of a hydrogen bond depends on the amount of protein in the helical structure. As discussed by others [see, *e.g.*, Baker and Habbard (1984)], the geometry of hydrogen bonding in α-helices differs significantly from the geometry in β-sheets. In particular, the length of hydrogen bonds in a helical structure is greater than in a β-sheet structure (Figure 3). This should lead to a larger enthalpy of hydrogen bonding in β-sheet structures (Wintrode *et al.*, 1994). The average hydrogen bond length in the interleukin-1β molecule is 2.98 Å. For comparison, the average length of a hydrogen bond is 3.10 Å in myoglobin and 3.06 Å in lysozyme. This difference in length correlates with the difference in the enthalpy of hydrogen bonding in these proteins (Figure 2b). It is not surprising that such a large difference in the energy of hydrogen bonding arises from changing the length of the bond by 0.12 Å. Estimations using the simplified approach of Hermans *et al.* (1984) predict a value of 1.6 for the ratio of the energies of hydrogen bonding with L_{HB} = 2.98 and 3.10 Å, which is in good agreement with our results.

Contribution of Hydration to the Entropy of Unfolding of Interleukin-1β. The entropy of protein unfolding can be represented as

$$\Delta^U_N S^{exp} = \Delta^U_N S^{hyd}_{npl} + \Delta^U_N S^{hyd}_{pol} + \Delta^U_N S^{cnf} \quad (11)$$

where $\Delta^U_N S^{hyd}_{npl}$ and $\Delta^U_N S^{hyd}_{pol}$ are the entropies of hydration of nonpolar and polar groups exposed to water upon unfolding, respectively, and $\Delta^U_N S^{cnf}$ is the configurational entropy of unfreezing the backbone chain, including the entropy of disruption of hydrogen bonds and of unpacking side chain groups. The hydration terms are assumed to be proportional to the change in water accessible surface area:

$$\Delta^U_N S^{hyd}_{pol} = \sum_i \Delta\hat{S}^{hyd}_i \Delta^U_N ASA_i \quad (12)$$

$$\Delta^U_N S^{hyd}_{npl} = \Delta\hat{S}^{hyd}_{arm} \Delta^U_N ASA_{arm} + \Delta\hat{S}^{hyd}_{alp} \Delta^U_N ASA_{alp} \quad (13)$$

where $\Delta\hat{S}^{hyd}_i$ are the hydration entropies of a given type of surface area on a per square angstrom basis (Privalov & Makhatadze, 1993). The hydration entropies of interleukin-1β are listed in Table 2. Knowing the entropies of hydration and the experimental entropies of interleukin-1β unfolding, one can get the conformational entropy change using eq 11. The values of $\Delta^U_N S^{cnf}$ obtained in this manner are listed in Table 2. At 25 °C, the conformational entropy of unfolding of interleukin-1β in vacuum is about 50 ± 5 J K^{-1} mol^{-1}. Assuming that an amino acid residue has on average about five rotatable bonds, the entropy of unfreezing one bond will be 10 J K^{-1} mol^{-1}. This is in a reasonable agreement with the previous estimates for the conformational entropy, both theoretical and experimental. For example, according to Novotny *et al.* (1989), one rotatable bond contributes about 9 J K^{-1} mol^{-1}. A similar number (18 J K^{-1} mol^{-1}) was obtained by Page and Jencks (1971) from an analysis of cyclization of model compounds. The estimate of the entropy change upon sublimation of linear alkanes gives a value of 14 J K^{-1} mol^{-1}/rotatable bond or about 70 J K^{-1}/mol of amino acid residues [entropy of fusion per C–C bond is 6 J K^{-1} mol^{-1} (Nicholls *et al.*, 1991) and entropy of vaporization is 8 J K^{-1} mol^{-1} (Ben-Naim & Marcus, 1984)]. Since about 60–70% of all residues are buried in a native protein structure (Miller *et al.*, 1987), this gives –45 J K^{-1} mol^{-1} of residues. According to Pickett and Sternberg (1993), the rotational entropy, calculated using Boltzmann sampling of the possible conformations, gives a value for the entropy of a side chain *relative to alanine* ranging from 7 (Val) to 28 (Arg) J K^{-1} mol^{-1}. For the absolute values of the entropies per amino acid residue, we have about 37–68 J K^{-1} mol^{-1}, by adding one rotatable bond for Ala and two rotatable bonds for the backbone. So it appears that after subtraction of the hydration entropies from the experimentally measured entropy of interleukin-1β unfolding, we are getting very reasonable numbers for the conformational entropy of unfolding a protein in vacuum.

Contribution of Hydration and Hydrogen Bonding to the Gibbs Energy of Unfolding of Interleukin-1β. The Gibbs energy of hydration can be obtained from the enthalpies and entropies of hydration using the equation:

$$\Delta_N^U G^{hyd}(T) = \Delta_N^U H^{hyd}(T) - T\Delta_N^U S^{hyd}(T) \qquad (14)$$

The values obtained for the Gibbs energy of hydration of polar, aliphatic, and aromatic groups upon unfolding of interleukin-1β are listed in Table 2. The latter two functions are different in their sign (Makhatadze & Privalov, 1994). The Gibbs energy of hydration of aliphatic groups is always positive, whereas the Gibbs energy of hydration of aromatic groups is negative. The Gibbs energy of hydration of aliphatic groups at 25 °C is 50 J mol^{-1} Å^{-2}, compared to −53 J mol^{-1} Å^{-2} for aromatic groups. Thus, only the hydration effect of aliphatic groups increases the stability of the protein. In contrast, the Gibbs energy of hydration of aromatic groups favors unfolding.

Before being exposed to solvent as a result of unfolding, the protein's nonpolar groups were in van der Waals contacts with other nonpolar groups in the native protein structure. The sum of these van der Waals interactions and the Gibbs energy of hydration of nonpolar groups corresponds to the Gibbs energy of the interactions commonly known as hydrophobic interactions, $\Delta_N^U G^{hph}$ (Kauzmann, 1959; Dill, 1990; Sharp *et al.*, 1991). The energy of van der Waals interactions, which we estimated from the enthalpy of sublimation of organic crystals, is always positive (Makhatadze & Privalov, 1993) and at 25 °C amounts to 126 J mol^{-1} Å^{-2} for aliphatic groups and 176 J mol^{-1} Å^{-2} for aromatic groups. Thus, the Gibbs energy of disruption of van der Waals interactions between aliphatic groups in the native state followed by their exposure to water will be positive, favoring the native state. The van der Waals interactions between aromatic groups are larger than the negative Gibbs energy of their hydration and are much stronger than the van der Waals interactions between the aliphatic groups (Makhatadze & Privalov, 1994), meaning that the hydrophobic effect of exposure of aromatic surface to water will also be stabilizing by about 126 J mol^{-1} Å^{-2} at 25 °C. Consequently, the Gibbs energy of disruption of the internal contacts between nonpolar groups followed by their exposure to water stabilizes the native protein structure. It has to be noted that the obtained value for the magnitude of the hydrophobic effect is in a fair agreement with the results of mutational analyses in proteins, which give a value of 220 ± 85 J mol^{-1} Å^{-2} [see Pace (1992) and Fersht *et al.* (1993)].

The Gibbs energy of disruption of hydrogen bonds between polar groups and the exposure of these groups to water are also represented by two terms. The enthalpy of hydrogen bonding considered above reflects the energy of internal hydrogen bonds, since the effect of hydration is taken into account separately. The total contribution of hydrogen bonding, $\Delta_N^U G_{hyd}^{HB}$, to the stability of the protein will consist of the energy of disruption of the internal hydrogen bonds followed by the hydration of groups involved in hydrogen bonding, $\Delta_N^U G_{pol}^{hyd}$. The sum of these two energies, ΔG_{hyd}^{HB}, corresponds to the stabilization effect of hydrogen bonding. This number for interleukin-1β can be compared to the hydrogen bond contribution to the stability of other proteins (Figure 2). The value obtained for interleukin-1β is close to that of ribonuclease A, about 25% larger than that for barnase, and almost twice that of myoglobin. It is notable that the difference increases with the difference in the secondary structure content (α-helix versus β-sheet) of the protein.

It is interesting that the Δg_{hyd}^{HB} follows the same dependence on the amount of helical structure, or the average hydrogen bond length, as the enthalpy of internal hydrogen bonding (Figure 2). Thus, the Gibbs energy of a hydrogen bond decreases with increasing hydrogen bond length and increasing helical structure content. This double correlation means that the $\Delta_N^U G_{pol}^{hyd}$ calculated per one hydrogen bond also correlates with f_α and L_{HB}.

As follows from the above discussion, both hydrogen bonding and hydrophobic interactions stabilize the compact protein conformation. The destabilizing action on the compact native state of protein comes from a thermal dissipative force, which is proportional to the gain of configurational entropy upon protein unfolding and the absolute temperature, $-T\Delta S^{cnf}$. Our estimated values of the contributions of different forces to the stability of interleukin-1β might carry absolute errors but appear to give a reasonable picture of relative magnitudes. The estimation of contributions of various factors to the stabilization of the native protein structure is not, however, the main result of the present analysis. The estimated values of interactions in proteins would certainly be improved by an expansion of the experimental data base and an increase in precision of the calorimetric experiments. The main result we see lies in the demonstration that the thermodynamic approach used for the problem of energetics of protein structure is not unrealistic, notwithstanding many concerns.

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