Proteins in Solution and in Membranes

Chapter 7

Proteins

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Proteins in Solutions and Membranes

- The folded conformations of a native protein gives it properties that are different from the unfolded polypeptide chain.
- The properties are NOT only the sum of all properties of the single aminoacids
- The compactness allows the proteins to rotate and diffuse rapidly.
- Domains of proteins are relatively resistant to protease.

Proteins in Solutions and Membranes

- Multidomain proteins can be separated by protease treatment.
- The separated domains can be reconstituted to form a functional protein
- The folded conformation of proteins brings residues into close proximity
- They are being held in place by the fold
- Their local relative concentration is so high that reactions occur between them.

Proteins in Solutions and Membranes

- Many of these properties are not evident in protein crystals
- Appear in solution or membranes
- Proteins need a certain flexibility
- Protein conformation is largely unaltered when in a crystal
- Exceptions intrinsically flexible sidechains and surface loops

Proteins in Solutions and Membranes

- The intermolecular forces of proteins in a crystal lattice are similar to the intra molecular forces of a folded protein
- Crystallization conditions favor the folded proteins
- Exception to this
- Very small proteins
- They have the most mobile conformations
- Glucagon: 29 aa
- Diluted solution--- random coil
- Concentrated solution --- trimeric helical structure

Proteins in Solutions and Membranes

- Conformations of small peptides in crystal structures need to be validated in solution
- Protein domains have only one compact folded structure
- Conformational changes in a protein are mainly used to rationalize unexpected protein behaviour
- Many conformational changes may involve localised alterations or changes in degree of flexibility.
- Structural rearrangements have been found only for quarternary structures

Aqueous solubility

- Some proteins aree extremely soluble
- Structural proteins are nearly insoluble
- Proteins interact with the solvent with their surfaces
- Globular proteins have charged and polar residues on their surfaces
- Solubility is goverend by their interactions with water
- Structural proteins interact with other proteins more strongly than with water

Aqueous solubility

- Solubility of a protein increases at pH values farther away from pI
- pI of a protein is the pH where the protein has zero net charge
- At extreme pH values proteins unfold ---affects solubility
- Most proteins can be solubilised in aqueous solution by adding detergents or chaotropic ions (urea, GdnHCl)

Aqueous solubility

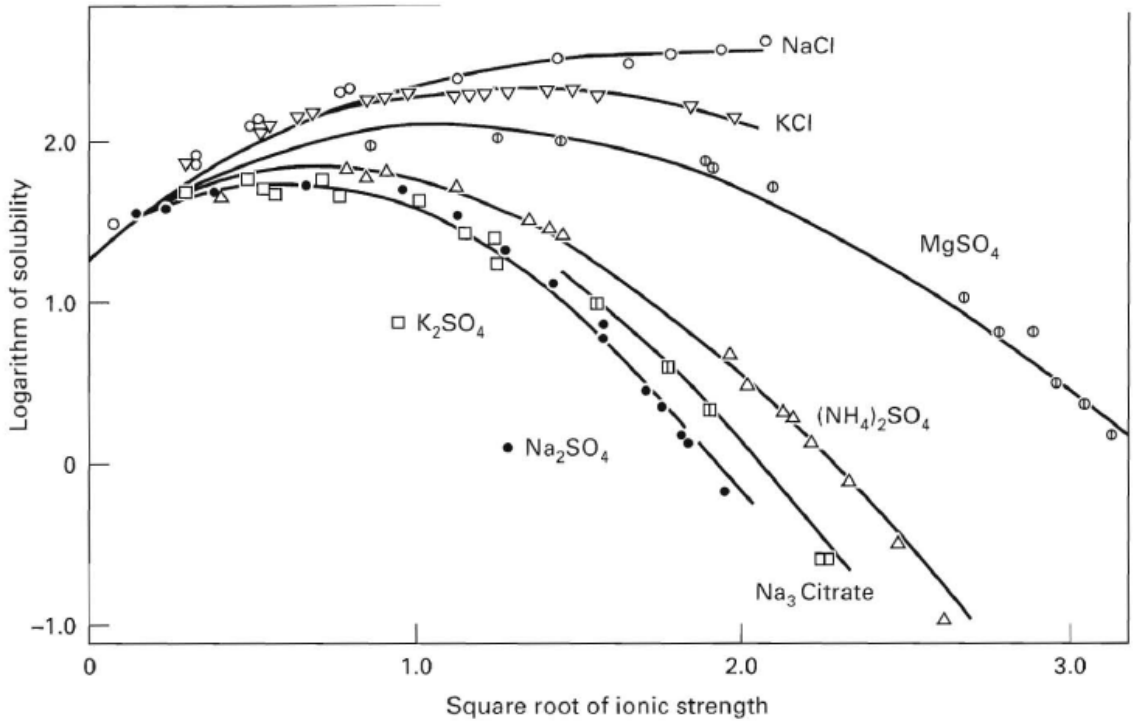
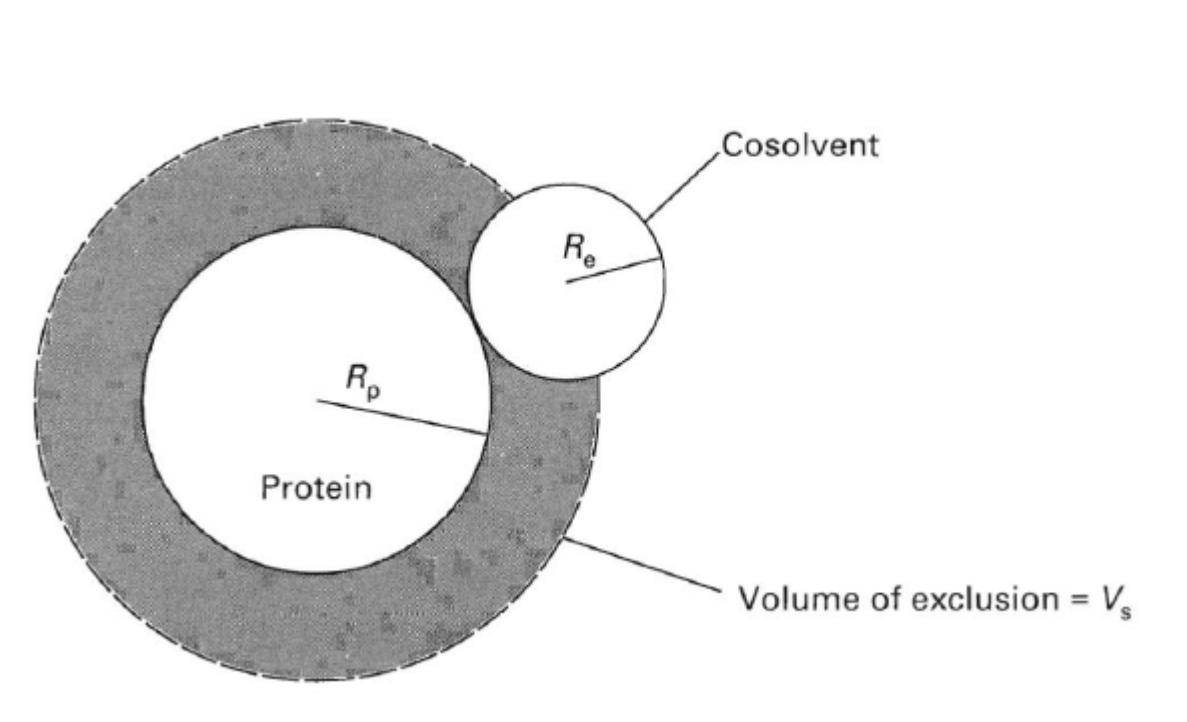


FIGURE 7.1
The solubility of hemoglobin (with carbon monoxide bound) in various electrolytes at different concentrations and 25°C. Solubility is expressed as grams per 1000 grams H_2O. (From A. A. Green, *J. Biol. Chem.* 95:47–66, 1932.)

Preferential Hydration



Hydrodynamic Properties in Aqueous Solution

Diffusion

$$\frac{\delta c}{\delta t} = D\frac{\delta^2 c}{\delta x^2} \qquad (7.1)$$

$$D = \frac{\overline{x^2}}{2t} \qquad (7.2)$$

Einstein-Sutherland equation

$$f = \frac{k_B T}{D} \qquad (7.3)$$

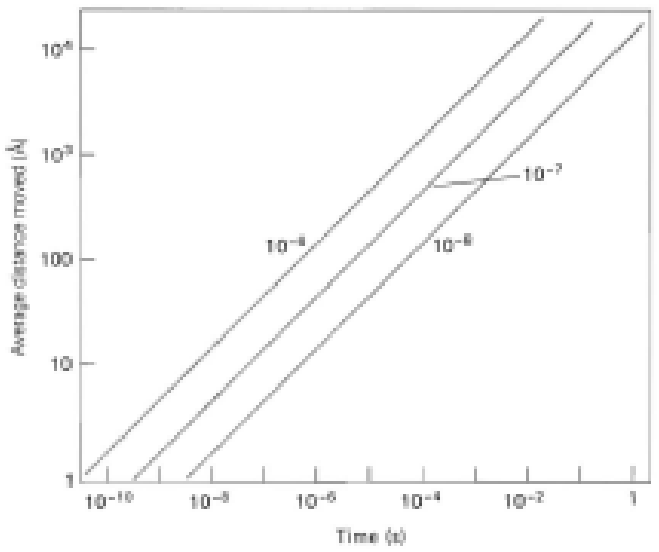


FIGURE 7.3
Average distance moved as a function of time by molecules with typical translational diffusion coefficients of 10^{-6}, 10^{-7}, and 10^{-8} cm^2/s. Values were calculated with Equation (7.2).

Table 7.1 Hydrodynamic Properties of Proteins of Known Structure

Protein (source)	Hydrodynamic Data: $s^0_{20,w}$[a] (S)	Hydrodynamic Data: $D^0_{20,w}$[b] (10^{-7} cm²/sec)	Hydrodynamic Data: $\bar{\nu}$[c] (ml/g)	Molecular Weight: Structure[d]	Molecular Weight: Measured[e]	f/f_0	Dimensions[g] (Å)
Pancreatic trypsin inhibitor (bovine)	1.0	12.9	0.718	6,520	6,670	1.321	29 × 19 × 19
Cytochrome *c* (equine)	1.83	13.0	0.715	12,310	11,990	1.116	25 × 25 × 37
Ribonuclease A (bovine)	1.78	10.7	0.703	13,690	13,600	1.290	38 × 28 × 22
Lysozyme (hen)	1.91	11.3	0.703	14,320	13,800	1.240	45 × 30 × 30
Myoglobin (sperm whale)	1.97	11.3	0.745	17,800	16,600	1.170	44 × 44 × 25
Adenylate kinase (porcine)	2.30	10.2	0.74	21,640	21,030	1.167	40 × 40 × 30
Trypsin (bovine)	2.50	9.3	0.727	23,200	23,890	1.187	50 × 40 × 40
Bence Jones REI (human)[h]	2.6	10.0	0.726	23,500	23,020	1.156	40 × 43 × 28
Chymotrypsinogen (bovine)	2.58	9.48	0.721	25,670	23,660	1.262	50 × 40 × 40
Elastase (porcine)	2.6	9.5	0.73	25,900	24,600	1.214	55 × 40 × 38
Subtilisin novo (B. amyloliq.)	2.77	9.04	0.731	27,530	27,630	1.181	48 × 44 × 40
Carbonic anhydrase (human)	3.23	10.7	0.729	28,800	27,020	1.053	47 × 41 × 41
Superoxide dismutase (bovine)	3.35	8.92	0.729	33,900	33,600	1.132	72 × 40 × 38
Carboxypeptidase A (bovine)	3.55	9.2	0.733	34,500	35,040	1.063	50 × 42 × 38
Phosphoglycerate kinase (yeast)	3.09	6.38	0.749	45,800	46,800	1.377	70 × 45 × 35
Concanavalin A	3.8	6.34	0.732	51,260	54,240	1.299	80 × 45 × 30
Hemoglobin, oxy (equine)[i]	4.22	6.02	0.750	64,610	67,980	1.263	70 × 55 × 55
Malate dehydrogenase (porcine)[k]	4.53	5.76	0.742	74,900	73,900	1.344	64 × 64 × 45
Alcohol dehydrogenase (equine)[k]	5.08	6.23	0.750	79,870	79,070	1.208	45 × 55 × 110
Lactate dehydrogenase (dogfish)[l]	7.54	4.99	0.74	146,200	141,000	1.273	74 × 74 × 84

Sedimentation analysis

$$\frac{dr}{dt} = \frac{M_w(1-\bar{v}\rho)}{N_A f}\omega^2 r \qquad (7.4)$$

The Svedberg Equation

$$s = \frac{M_w(1-\bar{v}\rho)}{N_A f} = \frac{M_w(1-\bar{v}\rho)}{DRT} \qquad (7.5)$$

Gel Filtration
Rotation

$$\tau_R = \frac{3V\eta_0}{k_B T}$$

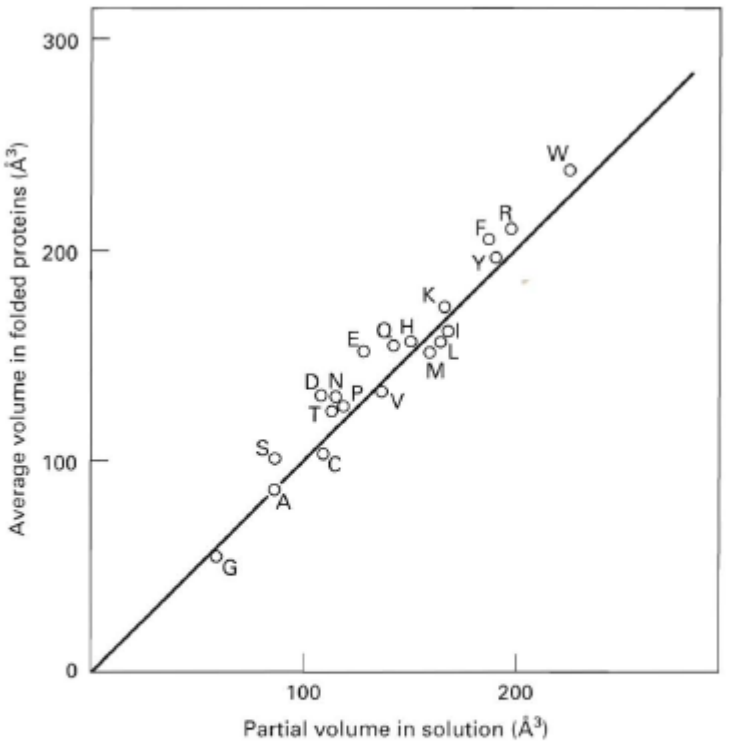


FIGURE 7.4
Correspondence between the average volume occupied by each amino acid residue in solution and in folded proteins. The line has a slope of unity. The values for the partial molar volumes in solution are from Table 4.3, those for folded proteins from Table 6.3.

Hydrodynamic properties

Table 7.2 *Examples of Translational and Rotational Diffusion Rates*

Molecule	Translational diffusion coefficient (10^{-7} cm^2/s)	Rotational relaxation time
H_2O	200	10^{-2} ns
Glycine	106[a]	
Alanine	91[a]	
Ala-Gly	72[a]	
Tryptophan		8.7 ns[b]
Globular proteins		
Myoglobin		30 ns[b]
Ribonuclease A	12.6[c]	22 ns[d]
Lysozyme	10.6[c]	30 ns[e]
Chymotrypsin		45 ns[e]
Immunoglobulin G	3.8[c]	504 ns[f]
Serum albumin	6.7[a]	125 ns[f]
Unfolded proteins		
Serum albumin	1.9[g]	
Pepsinogen	2.5[g]	
Chymotrypsinogen	3.2[g]	
Tropomyosin	2.2[a]	
Fibrinogen	2.0[h]	3.5 ms[h]
Myosin	0.84[c]	
Collagen		0.5 ms[h]
Poly(benzyl-Glu) ($M_w = 3.4 \times 10^5$)		
α-Helix	0.85[i]	
Random coil	1.30[i]	
Tobacco mosaic virus	0.3–0.4[c]	1.2–1.6 ms[c]

Spectral Properties - fluorescence

Table 7.3 *Exposure of Tyrosine Residues of Various Conformational States of Bovine Pancreatic Trypsin Inhibitor (BPTI)*

Form of BPTI[a]	Fractional Exposure of Tyr Residues (%)			
	Compared with R		Compared with Gly-Tyr-Gly	
	Comparison spectra[b]	Perturbation spectra[c]	Comparison spectra	Perturbation spectra
R	100	100	84	86
(5–30)	73	80	59	69
(30–51)	64	67	51	57
(30–51, 5–14) + (30–51, 5–38)	60	63	47	53
(30–51, 14–38)	49	49	37	42
(30–51, 5–55)	27	41	16	35
Refolded + (5–55, 14–38)	36	37	25	32
Native	36	35	25	30

Chemical Properties

Table 7.4 *Effects of Various Mutations of Ionized Residues on the Apparent pK_a Value of His 64 of Subtilisin at Low Ionic Strength*[a]

Mutant	Measured ΔpK_a[b]	Mean distance from charge to His 64 nitrogen atoms (Å)[c]	Effective dielectric constant, D_{eff}[d]
Asp 99 → Ser	−0.40	12.6	48
Glu 156 → Ser	−0.38	14.4	45
Ser 99 → Lys	(−0.25)	15.0	65
Ser 156 → Lys	(−0.25)	16.5	59
Lys 213 → Thr	+0.08	17.6	173
Asp 36 → Gln	−0.18	15.1	90
Asp 99 → Lys	−0.64	(13.8)	55
Gly 156 → Lys	−0.63	(15.5)	50
Asp 99 → Ser and Glu 156 → Ser	−0.63	(13.5)	57
Asp 99 → Lys and Glu 156 → Lys	−1.00	(14.7)	66

[d] The effective dielectric constant was calculated using the equation

$$D_{eff} = \frac{244}{(\Delta q) r (\Delta pK_a)}$$

where Δq is the change in number of charges and r is the distance in Å.

Chemical Properties

Table 7.5 *Relative Rates of Alkylation of Histidine and of Two His Residues of Ribonuclease A*

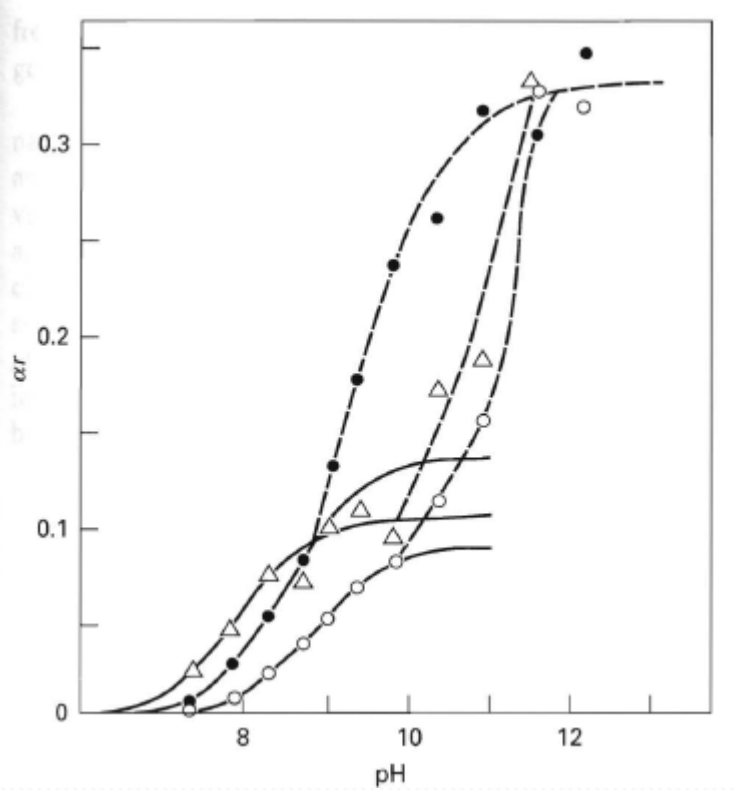
	Second-Order Rate Constant[a] (10^{-4} s^{-1} M^{-1})		
		Ribonuclease A[b]	
Alkylating reagent	L-Histidine	His 12	His 119
Iodoacetate		7.3	51.1
Iodoacetamide	0.012	1.1	0
Bromoacetate	0.086	20.5	184.5
L-α-Bromopropionate	0.0027	0.19	0.66
D-α-Bromopropionate	0.0028	4.16	1.84
D-α-Bromo-*n*-butyrate		3.60	1.11
β-Bromopyruvate		0	911
β-Bromopropionate	0.0229	0	6.33

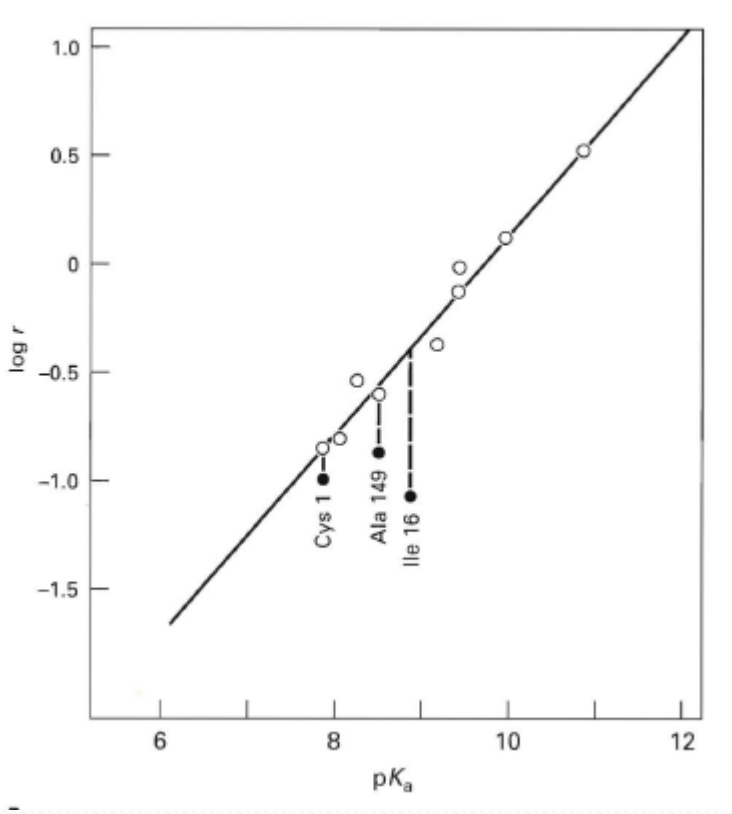
[a] Reactions were carried out at 25°C and pH 5.3–5.5.

[b] His 12 is always alkylated at atom $N^{\epsilon 2}$, His 119 at $N^{\delta 1}$; reaction of one atom inhibits reaction at the other.

From R. L. Heinrickson et al., *J. Biol. Chem.* 140:2921–2934 (1965); R. G. Fruchter and A. M. Crestfield, *J. Biol. Chem.* 242:5807–5812 (1967).

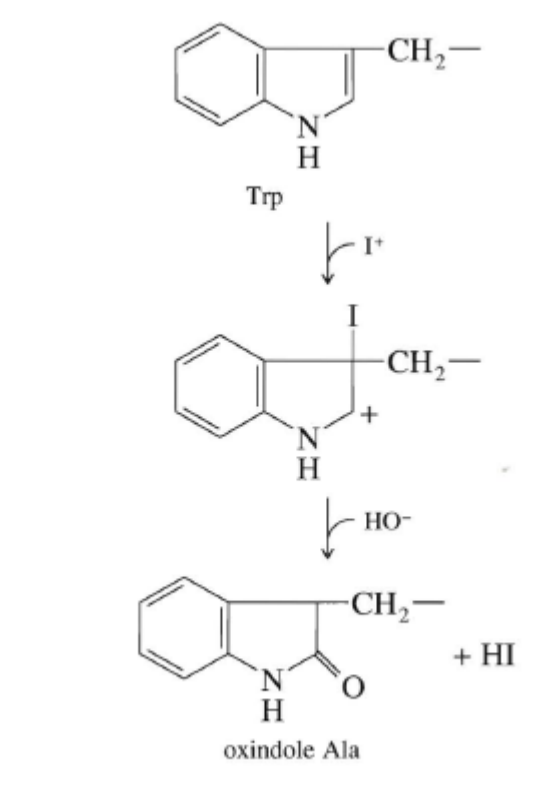
Chemical Properties





Competitive labeling of the three α-amino groups, of residues 1, 16, and 149, of α-chymotrypsin. *A:* Reactivities of the groups with acetic anhydride as a function of pH. The reactivities are relative to the nonionized standard and are expressed as αr, where α is the fraction of nonionized α-amino group and r is the relative reactivity of the nonionized form. The solid lines are the theoretical curves for the following pK_a and r values, respectively: 7.9 and 0.10 for

Chemical Properties



Chemical Properties

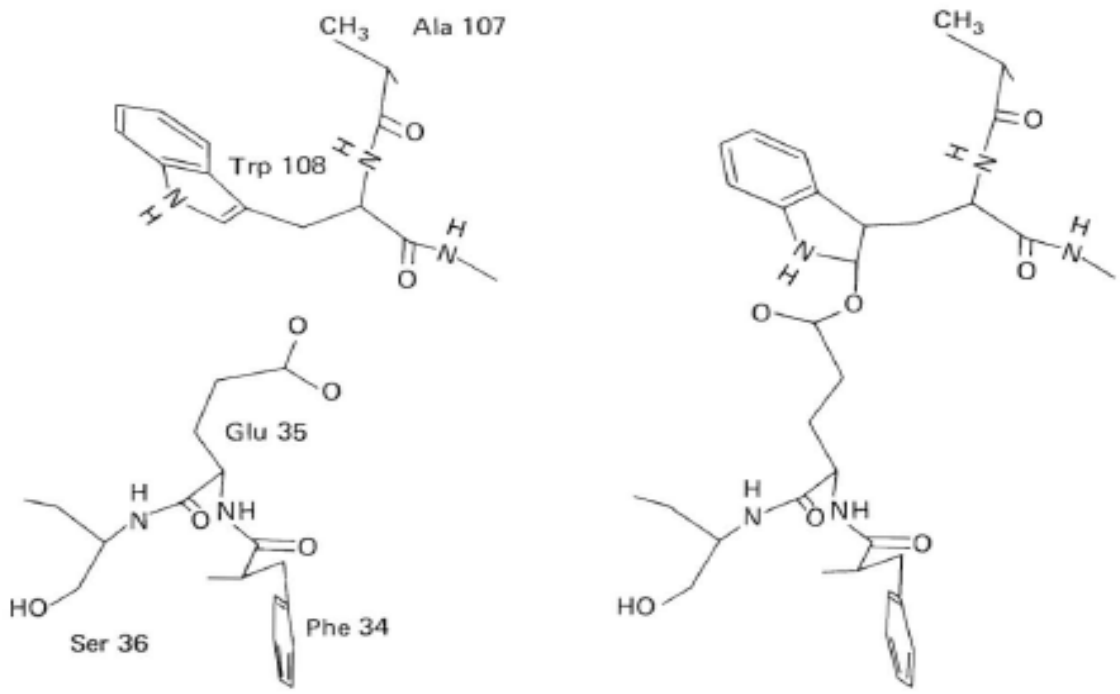
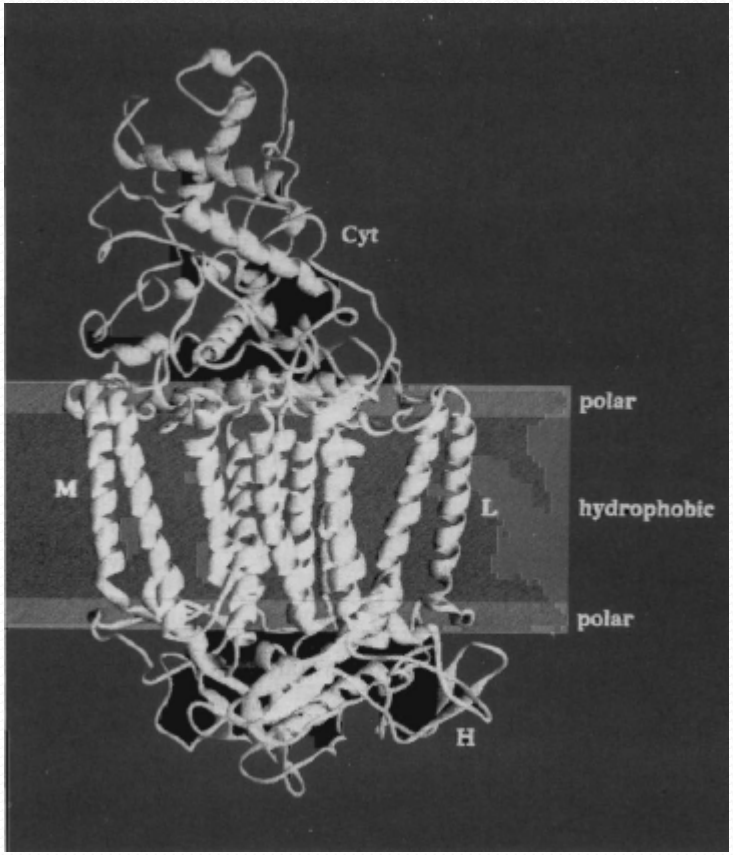


FIGURE 7.6
Covalent cross-link between Glu 35 and Trp 108 of hen lysozyme produced by iodine treatment. The positions of these two residues in native lysozyme are shown at *left*. Iodine presumably reacts initially with Trp 108, but then the adduct reacts preferentially with Glu 35 rather than with water, owing to the proximity of the Glu side chain. The structure of the cross-linked protein is shown at *right*. (Adapted from C. R. Beddell et al., *J. Mol. Biol.* 97:643–654, 1975.)

Membrane Proteins



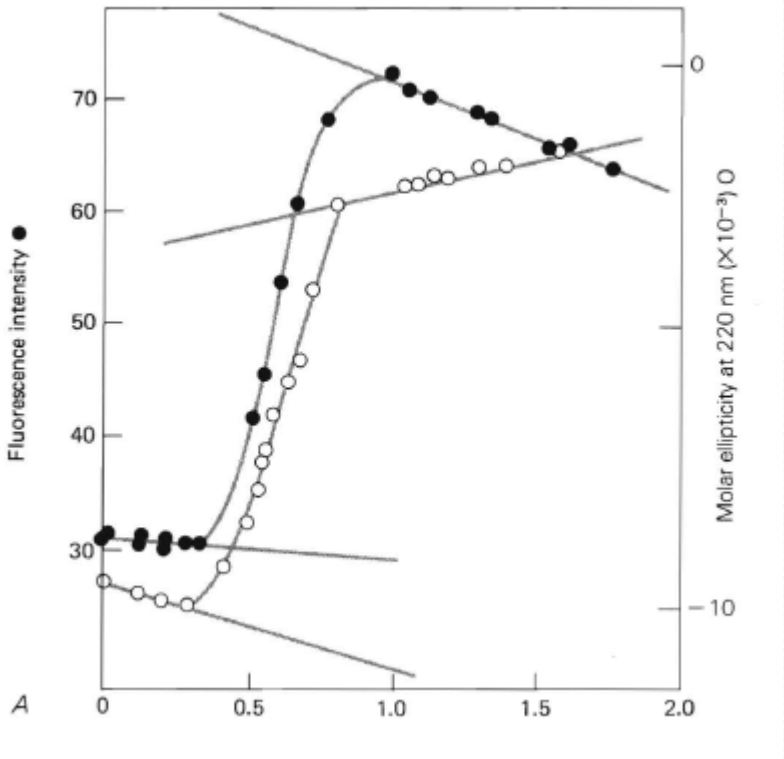
Side Chain Rotations

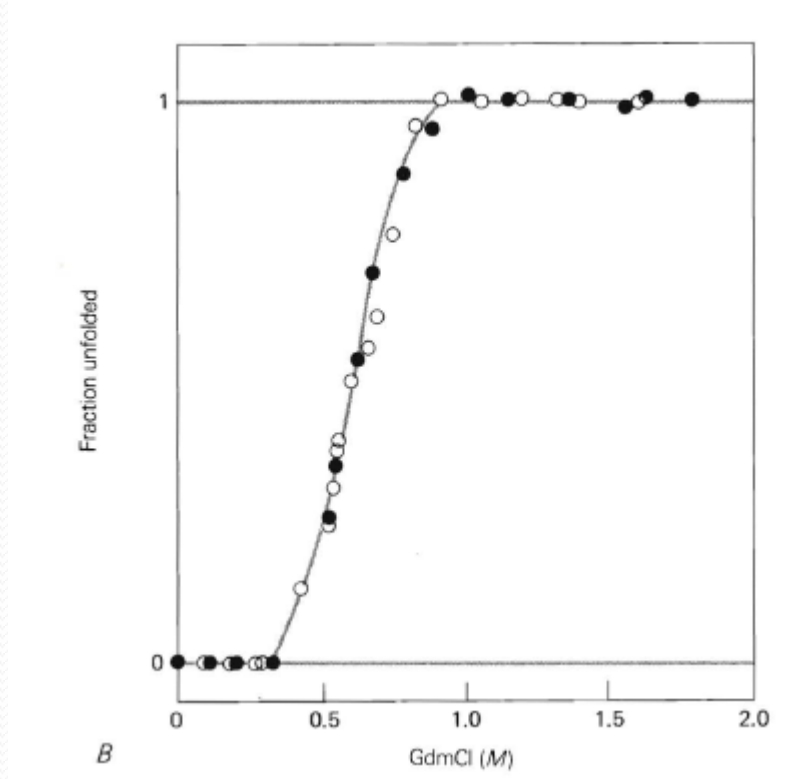
Table 7.6 *Rotation of Aromatic Rings in BPTI*

Residue	Frequency of 180° Rotations (s^{-1}) at Temperature of			Activation Parameters		
	4°C	40°C	80°C	Enthalpy $\Delta H^{\ddagger}$ (kcal/mol)	Entropy $\Delta S^{\ddagger}$ [cal/(mol · °C)]	Volume $\Delta V^{\ddagger}$ (Å^3)
Tyr 10	Rotating rapidly at all temperatures					
Tyr 21	Rotating rapidly at all temperatures					
Tyr 23	<5	3×10^2	5×10^4	26	35	
Tyr 35	<1	50	5×10^4	37	68	60
Phe 4	Rotating rapidly at all temperatures					
Phe 22	Rotating rapidly at all temperatures					
Phe 33	Rotating rapidly at all temperatures					
Phe 45	30	1.7×10^3	5×10^4	17	11	50

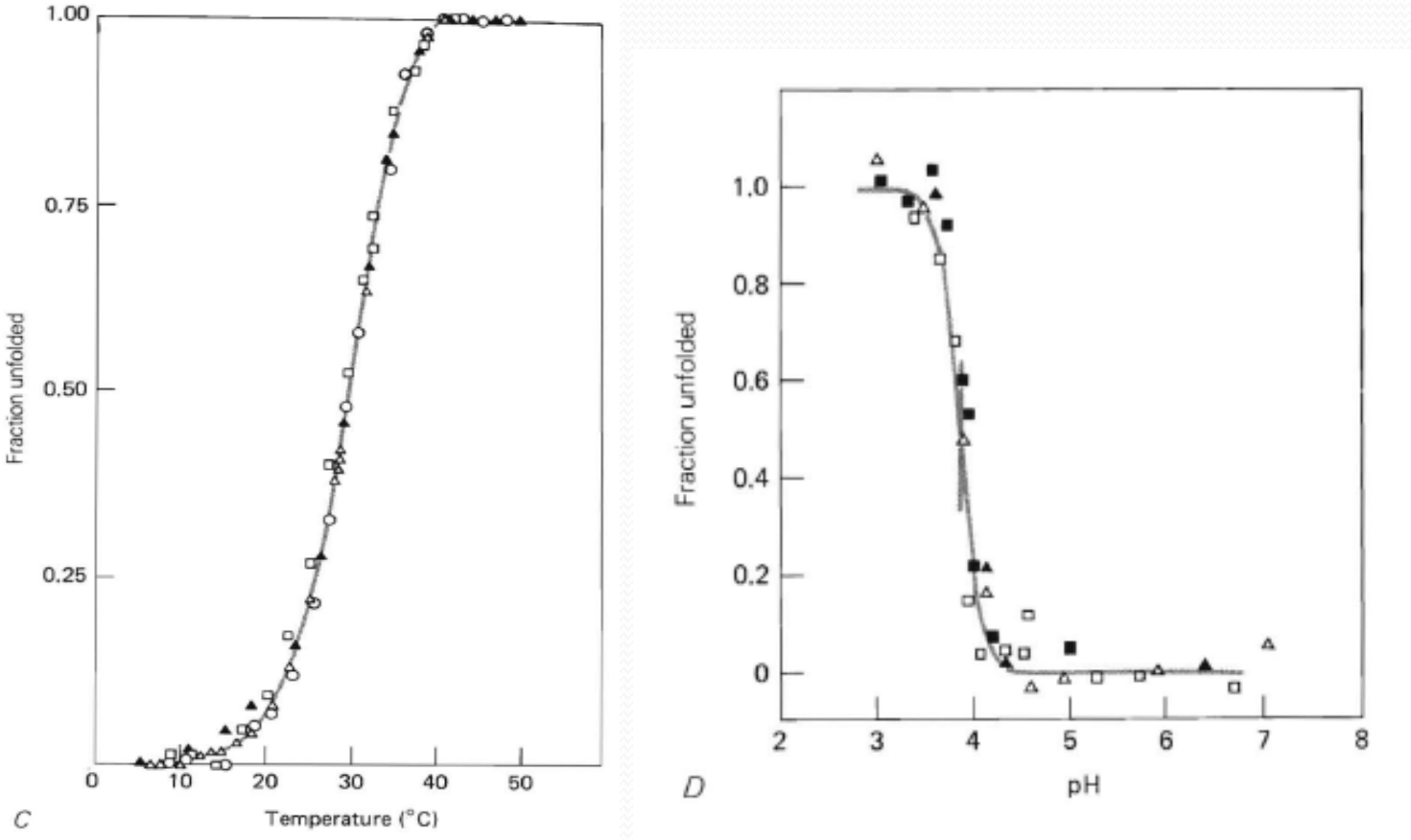
From G. Wagner et al., *Biophys. Struct. Mech.* 2:139–159 (1976); *J. Mol. Biol.* 196:227–231 (1987).

Reversible Unfolding transitions





Reversible Unfolding Transitions



Reversible Unfolding Transition

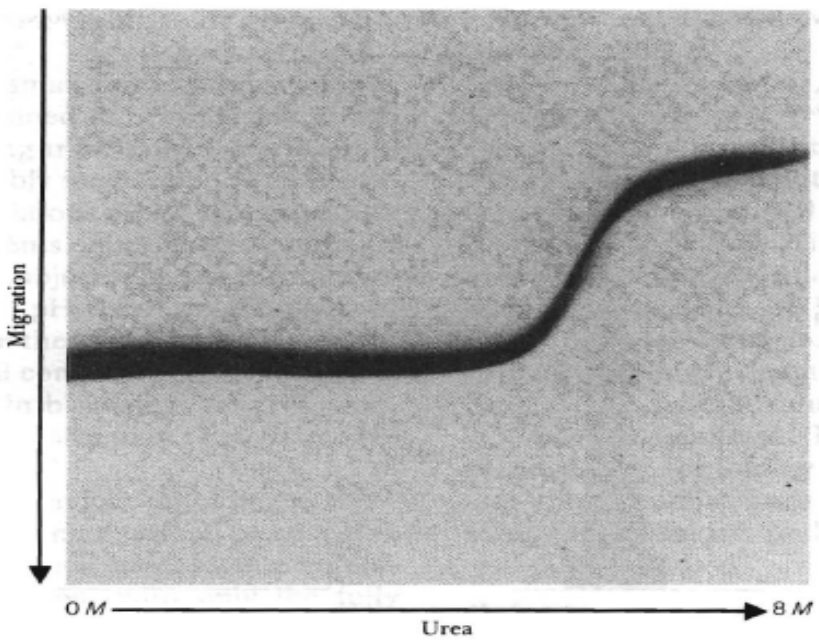
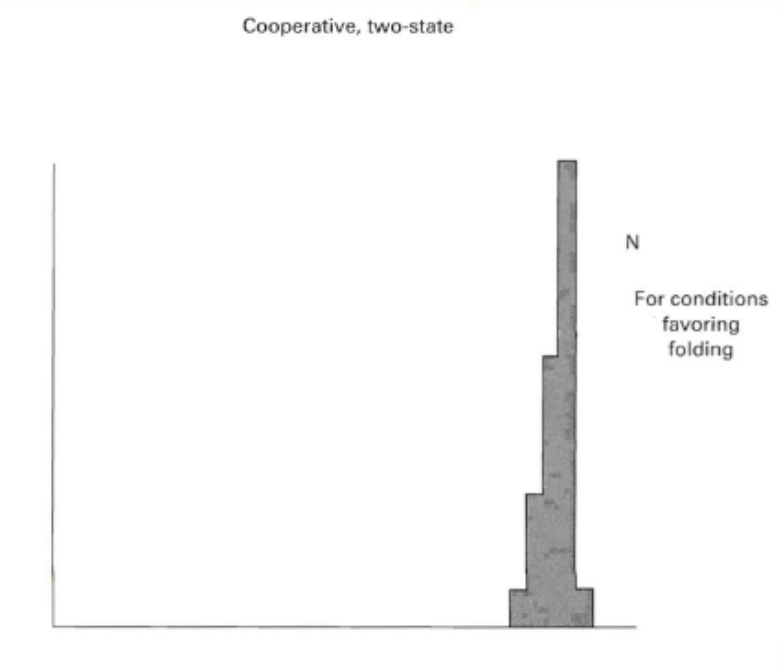
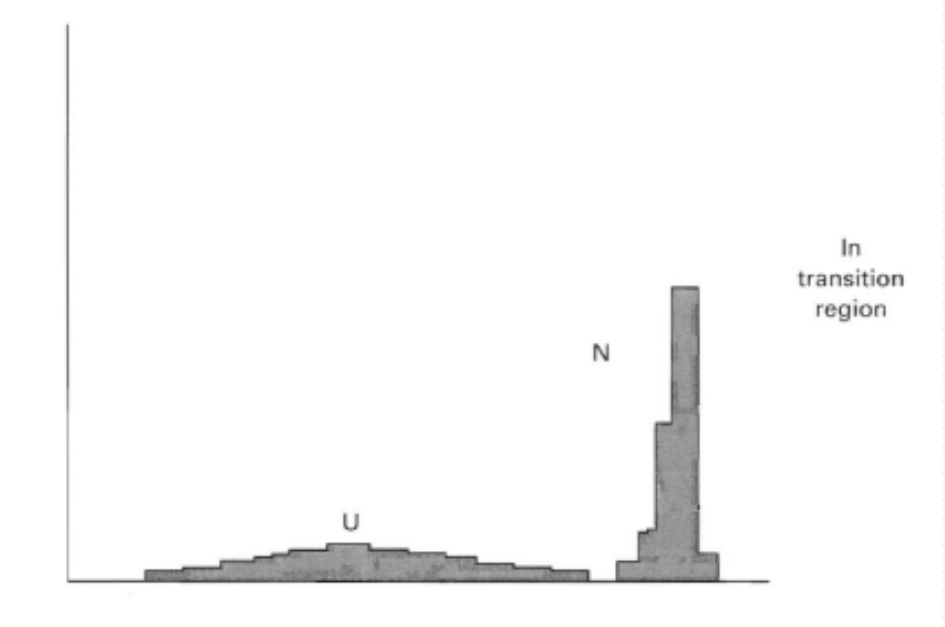


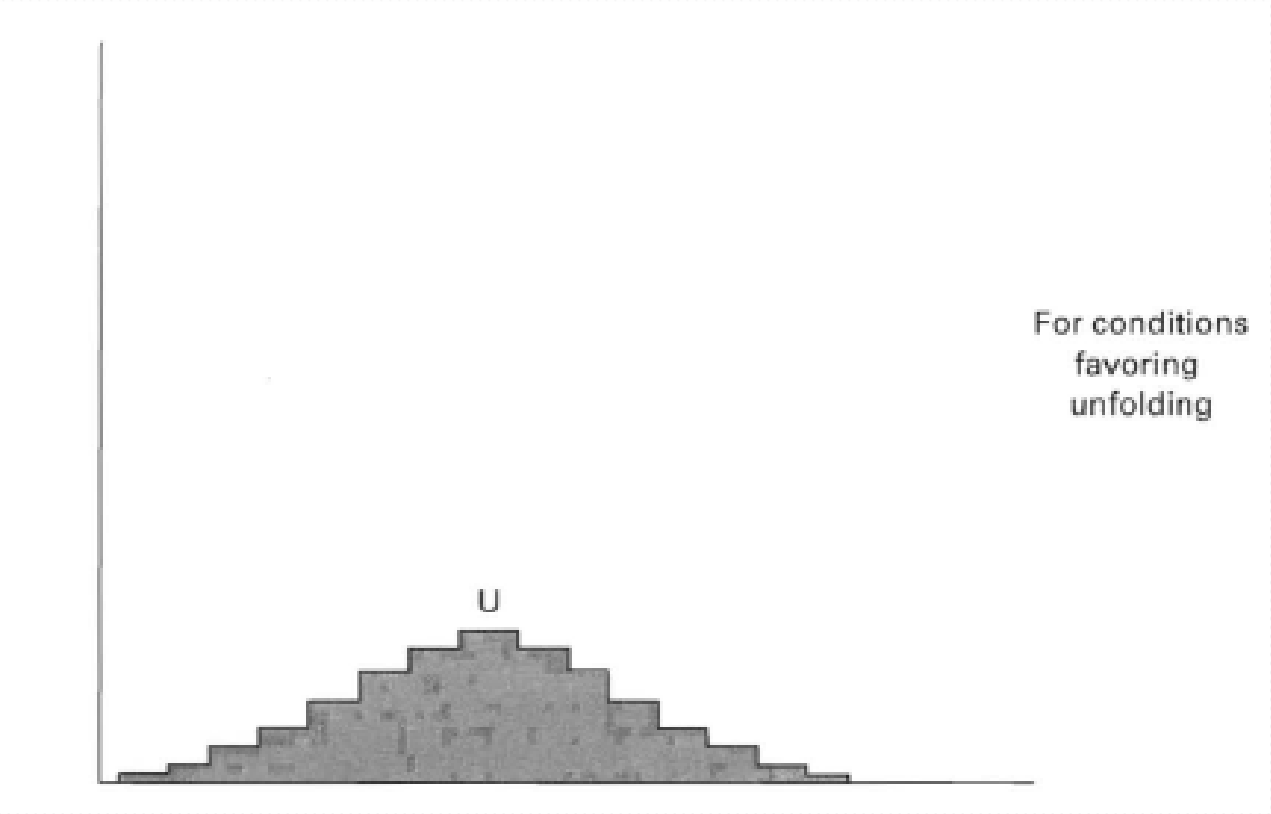
FIGURE 7.12
Transverse urea-gradient electrophoresis of cytochrome *c*. The folded protein was layered on the top of the polyacrylamide gel, which contained a linear gradient of urea from left to right. Electrophoresis at pH 4.0 was from top to bottom. At low urea concentrations, the protein remains folded and migrates rapidly; at high urea concentrations, it is unfolded and migrates more slowly. The same pattern is obtained starting with unfolded protein. This and the continuous band of protein through the abrupt unfolding transition indicate that unfolding and refolding were rapid relative to the time of electrophoresis. Therefore, the fraction of unfolding at equilibrium determined the rate of migration. The smooth shape of the transition, with a single inflexion point, indicates that only two conformational states with different electrophoretic mobilities were present to significant extents. (From T. E. Creighton, *J. Mol. Biol.* 129:235–264, 1979.)

Reversible Unfolding Transition

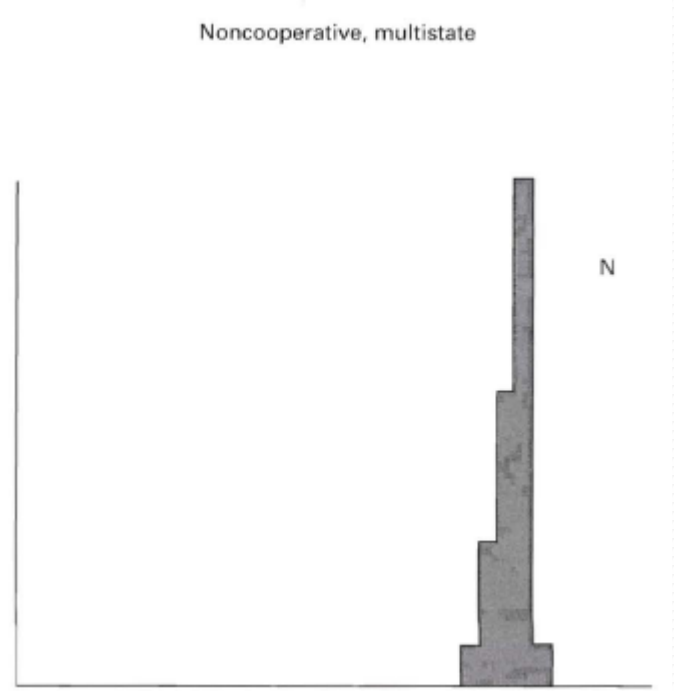


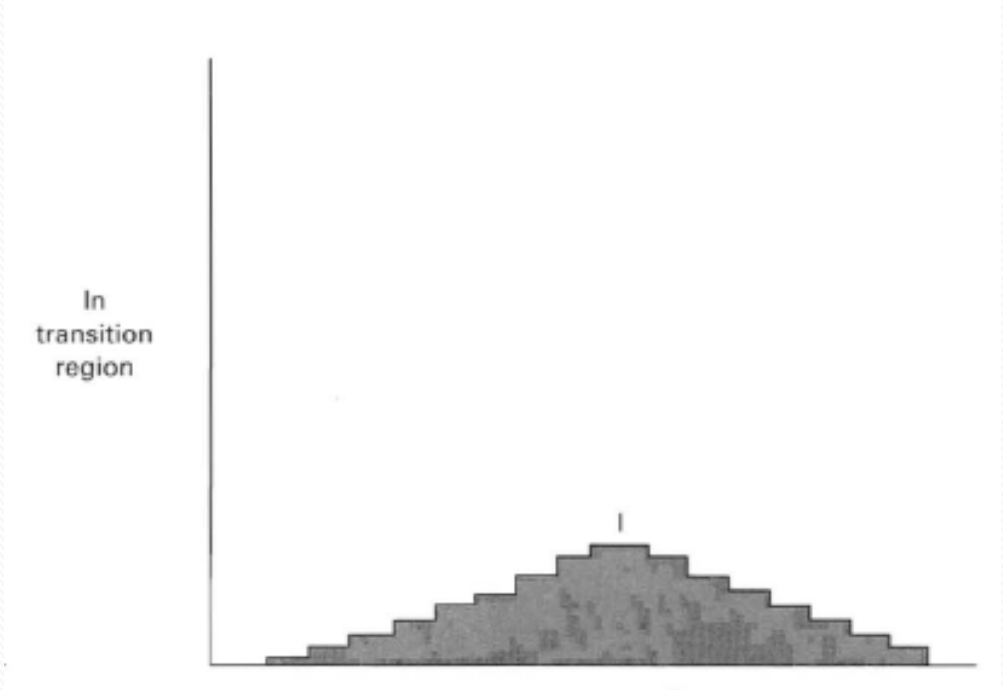


Reversible Unfolding Transition

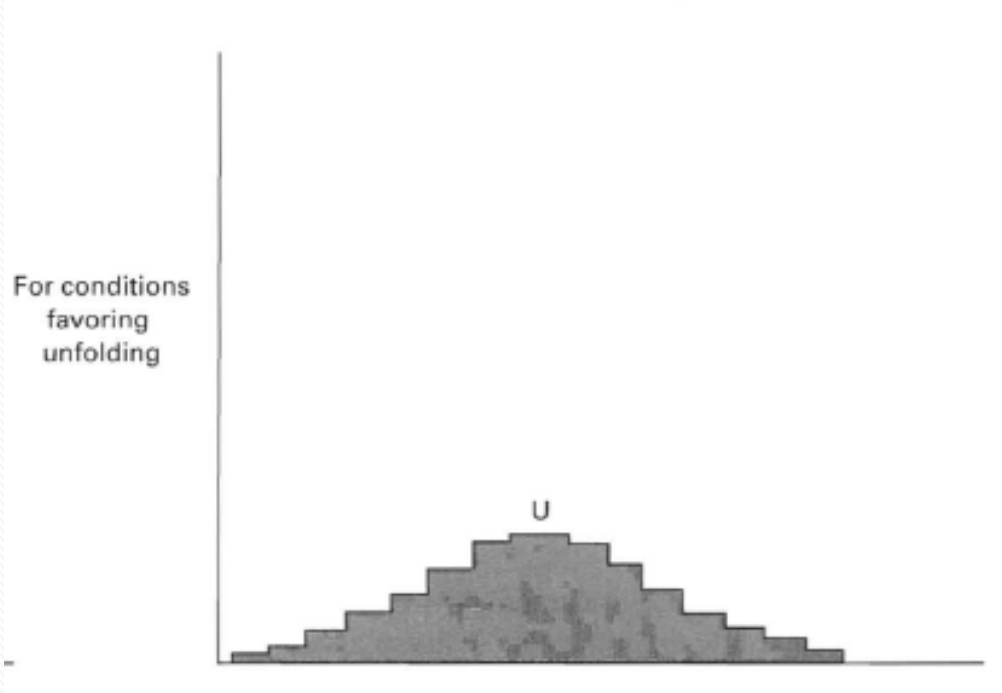


Reversible Unfolding Transition





Reversible Unfolding Transition



Reversible Unfolding Transition

For a two-state transition, the equilibrium constant between the N and *V states can be measured directly from the average fraction of unfolding (a) in the transition region:*

$$K_{eq} = \frac{[N]}{[U]} = \frac{1 - \alpha}{\alpha} \qquad (7.10)$$

Where the value of *a is significantly different from 0 or 1, the value of* K_{eq} *is known. This gives the free energy of N relative to that of U,* $\Delta G_{fold}'$ *under each set of conditions :*

$$\Delta G_{fold} = G_N - G_U = -RT \ln K_{eq} \qquad (7.11)$$

Reversible Unfolding Transition

$$\Delta G_{\text{fold}} = \Delta G_{\text{fold}}^{\text{H}_2\text{O}} + m\,[\text{denaturant}] \qquad (7.12)$$

Nature of the unfolded state

- Some proteins have been found in a state that is neither folded nor unfolded.
- The molten globule state:
- 1) the overall dimensions of the protein aer much less then for the rndom coil and only slightly larger than for the folded state
- 2) the average content of secondary structure is similar tot he folded state
- 3) the side chains are in homogenous surrounding
- 4) Many amide groups exchange hydrogens much more rapidly than they do in the folded state
- 5) the enthalpy of the molten globule is nearly the same as for the fully unnfolded state
- Interconversion of the MG state with the folded state are slow and cooperative. MG – unfolded are rapid and non cooperaive

Nature of the unfolded state

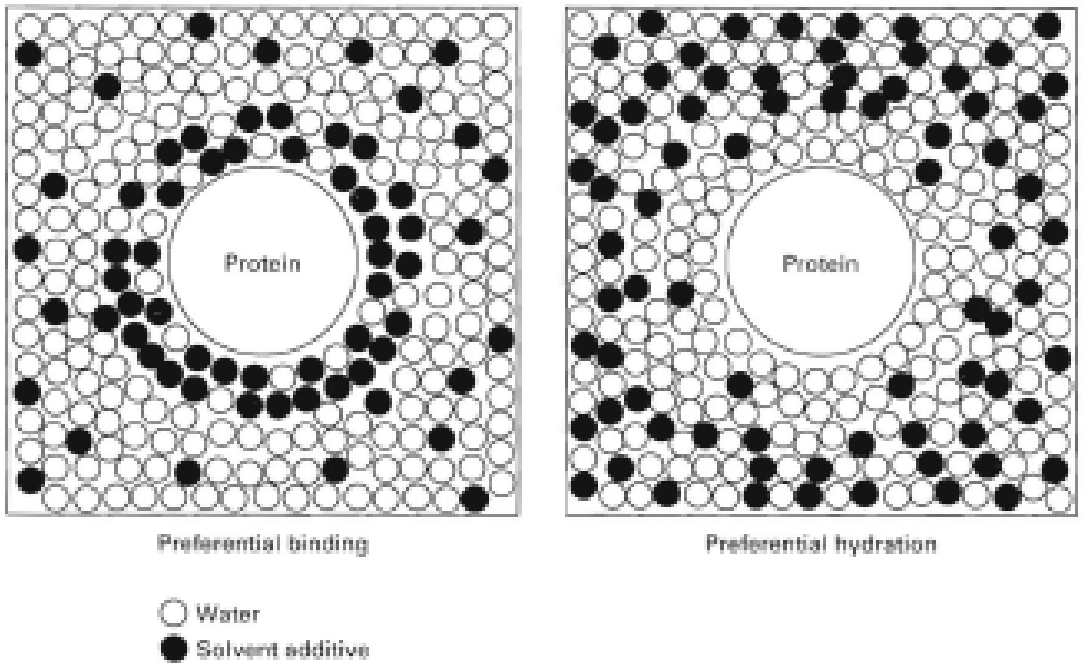


FIGURE 7.14
Schematic illustration of preferential binding and preferential hydration by solvent additives. In preferential binding, the additive occurs in the solvation shell of the protein at a greater local concentration than in the bulk solvent. Preferential hydration results from exclusion of the additive from the surface of the protein. (From S. N. Timasheff and T. Arakawa, in *Protein Structure: A Practical Approach*, T. E. Creighton, ed., pp. 331–345. IRL Press, Oxford, 1989.)

Nature of the unfolded state

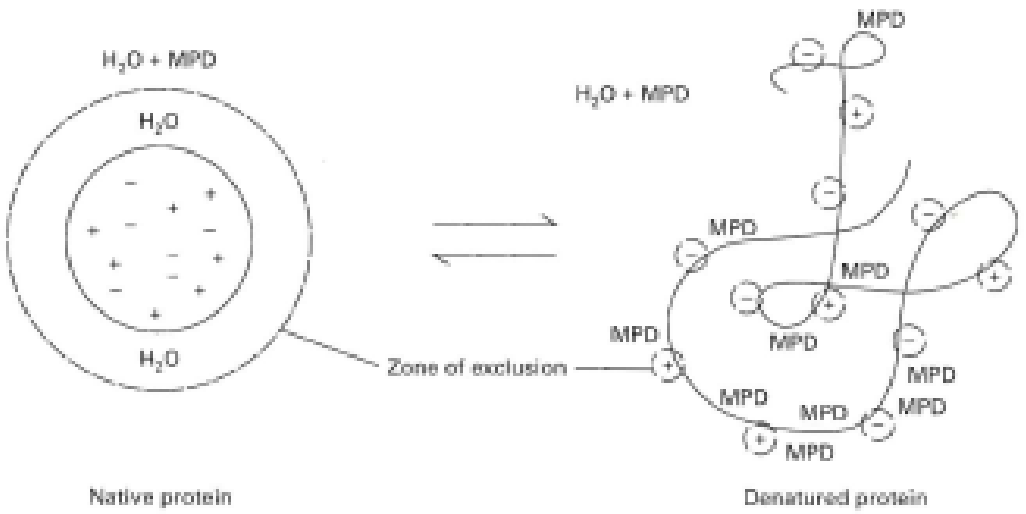
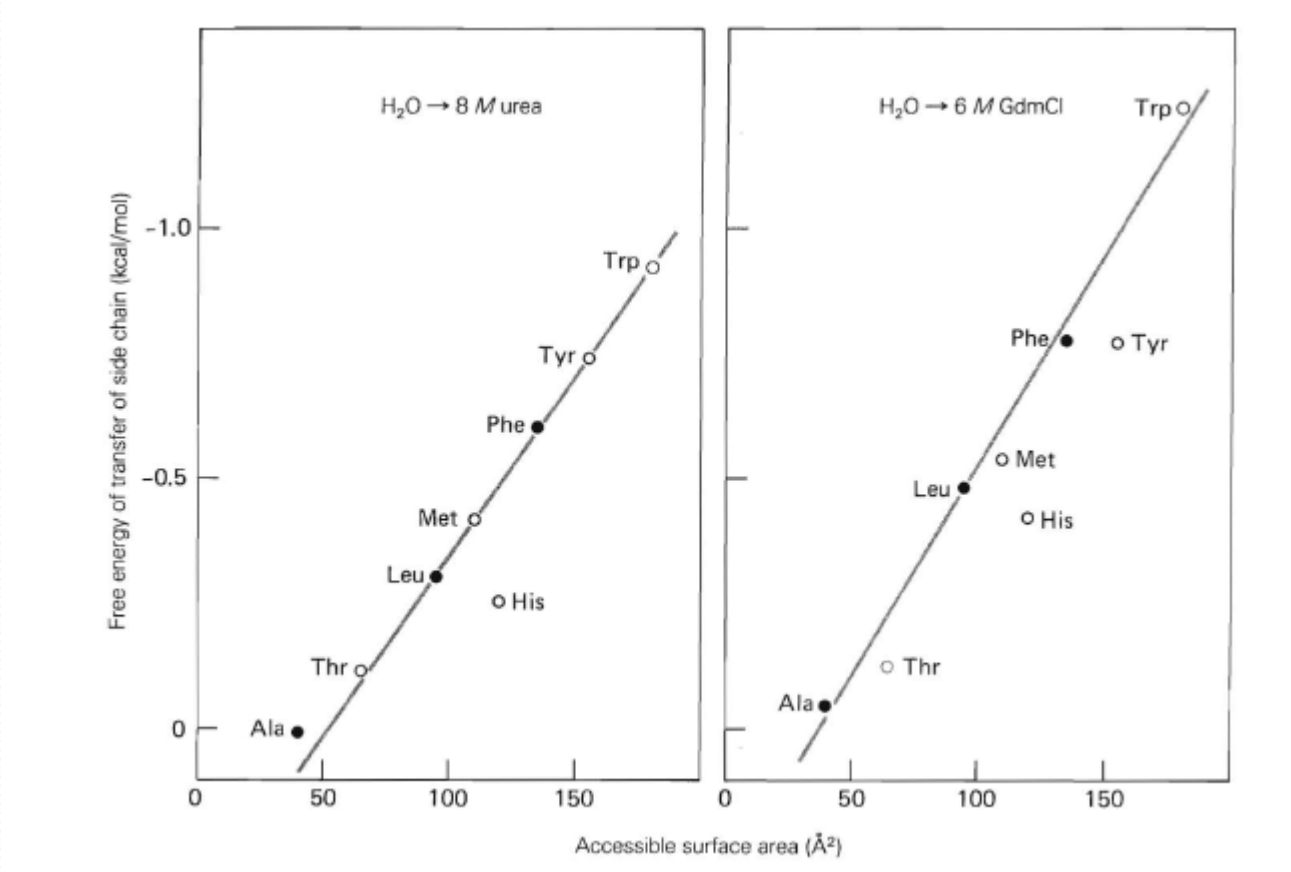


FIGURE 7.15
Schematic illustration of why a nonpolar additive, such as 2-methyl-2,4-pentanediol (MPD), decreases the solubility of a protein but destabilizes its folded conformation. In the folded state, the MPD is repelled by the high charge density on the protein surface, producing preferential hydration. This decreases the solubility of the folded conformation, and MPD is a potent agent for inducing crystallization of proteins. MPD decreases the stability of the folded state because the electrostatic repulsions are minimized in the unfolded state and because the MPD interacts favorably with the nonpolar surfaces that are exposed by unfolding. (From S. N. Timasheff and T. Arakawa, in *Protein Structure: A Practical Approach*, T. E. Creighton, ed., pp. 331–345. IRL Press, Oxford, 1989.)

Nature of the unfolded state



Nature of the unfolded state

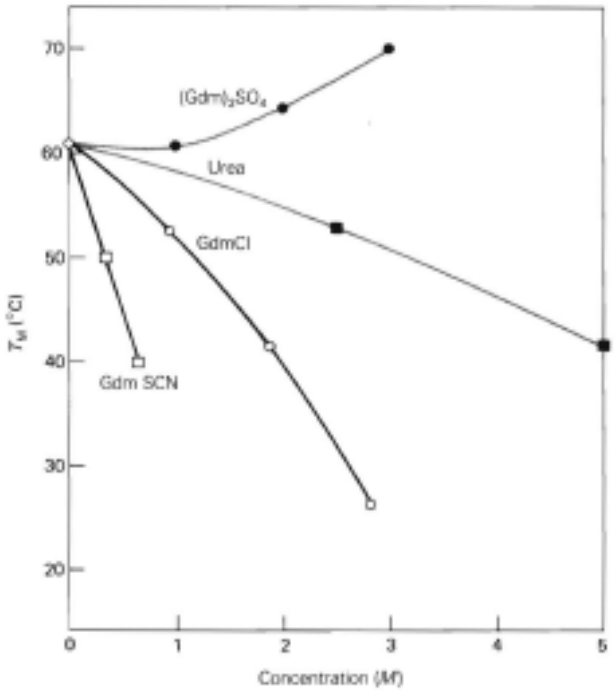
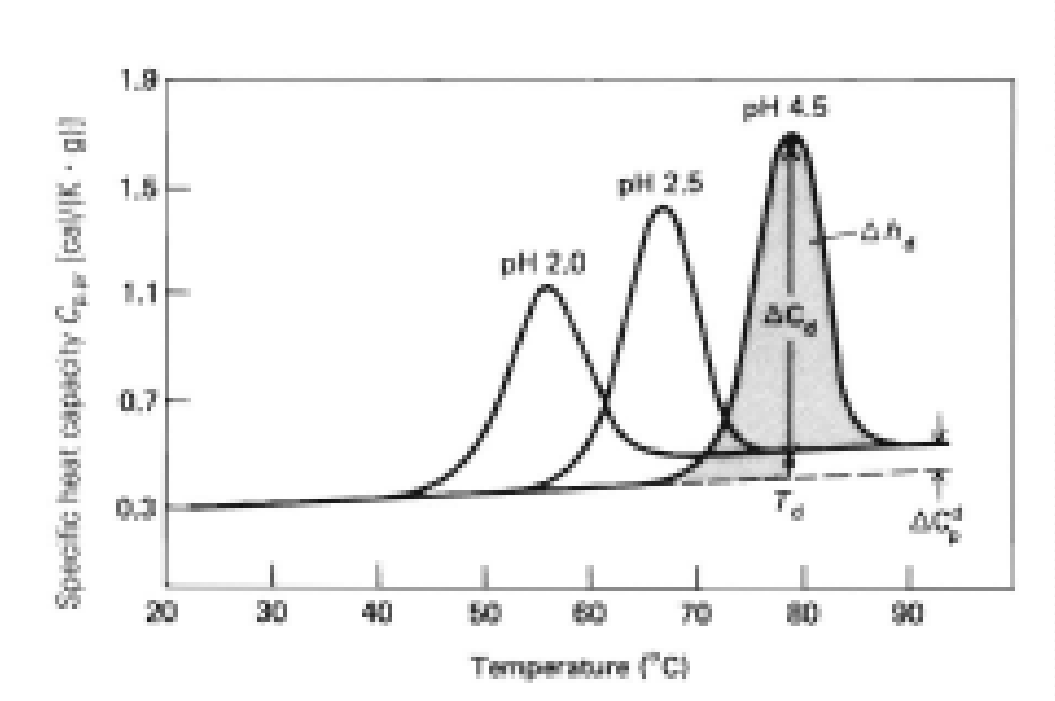


FIGURE 7.17
Thermal stability of ribonuclease A as a function of the concentration of urea and various guanidinium (Gdm^+) salts. The temperature at the midpoint of the thermal unfolding transition, T_m, is given. (Adapted from P. H. Von Hippel and K. Y. Wong, *J. Biol. Chem.* 240:3909–3923, 1965.)

Thermodynamics of Unfolding



Thermodynamics of Unfolding

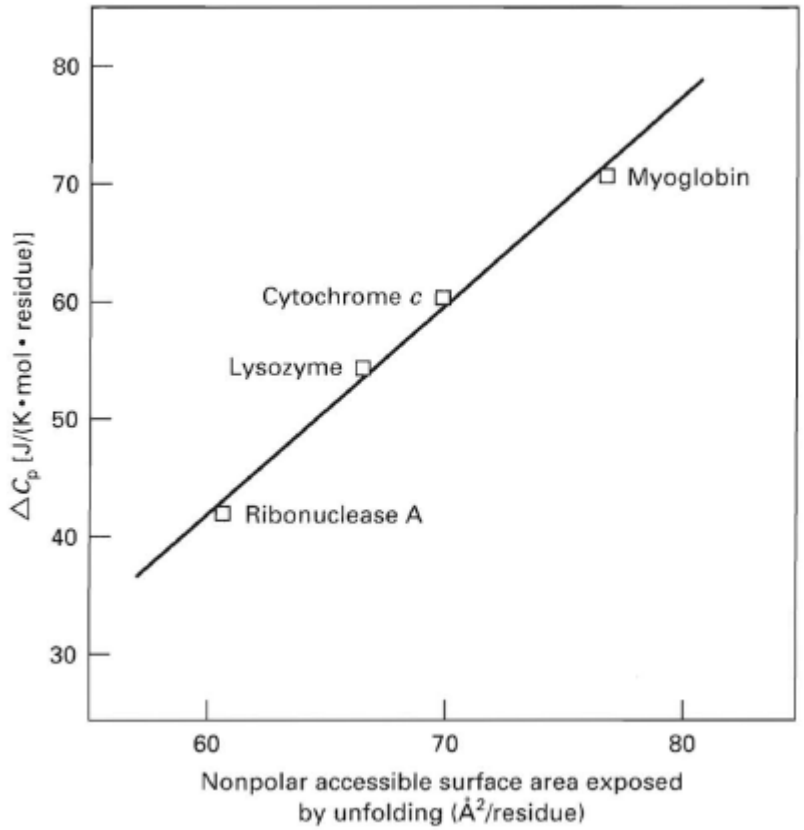
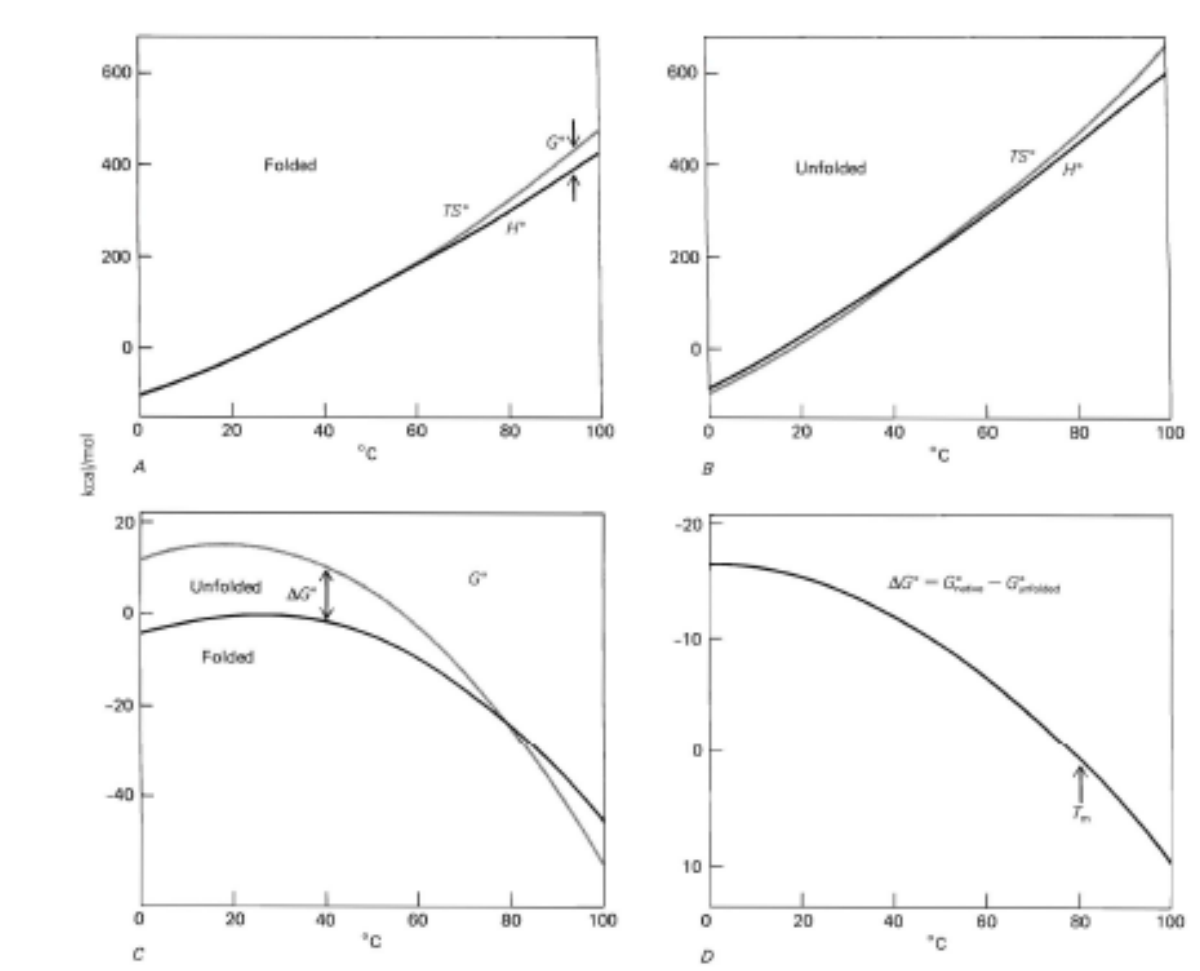


FIGURE 7.19

Relationship between the measured change in heat capacity upon unfolding of several proteins and the nonpolar surface area that is buried in the interior of the protein and is assumed to be exposed to solvent upon unfolding. Note that the relationship is not one of direct proportionality, in that it does not extrapolate to the origin. (Adapted from P. L. Privalov and G. I. Makhatadze, *J. Mol. Biol.* 213:385–391, 1990.)

Thermodynamics of Unfolding



Thermodynamics of Unfolding

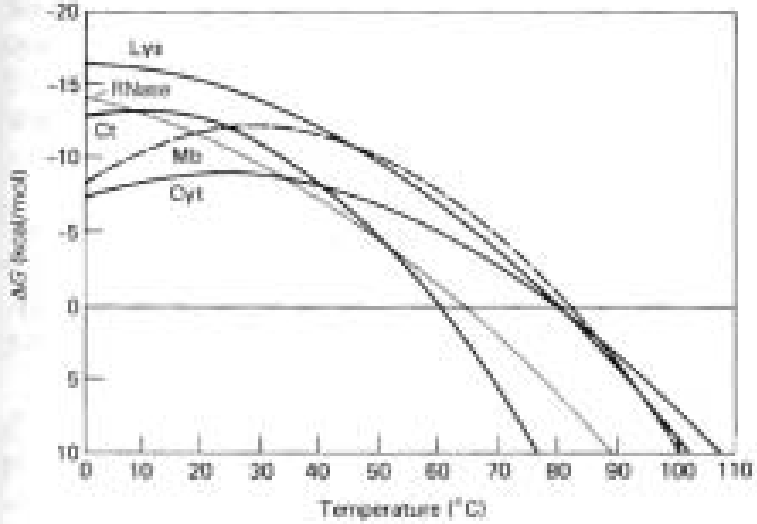


FIGURE 7.21
Temperature dependence of the difference in free energy between the folded and unfolded states of several proteins, expressed per mole of protein. Lys, hen lysozyme; RNase, ribonuclease A; Mb, metmyoglobin; Ct, α-chymotrypsin; Cyt, cytochrome *c*. The pH of each solution was that for which the protein is most stable. (Adapted from P. L. Privalov and N. N. Khechinashvili, *J. Mol. Biol.* 86:665–684, 1974.)

Thermodynamics of Unfolding

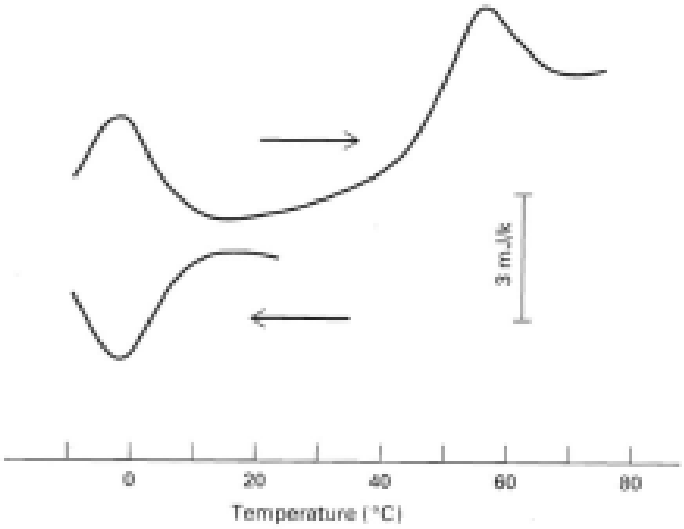


FIGURE 7.22
Unfolding of apomyoglobin at high and low temperatures measured calorimetrically. In the lower trace, folded apomyoglobin at room temperature was cooled to −10°C; the trough in the heat capacity is caused by the release of heat upon unfolding at −6°C. The cooled solution was then warmed, to produce the upper trace. The peak at −6°C corresponds to the uptake of heat as the apomyoglobin refolds; this is followed by a second peak of heat uptake, above 50°C, as the protein unfolds. (From Y. Griko et al., *J. Mol. Biol.* 202:127–138, 1988.)

Thermodynamics of Unfolding

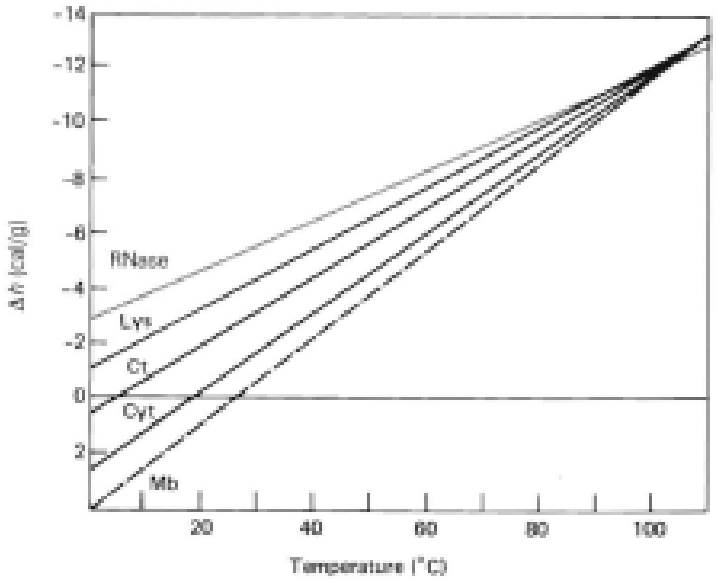


FIGURE 7.23
The specific enthalpy difference, Δh (per gram of protein), between the folded and unfolded states of five proteins: RNase, ribonuclease A; Lys, hen lysozyme; Ct, bovine α-chymotrypsin; Cyt, cytochrome *c*; Mb, metmyoglobin. The pH of each solution was that at which the protein is most stable. (Adapted from P. L. Privalov and N. N. Khechinashvili, *J. Mol. Biol.* 86:665–684, 1974.)

Thermodynamics of Unfolding

$$\Delta G_{\text{unfold}} = \Delta H^* - T\,\Delta S^* + \Delta C_p\left[(T - T^*) - T \ln \frac{T}{T^*}\right]$$

Thermodynamics of Unfolding

Table 7.7 *Free-Energy Contributions of Various Groups to the Stability of Cyclic Dipeptide Crystals in Water, Compared with Their Free Energy of Transfer to a Nonpolar Liquid*

Groups	Transfer from Water to	
	Cyclic dipeptide crystal[a] (kcal/mol)	Nonpolar liquid (kcal/mol)
	-0.38 ± 0.29	$+6.12$[b] $+0.55$ (hydrogen bonded)[b]
Apolar hydrogen, — CH	-0.31 ± 0.05	-0.45[c]
Phenyl ring	-1.37 ± 0.43	-2.58[c]
—OH	-0.07 ± 0.26	$+2.23$[c]

[a] From K. P. Murphy and S. J. Gill, *Thermochim. Acta* 172:11–20 (1990).

[b] From M. A. Roseman, *J. Mol. Biol.* 201:621–623 (1988).

[c] From D. J. Abraham and A. J. Leo, *Proteins: Struct. Funct. Genet.* 2:130–152 (1987).

Thermodynamics of Unfolding

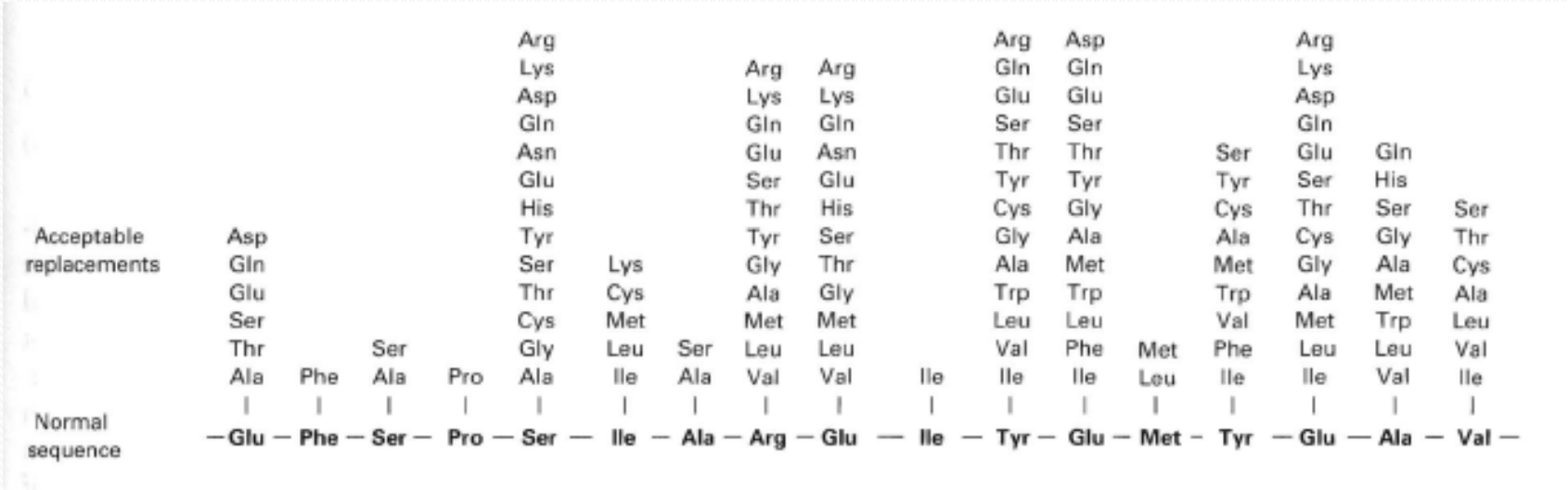
Contribution	$G^N - G^U$ (kcal/mol)
Greater conformational entropy of U[a]	+167
Net stabilizing interactions[b]	−198
Solvation of nonpolar surface in U[c]	+17
Net stability	−14

[a] $T\,\Delta S_{conf}$; $\Delta S_{conf} = 4.35$ cal/(K · mol · residue)

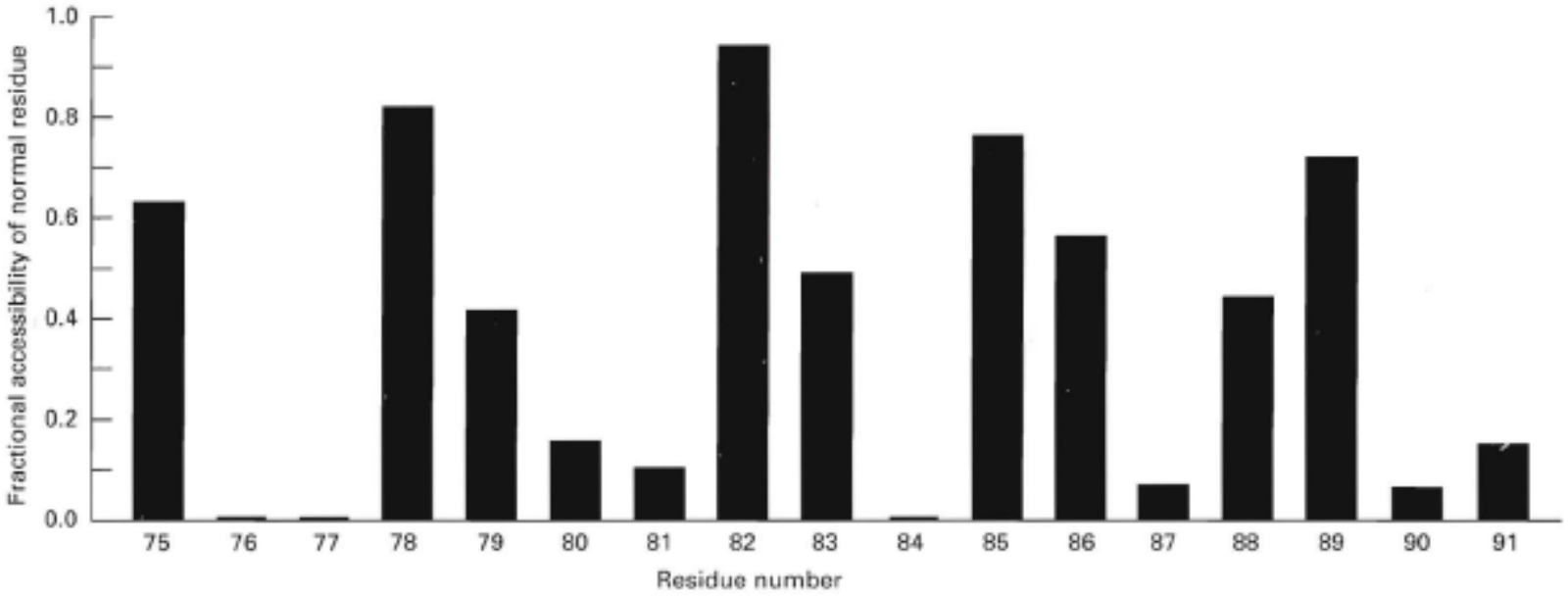
[b] Sum of van der Waals interactions in N, net greater stability of hydrogen bonds and other polar interactions in N relative to U, minus any conformational strain. Calculated from $\Delta H^* = 1.54$ kcal/(mol · residue).

[c] Favorable interactions of nonpolar surface with water at 25°C, calculated from $\Delta C_p[T - T^* - T \ln (T/T^*)]$, where $T^* = 112$°C and $\Delta C_p = 12.5$ cal/(K · mol · residue), the measured value for hen lysozyme.

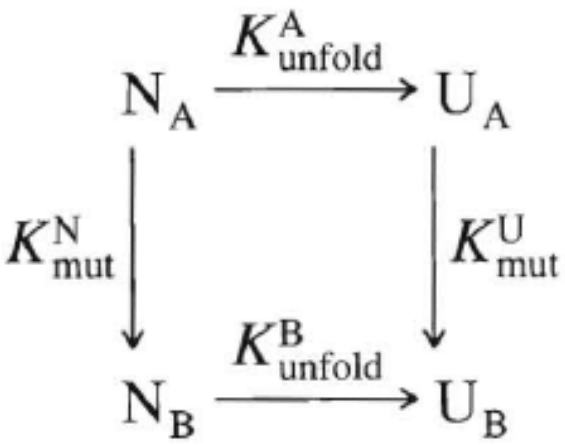
Thermodynamics of Unfolding



Thermodynamics of Unfolding



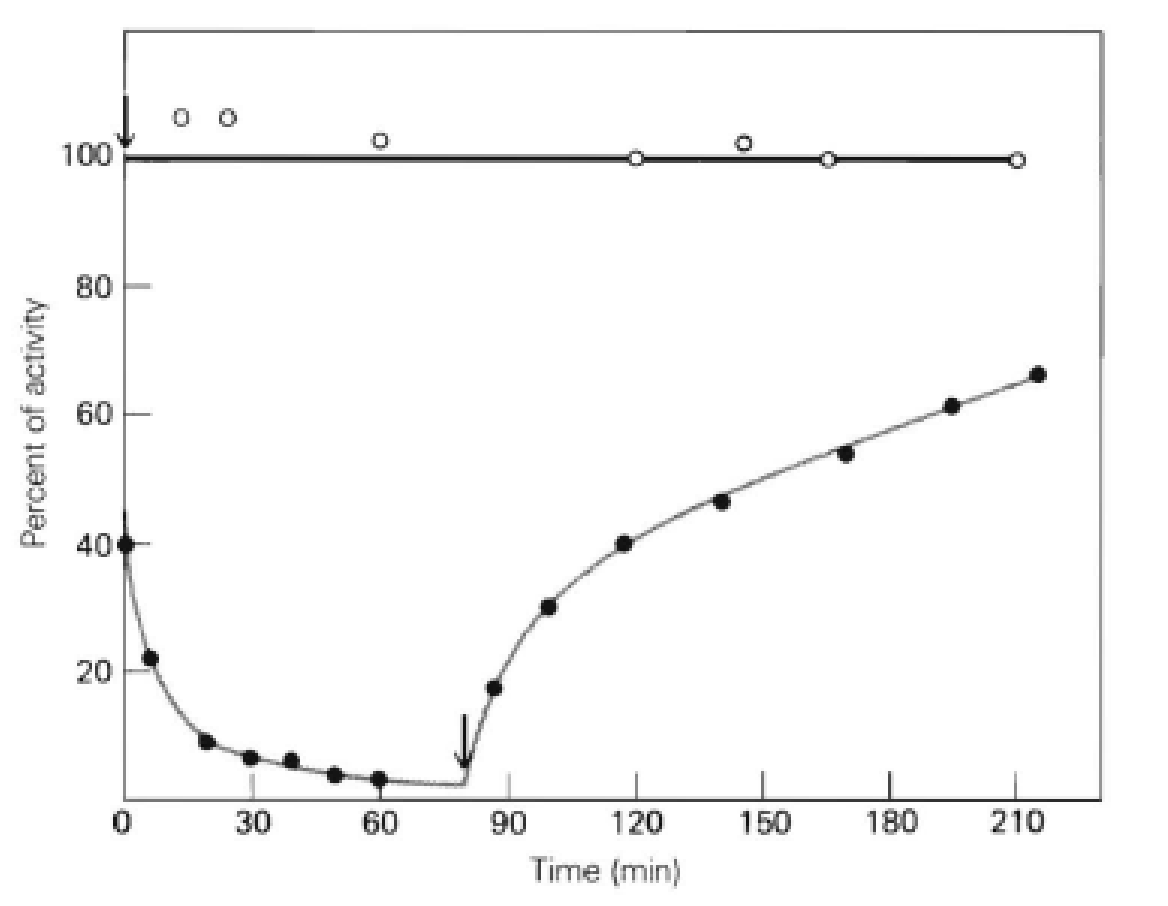
Thermodynamics of Unfolding



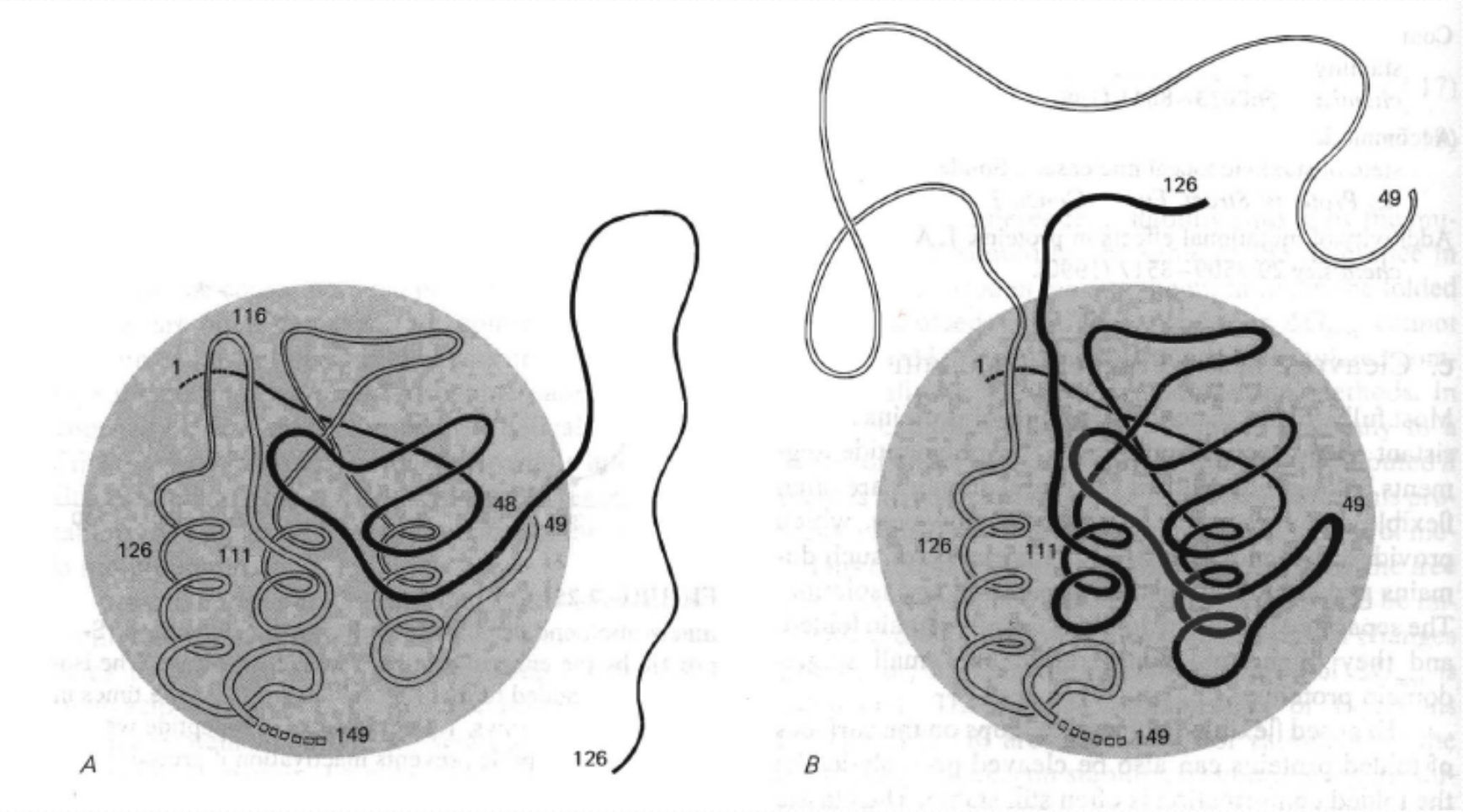
$$\frac{K^{A}_{unfold}}{K^{B}_{unfold}} = \frac{K^{N}_{mut}}{K^{U}_{mut}}$$

$$\Delta G_{unfold} = \Delta G_{mut}$$

Thermodynamics of Unfolding



Thermodynamics of Unfolding



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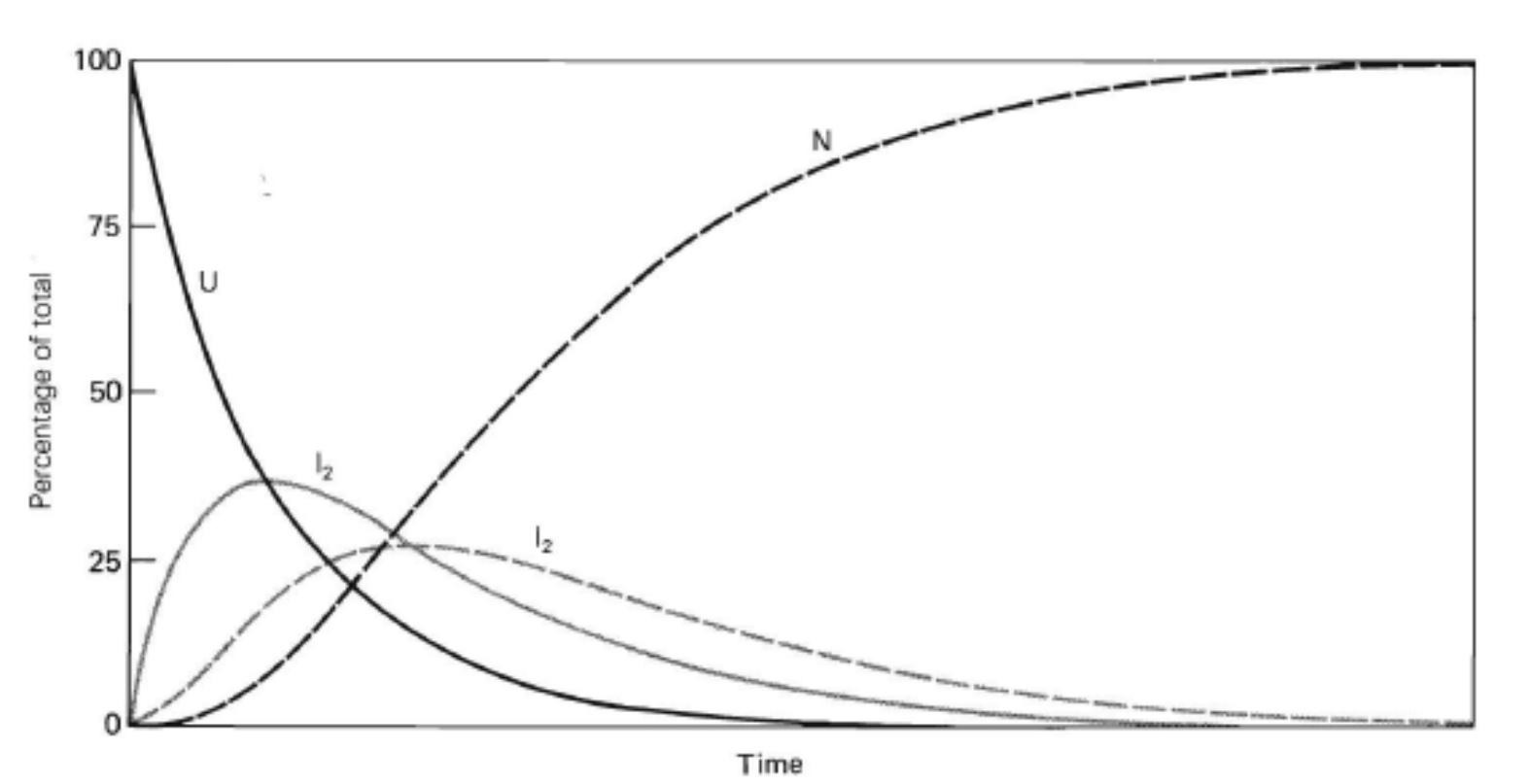

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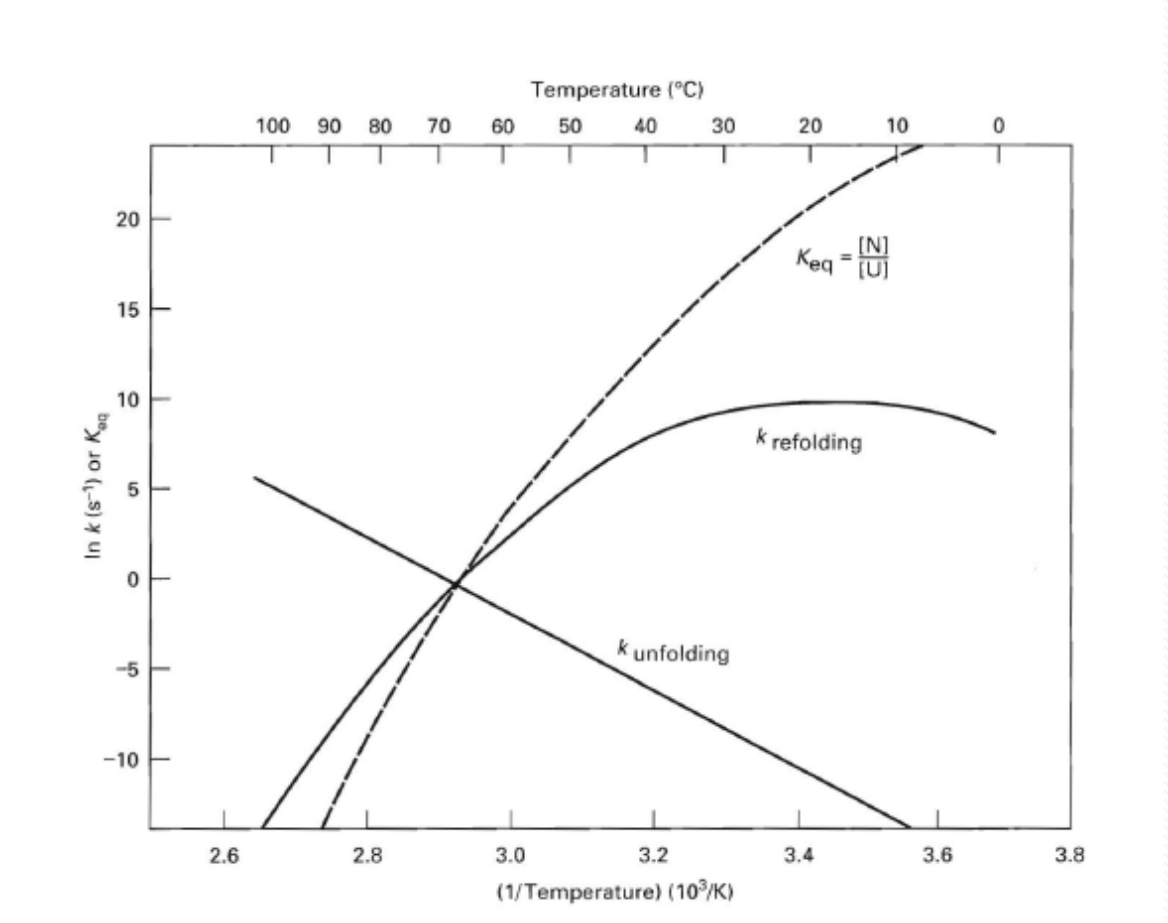

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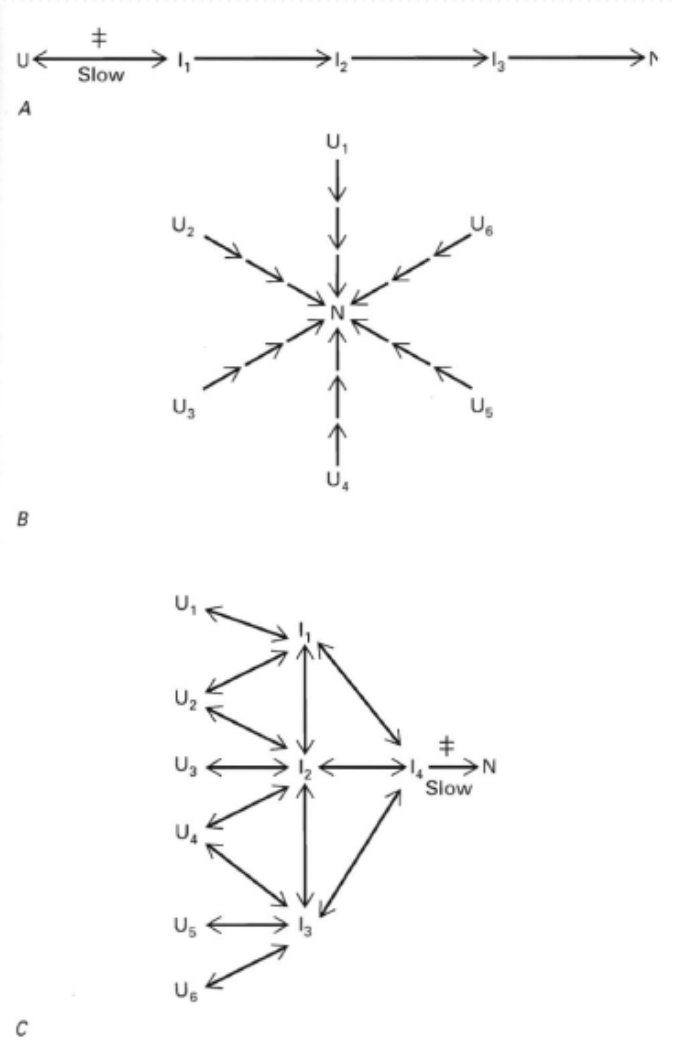


Thermodynamics of Unfolding

$$U \underset{}{\overset{K_{conf}}{\rightleftharpoons}} N \overset{K_N}{\underset{Ab}{\rightleftharpoons}} Ab \cdot N$$

$$K_N = \frac{[Ab \cdot N]}{[N]\,[Ab]}$$

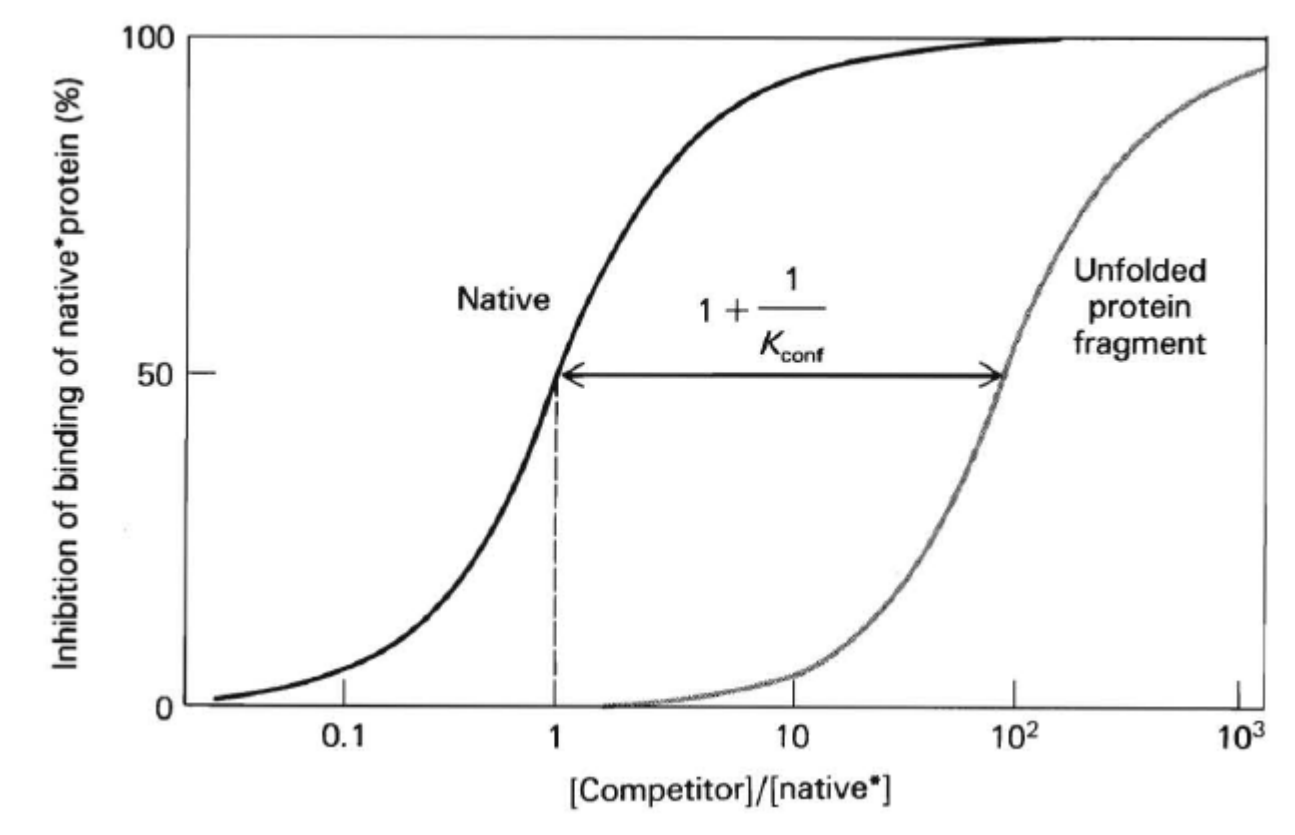
Therefore, the affinity of the polypeptide for the anti-N antibodies is lower by the factor $[1 + (1/K_{conf})]$. If K_{conf} is very small, this factor becomes $1/K_{conf}$ (Fig. 7.30).

Values of K_{conf} measured with a few unfolded proteins or fragments are in the range of $10^{-3} - 10^{-4}$. These

$$K_{app} = \frac{[Ab \cdot N]}{([U] + [N])[Ab]} = \frac{[Ab \cdot N]}{\left(1 + \frac{1}{K_{conf}}\right)[N][Ab]}$$

$$= \frac{K_N}{\left(1 + \frac{1}{K_{conf}}\right)} \qquad (7.24)$$

Thermodynamics of Unfolding



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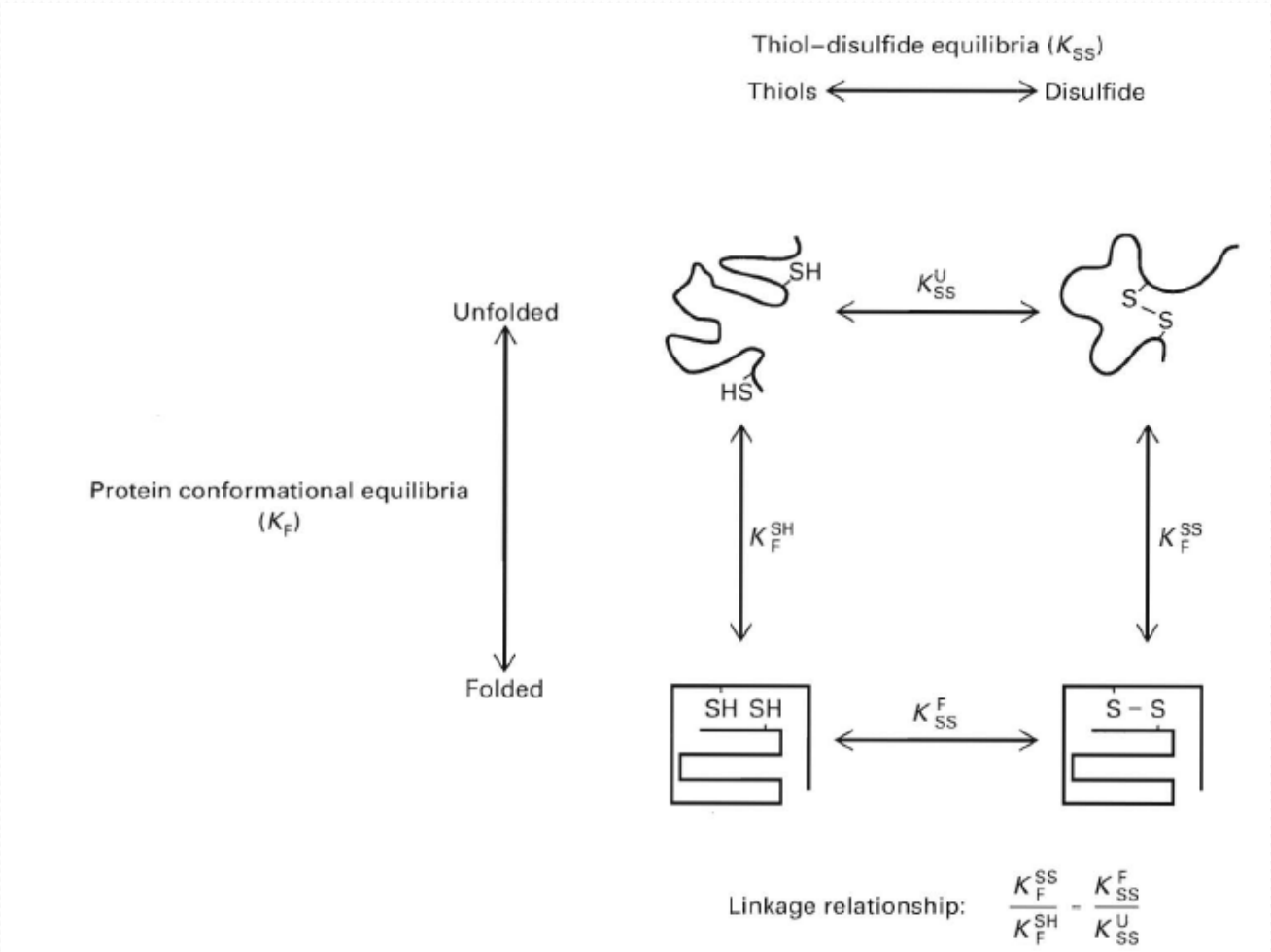


Thermodynamics of Unfolding

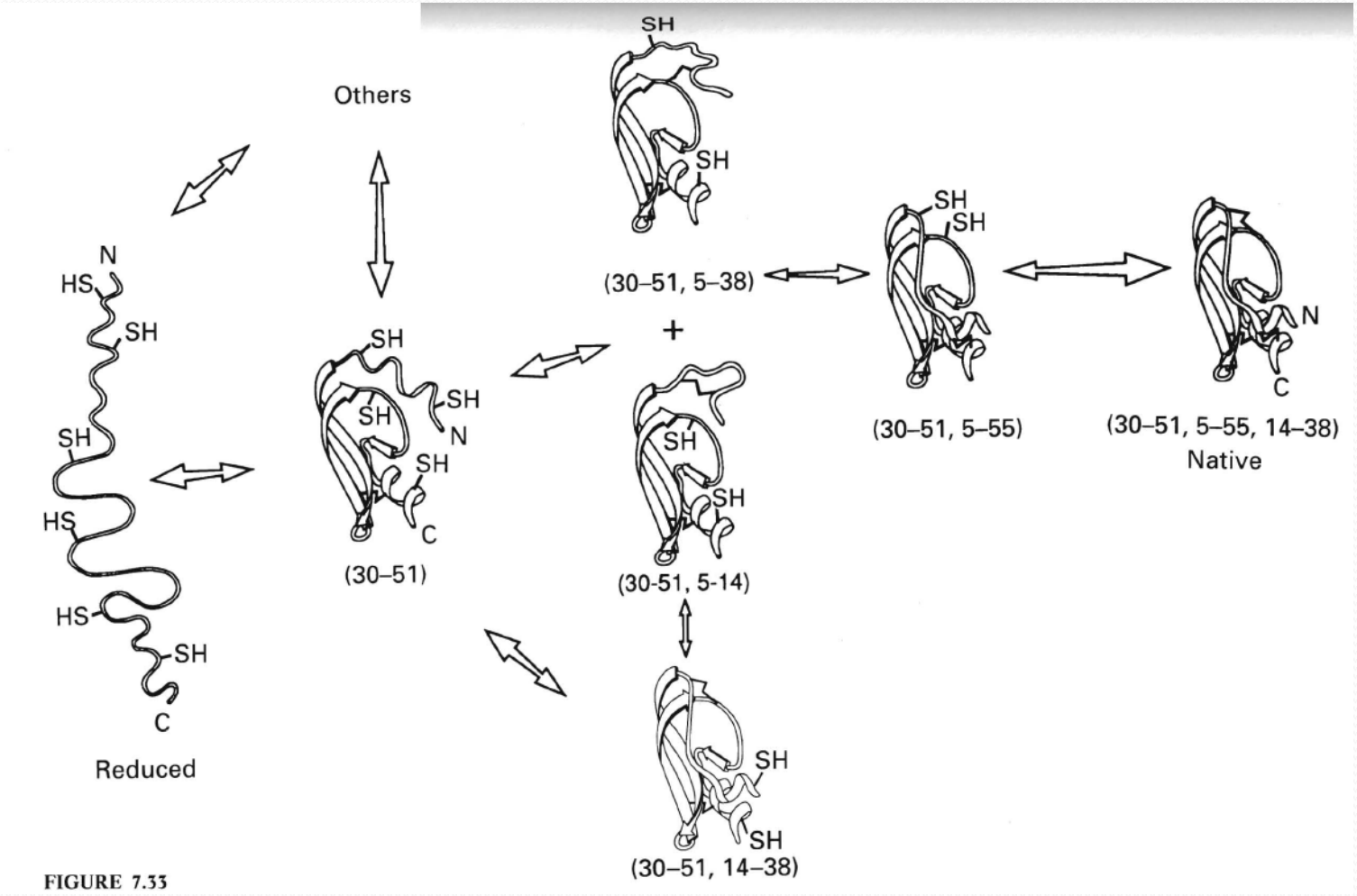
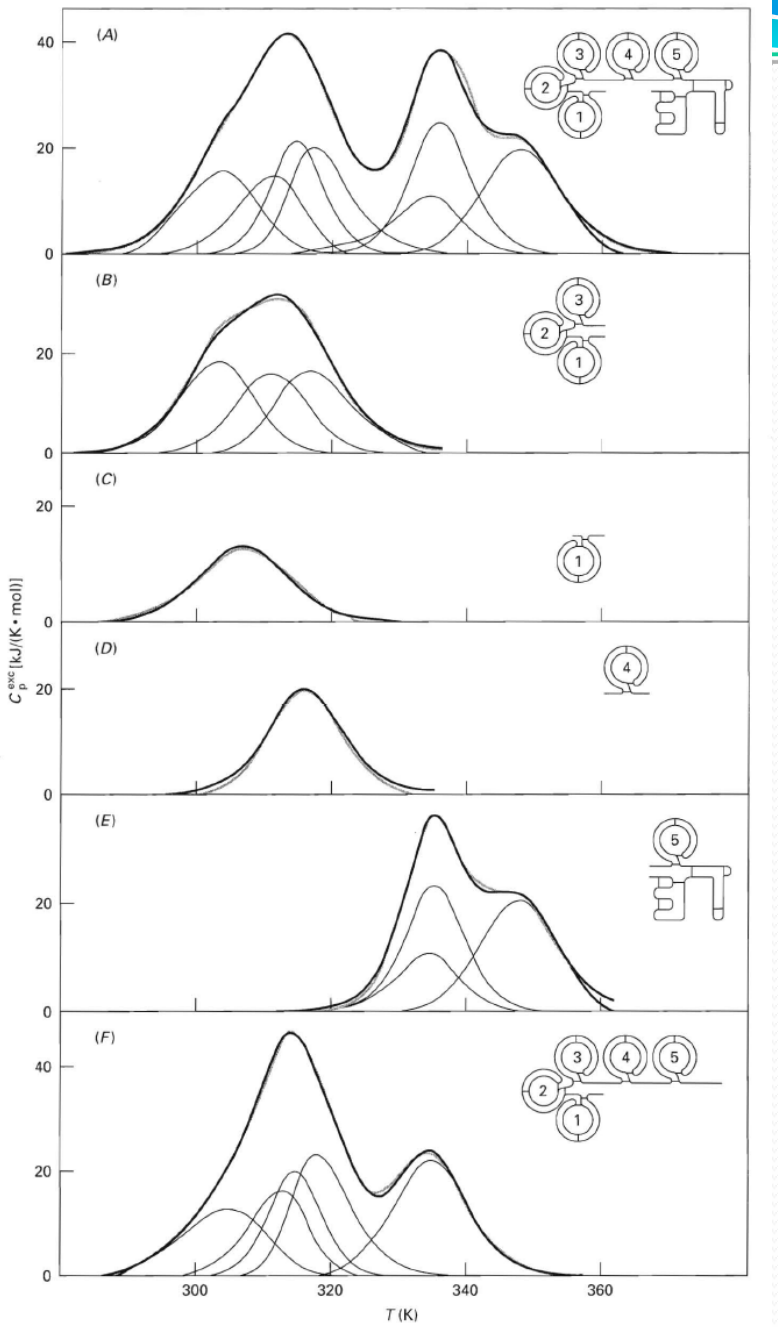


FIGURE 7.33


(A)
(B)
(C)
(D)
(E)
(F)
1
2
3
4
5
40
20
0
300
320
340
360
T (K)
C_p^{exc} [kJ/(K • mol)]

Biosynthetic folding

- Molecular chaperones
- Prolyl peptide isomerases
- Protein disulfide isomerase