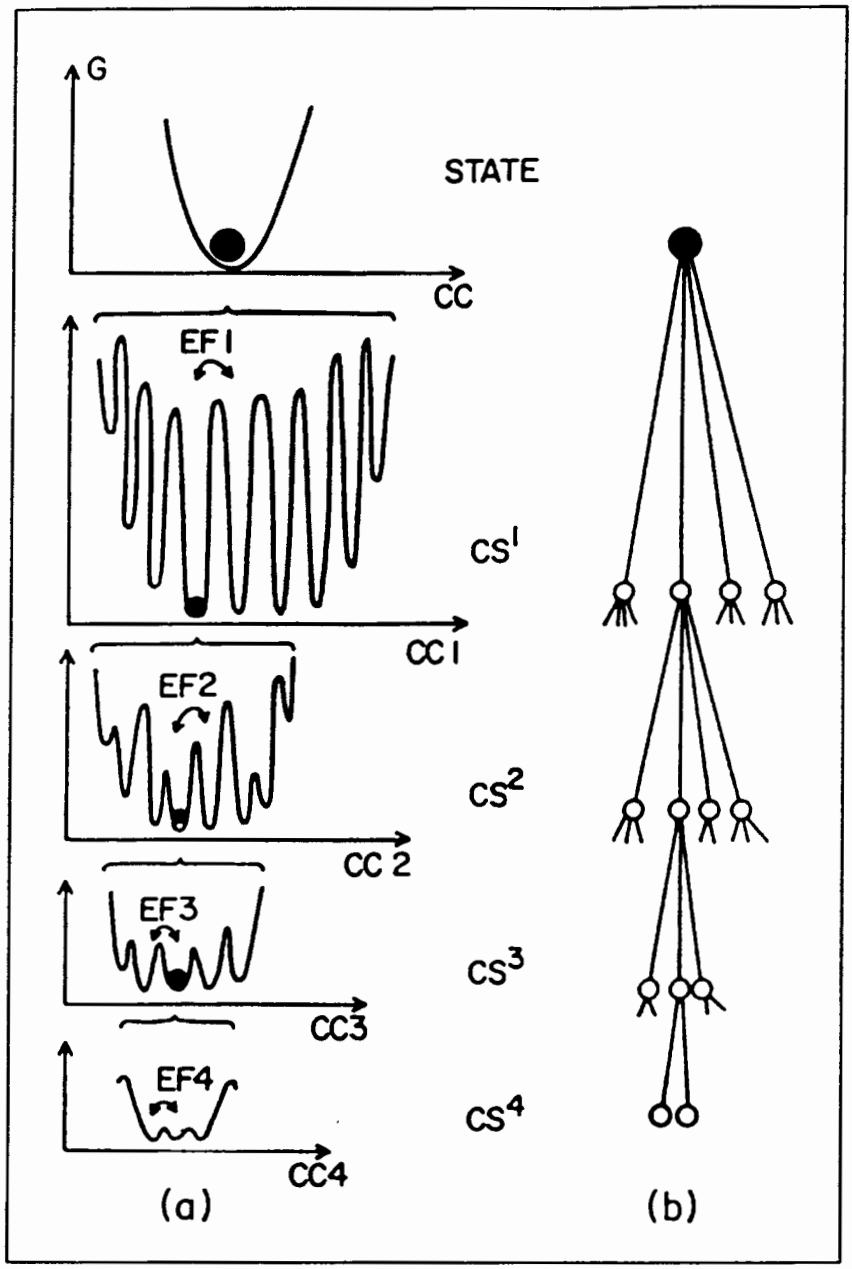


FIGURE 1–4. *Schematic diagram of conformational substates, CS, and equilibrium fluctuations, EF. The ordinate is the Gibbs free energy, the abscissae the conformational coordinates, CC 1–4. At room temperature the state CC appears to be located in a smooth well. As the temperature is lowered, the substructure of the energy landscape begins to appear with many minima representing closely similar forms in thermal equilibrium. As the temperature decreases, molecules are frozen into the individual minima, each of which develops additional structure. Within each tier of substates, the equilibrium fluctuations distribute the molecules among these minima. The final tier, CS^4, would be identifiable only at a temperature near absolute zero. This general description was set up to rationalize the data collected on the kinetics of the photodissociation and reassociation of the myoglobin-carbon monoxide complex as a function of temperature. (Reprinted with permission from Ansari et al., 1985)*

Mechanism of the Protein Folding Reaction

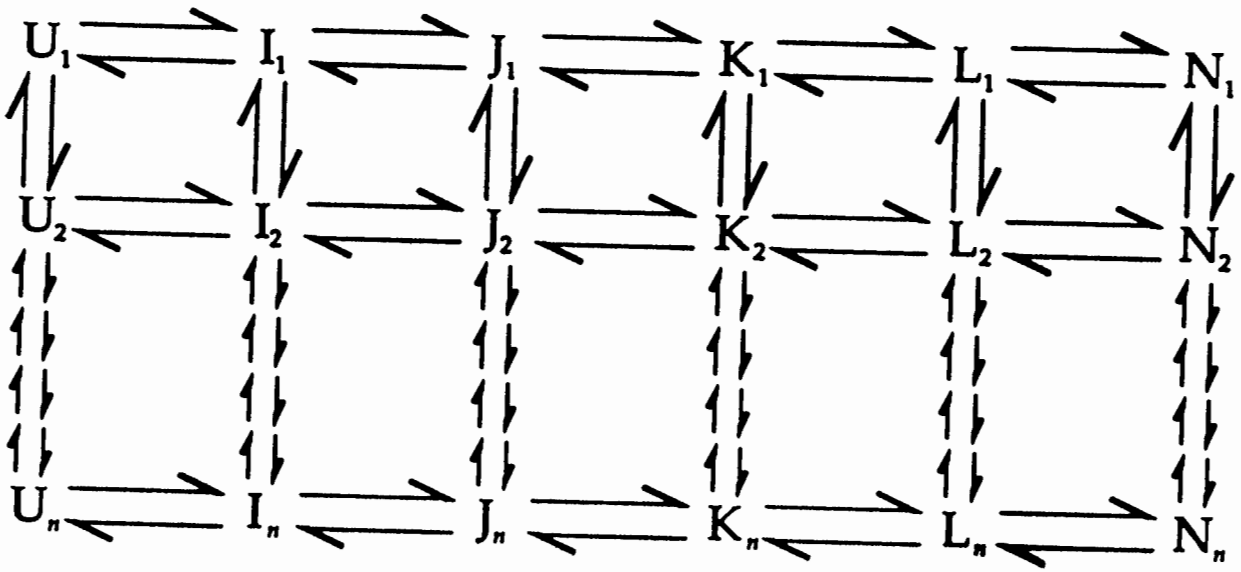
1. Levinthal Random Sampling
2. Sequential Folding Model
3. Nucleation/Growth Model
4. Diffusion–Collision–Adhesion
5. Framework Model
6. Hydrophobic Collapse Model
7. Jigsaw Puzzle Model

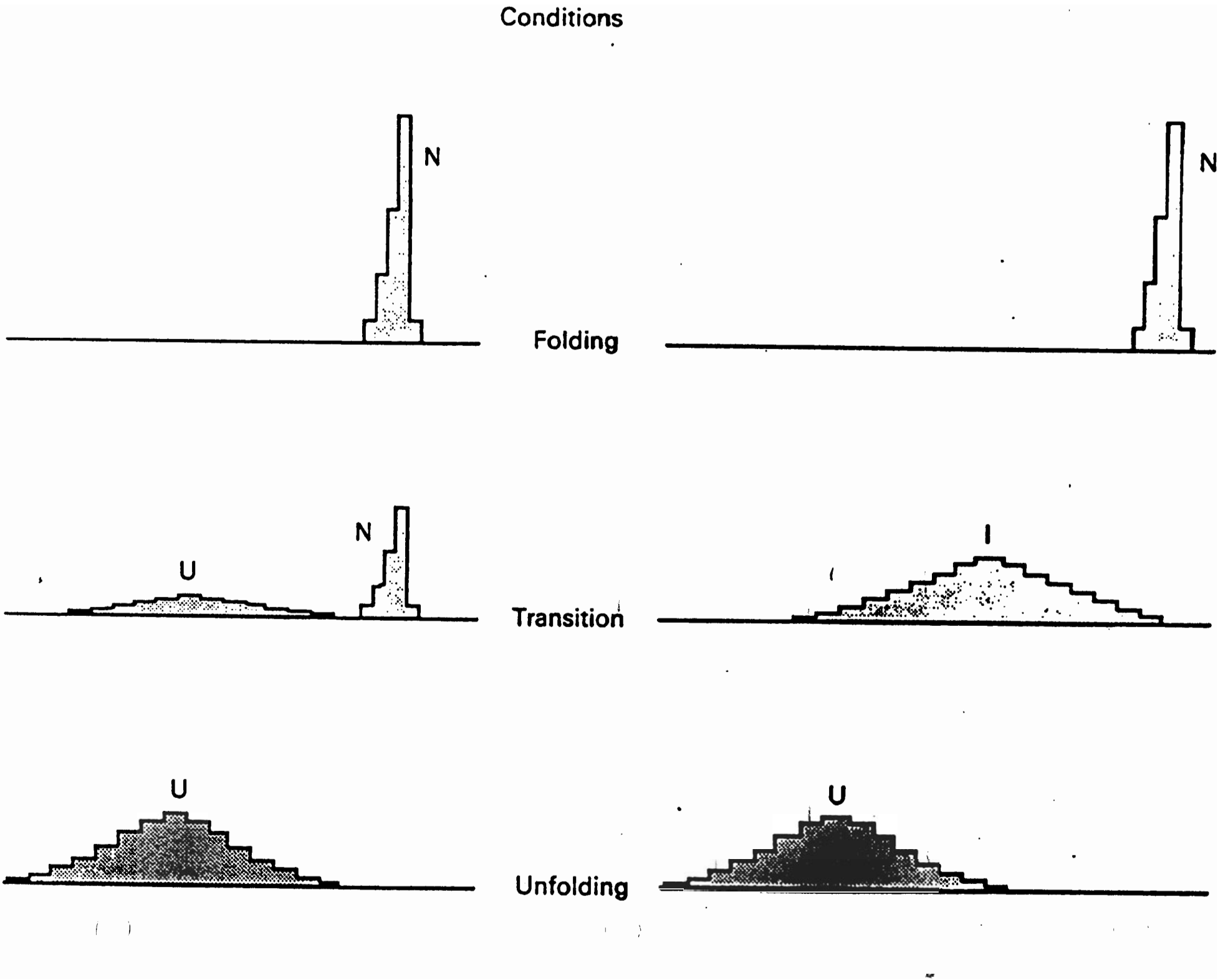
The elementary form of the folding reaction is deceptively simple:

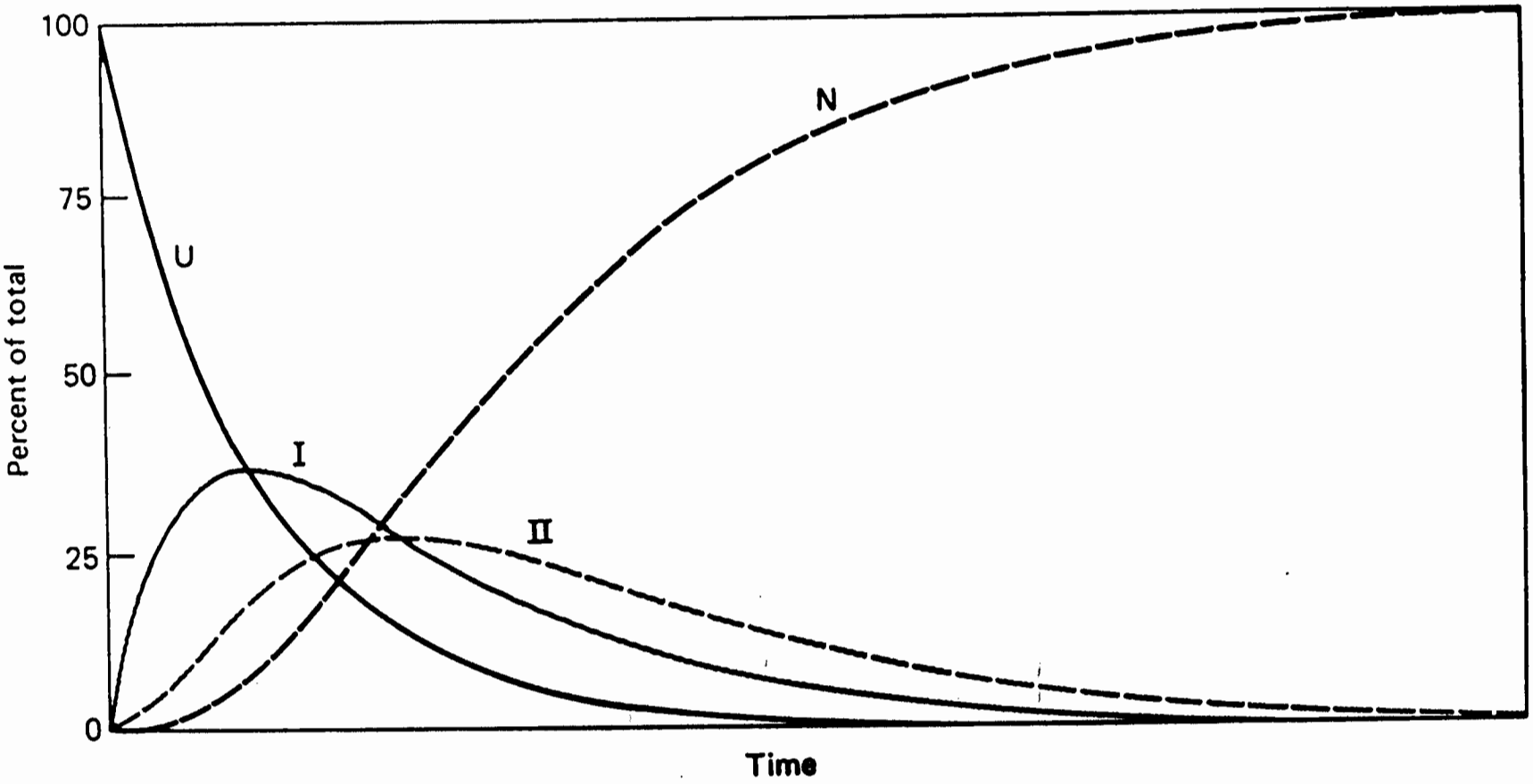
denatured or unfolded — U ⇌ N — native or folded

renaturation or folding

denaturation or unfolding

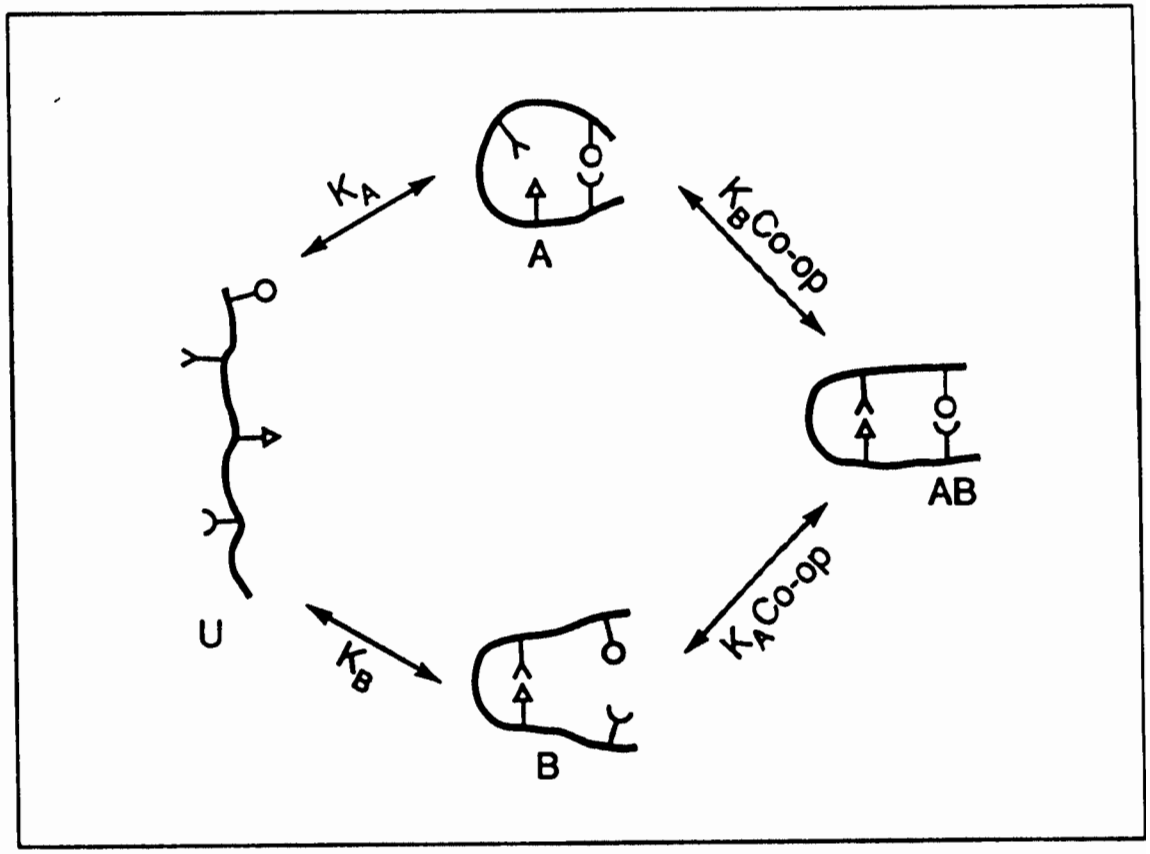



Conditions
N
N
Folding
N
U
I
Transition
U
U
Unfolding



Kinetics of a three-step sequential reaction, $U \rightarrow I \rightarrow II \rightarrow N$, in which all three steps have the same rate constant. The intermediates accumulate to relatively low concentrations.

Fig. 4. Schematic illustration of co-operativity between two interactions, with equilibrium constants K_A and K_B in the unfolded polypeptide chain, U. If both interactions are possible simultaneously, the presence of one will increase the proximity of the other pair of groups, and less conformational entropy of the polypeptide chain will need to be lost for them to interact. Consequently, each interaction will be stronger when the other is also present by the factor 'Co-op'. The effect must be mutual, due to the thermodynamic requirement that there be no free energy change around such a cycle.



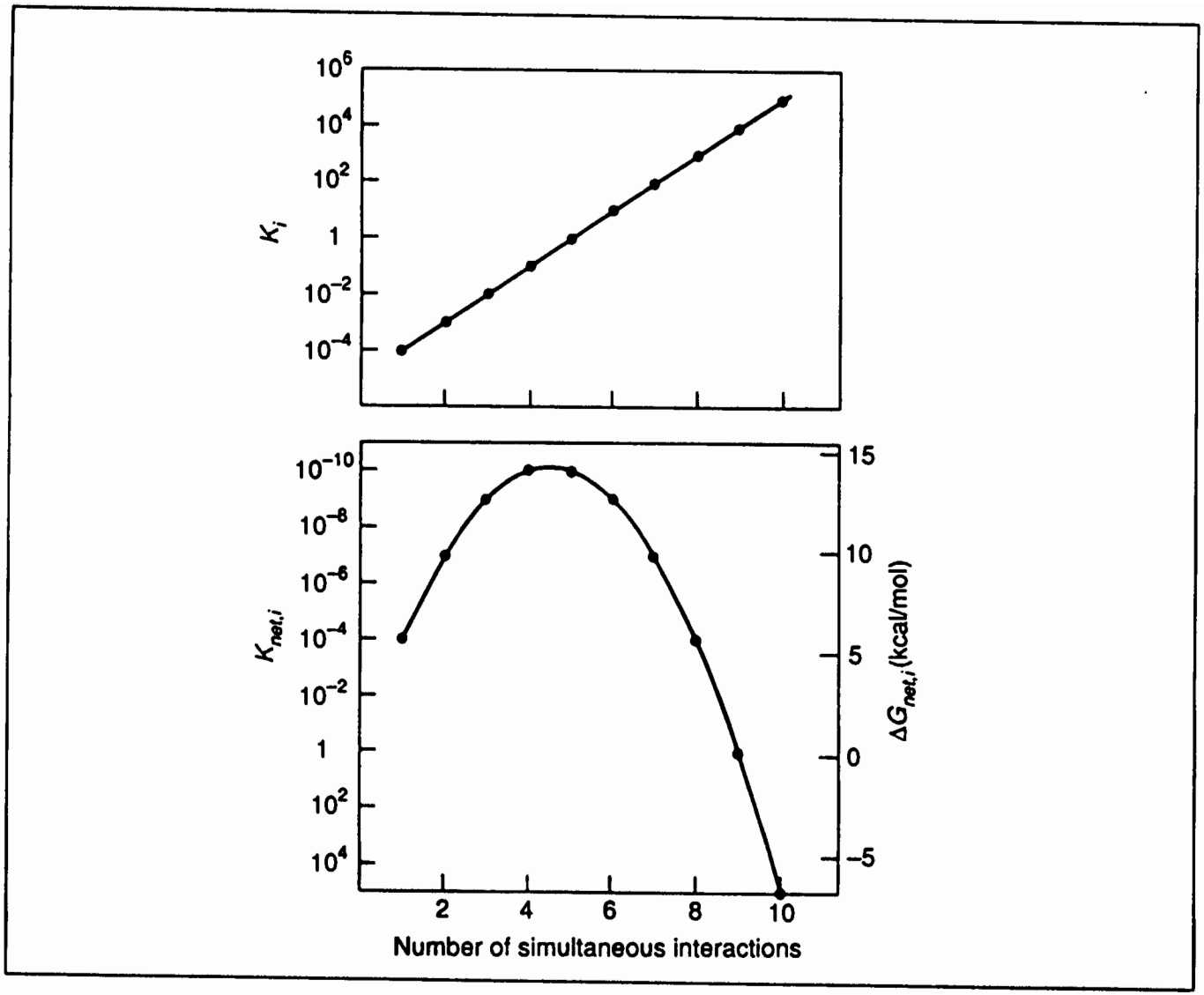
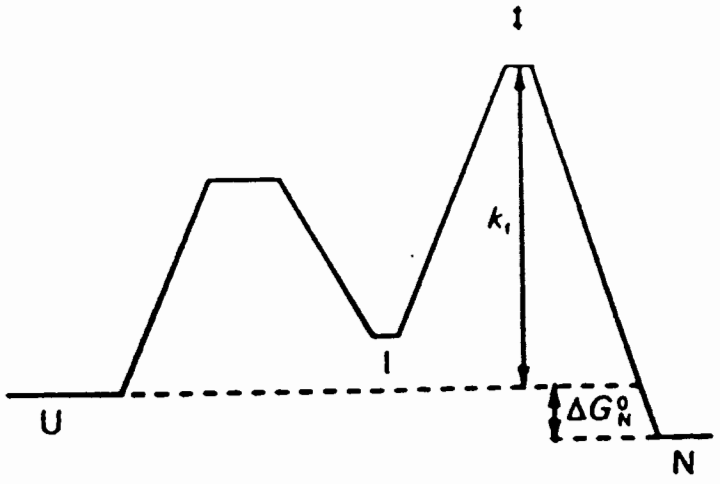
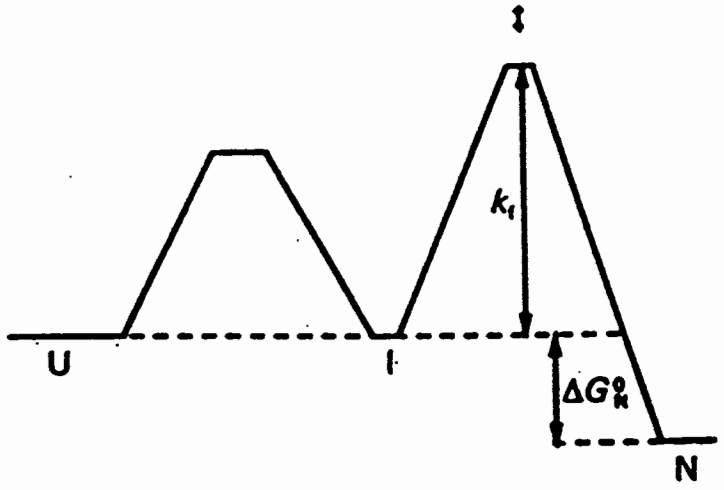


Fig. 5. Hypothetical illustration of the co-operativity of protein folding produced by multiple weak interactions. The equilibrium constant for forming the *i*th interaction, K_i, is shown at the *top*. Individual interactions are postulated to be weak in U, with equilibrium constants no greater than 10^{-4}; each interaction is postulated to be ten times stronger than the preceding one due to entropic co-operativity between them, as described in Fig. 4. The overall equilibrium constant between U with no interactions and each species with *i* interactions, $K_{net,i}$, is given at the *bottom*. The value of $K_{net,i}$ decreases initially, as each K_i is less than unity. As K_i becomes greater than unity, the value of $K_{net,i}$ increases. Only with 10 such interactions is $K_{net,i} > 1$ and the folded structure stable. The free energy of each state relative to U is given by $\Delta G_{net,i} = -RT \ln K_{net,i}$, with the scale on the *right* pertaining to 25 °C.

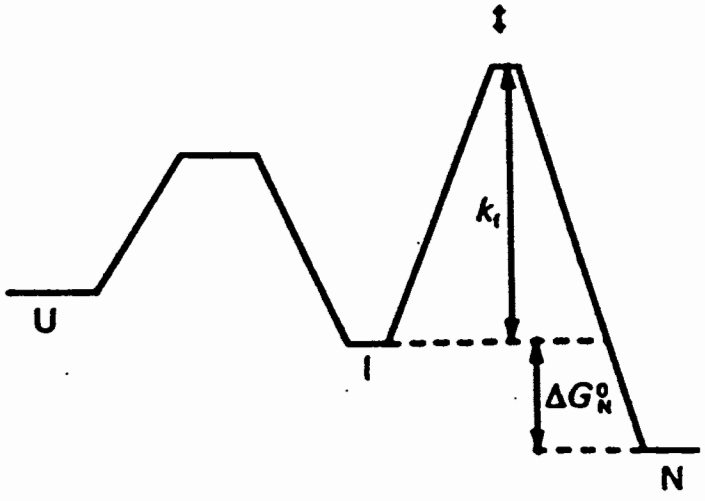
(a)



(b)



(c)



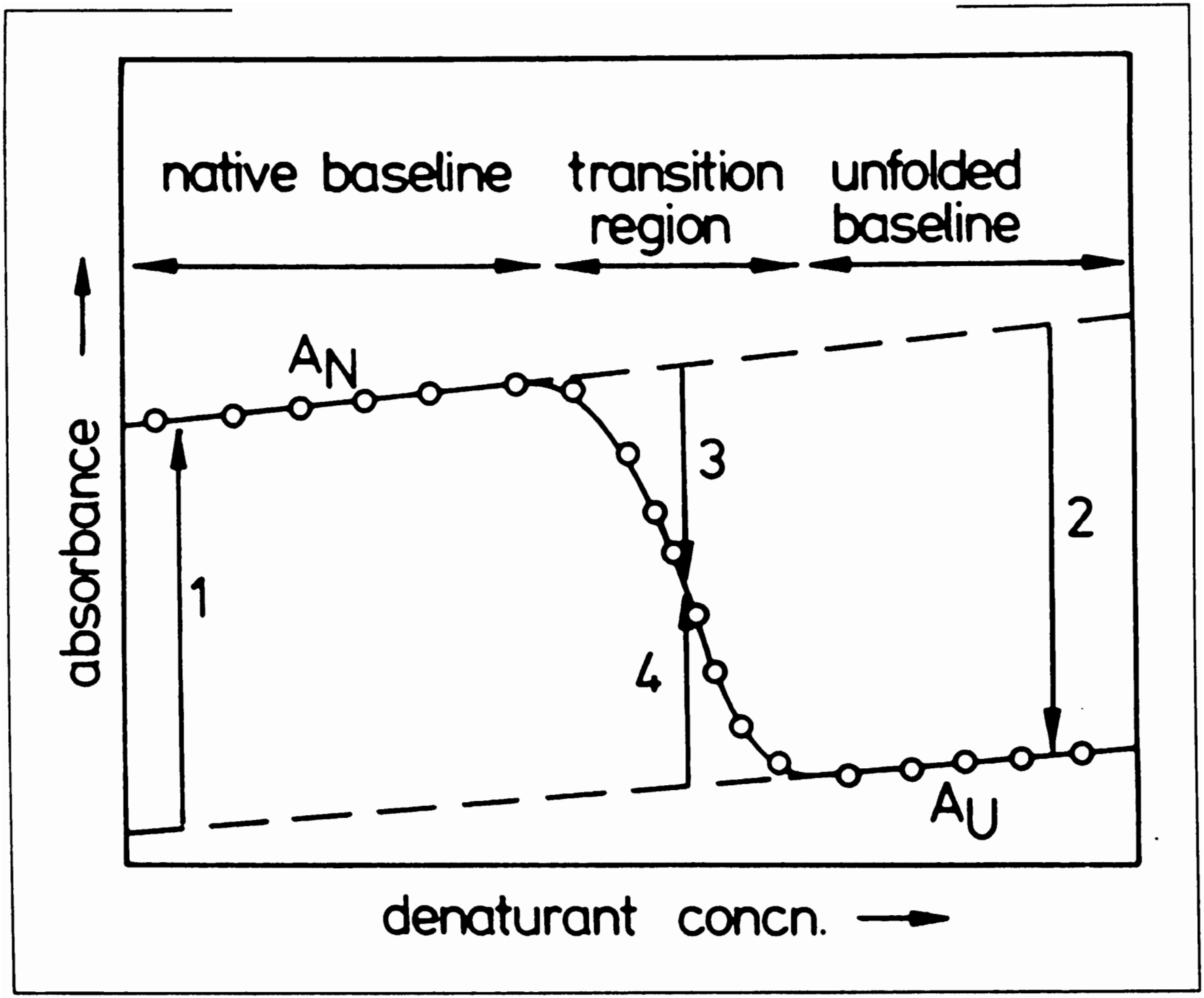
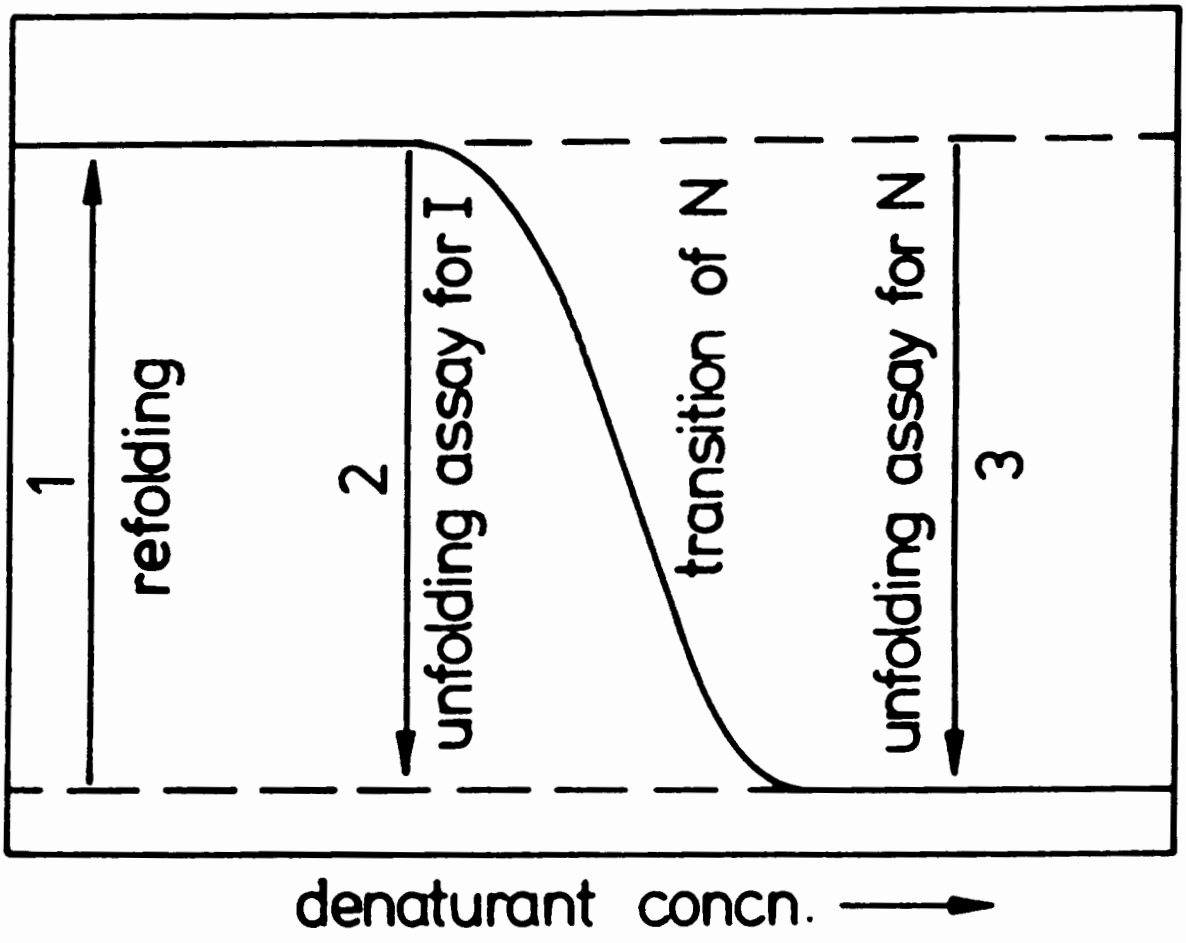


FIGURE 5–1. Schematic representation of a denaturant-induced unfolding transition

(A) Schematic unfolding transition.

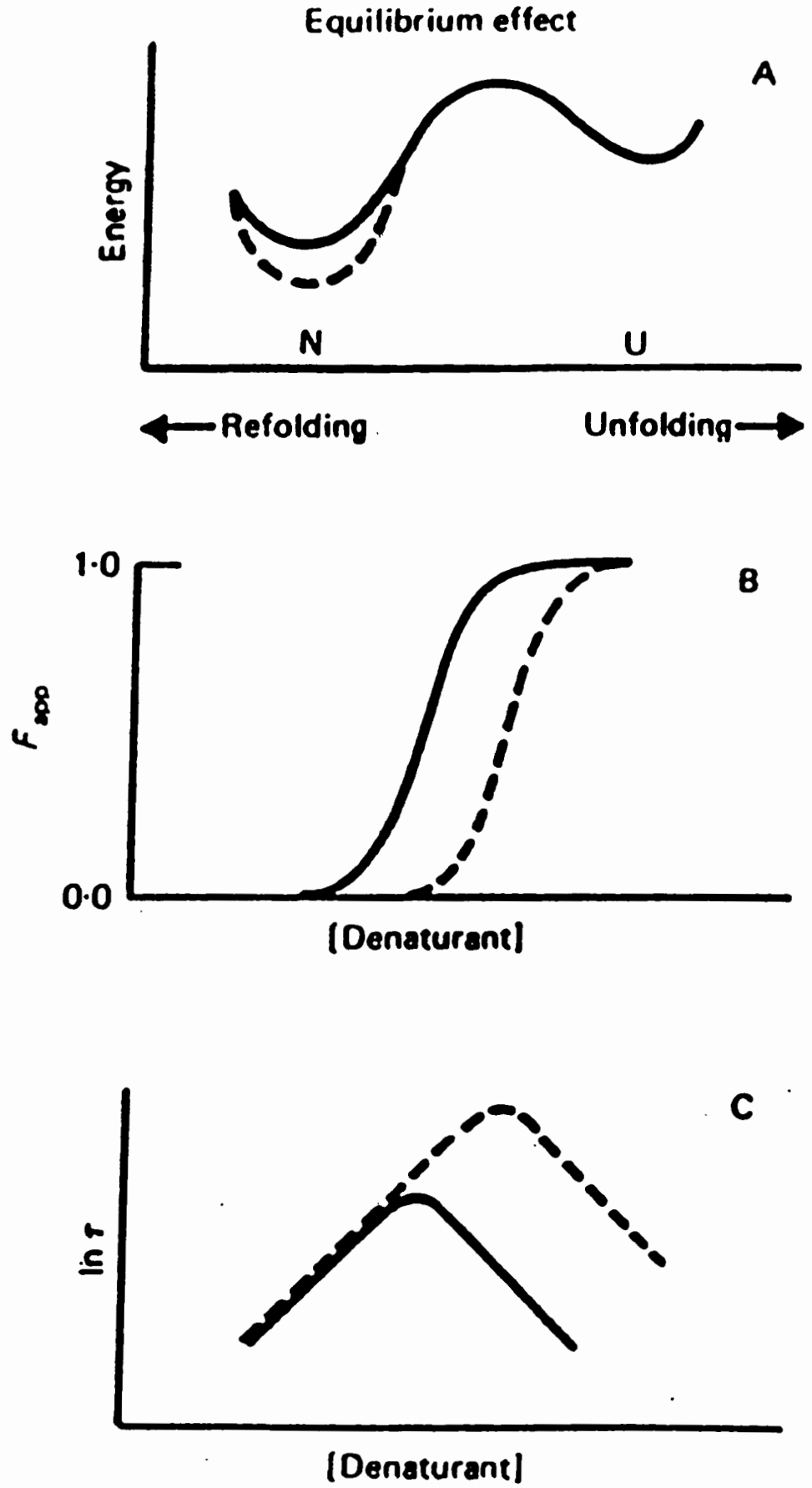


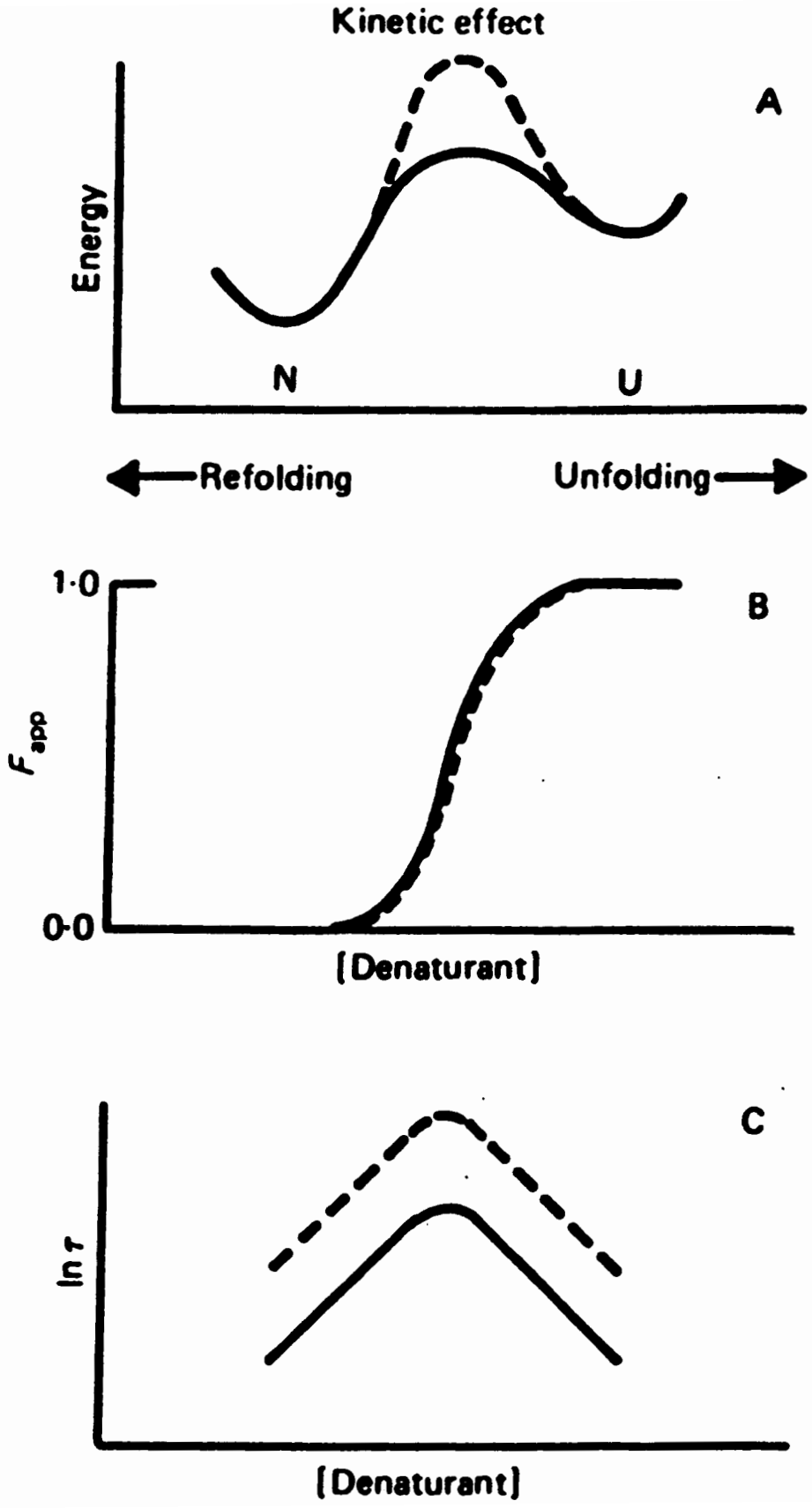
(B) Two-step procedure:

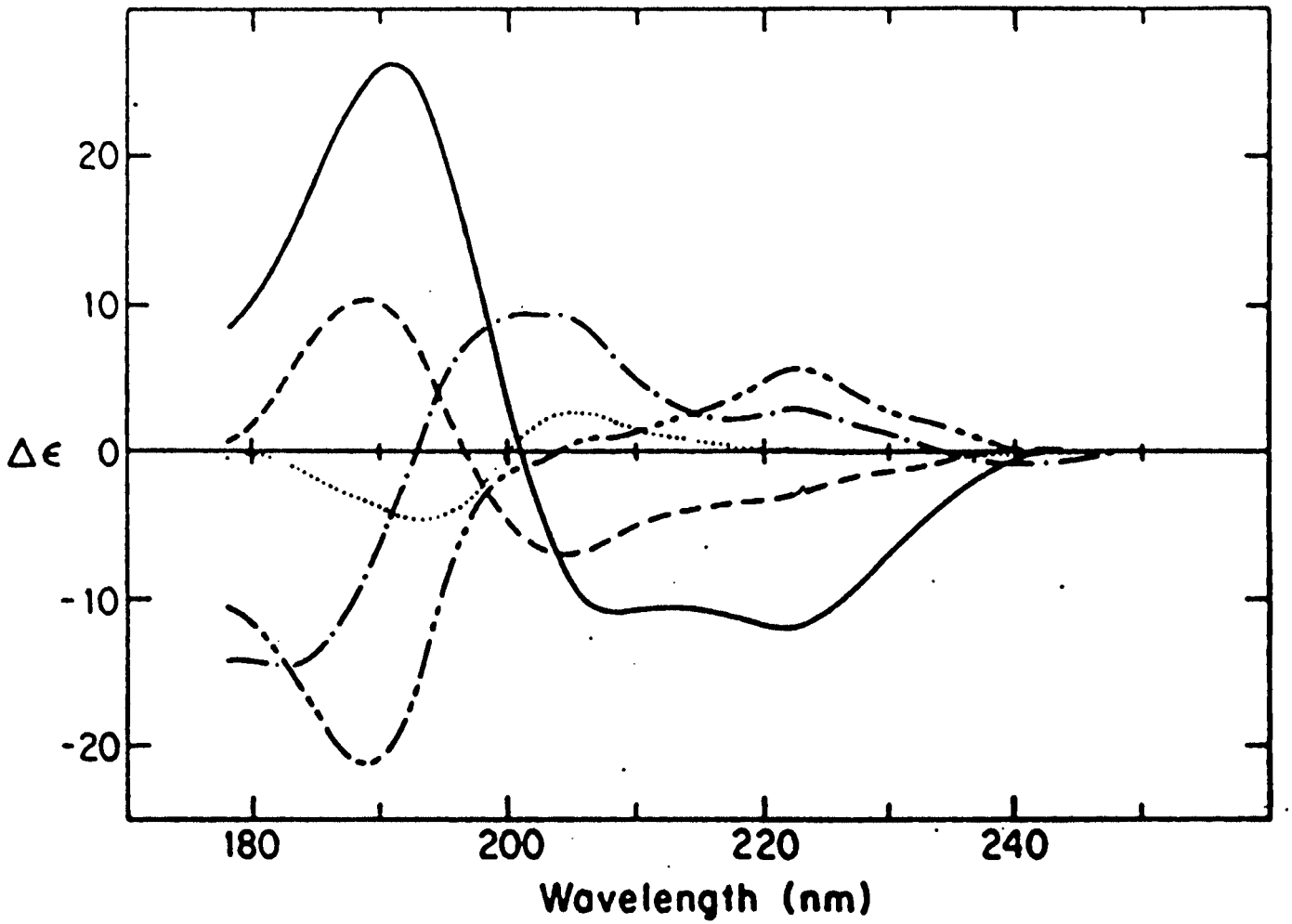
I: Refolding step.
At t = 0 : Start refolding in a test tube (cf. arrow 1)

II: Unfolding assay.
At $t = t_i$: jump to (2) or (3) to measure, respectively, the unfolding amplitude of the intermediate (I) or the native protein (N), as indicated by arrows (2) and (3).

FIGURE 5–5. Schematic outline of the method of using unfolding assays to measure the formation of intermediates or of native protein during refolding.


Equilibrium effect
A
Energy
N
U
Refolding
Unfolding
1·0
0·0
F_{app}
B
[Denaturant]
C
ln r
[Denaturant]


Kinetic effect
A
Energy
N
U
Refolding
Unfolding
1·0
B
F_{app}
0·0
[Denaturant]
C
ln τ
[Denaturant]



Secondary structure spectra for five major secondary structures from 178 to 260 nm: α-helix (——), antiparallel β-sheet (---), parallel β-sheet (-·-), β-turn (– -- –), other (random) structure (· · ·).

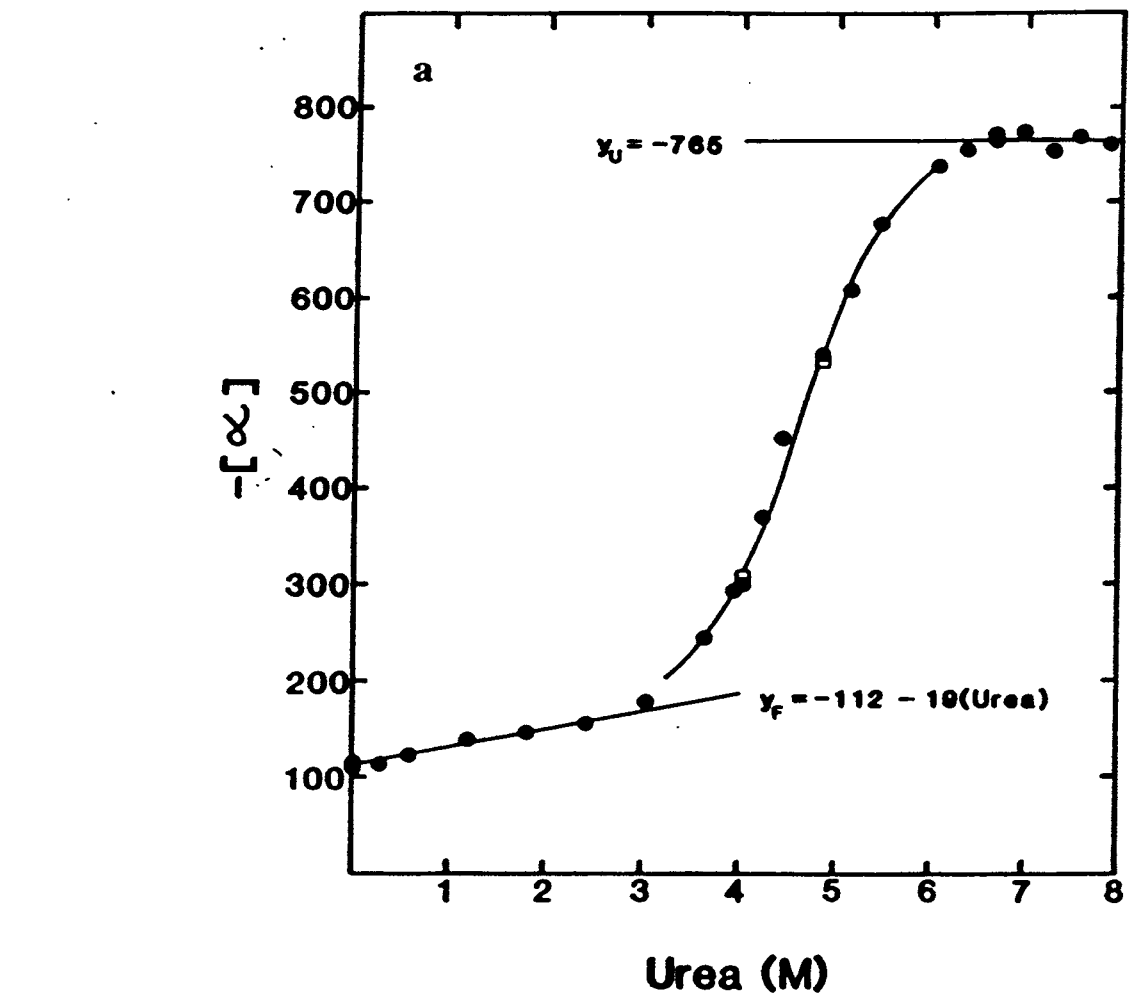
Analysis of a thermal unfolding curve

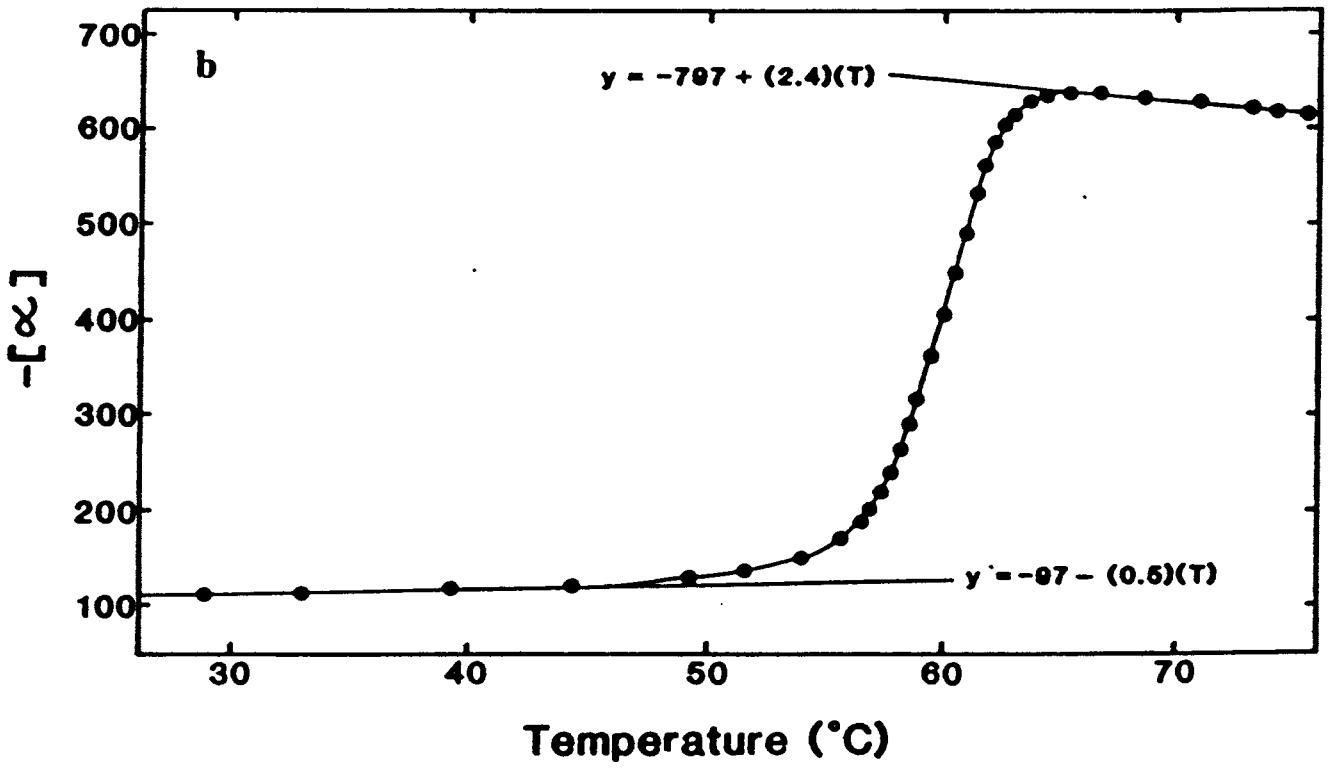
1. The data are from a thermal unfolding curve determined under the same conditions as the urea unfolding curve analysed in *Table 3*. Values of f_U, K and ΔG were calculated using Equations 2, 3 and 4, respectively. A least-squares analysis of the pre- and post-transition region gave $y_F = 87.2 + 0.66\,(T)$ and $y_U = 646$.

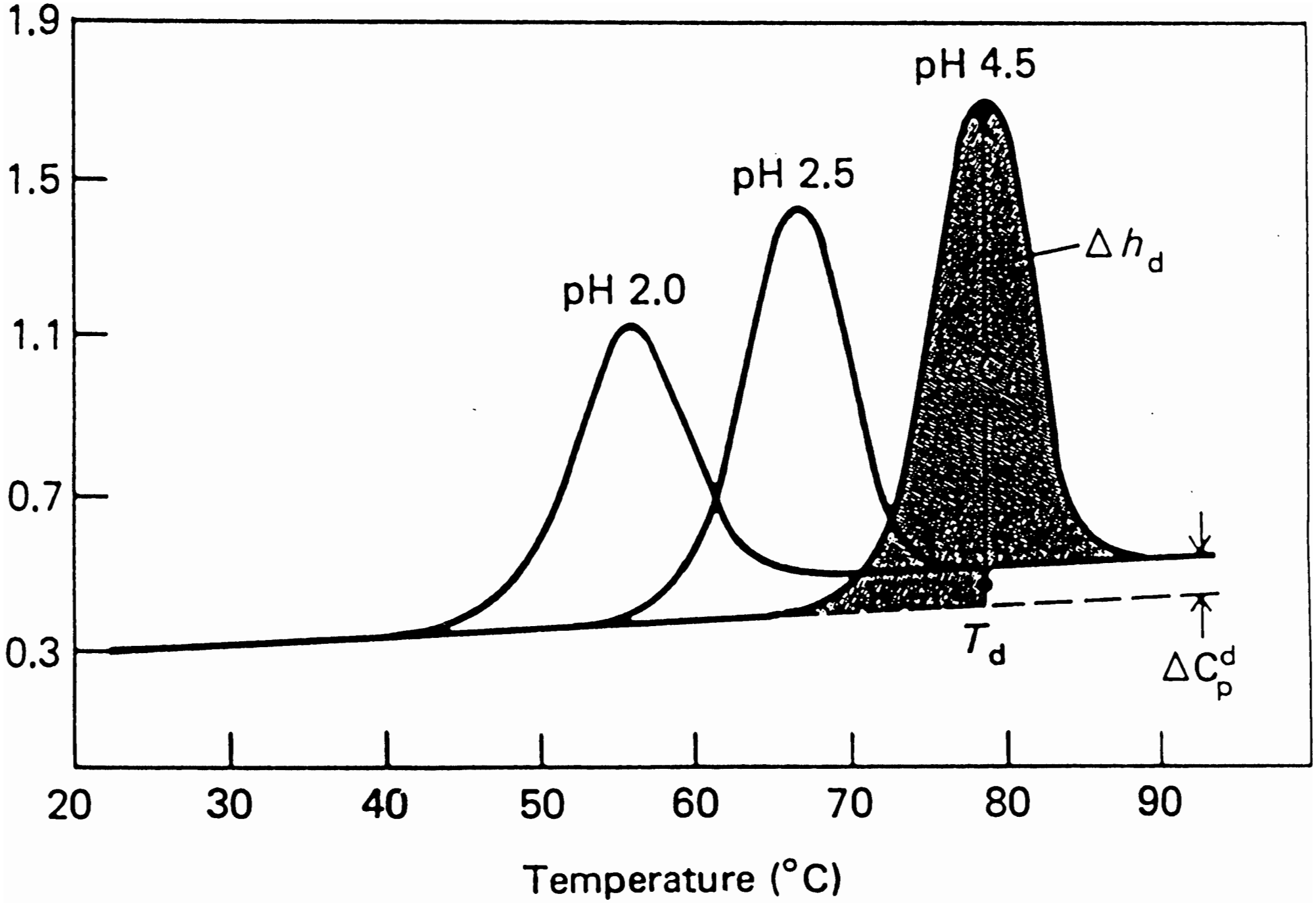
T (°C)	y[a]	f_U	K	ΔG (cal/mol)
16.2	98.1			
21.0	100.9			
25.6	103.7			
30.2	107.4			
45.4	221.3	0.197	0.245	890
46.3	263.9	0.277	0.383	610
47.2	313.9	0.371	0.589	337
48.1	367.6	0.472	0.894	72
49.0	422.2	0.575	1.353	−193
49.9	474.1	0.673	2.061	−464
50.8	518.5	0.757	3.123	−733
51.7	555.5	0.828	4.805	−1013
61.2	645.4			
65.5	646.3			
69.8	646.3			
73.8	645.4			

2. The slope of a plot of ΔG versus $T = -300.3$ cal/mol/deg, and $T = T_m = 48.3$°C at $\Delta G = 0$. Since $\Delta G = 0$ at T_m, $\Delta H_m = (T_m)(\Delta S_m)$. Therefore, $\Delta H_m = (48.3 + 273.2)(300.3) = 96.5$ kcal/mol. The slope of a van't Hoff plot (Equation 6) = −48631 K. Therefore, $\Delta H_m = -(1.987)(-48631) = 96.6$ kcal/mol. The values of ΔH obtained by taking the slope between individual points in the transition regions are: 99.9, 97.5, 94.7, 94.8, 96.7, 96.1 and 100.1 kcal/mol. Clearly, these data cannot be used to determine ΔC_p with Equation 7.
3. To estimate ΔG at 25°C, we use Equation 8. If we assume $\Delta C_p = 0$, the second term in the equation = 0, and $\Delta G(25°C) = 7.0$ kcal/mol. If we calculate ΔC_p using the rough rule of thumb mentioned in the text, ΔC_p = (12 cal/mol/deg/residue) × (104 residues) = 1250 cal/mol/deg. This leads to $\Delta G(25°C) = 7.0 - 1.1 = 5.9$ kcal/mol with Equation 8. Note that this is in excellent agreement with the value of $\Delta G(H_2O) = 5.7$ kcal/mol from the analysis of a urea unfolding curve in *Table 6*.

[a]The value of $-y$ is the specific rotation measured at 295 nm.


a
800
700
600
500
400
300
200
100
-[α]
$y_U = -765$
$y_F = -112 - 19(Urea)$
1
2
3
4
5
6
7
8
Urea (M)


b
700
600
500
400
300
200
100
-[α]
y = -797 + (2.4)(T)
y = -97 - (0.5)(T)
30
40
50
60
70
Temperature (°C)


1.9
1.5
1.1
0.7
0.3
pH 4.5
pH 2.5
pH 2.0
Δh_d
T_d
ΔC_p^d
20
30
40
50
60
70
80
90
Temperature (°C)