Electronic Supplementary Information

Two Different Regimes in Alcohol-Induced Coil-Helix Transition: Effects of 2,2,2-Trifluoroethanol on Proteins Being Either Independent of or Enhanced by Solvent Structural Fluctuations

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Table S1. The values of a_{33}, $\Delta\Gamma_{23}$, and unit conversion between mole fraction of TFE, x_{TFE}, and the molarity, M_{TFE}, shown in Figure 8. The helix contents at these TFE concentrations are calculated by Eqs. (19) and (20) with the reported values (ΔG_0 = 9.5 kJ mol^{-1}, m = 6 kJ mol^{-1} M^{-1}) for melittin. [1]

M_{TFE} mol L^{-1}	x_{TFE}	a_{33}	$\Delta\Gamma_{23}$	f_h
0.0315	0.000570	0.997	0.0644	0.0215
0.0525	0.000951	0.533	0.201	0.0226
0.106	0.00192	1.04	0.207	0.0256
0.279	0.00512	1.14	0.497	0.0388
0.545	0.0101	1.51	0.737	0.0719
0.793	0.0149	1.05	1.55	0.125

Table S1 (continued)

M_{TFE} mol L^{-1}	x_{TFE}	a_{33}	$\Delta\Gamma_{23}$	f_h
1.03	0.0195	1.02	2.06	0.203
1.39	0.0268	1.07	2.64	0.381
1.55	0.0302	0.827	3.81	0.478
1.67	0.0327	1.02	3.33	0.551
1.95	0.0388	0.917	4.33	0.711
2.23	0.0451	0.783	5.81	0.831
2.51	0.0516	0.668	7.67	0.908
2.79	0.0582	0.496	11.5	0.951
2.93	0.0615	0.460	13.0	0.965
2.93	0.0617	0.450	13.3	0.965
3.08	0.0654	0.402	15.6	0.976
3.20	0.0683	0.386	16.9	0.982
3.21	0.0688	0.396	16.6	0.982
3.36	0.0725	0.343	19.9	0.987
3.50	0.0763	0.313	22.8	0.991
3.52	0.0768	0.306	23.5	0.992
3.65	0.0803	0.298	24.9	0.994
3.79	0.0841	0.256	30.2	0.996
3.79	0.0841	0.253	30.5	0.996
3.94	0.0882	0.234	34.3	0.997
4.07	0.0920	0.235	35.3	0.998
4.09	0.0925	0.219	38.0	0.998
4.22	0.0962	0.213	40.3	0.998
4.34	0.0999	0.198	44.8	0.999
4.34	0.0999	0.188	47.1	0.999
4.65	0.109	0.175	54.1	0.999
4.91	0.118	0.169	59.3	1.00
5.20	0.127	0.160	66.4	1.00
5.50	0.137	0.156	71.8	1.00
5.64	0.142	0.169	67.9	1.00
5.90	0.152	0.163	73.6	1.00
6.19	0.163	0.168	75.2	1.00
6.46	0.174	0.175	75.2	1.00
6.74	0.186	0.181	75.9	1.00
7.07	0.200	0.189	76.3	1.00
8.44	0.271	0.271	63.4	1.00
9.87	0.370	0.515	39.1	1.00

Thermodynamic Equilibrium of Coil-Helix Transition

We note that Eq.(19) in the manuscript neglects a non-ideality of the protein solution as follows. Reaction Gibbs function, ΔG_r, for the equilibrium coil ⇌ helix of a protein is defined as

$$\Delta G_r = \mu_h - \mu_c, \qquad \text{(S1)}$$

where μ_h and μ_c are the chemical potentials of the helix and the coil, respectively. A virial expansion for expressing non-ideality of the protein solution gives their chemical potentials as [2]

$$\mu_h = \bar{\mu}_h + RT\ln(c_h) + 2RTMB_h c_h \qquad \text{(S2)}$$

and

$$\mu_c = \bar{\mu}_c + RT\ln(c_c) + 2RTMB_c c_c, \qquad \text{(S3)}$$

where $\bar{\mu}_h$ and $\bar{\mu}_c$ are the standard chemical potentials of the helix and the coil, respectively. c_h and c_c are the concentrations of the helix and the coil, respectively. B_h and B_c are the second virial coefficients of the helix and the coil, respectively; we assumed zero values for the higher-order terms of the coefficients such as the third virial coefficient. The second virial coefficient represents an effective intermolecular interaction of the protein; solvent effects are included. R is the gas constant. T is the thermodynamic temperature. The total concentration of the protein, c, is

$$c = c_h + c_c. \qquad \text{(S4)}$$

c_h and c_c are rewritten with use of f_h, a population of the helix or a fraction of the helix, as

$$c_h = f_h c \qquad \text{(S5)}$$

$$c_c = (1 - f_h)c. \qquad \text{(S6)}$$

With $\ln(c_h/c_c) = \ln(f_h/(1 - f_h))$, ΔG_r is given as

$$\begin{aligned}\Delta G_r &= \mu_h - \mu_c \\ &= \bar{\mu}_h + RT\ln(c_h) + 2RTMB_h c_h - (\bar{\mu}_c + RT\ln(c_c) + 2RTMB_c c_c) \\ &= (\bar{\mu}_h - \bar{\mu}_c) + RT\ln(c_h/c_c) + RTMc(f_h B_h - (1 - f_h)B_c) \\ &= (\bar{\mu}_h - \bar{\mu}_c) + RT\ln(f_h/(1 - f_h)) + RTMc(f_h B_h - (1 - f_h)B_c). \qquad \text{(S7)}\end{aligned}$$

ΔG_r at the equilibrium coil ⇌ helix is zero, and we define $\Delta G(c)$ as follows.

$$\Delta G(c) = -RT\ln(f_h/(1 - f_h)) - RTMc(f_h B_h - (1 - f_h)B_c) \qquad \text{(S8)}$$

$$\Delta G(c) = \bar{\mu}_h - \bar{\mu}_c \qquad \text{(S9)}$$

$\Delta G(c)$ is a function of c. When the protein solution is ideal (no intermolecular interaction; i.e., $B_h = 0$ and $B_c = 0$), μ_h and μ_c are represented as

$$\mu_h = \bar{\mu}_h + RT\ln(c_h) \qquad \text{(S10)}$$

$$\mu_c = \bar{\mu}_c + RT\ln(c_c). \qquad \text{(S11)}$$

And, $\Delta G_r = 0$ gives $\Delta G(c)$ as ΔG_{ideal}:

$$\Delta G_{ideal} = \bar{\mu}_h - \bar{\mu}_c = -RT\ln(f_h/(1 - f_h)), \qquad \text{(S12)}$$

where the subscript "ideal" indicates an ideal solution. Eq.(S12) indicates no protein-concentration dependence of ΔG_{ideal}. Eq.(S8) and Eq.(S9) are generalized formulas of the thermodynamic equilibrium of coil ⇌ helix, where the non-ideality is included. We write the non-ideal term in Eq.(S8) as $\Delta G_{non\text{-}ideal}$:

$$\Delta G(c) = \Delta G_{ideal} + \Delta G_{non\text{-}ideal} \qquad \text{(S13)}$$

$$\Delta G_{non\text{-}ideal} = -RTMc(f_h B_h - (1 - f_h)B_c). \qquad \text{(S14)}$$

These parameters require measurement of f_h at infinite dilution, while experiments by conventional spectroscopies such as circular dichroism are conducted at a finite concentration. Thus, concentration dependence should be involved in the experimentally determined population of the helix or a fraction of the helix. Here, we designate this as $f_h(c)$ and find

$$\lim_{c \to 0} f_h(c) = f_h. \qquad \text{(S15)}$$

The data experimentally available have been **"empirically"** treated as

$$-RT\ln(f_h(c)/(1 - f_h(c))) = A + mC_a, \qquad \text{(S16)}$$

where A and m values are constant, and C_a is the concentration of an additive such as alcohol in unit of molar. And, Eq.(S16) has also been deemed to be $\Delta G(c)$:

$$\Delta G(c) = A + mC_a \qquad \text{(S17)}$$

Accordingly, Eqs.(S12–17) give

$$\Delta G(c) = -RT\ln(f_h(c)/(1 - f_h(c)))$$

$$= -RT\ln(f_h/(1-f_h)) + \Delta G_{\text{non-ideal}} \qquad \text{(S18)}$$

$$RT\ln(f_h/(1-f_h)) - RT\ln(f_h(c)/(1-f_h(c))) = \Delta G_{\text{non-ideal}}. \qquad \text{(S19)}$$

$\Delta G_{\text{non-ideal}}$ reflects the difference between f_h and the experimentally given $f_h(c)$. Taken together, we used $\Delta G(c)$ in Eq.(S8) as ΔG in Eq.(19) of the manuscript with the assumption of $B_h = 0$ and $B_c = 0$:

$$\Delta G_{\text{non-ideal}} = 0 \text{ (i.e., } f_h(c) = f_h) \qquad \text{(S20)}$$

and

$$\Delta G(c) = \Delta G_{\text{ideal}}. \qquad \text{(S21)}$$

References

[1] N. Hirota, Y. Goto and K. Mizuno, *Protein Sci*., 1997, **6**, 416–421.

[2] B. Guo, S. Kao, H. McDonald, A. Asanov, L. L. Combs and W. W. Wilson, Correlation of Second Virial Coefficients and Solubilities Useful in Protein Crystal Growth. *J. Cryst. Growth*, 1999, **196**, 424–433.