LETTER

Why elliptical stability diagrams are not related to protein conformational disorder

Wiedersich *et al*. (1) try to prove that the pressure–temperature stability diagrams of simple proteins generically assume the shape of an ellipse because of correlated fluctuations in the transition volume and enthalpy ΔV, ΔH. The conventional view is that the reentrant phase behavior of proteins derives from a strongly temperature-dependent entropy and pressure-dependent volume due to hydrophobic interactions in the unfolded state (2). This leads to a recrossing of the stability line. Their treatment, first outlined in ref. 3, suffers from a basic statistical flaw. The authors consider fluctuations in the transition volume as being composed of volume fluctuations in the native (N) and denatured (D) states:

$$\delta\Delta V = \delta V_D - \delta V_N. \qquad \textbf{[1]}$$

From this they derive equations 10 and 12 of ref. 3 by ignoring cross-correlations:

$$\langle \delta\Delta V^2 \rangle = \langle \delta V_D^2 \rangle - \langle \delta V_N^2 \rangle. \qquad \textbf{[2]}$$

By squaring Eq. **1** and by taking the average one obtains instead:

$$\langle \delta\Delta V^2 \rangle = \langle \delta V_D^2 \rangle - 2\langle \delta V_N \times \delta V_D \rangle + \langle \delta V_N^2 \rangle. \qquad \textbf{[3]}$$

The cross-term vanishes because D and N belong to different statistical manifolds within the assumed two-state model. Eq. **2** thus violates the following statistical theorem: The variance of any linear combination of statistically independent variables is given by the sum (and not by the difference) of the variances of the individual variables. For this reason, equation 6 of ref. 1 does not follow from equation 5. Moreover, equation 3 of ref. 1 does not represent the correct ellipse condition, which is $\Delta\alpha^2\, T_0 - \Delta c_p \Delta\beta < 0$. The Δ implies a difference, which can be positive or negative. In conclusion, instead of revealing inherent conformational disorder in the native state, closed stability diagrams reflect changes in the thermodynamic susceptibilities and the fluctuation spectrum mainly due to exposure of hydrophobic residues.

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