Biochemistry

 Volume 36, Number 25 June 24, 1997

New Concepts in Biochemistry

High-Energy Channeling in Protein Folding†

Maria Silow and Mikael Oliveberg*

Biochemistry, Chemical Centre, P.O. BOX 124, S-221 00 Lund, Sweden

Received January 28, 1997; Revised Manuscript Received April 23, 1997[⊗]

ABSTRACT: Recent controversy about the role of populated intermediates in protein folding emphasizes the need to better characterize other events on the folding pathway. A complication is that these involve high-energy states which are difficult to target experimentally since they do not accumulate kinetically. Here, we explore the energetics of high-energy states and map out the shape of the free-energy profile for folding of the two-state protein U1A. The analysis is based on nonlinearities in the GdnHCl dependence of the activation energy for unfolding, which we interpret in terms of structural changes of the protein-folding transition state. The result suggests that U1A folds by high-energy channeling where most of the conformational search takes place isoenergetically at transition-state level. This is manifested in a very broad and flat activation barrier, the top of which covers more than 60% of the reaction coordinate. The interpretation favors a folding mechanism where the pathway leading to the native protein is determined by the sequence's ability to stabilize productive transition states.

To understand how unfolded proteins find their native conformation so rapidly is one of the major goals in folding studies. The question was raised by Levinthal (1968) who demonstrated that folding by random search of the conformational space is too slow to be accomplished within the life span of an organism. Several explanations have been centered around rapid formation of folding intermediates[1] since these are depicted to reduce the conformational freedom of the unfolded protein and, thus, speed up the search for productive interactions (Bryngelson *et al.*, 1995; Dill *et al.*, 1995; Karplus & Weaver, 1976; Sali *et al.*, 1994). However, the role of folding intermediates is controversial since proteins fold efficiently also without them. Intermediates that are populated under physiological conditions become destabilized and do not accumulate in the presence of denaturant (Matouschek *et al.*, 1990; Khorasanizadeh *et al.*, 1993; Oliveberg *et al.*, 1995; Oliveberg & Fersht, 1996a,b). This is seen most clearly at the midpoint for the unfolding transition where most proteins fold directly from the unfolded state and resolves the search problem without conformational restrictions at ground-state level [c.f. Abkevich *et al.* (1995) Gutin *et al.* (1995), and "type I" folding in Wolynes *et al.* (1996)]. The key to the folding problem may thus be found in the two-state process, and intermediates could just be a complication of multiple folding modules (Fersht, 1996; Tan *et al.*, 1996) or even off-pathway misfolds (Sosnick *et al.*, 1994; Kiefhaber, 1995; Silow & Oliveberg, 1997). But how is it possible to characterize two-state pathways where all partly folded states are unstable and never seem to accumulate? Fersht and co-workers have used stopped-flow kinetics to construct a free-energy profile for the folding reaction of the small two-state protein CI2 (Jackson & Fersht, 1991). The extent of interactions in the transition state for folding was then mapped out by studying the effects of point mutations on the activation energy (Itzhaki *et al.*, 1995). With CI2, it appears that secondary and tertiary interactions begin to form simultaneously in the transition state by so called

† Our work is supported by the Swedish Nautral Science Research Council and the Sven and Lilly Lawski Foundation for Natural Science.

* Corresponding author. Tel: +46-46-222-0107. Fax: +46-46-222-4534. Email: mikael.oliveberg@biokem.lu.se.

[⊗] Abstract published in *Advance ACS Abstracts*, June 1, 1997.

[1] Intermediates are here used to denote denatured conformations which are more stable than the unfolded state and, hence, accumulate in the refolding reaction. These also include compact disorganized globules.

S0006-2960(97)00210-9 CCC: $14.00

"nucleation condensation". It is argued that good nucleation sites are embryonic in unfolded polypeptides and become stable only after interacting with other parts of the protein (Fersht, 1996). If this happens first in high-energy conformations, the transition state is stabilized relative to the denatured ground state leading to an optimization of the folding rate. The mechanism is analogous to the principle of enzyme catalysis, where optimal rate is obtained when all intermediates on the reaction pathway are less stable than the starting materials and so do not accumulate (Fersht, 1974). In this study, we provide additional information about the folding energetics which extend the concept of nucleation condensation. With the spliceosomal protein U1A, the productive folding seems to take place predominantly at the top of a very broad and flat activation barrier. This implies that high-energy nucleation starts early in the folding process and then propagates isoenergetically at transition-state level until the protein is almost fully formed.

MATERIALS AND METHODS

Human U1A is a 102 residue protein without cysteines, expressed and purified as in Nagai *et al.* (1990). We used here a U1A mutant in which the semiburied phenylalanine 56 was substituted with a tryptophan. The F56W variant occurs naturally in U1A from potato (Simpson *et al.*, 1995) and produces large changes in fluorescence upon unfolding. This allows accurate detection of the folding process even at very low protein concentrations. Stability measurements were done by standard GdnHCl titration (Pace, 1986), using a Perkin−Elmer LS 50 luminescence spectrometer, and analyzed with the software package KaleidaGraph. Folding kinetics were studied by 1:10 dilution of denatured protein (in 5.1 M GdnHCl) with buffer and unfolding kinetics by 1:10 mixing of native protein (in buffer) into high [GdnHCl], using an SX.18MV stopped-flow instrument from Applied photophysics. Excitation was at 280 nm, and the emission was recorded with a 315 nm cut-off filter. Despite mixing solutions of rather different viscosity, the dead time was less than 5 ms. The buffer was 50 mM MES at pH 6.3, and the chemicals were of analytical grade (Sigma). The protein concentration was 1 μM unless otherwise stated. All instruments were thermostatted at 25 °C.

U1A May Either Fold Rapidly in a Two-State Process, or More Slowly from an Off-Pathway Aggregate, Depending on Solvent Conditions and Protein Concentration

In a two-state process, the equilibrium constant for unfolding (K_{D-N}) is given by the ratio of the unfolding and refolding rate constants (k_u and k_f) (Jackson & Fersht, 1991),

$$K_{D-N} = \frac{[D]}{[N]} = \frac{k_u}{k_f} \Rightarrow \log k_f = \log k_u - \log K_{D-N} \quad (1)$$

where D and N are the denatured and native protein respectively. The folding reaction of U1A was tested by eq 1 as follows.

Determination of log K_{D-N} vs [GdnHCl]. The GdnHCl dependence of the free-energy for unfolding ($m_{D-N} = \partial\Delta G_{D-N}/\partial[\mathrm{GdnHCl}]$) at the midpoint for the unfolding transition ($\mathrm{GdnHCl}_{1/2}$) was obtained from titration data according to (Pace, 1986)

$$\int f(\mathrm{GdnHCL}) = [(a_N + b_N[\mathrm{GdnHCl}]) + (a_D + b_D[\mathrm{GdnHCl}])10^{-m_{D\text{-}N}[[\mathrm{GdnHCl}] - \mathrm{GdnHCl}_{1/2}]/[2.3RT]}]/[1 + 10^{-m_{D\text{-}N}[[\mathrm{GdnHCl}] - \mathrm{GdnHCl}_{1/2}]/[2.3RT]}] \quad (2)$$

where $\int f(\mathrm{GdnHCl})$ is the area under the fluorescence spectrum between 320 and 370 nm, and ($a_D + b_D$[GdnHCl]) and ($a_N + b_N$[GdnHCl]) are baselines. The equilibrium constant for unfolding (log K_{D-N}) at each concentration of GdnHCl was then estimated by linear extrapolation (Schellman, 1978),

$$\log K_{D-N} = -(\Delta G_{D-N}^{H2O} + m_{D-N}[\mathrm{GdnHCl}])/2.3RT \quad (3)$$

where $\Delta G_{D-N}^{H_2O}$ is the protein stability in the absence of GdnHCl (Table 1, experimental data not shown). From mass action, the sensitivity to denaturant (m_{D-N}) reflects thenumber of GdnHCl binding sites that becomes accessible upon unfolding. Generally, m_{D-N} is considered to represent a measure of changes in solvent exposure upon denaturation (Pace, 1986).

Determination of the Kinetics and Test of the Two-State Criteria (Eq 1). The unfolding rate constant (log k_u vs [GdnHCl]) was measured by stopped-flow. Data obtained between 5−7 M GdnHCl (Figure 1) fit the polynomial function

$$\log k_u = -4.2(\pm 0.12) + 1.25(\pm 0.04)[\mathrm{GdnHCl}] - 0.061(\pm 0.003)[\mathrm{GdnHCl}]^2 \quad (4)$$

and eq 4 was used to represent log log k_u in eq 1. Finally, the refolding rate constant expected for a two-state process (log k_f^{calc}) was calculated from (eqs 1, 3, and 4)

$$\log k_f^{calc} = 2.65(\pm 0.18) - 0.43(\pm 0.09)[\mathrm{GdnHCl}] - 0.061(\pm 0.003)[\mathrm{GdnHCl}]^2 \quad (5)$$

and the result was compared with experimental values for log k_f vs [GdnHCl]. These were obtained by fitting of a second-order polynomial to the refolding data in Figure 1:

$$\log k_f = 2.50(\pm 0.02) - 0.36(\pm 0.02)[\mathrm{GdnHCl}] - 0.070(\pm 0.006)[\mathrm{GdnHCl}]^2 \quad (6)$$

At low concentrations of protein (1 μM), the observed refolding rate constant (eq 6) fits within the experimental error that calculated from log K_{D-N} and log k_u (eqs 1 and 5). The good agreement of the two curves, and the parameters in Table 1, suggest that the refolding of U1A takes place directly from D and without the accumulation of a folding intermediate (Jackson & Fersht, 1991). This comprises also that D and N interconvert over a common transition state at all concentrations of GdnHCl. At higher protein concentrations, U1A forms an intermediate-like aggregate in the dead time of the stopped-flow instrument which leads to a retardation of the refolding rate at [GdnHCl] < 1.5 M (Figure 1) (Silow & Oliveberg, 1997). In this study, we discuss only the fast two-state folding of the monomeric protein.

Monomeric U1A Folds Mainly by High-Energy Conformations in a Two-State Process

The activation barrier for unfolding ($\Delta G_{‡-N}$) is defined here as the free-energy difference between N and an apparent

Table 1: Fast Folding of U1A is a Two-State Process Which Seems To Take Place Directly from the Denatured State[a]

	$\Delta G^{H_2O}_{D-N}$ (kcal/mol)	$GdnHCl_{1/2}$ (M)	m_{D-N}
equilibrium unfolding	9.3 ± 0.2[b]	4.07 ± 0.02[b]	-2.3 ± 0.1[b]
kinetics	9.1 ± 0.2[c]	4.08[d]	-2.2 ± 0.1[e]

[a] This is supported by comparison of equilibrium and kinetic data (eq 1), which yield the same parameters for the protein stability (ΔG_{D-N}). $\Delta G^{H_2O}_{D-N}$ is the stability in pure water, $GdnHCl_{1/2}$ is the GdnHCl concentration at which $\Delta G_{D-N} = 0$, and m_{D-N} is the GdnHCl dependence of ΔG_{D-N}. [b] The value is determined by standard GdnHCl denaturation experiments (eqs 2 and 3). [c] $\Delta G^{H_2O}_{D-N} = -2.3RT \log K_{D-N} = -2.3RT(\log k_u - \log k_f)$ at [GdnHCl] = 0 M (eqs 1, 4, and 6). The derivation does not take into account the cis−trans equilibria of the prolines in D, which contribute to a small underestimate of $\Delta G^{H_2O}_{D-N}$ (Jackson & Fersht, 1991). The difference is within the experimental error. [d] Obtained from the intersect between the fits in Figure 1 where $\log k_f = \log k_u$ (eqs 4 and 6). [e] $m_{D-N} = m_{‡-N} - m_{D-‡}$ (eqs 1, 3, and 7; Jackson & Fersht, 1991).

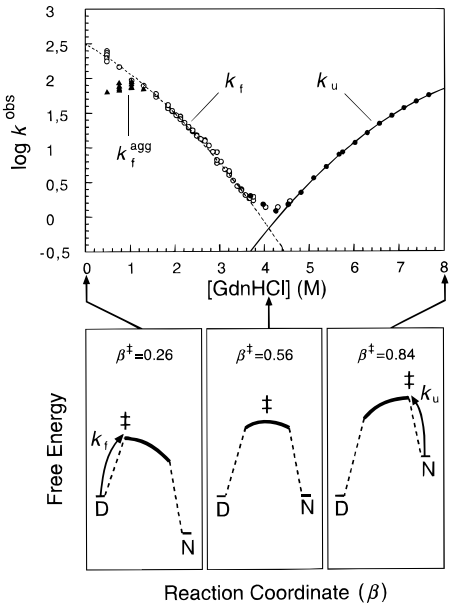

FIGURE 1: GdnHCl dependence of the rate constants for folding and unfolding of U1A, together with free-energy profiles for the folding reaction. Top panel. The rate constants were obtained by fitting of exponential functions to stopped-flow data and are in units of inverse seconds. The left arm of the v-shaped plot shows the refolding rate constant (k_f, ○), and the right arm shows the unfolding rate constant (k_u, ●). The curves are polynomial functions (eqs 4 and 6) which represent two-state folding directly from the denatured state (eq 1). The matching curvatures of log k_f and log k_u are suggested to result from structural changes of the transition state. The deviation from two-state folding observed at protein concentrations above ~3 μM (k_f^{agg}, ▲) is caused by transient aggregation of denatured protein under refolding conditions (Silow & Oliveberg, 1997). Bottom panels. Free-energy profiles for folding of the U1A monomer, obtained from the data in the top panel and eqs 7 and 8. The curvature of log k_f and log k_u indicates large movements of the transition state (‡) along the top of a very broad energy barrier. The degree of solvent exposure of ‡ is given by $\beta^‡$ (eq 8). In pure water, ‡ is relatively expanded (left panel), whereas at higher [GdnHCl], ‡ becomes more consolidated (right panel). This because the conformations along the reaction coordinate are destabilised by GdnHCl in proportion to their solvent exposure which causes the highest part of the energy barrier to move (Figure 2).

transition state for unfolding (‡). We use the term apparent transition state to emphasis that in high-dimensional reactions, like protein folding, all partly structured conformations represent ensemble averages. The transition-state ensemble may then be visualized as the saddle point in the free-energy landscape which is defined by the Boltzmann distribution of entities (Bryngelson *et al.*, 1995; Matthews & Fersht, 1995). Similar to the free-energy of unfolding, $\Delta G_{‡-N}$ is expected to describe an approximately linear dependence on [GdnHCl], where the gradient $m_{‡-N}$ reflects the change in solvent-accessible surface area upon activation (Tanford, 1970; Matouschek & Fersht, 1993; Parker *et al.*, 1995). $m_{‡-N}$ is obtained from the unfolding rate constant by

$$\log k_u = \log \frac{k_B T \kappa}{h} - \frac{\Delta G_{‡-N}}{2.3RT} \Rightarrow \frac{\partial \log k_u}{\partial [\mathrm{GdnHCl}]} = \frac{m_{‡-N}}{2.3RT} \quad (7)$$

where the left hand expression is Eyrings rate equation. The pre-exponential factor, $k_B T\kappa/h \approx 10^{13}$ s^{-1}, is the maximum rate of an elementary reaction step in small molecules (the vibrational frequency). This is most likely an overestimate for structural diffusion in proteins (Hagen *et al.*, 1996), but the term cancels upon derivation and will not affect our derivation of kinetic *m* values.

The proportionality between structural changes and *m* values allows a crude determination of the extent of structure in the transition state (‡) according to

$$\beta^‡ = 1 - \frac{m_{‡-N}}{m_{D-N}} \quad (8)$$

where m_{D-N} is the value for the D ⇄ N transition (eq 3), and $\beta^‡$ is a reaction coordinate for folding based on solvent exposure of the polypeptide (Tanford, 1970; Matouschek & Fersht, 1993; Parker *et al.*, 1995). Thus, if the structure of the transition state resembles the denatured state, $\beta^‡ = 0$, whereas if it resembles the native state, $\beta^‡ = 1$. In most other studies, m_{D-N} and $m_{‡-N}$ are approximately independent of [GdnHCl] (Jackson & Fersht, 1991; Muñoz *et al.*, 1994; Kiefhaber, 1995), although in some cases $m_{‡-N}$ decreases slightly at high concentrations of denaturant causing a curvature in plots of log k_u (eq 7) (Matouschek & Fersht, 1993). The reason for this change is unclear, mainly because the binding of denaturants like GdnHCl is poorly understood (Schellman, 1978; Pace, 1986). Since the equlibrium value m_{D-N} shows much smaller variations, however, it has been speculated that the curvature is due to changes in the structure of the transition state upon addition of denaturant (Matouschek & Fersht, 1993).

With U1A, the curvature of log k_u vs [GdnHCl] is unusually pronounced (Figure 1, eq 4). Notably, the folding reaction displays precisely the opposite curvature (eq 6), resulting in a constant value of m_{D-N} according to eq 1. The behavior is unlikely due to intrinsic effects of the GdnHCl binding. This since binding models (Schellman, 1978; Pace, 1986) and experimental findings (Pace *et al.*, 1990; Johnson & Fersht, 1995) suggest a decreasing sensitivity to denaturant with increasing [GdnHCl], as opposed to the accelerating GdnHCl dependence of log k_f in Figure 1. We conclude, therefore, that the coupled curvatures of log k_u and log k_f result from a shift of the structure of a common transition state and that $\beta^‡$ (eq 8) describes the movement of this transition state on the D ⇄ N reaction coordinate. As the value of $\beta^‡$ varies from 0.26 in pure water to 0.84 at [GdnHCl] = 8 M, the transition state appears to undergo large conformational changes upon destabilization. The behavior suggests a reaction profile with a very broad energy

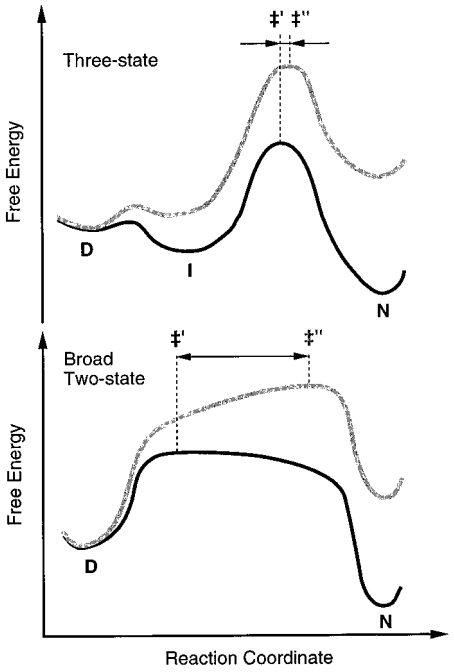

FIGURE 2: Movements of the transition state (‡) with [GdnHCl] can be explained by simple binding theory. The figure shows two types of reaction profiles for folding and how they change upon destabilization by GdnHCl. Profiles under native and denaturing conditions are shown in black and grey respectively. GdnHCl favors protein conformations which provide the highest number of GdnHCl binding sites. Therefore, the compact native structure (N) becomes destabilised relative to the extended and solvent accessible denatured state (D). Partly structured conformations, like folding intermediates (I), are destabilized by GdnHCl in proportion to their solvent exposure. The solvent exposure is here decreasing linearly along the reaction coordinate between D and N. Top. Three-state proteins have relatively narrow and highly structured activation barriers. The sharp activation barrier results in small movements of the transition state along the reaction coordinate (‡′,‡″) upon destabilization. Two-state kinetics is seen at [GdnHCl] where I is less stable than D. It is predicted that two-state proteins which fold via high-energy intermediates have related reaction profiles, i.e., sharp activation barriers which show small changes with [GdnHCl]. Bottom. The large changes in solvent exposure of the transition state of U1A suggests a very broad and flat activation barrier. Under native conditions, the transition state of U1A is rather solvent accessible and close to D on the reaction coordinate (‡′). Upon addition of GdnHCl, the structured end of the broad activation barrier experiences a greater destabilization than the less structured end. This causes its highest point to move along the reaction coordinate towards N (‡″).

barrier where the more consolidated conformations experience greater destabilization upon addition of GdnHCl (Figures 1 and 2). Although the height of the activation barrier is uncertain and relies on the applicability of transition-state theory, the width and top surface are easily obtained from the kinetic *m* values, which allow free-energy extrapolation of a compact transition state to low [GdnHCl] and *vice versa* (eqs 3 and 7). It is important to point out that low-dimensional free-energy extrapolations give still a limited picture of the high-dimensional energy landscape for folding. For example, transition states observed at high [GdnHCl] may not be on the pathway at low [GdnHCl] if other folding channels open up under stabilizing conditions, i.e., the protein crosses the broad activation barrier diagonally. It is also difficult to distinguish experimentally whether the movement of the transition state is smooth or proceeds over a series of discrete free-energy maxima along the reaction coordinate (Matthews & Fersht, 1995). At the extreme, however, changes between few and well-separated barriers are identified by sharp kinks in the chevron plot, as observed in the unfolding reaction of Arc repressor (Jonsson *et al.*, 1996). Despite these limitations, the reaction profile obtained here provides a useful description of the macroscopic pathway for comparisons with theory. This is nicely illustrated by recent calculations on folding kinetics by Finkelstein and Badredtinov (1997) and by Plotkin *et al.* (1997). The latter study yields also large movements of the transition state upon destabilization.

Is the Conformational-Search Problem in Protein Folding Resolved at Transition-State Level?

Although high-energy folding is a feature of all two-state proteins, previously studied proteins seem to have more narrow and pointed activation barriers than U1A. This is indicated by the lack of curvature in plots of log k_u vs [denaturant] (Jackson & Fersht, 1991; Viguera *et al.*, 1994; Kragelund *et al.*, 1995). With narrow activation barriers, only a limited part of the folding reaction takes place at transition-state level and there may be substantial free-energy dips in other parts of the reaction profile. Such local free-energy minima would represent on-pathway intermediates which, although they do not populate, may indicate a folding mechanism similar to that of three-state proteins (Figure 2). With U1A, on the other hand, we observe a reaction where most of the structure forms at transition-state level. The high-energy nucleation is here likely to start early in the folding process and to propagate isoenergetically by a series of increasingly structured transition states, until the protein finally drops down in the potential well of the native conformation (Figure 2). U1A may thus represent a new class of proteins which fold by high-energy channeling. The concept implicates a mechanism where the pathway leading to the native protein is determined by the sequence's ability to stabilize productive transition states and not by conformational restrictions in the denatured state or in high-energy intermediates. In a highly evolved system, this would be manifested in a low and flat transition barrier where losses in conformational freedom are precisely balanced by gains in intramolecular interactions. If the nucleation is too strong, this would cause overcompensation and stabilization of intermediates which slow down the folding process.

ACKNOWLEDGMENT

We thank Dr. Kiyoshi Nagai and co-workers for generously providing the U1A clones and Dr. Alan Fersht and his group for help with expression systems.

REFERENCES

Abkevich, V. I., Gutin, A. M., & Shakhnovich, E. I. (1995) *Protein Sci. 4*, 1167−1177.

Bryngelson, J., Onuchic, J. N., Socci, N. D., & Wolynes, P. (1995) *Proteins 21*, 167−195.

Dill, K. A., Bromberg, S., Yue, K., Fiebig, K. M., Yee, D. P., Thomas, P. D., & Chan, H. S. (1995) *Protein Sci. 4*, 561−602.

Fersht, A. R. (1974) *Proc. R. Soc. London, Ser. B 187*, 397−407.

Fersht, A. R. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 10869−10873.

Finkelstein, A. V., & Badredtinov, A. Y. (1997) *Folding Des. 2*, 115−121.

Gutin, A. M., Abkevich, V. I., & Shakhnovich, E. I. (1995) *Biochemistry 34*, 3066−3076.
Hagen, S. J., Hofrichter, J., Szabo, A., & Eaton, W. A. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 11615−11617.
Itzhaki, L. S., Daniel, E. O., & Fersht, A. R. (1995) *J. Mol. Biol. 254*, 260−288.
Jackson, S. E., & Fersht, A. R. (1991) *Biochemistry 30*, 10428−10435.
Johnson, C. M., & Fersht, A. R. (1995) *Biochemistry 34*, 6975−6804.
Jonsson, T., Waldburger, C. D., & Sauer, T. (1996) *Biochemistry 35*, 4795−4802.
Karplus, M., & Weaver, D. C. (1976) *Nature 260*, 404−406.
Khorasanizadeh, S., Peters, I. D., Butt, T. R., & Roder, H. (1993) *Biochemistry 32*, 7054−7063.
Kiefhaber, T. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 9029−9033.
Kragelund, B. B., Robinson, C. V., Knudsen, V., Dobson, C. M., & Poulsen, F. M. (1995) *Biochemistry 34*, 7217−7224.
Levinthal, C. (1968) *J. Chim. Phys. 85*, 44−45.
Matouschek, A., & Fersht, A. R. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 7814−7818.
Matouschek, A., Kellis, J. T., Jr., Serrano, L., Bycroft, M., & Fersht, A. R. (1990) *Nature 346*, 440−445.
Matthews, J. M., & Fersht, A. R. (1995) *Biochemistry 34*, 6805−6814.
Muñoz, V., Lopez, E. M., Jager, M., & Serrano, L. (1994) *Biochemistry 33*, 5858−5866.
Nagai, K., Oubridge, C., Jessen, T.-H., Li, J., & Evans, P. R. (1990) *Nature 348*, 515−520.
Oliveberg, M., & Fersht, A. R. (1996a) *Biochemistry 35*, 2726−2737.
Oliveberg, M., & Fersht, A. R. (1996b) *Biochemistry 35*, 2738−2749.
Oliveberg, M., Tan, Y. J., & Fersht, A. R. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 8926−8929.
Pace, C. N. (1986) *Methods Enzymol. 131*, 266−279.
Pace, C. N., Laurents, D. V., & Thomson, J. A. (1990) *Biochemistry 29*, 2564−2572.
Parker, M. J., Spencer, J., & Clarke, A. R. (1995) *J. Mol. Biol. 253*, 771−786.
Plotkin, S. S., Wang, J., & Wolynes, P. G. (1997) *J. Chem. Phys. 106*, 1−17.
Sali, A., Schaknovich, E. I., & Karplus, M. (1994) *Nature 369*, 248−251.
Schellman, J. A. (1978) *Biopolymers 17*, 1305−1322.
Silow, M., & Oliveberg, M. (1997) *Proc. Natl. Acad. Sci. (*in press).
Simpson, G. G., Clark, G. P., Rothnie, H. M., Boelens, W., van Venrooij, W., & Brown, J. W. S. (1995) *EMBO J. 14*, 4540−4550.
Sosnick, T. R., Mayne, L., Hiller, R., & Englander, S. W. (1994) *Nat. Struct. Biol. 1*, 149−156.
Tan, Y. J., Oliveberg, M., & Fersht, A. R. (1996) *J. Mol. Biol.* 264, 377−389.
Tanford, C. (1970) *Adv. Protein Chem. 24*, 1−95.
Viguera, A. R., Martinez, J. C., Filimonov, V. V., Mateo, P. L., & Serrano, L. (1994) *Biochemistry 33*, 2142−2150.
Wolynes, P. G., Luthey-Schulten, Z., & Onuchic, J. N. (1996) *Chem. Biol. 3*, 425−432.

BI970210X