Redesigning the Folding Energetics of a Model Three-helix Bundle Protein by Site-directed Mutagenesis*

Dahabada H. J. Lopes‡§§, Alex Chapeaurouge‡‡§¶¶, Gavin A. Manderson**, Jonas S. Johansson**‡‡‡, and Sérgio T. Ferreira‡‡

From the ‡Departamento de Bioquímica Médica, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-580, Brazil, the §Departamento de Fisiologia e Farmacodinâmica, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, RJ 21045-900, Brazil, and the ¶¶Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Because of their limited size and complexity, de novo designed proteins are particularly useful for the detailed investigation of folding thermodynamics and mechanisms. Here, we describe how subtle changes in the hydrophobic core of a model three-helix bundle protein (GM-0) alter its folding energetics. To explore the folding tolerance of GM-0 toward amino acid sequence variability, two mutant proteins (GM-1 and GM-2) were generated. In the mutants, cavities were created in the hydrophobic core of the protein by either singly (GM-1; L35A variant) or doubly (GM-2; L35A/I39A variant) replacing large hydrophobic side chains by smaller Ala residues. The folding of GM-0 is characterized by two partially folded intermediate states exhibiting characteristics of molten globules, as evidenced by pressure-unfolding and pressure-assisted cold denaturation experiments. In contrast, the folding energetics of both mutants, GM-1 and GM-2, exhibit only one folding intermediate. Our results support the view that simple but biologically important folding motifs such as the three-helix bundle can reveal complex folding plasticity, and they point to the role of hydrophobic packing as a determinant of the overall stability and folding thermodynamics of the helix bundle.

Proteins that explore multiple regions of the free energy surface by redesign represent versatile tools for the elucidation of folding mechanisms. Prominent examples of such proteins are the small IgG binding domain of protein G (1) and the formin-binding protein 28 WW domain (2). Kinetic folding studies of these proteins revealed that the folding pathway could be substantially changed upon the introduction of mutations in the hydrophobic core. The folding of protein G switched to that of the structurally related protein L, while the free energy surface of the formin-binding protein could be tuned from three-state to two-state kinetics. In the present study, we have chosen a thermodynamic approach and addressed the question of whether a simple folding motif like the three-helix-bundle reveals folding plasticity in terms of different populations of equilibrium folding intermediates upon the introduction of single and double mutations in the hydrophobic core. Investigating the folding of small (<100 amino acids) single domain proteins is especially intriguing. Single domain proteins are generally considered to be fast, two-state folders. However, increasing experimental (3, 4) and theoretical (5, 6) evidence supports the notion that the folding of these molecules may also proceed via well defined, partially folded kinetic or thermodynamic intermediate states. Recently, we showed that a de novo designed native-like three-helix bundle protein, αβ-1, exhibits two partially folded equilibrium intermediate states (7). Here, we use a similar de novo designed three-helix bundle protein (GM-0) as a model system for dissecting its folding energetics and to examine the influence of changes in the packing of the hydrophobic core. The design of GM-0 was based on the classic heptad repeat motif found in coiled-coil proteins and consists of six distinct hydrophobic core layers, each comprising three amino acids. The “mutant” GM-1 carries a leucine to alanine substitution at one of the hydrophobic d positions (L35A), whereas GM-2 has an additional isoleucine to alanine replacement at heptad position a of helix II (L35A/I39A). The introduction of the smaller hydrophobic side chains of Ala was intended to create cavities in the core region of the variant proteins, thus destabilizing their native states and possibly their equilibrium folding intermediates. Remarkably, the present findings indicate that the helix bundle GM-0 populates two equilibrium intermediate states, whereas only one folding intermediate is populated in both hydrophobic core mutants.

**EXPERIMENTAL PROCEDURES**

Reagents—Bis-ANS1 was from Molecular Probes (Eugene, OR). All other reagents were of the highest analytical grade available. Prediluted water was filtered and deionized through a Millipore purification system.

Protein Synthesis—The design and synthesis of GM-0 was based on that of the previously described de novo designed protein αβ-1 (8), with two amino acid replacements at positions 42 (leucine to methionine) and 51 (cysteine to valine). The amino acid sequence of GM-0 is shown in Scheme 1 in single-letter codes. The helical diagram of GM-0 (Scheme 1) depicts the coiled-coil heptad repeat positions a and d and identifies the location of the residues replaced in GM-1 and GM-2. The N terminus of the protein is acetylated, and the C terminus is amidated to avoid charge repulsion.

GdnHCl Denaturation—GM-0, GM-1, and GM-2 (5 μM for all proteins) were incubated for 2 h at 23 °C in 20 mM Tris-HCl, 130 mM NaCl, pH 7, in the presence of increasing concentrations of GdnHCl.

1 The abbreviations used are: bis-ANS, 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid; GdnHCl, guanidine hydrochloride.
measurements showed that no further spectroscopic changes (CD and fluorescence) took place after 2 h of incubation with GdnHCl (not shown). Unfolding was monitored by the loss of CD signal at 222 nm and/or by the red shift of the intrinsic fluorescence emission.

Circular Dichroism—Far-UV CD spectra were recorded on a model 62 DS spectropolarimeter (Avid, Lakewood, NJ) using 2-mm path length quartz cells, a bandwidth of 1 nm, a scan step of 0.5 nm, and a time constant of 3 s. The cell holder was maintained at 25 ± 0.1 °C.

Fluorescence Measurements—Unless otherwise indicated, fluorescence emission spectra were measured at 25 °C on an ISS-PC spectrofluorometer (ISS Inc., Champaign, IL). For intrinsic fluorescence measurements, excitation was at 280 nm, and emission spectra were recorded from 300 to 420 nm. Bis-ANS fluorescence was excited at 375 nm and spectra were measured after 10 min of equilibration at each pressure. Control measurements showed that no additional fluorescence changes took place after 10 min at each pressure step. All experiments were performed in 20 mM Tris-HCl, pH 7, containing 130 mM NaCl. Unless otherwise indicated, the protein concentration in all experiments was 2 μM (determined using ε280 = 5,700 M⁻¹ cm⁻¹), and the concentration of bis-ANS, when used, was 3 μM. At temperatures below 15 °C the windows of the pressure bomb were flushed with nitrogen to prevent water condensation. Fluorescence spectral centers of mass (intensity-weighted average emission wavelengths) were calculated with software provided by ISS Inc. as shown in Equation 1,

\[ \lambda_{av} = \sum \lambda I(\lambda) / \sum I(\lambda) \]  

where \( \lambda \) is the emission wavelength and \( I(\lambda) \) represents the fluorescence intensity at wavelength \( \lambda \).

Determination of the Equilibrium Constant and Volume Change of Unfolding—The thermodynamic equilibrium between the native-like state and the unfolded protein is described by the relation depicted in Equation 2,

\[ K = \alpha / (1 - \alpha) \]  

where \( K \) is the equilibrium unfolding constant and \( \alpha \) is the degree of unfolding. The effect of hydrostatic pressure on the unfolding equilibrium can be described by Ref. 7 and shown in Equation 3,

\[ K_p = K_0 \exp(p \Delta V / R T) \]  

where \( K_p \) is the equilibrium unfolding constant at pressure \( p \), \( K_0 \) is the equilibrium constant at atmospheric pressure, \( \Delta V \) is the molar volume change of folding, and \( R \) and \( T \) have their usual meanings.

In the present work, changes in the spectral center of mass of the intrinsic fluorescence emission of the different proteins were used to monitor the degree of unfolding. Shifts in the spectral center of mass were converted into the extent of denaturation \((\alpha_p)\) at each pressure according to the following phenomenological relationship (7), seen in Equation 4,

\[ \alpha_p = 1 / (1 + (\lambda_{av} - \lambda_{av}^0)/(\lambda_{av} - \lambda_{av}^0)) \]  

where \( \lambda_{av} \) and \( \lambda_{av}^0 \) are the spectral centers of mass of native-like and fully unfolded proteins, respectively, and \( \lambda_{av} \) represents the spectral center of mass at pressure \( p \). The standard volume change was obtained from the slope of the linear plot of ln \((\alpha_p / (1 - \alpha_p))\) versus pressure according to equations 2 and 3, whereas the equilibrium constant at atmospheric pressure was obtained from the intercept on the \( y \)-axis in this plot.

At constant pressure, the temperature dependence of the equilibrium constant for an unfolding transition can be described by the van't Hoff equation in Equation 5,

\[ \Delta G / T = (1 / T) \Delta H - \Delta S = -R \ln K_p \]  

where \( K_p \) is the equilibrium constant for unfolding at temperature \( T \), and \( \Delta G \) is the corresponding Gibbs free energy change. From a plot of \( G / T \) versus the inverse temperature, the changes in enthalpy (\( \Delta H \)) and entropy (\( \Delta S \)) of unfolding can be extracted.

RESULTS

Unfolding by GdnHCl—The equilibrium unfolding of GM-0 by GdnHCl, as monitored by the red-shift of the intrinsic fluorescence emission, displayed a sigmoidal profile typical of that expected for a two-state unfolding transition (Fig. 1). Replacement of Leu-35 (GM-1 variant) and Leu-35/Ile-39 (GM-2 variant) by smaller side-chain alanine residues resulted in major decreases of 3.6 kcal/mol in the free energy of unfolding of GM-0 (Table 1). A number of studies have shown that cavity-creating mutations or packing defects are energetically unfavorable (for examples, see Refs. 10 and 11). Leucine to alanine or isoleucine to alanine mutations in the protein (11), a value that is very similar to the observed decrease in stability of GM-1 in comparison with GM-0 (Table 1). Interestingly, the double mutant GM-2 showed only a slight additional decrease in stability as compared with GM-1.

Of note is that all three proteins displayed highly cooperative, two-state unfolding transitions without an indication of the existence of equilibrium intermediate states. Analysis of the equilibrium unfolding data with a two-state model yielded Gibbs free energy changes of unfolding of 6.8, 3.4,
...and 3.1 kcal/mol for GM-0, GM-1, and GM-2, respectively (Table I).

Pressure Denaturation of GM-0, GM-1, and GM-2—Application of increasing hydrostatic pressure to GM-0, GM-1, and GM-2 caused pronounced red shifts of the intrinsic fluorescence emission of the three proteins (Fig. 2), indicating increased exposure of the single Trp residue located in the hydrophobic core of the proteins (heptad position a in helix 2) to the aqueous medium. For the three proteins, the spectral centers of mass initially increased with pressure and reached well defined plateaus at high pressures (>3 kilobar in the case of GM-0 and >2 and 1.5 kilobar for GM-1 and GM-2, respectively; Fig. 2). Of note is that the fully unfolded proteins (i.e. in the presence of 6 M GdnHCl) exhibited spectral centers of mass of 355 nm (see Fig. 4), whereas the pressure-denatured states reached plateau values at 342, 348, and 349 nm for GM-0, GM-1 and GM-2, respectively (Fig. 2). Therefore, the pressure-denatured states of these proteins can be interpreted as partially unfolded intermediate states. In addition, the pressure denaturation curves of all three proteins measured at higher concentrations (10 μM; Fig. 2, open symbols) did not exhibit any shifts when compared with the transitions observed at low protein concentrations (2 μM; Fig. 2, closed symbols). These findings support the view that the plateaus of the pressure denaturation curves can be interpreted as equilibrium folding intermediates and rule out possible contributions from protein aggregation. Importantly, the transitions were fully reversible for the three proteins, as indicated by the fact that the spectral centers of mass recovered completely to their original values upon decompression (asterisks in Fig. 2).

Cold Denaturation of GM-0, GM-1, and GM-2—It is well known that elevated hydrostatic pressures cause a depression in the freezing point of water (12). This allowed us to carry out pressure-assisted cold denaturation experiments on GM-0 and its cavity-creating variants in the absence of cosolvent additives. Fig. 3 shows cold denaturation of GM-0, GM-1 and GM-2 at 3.5 kilobar of pressure as monitored by the red-shifts of their intrinsic fluorescence emission. Decreasing temperature caused a progressive red shift in the fluorescence emission of GM-0, and the spectral center of mass reached a plateau at 347 nm at −5 °C. Comparison of the spectral center of mass of the cold denatured state of GM-0 with that of the fully unfolded protein (in 6 M GdnHCl) revealed a difference of ~8 nm (Fig. 4), indicating that another equilibrium folding intermediate was stabilized at low temperature. Upon the return of the sample to room temperature, the fluorescence spectral center of mass returned to its original value, reflecting the complete reversibility of the process. Interestingly, the fluorescence emission spectra of GM-1 and GM-2 were unaffected by decreasing temperature, with no red shifts of the spectral centers of mass down to −10 °C (Fig. 3). These results indicate that GM-0 undergoes cold denaturation under pressure, reaching a state that is different from the fully unfolded state, whereas GM-1 and GM-2 do not undergo cold denaturation in the temperature range down to −10 °C. Thermodynamic parameters obtained from cold denaturation data for GM-0 are shown in Table I.

Bis-ANS Binding Studies—To further characterize the folding intermediates of the three-helix bundle proteins, we have used the amphiphilic dye bis-ANS. Bis-ANS is a fluorescent probe whose quantum yield increases markedly with the increasing hydrophobicity of the environment. Because of its non-covalent binding to exposed hydrophobic domains on proteins, bis-ANS has been extensively used to probe conformational changes and folding of a number of proteins (for recent...
examples, see Refs. 7 and 13–15). Surface-exposed hydrophobic domains are frequently found in folding intermediates such as molten globule states. These conformations are characterized by organized, native-like secondary structures but lack well defined tertiary interactions (16). For GM-0, the fluorescence intensity of bis-ANS increased 2-fold in the transition from the native-like to the pressure-denatured state, and an additional marked increase (2-fold) occurred in the cold-denatured state (Fig. 5A). A significant blue shift of the emission of bis-ANS was also observed in the cold-denatured state relative to the native and pressure-denatured states (Fig. 5A). These results indicate that the pressure- and cold-denatured states of GM-0 bear features that are typical of molten globule states. By contrast, no significant increases in bis-ANS binding were detected when GM-1 and GM-2 were compressed and subjected to low temperatures under the same conditions (Fig. 5B).

It is interesting to note that the individual free energy changes of the transitions to the pressure-denatured states of GM-0, GM-1, and GM-2 and the cold-denatured state of GM-0 are lower than the overall free energy change (ΔG_m) obtained from the full unfolding of the three proteins at high concentrations of GdnHCl (Table I). These observations support the idea that equilibrium folding intermediates are stabilized under these experimental conditions.

DISCUSSION

In this work, we investigated the equilibrium unfolding of distinct de novo designed three-helix bundle proteins using hydrostatic pressure and low temperatures to detect the existence of partially folded intermediates. Hydrophobic residues in the core region of GM-0 were mutated to evaluate their contributions to the folding plasticity of the protein. Based on pressure and cold denaturation studies, we conclude that native-like GM-0 populates two partially folded equilibrium intermediate states, whereas the mutants GM-1 and GM-2 (in which specific leucine and isoleucine residues were replaced by alanine residues) reveal only one folding intermediate under identical experimental conditions.

It is interesting to compare our findings to those reported for similar experiments on natural proteins. Studies on ubiquitin, a small single-domain protein, revealed an obligatory kinetic folding intermediate stabilized at low GdnHCl concentrations (3). Interestingly, upon replacement of the hydrophobic core residue valine 26 by alanine or glycine, the folding behavior of ubiquitin changed to a cooperative two-state transition (3). This observation was explained by the destabilization of the folding intermediate in the mutant proteins to such an extent that it was no longer observable. In addition, the authors also predicted that some proteins with apparent two-state folding may exhibit more complex transitions under well chosen stabilizing conditions. Good examples of this situation are the immunity proteins Im7 and Im9 (4, 17). These four-helix bundle proteins exhibit ~60% sequence identity to each other but fold by different mechanisms. Kinetic experiments carried out under physiological conditions revealed a productive on-pathway folding intermediate for Im7, whereas Im9 appeared to be a simple two-state folder (4). Extending those studies to acidic pH, however, revealed intermediate states for the folding transition of Im9 as well (17). It was concluded that intermediate formation is a general characteristic of immunity protein folding, depending on the range of folding conditions explored. Whereas the above mentioned studies focused on the population or not of kinetic folding intermediates, the literature also holds examples of changes in the folding thermodynamics of proteins upon subtle differences in physicochemical conditions or point mutations (18–20). Equilibrium folding studies of hen egg white lysozyme, for example, revealed an intermediate stabilized in the GdnHCl-induced denaturation at pH 0.9 as evidenced by CD-spectroscopy (18). In contrast, (un)folding of lysozyme induced by GdnHCl at a slightly higher pH (2.2) shows a single cooperative transition. Also striking is the effect of a single mutation on the denaturation mechanism of the transcription regulator factor for inversion stimulation; a proline to alanine mutation at a central helix is sufficient to alter the equilibrium folding transition of the 22-kDa protein from two-state to three-state while its biological function is maintained (20).

Application of hydrostatic pressure usually brings about reversible partial unfolding of proteins in aqueous solution, because the unfolded state occupies a smaller volume than the native state. The volume reduction is likely due to electrostriction and to hydrophobic solvation of the unfolded protein, as well as to the existence of packing defects and void volumes in the folded native state (21). The contribution of electrostriction to the volume change is considered to be relatively small, as most of the charged side chains in proteins are usually surface-exposed in their native states (22). Controversy still exists as to whether the exposure of hydrophobic groups to water brings about negative or positive contributions to the volume change. Early studies based on the transfer of model hydrophobic compounds from non-polar solvents to water concluded that the exposure of buried hydrophobic residues provides a negative volume change (23). A decade later, however, Masterton and Seiler presented evidence indicating that interactions between aliphatic groups and water yield a positive ΔV (24). Recent pressure denaturation studies on site-directed mutants of monomeric staphylococcal nuclease in which internal cavity sizes were altered by amino acid substitution support the view that the loss of internal void volume is the major driving force of pressure-induced unfolding (25). Substitution of a central valine to smaller glycine or alanine residues yielded changes in ΔV upon pressure denaturation that were very similar to the differences between the van der Waals volumes of the individual amino acid side chains. A similar conclusion was reached in a recent investigation of the pressure denaturation of two naturally occurring variants of bovine β-lactoglobulin, which differ by the substitution of a valine residue by an alanine residue in the hydrophobic core of the protein (26). To estimate the expected differences in volume changes (ΔΔV) for the substitution of Leu-35 and Leu-35/Ile-39 residues of GM-0 by alanine residues of GM-1 and GM-2, respectively, we used the van der Waals volumes of the corresponding amino acid side chains (27). As indicated in Table I, the predicted values of ΔΔV for GM-1 and GM-2 were 34 and 69 ml/mol, respectively. In the case of GM-1, this compares well with the experimentally ob-
Redesigning the Folding Energetics of a Model Protein

Fig. 5. Pressure and cold denaturation monitored by bis-ANS fluorescence. A, bis-ANS fluorescence emission spectra in the presence of native (trace 1), pressure-denatured (3.5 kilobar) (trace 2), and cold-denatured GM-0 (trace 3). B, bis-ANS fluorescence emission spectra in the presence of native (c), pressure-denatured (3.5 kilobar) (c) and cold-denatured GM-1 (c).

served volume change of 32 ml/mol, indicating that the solvent inaccessible cavity contributes significantly to the value of ΔV. More complex is the situation for GM-2. Clearly, the experimentally determined ΔΔV of 35 ml/mol is significantly greater than the predicted value (69 ml/mol) and very close to that of GM-1. Most likely, the hydrophobic core region of GM-2 has adapted to the amino acid substitutions by repacking of the side chains. Side chains in protein cores contact, on average, two or three other side chains, rendering protein cores a multibody problem (28). Thus, predicting the possible effects of substitutions is rather difficult. In addition, de novo designed proteins seem to be more flexible and malleable than their natural counterparts. For example, NMR analysis of the structure of αD, a de novo designed three-helix-bundle protein, showed that the protein adjusted in different ways to either alanine to leucine or alanine to isoleucine substitutions at the same position in the hydrophobic core (29). Whereas the leucine mutant showed reduced dispersion of the amide backbone and methyl side-chain resonances, the isoleucine mutant yielded well defined chemical shift dispersions, an indicator of native core packing.

Cold denaturation has been observed for a variety of native (for examples, see Refs. 30 and 31) and de novo designed proteins (7, 14, 32). Hydrophobic interactions, which are believed to be the major stabilizing forces of protein cores, are destabilized at low temperatures, leading to protein unfolding (33). Therefore, the partial unfolding of GM-0 at low temperature (Fig. 3) suggests that a hydrophobic collapse step is involved in the folding process. Consistent with the reduced hydrophobic packing of their internal residues, GM-1 and GM-2 did not undergo cold denaturation under the same conditions as GM-0. This shows that even subtle, conservative amino acid substitutions in the hydrophobic core may lead to profound changes in the folding mechanism of a protein, including the existence or not of a hydrophobic collapsed folding intermediate in equilibrium.

A number of simulation studies of the folding of natural and designed helical proteins are consistent with the idea that single domain proteins can exhibit non-cooperative folding trajectories (5, 6). For example, different folding scenarios have been obtained in simulations in which only the difference between the strength of native and non-native contacts in the protein was manipulated (5). It thus appears that folding can change from a cooperative two-state transition to one that includes up to two kinetic intermediate states. In addition, a rigorous thermodynamic calculation of the folding free energy of a three-helix bundle protein provided a detailed description of an equilibrium intermediate state characterized by strong interactions between two helices before the third helix docks onto this subdomain to complete the fold (6). The latter study is of particular interest to our findings in that the partial denaturation of GM-0 to the cold denatured state (Ic) involves an on-pathway pressure-denatured intermediate (Ic). Changes in protein folding pathways can also be rationalized by globally considering the free energy surface (34). Slight modifications in the amino acid sequence of a protein may have a small impact on the topography of the free energy surface. However, different parts of the unfolded free energy basin may be populated upon mutation and folding may follow a different path toward the global energy minimum. If, for example, such an alternative pathway is characterized by an intermediate basin, a two-state folder switches to a three-state folding transition (34).

In conclusion, our results support the notion that de novo protein design provides a valuable platform for characterizing the equilibrium folding transitions of biologically relevant structured motifs and might eventually also aid in the understanding of misfolding events and protein aggregation phenomena in a number of human diseases (35).

REFERENCES
1. Nauli, S., Kuhlman, B., and Baker, D. (2001) Nat. Struct. Biol. 8, 602–605
2. Nguyen, H., Jager, M., Moretto, A., Gruebele, M., and Kelly, J. W. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 3948–3953
3. Khorasanizadeh, S., Peters, I. D., and Roder, H. (1996) Nat. Struct. Biol. 3, 193–205
4. Capaldi, A. P., Shastry, M. C., Kleanthous, C., Roder, H., and Radford, S. E. (2001) Nat. Struct. Biol. 8, 68–72
5. Zhou, Y., and Rapsis, M. (1999) Nature 401, 400–403
6. Rozko, E. M., and Brooks, C. L., III (1995) Science 269, 393–396
7. Chapeaurouge, A., Johansson, J. S., and Ferreira, S. T. (2001) J. Biol. Chem. 276, 14861–14866
8. Johansson, J. S., Gibney, B. R., Skalicky, J. J., Wand, A. S., and Dutton, P. P. (1998) J. Am. Chem. Soc. 120, 3881–3886
9. Paladini, A. A., Jr., and Weber, G. (1981) Biochemistry 20, 2587–2593
10. Lim, W. A., Farruggio, D. C., and Sauer, R. T. (1992) Biochemistry 31, 4324–4333
11. Jackson, S. E., Moracci, M., Shasby, N., Johnson, C. M., and Fersht, A. R. (1993) Biochemistry 32, 11259–11269
12. Bridgman, P. W. (1931) The Physics of High Pressure, pp. 233–243, G. Bell and Sons, Ltd., London
13. Seale, J. W., Martinez, J. L., and Horwitz, P. M. (1995) Biochemistry 34, 7443–7449
14. Chapeaurouge, A., Johansson, J. S., and Ferreira, S. T. (2002) J. Biol. Chem. 277, 16473–16483
15. Martins, S. M., Chapeaurouge, A., and Ferreira, S. T. (2002) Eur. J. Biochem. 269, 5484–5491
16. Pitay, O. B. (1995) Trends Biochem. Sci. 20, 376–379
17. Gorski, S. A., Capaldi, A. P., Kleanthous, C., and Radford, S. E. (2001) J. Mol. Biol. 312, 849–863
18. Sasaahara, K., Demura, M., and Nitta, K. (2002) Proteins 49, 472–482
19. Godbole, S., Dang, A., Garbin, K., and Bowler, B. E. (1997) Biochemistry 36, 119–126
20. Hobart, S. A., Meinhold, D. W., Osuna, R., and Colon, W. (2002) Biochemistry 41, 13744–13754
21. Mochavare, V. V., Heremans, K., Frank, J., Masson, P., and Balny, C. (1996) Proteins 24, 81–91
22. Weber, G., and Drickamer, H. G. (1983) Q. Rev. Biophys. 16, 89–112
23. Kaszmann, W. (1959) in Advances in Protein Chemistry (Anfinsen, J. C. B., Bailey, K., Anson, M. L., and Edsall, M. T., eds) pp. 1–66, Academic Press, New York
24. Masterton, W. L., and Seiler, H. (1960) J. Chem. Phys. 22, 4257–4262
25. Frye, K. J., and Royer, C. A. (1998) Protein Sci. 7, 2217–2222
26. Botelho, M. M., Valente-Mesquita, V. L., Oliveira, K. M., Polikarpov, I., and
Ferreira, S. T. (2000) Eur. J. Biochem. 267, 2235–2241
27. Richards, F. M. (1974) J. Mol. Biol. 82, 1–14
28. Eastwood, M. P., and Wolynes, P. G. (2001) J. Chem. Phys. 114, 4702–4716
29. Walsh, S. T., Sukharev, V. I., Betz, S. F., Vekshin, N. L., and DeGrado, W. F. (2001) J. Mol. Biol. 305, 361–373
30. Antonino, L. C., Kautz, R. A., Nakano, T., Fox, R. O., and Fink, A. L. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7715–7718
31. Lassalle, M. W., Li, H., Yamada, H., Akasaka, K., and Redfield, C. (2003) Protein Sci. 12, 66–72
32. Boice, J. A., Dieckmann, G. R., DeGrado, W. F., and Fairman, R. (1996) Biochemistry 35, 14480–14485
33. Privalov, P. L. (1996) Crit. Rev. Biochem. Mol. Biol. 25, 281–305
34. Gruebele, M. (2002) Curr. Opin. Struct. Biol. 12, 161–168
35. Ferreira, S. T., and De Felice, F. G. (2001) FEBS Lett. 498, 129–134
Redesigning the Folding Energetics of a Model Three-helix Bundle Protein by Site-directed Mutagenesis

Dahabada H. J. Lopes, Alex Chapeaurouge, Gavin A. Manderson, Jonas S. Johansson and Sérgio T. Ferreira

J. Biol. Chem. 2004, 279:10991-10996.
doi: 10.1074/jbc.M308174200 originally published online December 30, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M308174200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 33 references, 5 of which can be accessed free at http://www.jbc.org/content/279/12/10991.full.html#ref-list-1