Protein Internal Flexibility and Global Stability: Effect of Urea on Hydrogen Exchange Rates of Bovine Pancreatic Trypsin Inhibitor†

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ABSTRACT: The hydrogen isotope exchange kinetics of buried NH protons in bovine pancreatic trypsin inhibitor (BPTI) was measured in 8 M urea at 30 °C and pH 3.5. The data were analyzed by the two-process model in which slower exchanging protons utilize an unfolding mechanism and more rapidly exchanging protons exchange from the folded state. Urea accelerates the set of protons exchanging by the unfolding mechanism, all of which have approximately the same exchange rate constants in urea. For protons in this set, the ratio of exchange rate constants in the presence and absence of urea is used to estimate $\Delta\Delta G_{(0\rightarrow 8M\ urea)}$ = 6.6 kcal/mol. For the set of protons exchanging from the folded state, 8 M urea either has no effect or slows exchange. Slowing of exchange by urea implies binding of urea to sites at or near the exchanging proton. Some buried protons exchanging from the folded state have diminished rates in 8 M urea, meaning that urea is accessible to these buried sites. Several unassigned side-chain NH's of arginine or lysine are highly protected from exchange by urea, suggesting that they are the location of urea binding sites on the surface of the molecule.

The internal motions underlying hydrogen exchange in a native protein are local excursions from an average approximated by the crystal structure. More rapid rates of hydrogen isotope exchange imply greater motion in the region of the exchanging proton. The observed rate constant at one pH and temperature, k_{obs}, does not, however, yield a frequency nor an amplitude of the putative fluctuations. Rather, k_{obs} is compared to the model compound exchange rate constant, k_{cx}. Model compounds are oligopeptides or other secondary amides that have the same chemical reaction for exchange (attack by H^+ or OH^- and then H-bonding to water followed by hydrogen isotope exchange), but no contribution from protein tertiary structure. The ratio of k_{obs} to k_{cx} at the same pH and temperature is expressed as the probability, β, for exposure of that proton to solvent for exchange or in the reciprocal as the factor expressing its protection from exchange. Values of β for different protons in one protein at a single pH and temperature may vary between 0.5 and $<10^{-8}$.

The interpretation of hydrogen exchange kinetics, then, requires explanation of the large differences in β values among the many exchanging protons in a protein and of the protein conformational fluctuations supporting exchange. The first step in analyzing hydrogen exchange data is the determination of which protons exchange from the interconverting ensemble of folded states and which exchange only when the protein first unfolds. The dual pathway of exchange is formalized in the two-process model (Woodward & Hilton, 1980; Woodward et al., 1982). Denaturants and/or mutations will affect each process differently. Destabilization of the protein by denaturant or mutation will accelerate exchange by the unfolding mechanism by a factor proportional to the unfolding/folding equilibrium constant, but it will have only local effects on fluctuations responsible for exchange from the folded state. That is, exchange from the folded state is not correlated with global stability. Exchange by the unfolding mechanism, accordingly, is correlated with global stability.

In this article, we examine the effect of the chemical denaturant, urea, on hydrogen exchange kinetics of BPTI. In other studies, we show the effect of single site mutations on folding thermodynamics and hydrogen exchange behavior (Kim et al., 1993b) and aromatic ring flip rates (K.-S. Kim, unpublished results). Urea and mutational effects on hydrogen exchange affirm the two-process model, and the separation of effects on each process leads to several conclusions. Exchange rates identify submolecular motional domains that are key structural elements in protein folding and function (Kim et al., 1993b). Protons exchanging by the unfolding mechanism may be used to estimate $\Delta\Delta G_{(WT\rightarrow destabilized\ protein)}$, the change in the folding/unfolding free energy difference between WT and denaturant- or mutant-destabilized protein. Internal motions in folded proteins permit the access of some buried sites to urea.

MATERIALS AND METHODS

NMR spectra were acquired as described in Kim et al. (1993b). Assignments were made in 90% ^1H_2O/10% ^2H_2O. Hydrogen exchange in urea was initiated by dissolving protein (4.5 mM) in deuterated 8.0 M urea solution (99% ^2H_2O) and adjusting the pH to 3.5 with ^2HCl (glass electrode reading). Urea was deuterated by dissolution in ^2H_2O and lyophilization. For the first 30 min, one-dimensional ^1H NMR spectra were recorded after equilibrating the sample in the NMR probe at 30 °C for 5 min. The magnitude mode COSY spectra were recorded thereafter with a 33-min data collection time. Hydrogen exchange was followed up to 21 days. Exchange rate constants were derived from a least-squares fit of cross-peak signal intensity by the equation, $\ln (I) = -kt + C$, where I is the signal intensity normalized by the internal standard (non-exchangeable proton) at time t, k is the first-order exchange rate constant (min^{-1}), t is the elapsed time (min) after the protein was dissolved in ^2H_2O, and C is the logarithmic value of the initial intensity of the signal at dead time.

RESULTS

Bovine pancreatic trypsin inhibitor (BPTI) COSY cross peaks in 8 M urea were assigned by comparison to COSY

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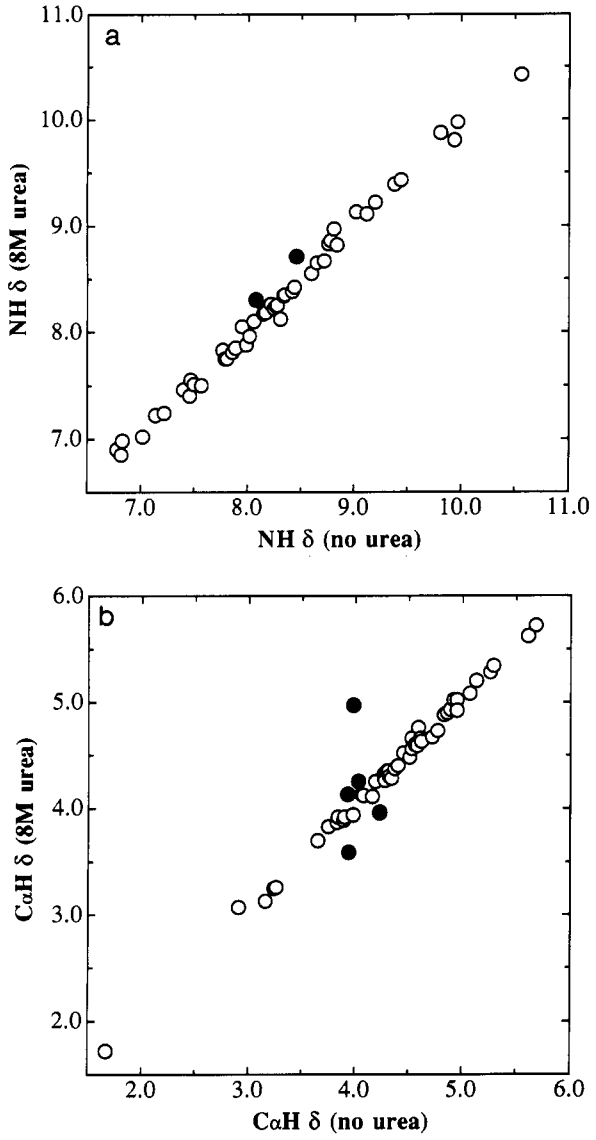

FIGURE 1: Comparison of chemical shifts of BPTI in ^2H_2O with and without 8 M urea at pH 3.5 and 30 °C. Proton chemical shifts (δ) in ppm from TSP are given for (a) NH resonances and (b) α-CH resonances. Protons with chemical shift differences >0.2 ppm are indicated by filled circles. A table of chemical shifts in 8 M urea is available as supplementary material.

spectra in water and confirmed by NOESY and HOHAHA connectivities. Assignments in water were previously made by Wagner et al. (1987). Few chemical shifts are different in the presence and absence of 8 M urea (Figure 1). Differences of >0.2 ppm, indicated by filled circles, are observed for β-sheet NH protons exposed to solvent (residues 30 and 32), α-CH protons located in the flexible loops (37 and 39), and α-CH protons at the C-terminus (53, 57, and 58). The largest differences are observed for the α-CH of Arg 39 and Arg 53.

Exchange rate constants of backbone NH protons with and without 8 M urea at 30 °C and pH 3.5 are given in Table I. Rate constants of protons exchanging too slowly to be measured at 30 °C in the absence of urea were extrapolated from high-temperature data as described in Kim et al. (1993b). Protons not listed in Table I exchange too rapidly to be measured under these conditions and are almost all on the surface of the protein.

Urea also slows the exchange of several side-chain NH protons which usually exchange too rapidly to be measured in the time window of these experiments. Figure 2 shows a portion of the COSY spectrum in 8 M urea taken 24 h after the protein was dissolved in ^2H_2O. Several unassigned resonances in the spectral region of lysine and arginine side-chain NH (top) are observed in urea after 24 h; without urea these exchange in less than 1 h.

Table I: Hydrogen Exchange Rate Constants (min^{-1}) of BPTI Backbone NH with and without 8 M Urea at 30 °C and pH 3.5

residue number	no urea	8 M urea
5	3.8×10^{-4}	5.0×10^{-4}
6	2.2×10^{-4}	5.0×10^{-5}
7	1.2×10^{-4}	1.6×10^{-4}
10	4.7×10^{-4}	4.0×10^{-4}
14	3.3×10^{-3}	1.3×10^{-4}
16	5.8×10^{-4}	5.4×10^{-4}
17		8.2×10^{-2}
18	5×10^{-7} [a]	2.7×10^{-5}
19	4.6×10^{-3}	1.1×10^{-3}
20	9×10^{-8} [a]	5.8×10^{-5}
21	2×10^{-9} [a]	4.0×10^{-5}
22	2×10^{-9} [a]	5.3×10^{-5}
23	2×10^{-9} [a]	5.0×10^{-5}
24	7×10^{-7} [a]	3.0×10^{-5}
25		2.2×10^{-3}
27	1.2×10^{-3}	4.0×10^{-4}
28	4.1×10^{-4}	4.2×10^{-5}
29	3×10^{-6} [a]	3.4×10^{-5}
30		3.4×10^{-3}
31	5×10^{-7} [a]	3.2×10^{-5}
32	1.9×10^{-3}	3.8×10^{-4}
33	6×10^{-7} [a]	5.5×10^{-5}
34	2.3×10^{-3}	2.1×10^{-4}
35	2×10^{-6} [a]	5.5×10^{-5}
36	7.7×10^{-5}	
38		4.8×10^{-4}
41	4.0×10^{-4}	1.9×10^{-4}
44	2×10^{-6} [a]	7.0×10^{-5}
45	6×10^{-7} [a]	5.8×10^{-5}
51	5.1×10^{-5}	3.6×10^{-5}
52	2.6×10^{-5}	1.0×10^{-4}
53	4.6×10^{-5}	2.1×10^{-4}
54	2.4×10^{-4}	1.0×10^{-4}
55	1.8×10^{-5}	1.5×10^{-4}
56	8.0×10^{-4}	1.6×10^{-3}

[a] Hydrogen exchange rates are extrapolated from higher temperature data as described in Kim et al. (1993b).

DISCUSSION

Two-Process Model of Hydrogen Exchange in Proteins. In a native protein at a given temperature and pH, the observed hydrogen exchange rate constant of one proton is the sum of the rate constants for exchange by two pathways (Woodward & Hilton, 1980; Woodward et al., 1982), as summarized in the Appendix. There are several practical consequences of dual pathways for exchange. First, at any pH and temperature, some protons may exchange by the unfolding mechanism while at the same time, in the same protein, other protons exchange by the native-state mechanism. Second, the range of k_{obs} values that are actually measured for a protein depends on the magnitude of K_{eq}, the equilibrium constant for unfolding/folding. An unstable protein has a relatively large K_{eq}, so that for many of its protons at neutral pH, $k_u \gg k_f$ (see the Appendix); these protons will all exchange by the unfolding mechanism, and the slow end of the distribution of k_f will not be observed. For a very stable protein such as BPTI, K_{eq} is small and a large range of k_f values is observed.

The two exchange mechanisms can be distinguished by their temperature dependence (Woodward et al., 1982). Exchange by the unfolding mechanism (eq 3) will have an apparent activation energy approximately equal to the enthalpy of the

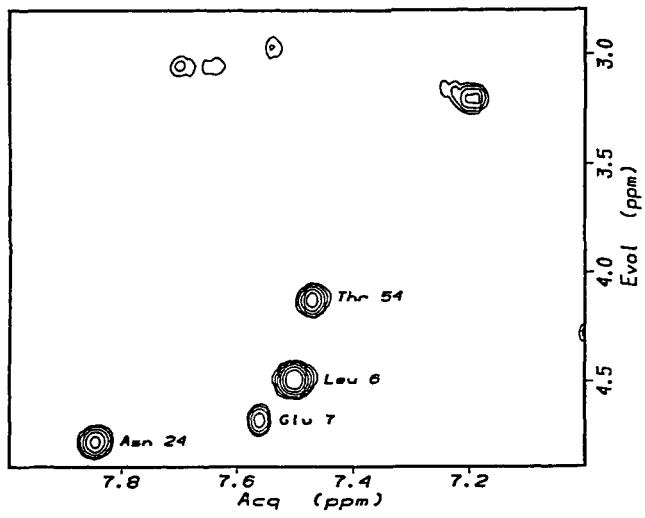

FIGURE 2: Part of the COSY spectrum of BPTI in 8 M urea taken 24 h after dissolution in ^2H_2O. Lysine amine or arginine guanido NH resonances are unlabeled cross peaks at the top.

unfolding equilibrium constant plus 13–17 kcal/mol (for k_{cx}), and so the unfolding mechanism is sometimes referred to as the high activation energy mechanism. Rate constants for exchange from the folded state typically have activation energies between 17 and 40 kcal/mol: about 17 kcal/mol for k_{cx} and 0–23 kcal/mol for the conformational process(es). Because of its larger temperature coefficient, exchange by the unfolding mechanism tends to dominate at higher temperatures, and as the temperature is raised, some protons switch from the folded-state mechanism to the unfolding mechanism. This is reflected in curved Arrhenius plots (ln k_{obs} versus $1/T$) in which the high-temperature slope gives an activation energy of approximately the sum of the unfolding enthalpy and the activation energy for k_{cx} and the low-temperature slope gives an activation energy in the range 17–40 kcal/mol (Woodward & Hilton, 1980; Wedin et al., 1982). A switch in mechanism is also observed for some of the slower exchanging protons in destabilized mutants of BPTI (Tao et al., 1993; Kim et al., 1993b).

Exchange rate constants of several slower exchanging protons in BPTI at pH 3.5 are reported at 36 and 68 °C (Hilton & Woodward, 1979; Wagner, 1983) and at 45 °C (Richarz et al., 1979). We have examined whether these data can be represented by simulations derived from a simple two-process model, assuming for the high-temperature (unfolding) mechanism one activation energy for all protons, and for the low-temperature (folded state) mechanism a different activation energy for each proton (in the range 17–40 kcal/mol). Since the enthalpy of unfolding/folding is temperature dependent (observed as the characteristically large denaturational heat capacity change), the assumption of a temperature-independent unfolding enthalpy is only an approximation; however, it seems a reasonable simplification given the limited data and the small temperature interval. In Figure 3, the dotted line labeled k_u indicates the temperature dependence of the WT rate constant for exchange by the unfolding mechanism only. This line is the fit over the range 45–74 °C to the observed rate constants for Tyr 21, Phe 22, and Tyr 23. The apparent activation energy is 78 kcal/mol;[1] it is extrapolated by the dotted line to lower temperatures.

[1] At higher temperatures (68–80 °C), a higher activation energy (88 kcal/mol) is reported (Roder et al., 1985). The higher activation energy at higher temperature may reflect the temperature dependence of the unfolding/folding enthalpy change.

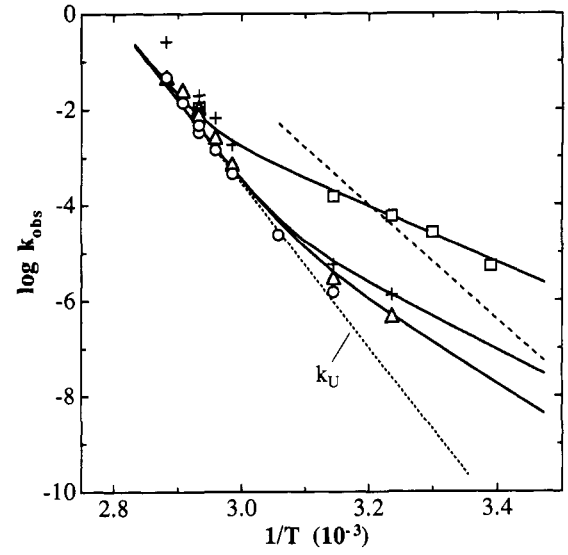

FIGURE 3: Temperature dependence of NH exchange rate constants of residues 31, 45, and 52 at pH 3.6. Solid lines show simulated curves of k_{obs} as a function of temperature, assuming that $k_{obs} = k_f + k_u$ and that at each temperature k_u is given by the dotted line while k_f is given by extrapolation of the observed rate constant at 36 °C, using an activation energy of 40, 32, or 27 kcal/mol for Gln 31, Phe 45, or Met 52, respectively. The dashed line is the temperature dependence of k_u for the destabilized mutant G37A, discussed in the text. Experimental data show the average of observed rate constants for Tyr 21, Phe 22, and Tyr 23 (○) and observed rate constants of Gln 31 (△), Phe 45 (+), and Met 52 (□) (Richarz et al., 1979; Hilton & Woodward, 1979; Wagner, 1983).

Since experimental values of the rate constants for 21–23 are very similar, their average is shown by the open circles in Figure 3.

Simulated curves for log $k_{obs,i}$ versus $1/T$ are given in Figure 3 for Gln 31, Phe 45, and Met 52; they are calculated by assuming $k_{obs,i}$ is the sum of $k_{u,i}$ and $k_{f,i}$ eq 1 (Appendix). At any temperature, $k_{u,i}$ is given by the dotted line in Figure 3, and $k_{f,i}$ is calculated using the experimental rate constant at 36 °C and activation energies of 40, 32, and 27 kcal/mol for Gln 31, Phe 45, and Met 52, respectively. The simulated curves give a reasonable fit to the experimental rate constants of 31, 45, and 52 at temperatures other than 36 °C, shown as data points. The deviation of the high-temperature experimental points of Phe 45 (+) from the simulated curve can be explained by a nearest-neighbor effect; the model peptide value of k_{cx} for Phe 45 (Molday et al., 1972) is twice the average model compound exchange rate of Tyr 21, Phe 22, and Tyr 23.

While the simulated curves in Figure 3 do not prove the two-process model, they illustrate several important features of the model. (1) The relative values of $k_{f,i}$ and $k_{u,i}$ may change with temperature or pH, and i may switch exchange mechanism as temperature or pH is varied. (2) If exchange from the folded state is negligible for the slowest exchanging protons, their exchange in WT and mutants can be used to determine $\Delta\Delta G_{(WT \rightarrow mut)}$, the change in overall stability due to the mutation. (3) The behavior of the slowly exchanging protons in destabilized mutants can be predicted. In Figure 3, for example, the dashed line represents k_u versus $1/T$ for destabilized mutant G37A, obtained as the rate constants of 21, 22, and 23 in G37A (Kim et al., 1993b); the position and slope of the line depend on K_{eq} for the mutant. The dashed line representing k_u for the mutant is higher and has a smaller slope than for WT (dotted line) because the mutant has a larger equilibrium constant and a smaller enthalpy change for the folding/unfolding reaction (eq 3, Appendix). In this

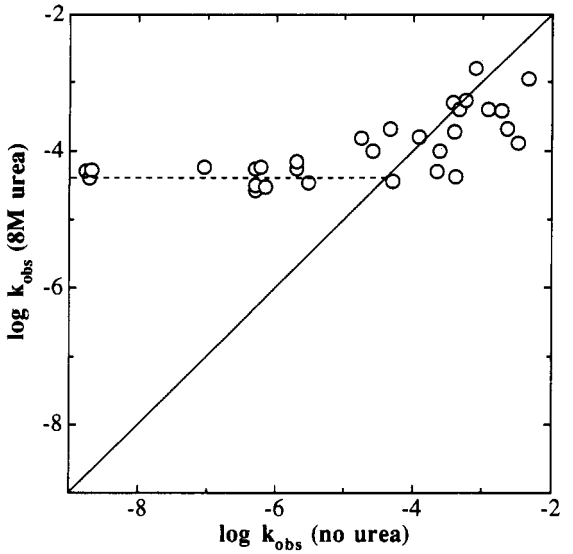

FIGURE 4: Comparison of BPTI hydrogen exchange rate constants with and without 8 M urea at pH 3.5 and 30 °C. Data are taken from Table I. Symbols indicate the exchange rate constant for the same proton in urea (y-axis) and in the absence of urea (x-axis).

mutant, $k_u > k_f$ for Gln 31 and Phe 45, and 31 and 45 will exchange by unfolding over the whole temperature range. That is, 31 and 45 exchange by different mechanisms in mutant and WT. However, for Met 52 in the mutant at temperatures <35 °C, $k_f > k_u$ and this proton is expected to switch exchange mechanism around 35 °C [see also Tao et al., (1993)].

Urea Effects on Hydrogen Exchange. Comparison of exchange rates in the presence and absence of 8 M urea is shown in Figure 4. Each circle represents one proton plotted to indicate its exchange rate constant with urea (y-axis) and without urea (x-axis). Protons exchanging faster, slower, or the same in urea will fall, respectively, to the left, to the right, or on the diagonal. Two urea effects are observed. In urea the slowest exchanging protons are accelerated, and all have approximately the same $k_{obs} \cong 5 \times 10^{-5}$ min^{-1}. In contrast, the more rapidly exchanging protons have either the same or slower exchange rates in urea. These two behaviors reflect the different effect of urea on the two exchange mechanisms. The substantial destabilization of the protein by urea increases the equilibrium constant for cooperative unfolding and thereby increases k_u (eq 3, Appendix). A large urea effect is therefore observed for the slower exchanging protons for which k_f is small. In the absence of urea $k_u < k_f$, but in 8 M urea $k_u > k_f$; addition of urea switches the exchange mechanism for these protons. For the more rapidly exchanging protons, k_f is large, and even in the presence of 8 M urea $k_u \ll k_f$. These protons still exchange from the folded state in 8 M urea. Some protons exchanging from the folded state do not have altered exchange rates in 8 M urea, and a number of protons have slower exchange rates. In poly(D,L-alanine), base-catalyzed exchange rates are decreased about 2.5-fold by the addition of 8 M urea (Loftus et al., 1986). At pH 3.5 exchange is based-catalyzed, and the slower exchange of the protons to the right of the diagonal in Figure 4 is explained by a decrease in k_{cx} of the protons exposed to urea in the folded state.

These results are consistent with our previous studies of BPTI hydrogen exchange in urea (Hilton et al., 1981). The unfolding midpoint temperature of BPTI is lowered by >15 °C by the addition of 8 M urea at pH 4–8. Nevertheless, 8 M urea slows bulk tritium–hydrogen exchange at pH 6.5 and 20 or 35 °C. Under these conditions, BPTI in 8 M urea is far from the temperature-induced unfolding, and no exchange by the unfolding mechanism is expected in the tritium experiment. We took these results as a demonstration that global stability is not correlated with exchange by the folded-state mechanism, and this is corroborated by the present work. Delepierre et al. (1983) also showed, with lysozyme, that the addition of 3 M urea accelerates the high activation energy exchange rates of individual Trp NH (exchange by cooperative unfolding), but not low activation energy exchange rates of the same NH (exchange from the folded state).

The two-process model provides a method for estimation of the change in ΔG for the N $\Leftrightarrow$ D reaction due to addition of a denaturing chemical or introduction of a destabilizing mutation. For protons exchanging *only* by the unfolding mechanism, from eqs 1 and 3 (Appendix),

$$k_{obs}/k_{obs,D,i} \cong K_{eq}/K_{eq,D} \quad (4)$$

where the subscript D indicates the rate constant or equilibrium constant in the presence of denaturant or in a destabilizing mutant protein. Then

$$\Delta\Delta G \cong -RT \ln (k_{obs,i}/k_{obs,D,i}) \quad (5)$$

where $\Delta\Delta G$ is the change in ΔG due to the denaturant or the mutation at that pH and temperature. A value of 6.6 kcal/mol for $\Delta\Delta G_{(0\rightarrow 8M\ urea)}$ at 30 °C and pH 3.5 is obtained by taking k_{obs} as the average of the rate constants of 21–23 without urea (Table I) and by taking $k_{obs,D}$ as 2.5 times the average of the rate constants of 21–23 with urea (Table I). The correction of 2.5 for the rate constants in urea is for the effect of 8 M urea on k_{cx} (Loftus et al., 1986). The value of $\Delta\Delta G_{(WT\rightarrow mut)}$ for BPTI mutants is estimated similarly from the ratio of exchange rate constants of 21–23 peptide amide NH's, and these are in reasonable agreement with the values obtained from calorimetry (Kim et al., 1993a,b).

The effect of 8 M urea on rates of exchange from the folded state is variable. Protons that have the same exchange rates with and without urea are 5, 7, 10, 16, 41, and 54 (Table I). Two (5 and 7) are in the N-terminal 3_{10} helix, two (10 and 41) are in the flexible loops and H-bonded to buried waters, one (16) is in the flexible loops and H-bonded to a buried backbone O, and one (54) is in the middle of the C-terminal α-helix. Slower exchange in urea indicates urea binding in the vicinity of the exchanging proton. Residues for which exchange is slower in urea by a factor >4 are 6, 14, 17, 19, 25, 28, 30, 32, 34, and 38 (Table I). Six of these (17, 19, 25, 30, 32, and 34) are on the surface of the molecule, are not intramolecularly H-bonded, and are the only surface protons we observe in these experiments. Surface peptide groups are expected to bind urea; crystallographic studies of diketopiperazine show multiple hydrogen bonding of urea with amide groups (Thayer et al., 1993). Of the other four protons that are slower exchanging in urea, two (14 and 38) are buried in the flexible loops and H-bonded to buried waters (and their side chains form one of the disulfide bonds), one (28) is in the turn between the central strands of β-sheet, and one (6) is the middle NH proton hydrogen bonded in the N-terminal helix. To summarize, exchange of all of the surface sites measured here is slowed by urea; some buried NH's in the flexible loops are slowed by urea, but other nearby NH's in the same loops are not affected by addition of 8 M urea. In the N-terminal 3_{10} helix, exchange of one buried NH (6) is slowed by urea while the two flanking buried NH's in the same helix (5 and 7) are not affected by urea; exchange of one buried residue (28) in a turn is accelerated by urea.

A protective, or slowing, effect of urea on the exchange of buried protons implies that urea is accessible to those buried

regions, presumably through local fluctuations. In denaturation models involving the selective binding of urea to protein (Schellman, 1987; Makhatadze & Privalov, 1992), binding of urea stabilizes both folded and unfolded states, but the larger number of binding sites exposed in the unfolded state favors denaturation. Makhatadze and Privalov (1992) showed that one-half of the urea binding sites are available in the folded state compared with the unfolded state. Our results suggest that some binding sites in folded BPTI are buried in the static crystal structure, but are accessible to urea in the more flexible regions of its dynamic structure.

A significant decrease in exchange rate is also observed in several unassigned side-chain NH protons of arginine and/or lysine (Figure 2). The slowed exchange of BPTI arginine/lysine side-chain NH's in 8 M urea may reflect specific binding of urea to amino or guanido groups. This suggestion is consistent with the observation that the most significant changes in chemical shifts of α-CH protons in urea (Table I) are those of Arg 39 and Arg 53.

APPENDIX

For each labile proton i in a native protein, exchange may occur by a native-state mechanism or by an unfolding mechanism:

$$k_{obs,i} = k_{f,i} + k_{u,i} \quad (1)$$

where $k_{obs,i}$ is the observed exchange rate constant, $k_{f,i}$ is the rate constant for exchange of i from the compact folded state, and $k_{u,i}$ is the rate constant for exchange of i by the unfolding mechanism.

For proton i exchanging from the folded state,

$$k_{f,i} = \beta_i k_{cx,i} \quad (2)$$

where β_i is the probability that i is exposed to water and catalyst and $k_{cx,i}$ is the chemical exchange rate constant for proton i in an oligopeptide of equivalent sequence; $k_{cx,i} = k_{H,i}[H^+] + k_{OH,i}[OH^-]$, where k_H and k_{OH} are the second-order rate constants for acid- and base-catalyzed exchange, respectively. The reaction is first-order in catalyst. In the base-catalyzed regime (pH > 3), an increase in pH of 1 unit increases k_{cx} by an order of magnitude; in the acid-catalyzed regime (pH < 3), a decrease in pH of 1 unit similarly increases k_{cx}. The activation energies of k_H and k_{OH} are 13 and 17.4 kcal/mol, respectively (Englander et al., 1979). The mechanism of exchange from the folded state is not certain, as discussed in Kim et al. (1993b).

For proton i exchanging by the unfolding mechanism,

$$k_{u,i} \cong [k_1 k_{cx,i}/(k_{-1} + k_{cx,i})] \cong K_{eq} k_{cx,i} \quad (3)$$

where k_1 and k_{-1} are protein unfolding and refolding rate constants and K_{eq} is the unfolding equilibrium constant. Under native conditions $k_{-1} \gg k_{cx}$, and $k_{u,i}$ is approximately the product of K_{eq} and $k_{cx,i}$. If $k_{-1} \ll k_{cx}$, then $k_{u,i} \cong k_1$; this case, sometimes called an EX1 mechanism, has only been observed over a narrow range of high pH and temperature for the slowest exchanging protons in BPTI (Woodward & Hilton, 1980; Roder et al., 1987).

When β_i is much smaller than the equilibrium constant of cooperative unfolding, $k_{u,i} \gg k_{f,i}$ and exchange of proton i occurs only by the unfolding mechanism. In the same protein, other buried protons with greater local flexibility may have $k_u \ll k_f$, and they exchange from the native state.

SUPPLEMENTARY MATERIAL AVAILABLE

Table of NH and α-CH chemical shifts of BPTI in 8 M urea (2 pages). Ordering information is given on any current masthead page.

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