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### Coupling of Conformational Folding and Disulfide-Bond Reactions in Oxidative Folding of Proteins<sup>†</sup>

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**ABSTRACT:** The oxidative folding of proteins consists of conformational folding and disulfide-bond reactions. These two processes are coupled significantly in folding-coupled regeneration steps, in which a single chemical reaction (the “forward” reaction) converts a conformationally unstable precursor species into a conformationally stable, disulfide-protected successor species. Two limiting-case mechanisms for folding-coupled regeneration steps are described. In the folded-precursor mechanism, the precursor species is preferentially folded at the moment of the forward reaction. The (transient) native structure increases the effective concentrations of the reactive thiol and disulfide groups, thus favoring the forward reaction. By contrast, in the quasi-stochastic mechanism, the forward reaction occurs quasi-stochastically in an unfolded precursor; i.e., reactive groups encounter each other with a probability determined primarily by loop entropy, albeit modified by conformational biases in the unfolded state. The resulting successor species is initially unfolded, and its folding competes with backward chemical reactions to the unfolded precursors. The folded-precursor and quasi-stochastic mechanisms may be distinguished experimentally by the dependence of their kinetics on factors affecting the rates of thiol–disulfide exchange and conformational (un)folding. Experimental data and structural and biochemical arguments suggest that the quasi-stochastic mechanism is more plausible than the folded-precursor mechanism for most proteins.

Oxidative folding is defined as the composite process by which a reduced, unfolded protein regains both its native disulfide bonds (disulfide-bond regeneration) and its native structure (conformational folding) (1, 2). These two processes are coupled significantly in folding-coupled regeneration steps, in which a single chemical reaction (the “forward” reaction) converts a conformationally unstable precursor species into a conformationally stable, disulfide-protected

successor species (Figure 1). The forward reaction may be either a disulfide-bond reshuffling (Figure 1a) or the final step of a disulfide-bond oxidation (Figure 1b); hence, folding-coupled regeneration steps may be further specified as folding-coupled reshuffling or oxidation steps.

The formation of stable tertiary structure drastically alters the rates of disulfide-bond rearrangements in oxidative folding by changing the accessibility, proximity, and reactivity of the thiols and disulfide bonds (1). Accordingly, oxidative folding may be divided into pre- and postfolding stages. The prefolding stage is characterized by rapid interconversions of unstructured disulfide species by disulfide reshuffling (Figure 2), at rates determined largely by loop entropy (3, 4) and the conformational biases of the protein

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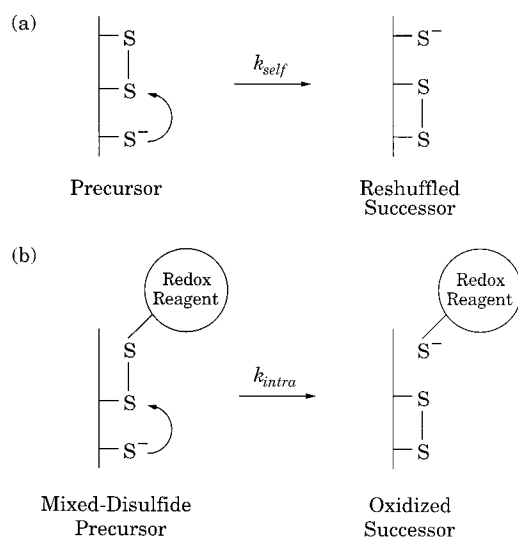


FIGURE 1: Chemistry of two types of folding-coupled regeneration steps. (a) In a folding-coupled reshuffling step, a thiolate in a conformationally unstable precursor species attacks an intraprotein disulfide bond, leading to thiol–disulfide exchange. (b) In the conformationally analogous folding-coupled oxidation step, the thiolate attacks a mixed disulfide bond. The reshuffling and oxidation rate constants are denoted as  $k_{\text{self}}$  and  $k_{\text{intra}}$ , respectively. The corresponding backward chemical reactions (back-reactions) are back-reshuffling and the attack of a redox reagent thiolate on an intraprotein disulfide bond, respectively (i.e., the first step in disulfide-bond reduction).

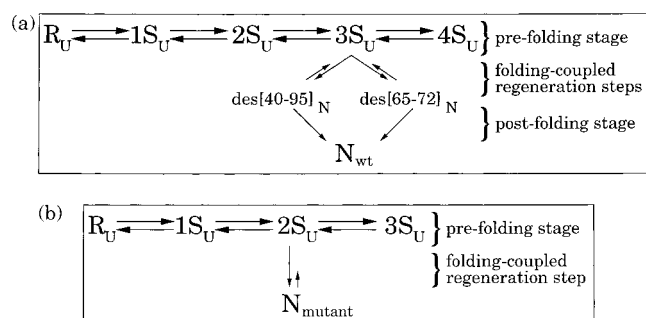


FIGURE 2: (a) Oxidative folding of the four-disulfide protein RNase A. The various  $nS_U$  ensembles ( $1S_U$ – $4S_U$ ) represent ensembles of disulfide species with  $n$  disulfide bonds; the equilibration of disulfide species within each  $nS_U$  ensemble (by reshuffling reactions) is more rapid than that between  $nS_U$  ensembles (by redox reactions) (1). These  $nS_U$  species are largely unstructured, but exhibit some conformational order; by contrast,  $\text{des}[40-95]_N$ ,  $\text{des}[65-72]_N$ , and the native protein  $N_{\text{wt}}$  are folded globally (1). The prefolding stage consists of reshuffling reactions within each  $nS_U$  ensemble and redox reactions between these ensembles. The postfolding stage corresponds to the oxidation reactions that convert the  $\text{des}$  species to the native protein. The folding-coupled regeneration steps are the single reshuffling reactions that convert unfolded precursor species in the  $3S_U$  ensemble to the folded  $\text{des}$  species, as in Figure 1a. (b) Oxidative folding of the three-disulfide mutants, C40A/C95A and C65S/C72S, of RNase A, corresponding to the  $\text{des}[40-95]$  and  $\text{des}[65-72]$  species of wild-type RNase A. Since these mutant proteins have no structured disulfide intermediates in oxidative folding, the folding-coupled regeneration steps are the single oxidation reactions that convert an unfolded, mixed-disulfide  $\text{des}$  species in the  $2S_U$  ensemble to the folded native protein  $N_{\text{mutant}}$ , as in Figure 1b. Further information about these kinetic models of oxidative folding is found in recent reviews (1, 2).

(5, 6). By contrast, the postfolding stage is characterized by folded, native-like disulfide species (Figure 2a), in which the reaction rates may deviate strongly from the corresponding rates in unstructured disulfide species (2). In the

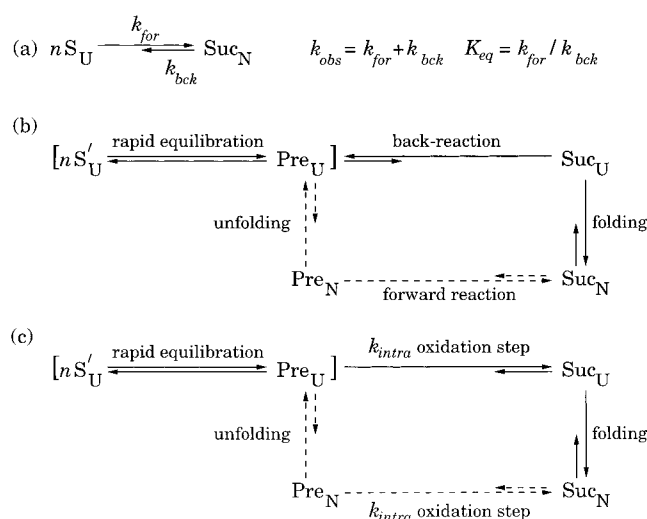


FIGURE 3: (a) Generic folding-coupled step, as observed experimentally. The folded successor species is produced from the unstructured  $nS_U$  ensemble with an apparent first-order kinetic rate constant  $k_{\text{obs}}$ .  $Suc_N$  is assumed to be more stable than  $nS_U$ , implying that the equilibrium ratio  $K_{\text{eq}}$  is greater than 1. (b) Expanded version of Figure 3a. The  $nS_U$  ensemble is expanded to show  $Pre_U$  (the immediate precursors of  $Suc_N$ ) in rapid equilibrium with the other  $nS_U$  species (denoted as  $nS'_U$ );  $Pre_U$  represents the disulfide species that can be converted to the successor species by a single chemical reaction, e.g., those of Figure 1. The quasi-stochastic and preformed structure mechanisms are shown with solid and dashed reaction arrows, respectively. The dominant reactions are labeled and denoted with longer arrows; by assumption, the  $Suc_U$  and  $Pre_N$  species are less stable than the  $nS_U$  and  $Suc_N$  species, respectively. In the  $Suc_U$  species, the main competing processes are folding and the backward chemical reaction, whereas in the  $Pre_N$  species, unfolding and the forward reaction compete. The kinetics of these mechanisms is analyzed in the two Appendices of the Supporting Information. (c) Under strongly oxidizing conditions and in the absence of free protein thiol, the back-reaction of a folding-coupled oxidation step (i.e., disulfide-bond reduction) is slow compared to the forward reaction (Figure 1b) and conformational folding.

prefolding stage, conformational folding has not yet occurred, whereas folding is more or less completed in the postfolding stage; thus, neither stage couples conformational folding and disulfide-bond reactions significantly. Rather, such coupling occurs at the boundary between these two stages, when folded disulfide species are produced from unfolded precursors (Figure 3a). We characterize the coupling between the disulfide-bond reactions and conformational folding in these folding-coupled regeneration steps. More specifically, we characterize the conformational ensemble at the instant when the forward reaction of a folding-coupled regeneration step occurs, i.e., when its chemical transition state is surmounted.

It is worth noting that conformational unfolding may be involved in disulfide-bond reactions of the postfolding stage. For example, a structured intermediate may bury its thiols in stable tertiary structure, inhibiting the formation of further disulfide bonds; in such cases, local or global conformational unfolding may be required to expose the thiols so that they can be oxidized to form further native disulfide bonds. However, the conformational ensemble of disulfide-bond reactions in the postfolding stage lies outside the scope of this article, which is concerned only with folding-coupled regeneration steps.

Two limiting-case mechanisms<sup>1</sup> may be discerned for folding-coupled regeneration steps (Figure 3b). In the folded-precursor mechanism, folding precedes the forward reaction;

thus, the forward reaction occurs in a folded precursor species, i.e., the transition-state ensemble of the forward reaction is characterized by native tertiary structure (Figure 3b). The (transient) native structure of the precursor accelerates the forward reaction by increasing the effective concentrations of the reactive thiol and disulfide groups. By contrast, in the quasi-stochastic mechanism, folding follows the forward reaction. Thus, the forward reaction occurs quasi-stochastically in an unfolded precursor; i.e., the reactive groups encounter each other with a statistical probability determined primarily by loop entropy, albeit possibly modified by residual conformational biases in the unfolded state. The resulting successor species is initially unfolded, and its folding competes with the back-reactions converting it to unfolded precursors (Figure 3b).

The principal conclusion of this article is that the quasi-stochastic mechanism is more plausible than the folded-precursor mechanism for most proteins. In the first section, structural and biochemical considerations are cited to argue that the folded-precursor mechanism is much less likely than the quasi-stochastic mechanism for most proteins. The second section summarizes a kinetic analysis (see Appendices A and B of the Supporting Information) that demonstrates that the folded-precursor and quasi-stochastic mechanisms predict a different dependence of  $k_{\text{obs}}$  (Figure 3a) on factors affecting the rates of thiol–disulfide exchange and conformational (un)folding. Measurements of the dependence of  $k_{\text{obs}}$  on such factors appear to support the quasi-stochastic mechanism over the folded-precursor mechanism. The third section compares the experimental values of  $k_{\text{obs}}$  for the folding-coupled regeneration steps in bovine pancreatic ribonuclease A (RNase A)<sup>2</sup> with those predicted by the quasi-stochastic and folded-precursor mechanisms. Again, the predictions of the quasi-stochastic mechanism appear to fit the data better than do those of the folded-precursor mechanism. Throughout this paper, folding-coupled reshuffling steps (Figure 1a) and folding-coupled oxidation steps (Figure 1b) are often discussed separately, although they are conformationally analogous (Figure 1). This is done partly for clarity and partly because their back-reactions are chemically different; the back-reaction of Figure 1a is an intramolecular reaction, whereas the back-reaction of Figure 1b is a bimolecular reaction involving the redox reagent.

The principal conclusion pertains to the oxidative folding of single-domain proteins, i.e., those that undergo global conformational folding in a two-state fashion. For simplicity, we assume that all disulfide species are either conformationally stable or unstable, i.e., that the free energies of their folded and unfolded states differ significantly (e.g., by  $\geq 4$  kcal/mol). We neglect the more complicated case when disulfide species are marginally stable, i.e., when their folded and unfolded states have comparable free energies (e.g., differing by  $< 4$  kcal/mol). Thus, a folding-coupled regeneration step may be defined as a single chemical reaction that converts a conformationally unstable precursor into a conformationally stable successor species. We likewise neglect

the case when the experimental conditions are sufficiently stabilizing that all disulfide species (even the fully reduced species) are folded globally since, under such conditions, folding-coupled regeneration steps are not possible.

### Structural and Biochemical Considerations

The quasi-stochastic mechanism is at least feasible for folding-coupled reshuffling steps (Figure 1a), since its two component reactions (quasi-stochastic disulfide reshuffling in an unstructured disulfide species and folding of a conformationally stable species) have often been observed experimentally (1, 2). Moreover, the putative competition between folding and back-reshuffling in the unfolded successor  $\text{Suc}_U$  (Figure 3b) has been observed experimentally in the unblocked des species of RNase A, which are lyophilized at low pH (where they are unfolded) after their isolation (7). If the lyophilized des species are redissolved directly into folding conditions at pH 8, a significant fraction undergoes reshuffling to unstructured 3S species (7). Thus, under such conditions, disulfide reshuffling competes with the conformational folding that would have protected the disulfide bonds, at least in some subpopulations, e.g., in a slow-folding fraction characterized by a nonnative cis–trans isomer of an essential proline (8, 9). By contrast, if the lyophilized des species are first equilibrated on ice at pH 5 (which permits folding but not reshuffling), very little ( $< 2\%$ ) unstructured species is produced upon a subsequent jump to pH 8 (unpublished observations). Presumably, the disulfide bonds remain protected when the pH is increased because conformational folding has been completed.

Similar arguments suggest that the quasi-stochastic mechanism is also plausible for the conformationally analogous folding-coupled oxidation steps (Figure 1b). However, the back-reaction (reduction) is generally much slower than conformational folding, because of the low concentrations of reducing agent under typical oxidative folding conditions. Hence, the back-reaction for folding-coupled oxidation steps generally does not compete significantly with conformational folding (Figure 3c). Under these conditions, the quasi-stochastic mechanism predicts that the reaction rate  $k_{\text{obs}}$  is largely independent of factors (e.g., denaturants) that affect only the rate of conformational folding.

By contrast, the folded-precursor mechanism seems unlikely to be preferred over the quasi-stochastic mechanism for folding-coupled reshuffling steps (Figure 3b). Such a preference of the folded-precursor mechanism would seem to require that the rate of the forward reaction ( $\text{Pre}_N \rightarrow \text{Suc}_N$ ) be comparable to (or faster than) the rate of unfolding ( $\text{Pre}_N \rightarrow \text{Pre}_U$ ) in the folded precursor species (Figure 3b). However, the unfolding rate for conformationally unstable proteins is typically on the millisecond time scale (10), which is much faster than typical  $k_{\text{self}}$  and  $k_{\text{intra}}$  rates. For example, in the des[40–95] species of RNase A, the  $k_{\text{intra}}$  rate constant at 25 °C and pH 8 equals  $67 \text{ min}^{-1}$  (7), roughly 100-fold slower than typical unfolding rates (10). Given that this  $k_{\text{intra}}$  rate is unusually high due to proximity effects in the conformationally stable des[40–95] species, the forward reaction seems even less likely to compete with unfolding in a conformationally unstable precursor species  $\text{Pre}_N$ . Similar arguments make the folded-precursor mechanism also unlikely for the conformationally analogous folding-coupled oxidation steps (Figure 1).

<sup>1</sup> The term mechanism is used instead of pathway to describe these alternatives, since “pathway” already has a technical meaning for oxidative folding (1, 2).

<sup>2</sup> Abbreviations: RNase A, bovine pancreatic ribonuclease A; des-[x–y], disulfide species of RNase A with all the native disulfide bonds except that between Cys-x and Cys-y.



The folded-precursor mechanism seems especially implausible for folding-coupled reshuffling steps, since it requires the conformational folding of a precursor species with a nonnative disulfide bond. Such a misfolded species (i.e., one with a nonnative disulfide bond involved in stable tertiary structure) has hitherto not been observed (1). Furthermore, the reshuffling reaction itself should generally involve a large deformation of the native topology, since thiol–disulfide exchange reactions require that the three reacting sulfur atoms be adjacent at the moment of the reaction (11). Such deformations are unlikely to be compatible with conformational folding to the native protein; atomic-force microscopy experiments and computer simulations of the force-mediated unfolding of protein domains indicate that even small deformations of the topology may suffice for global unfolding (12–14).

#### *Kinetics of the Two Mechanisms*

Kinetic analyses of the folded-precursor and quasi-stochastic mechanisms were carried out (see Appendices A and B of the Supporting Information). These analyses indicate that the folded-precursor mechanism is sensitive to factors (e.g., denaturants) affecting conformational folding and/or stability under all experimental conditions, whereas the quasi-stochastic mechanism is not sensitive to such factors when the subsequent conformational folding is much faster than the reshuffling reaction from the unstructured ensemble. More generally, the folded-precursor and quasi-stochastic mechanisms can be distinguished experimentally by the dependence of  $k_{\text{obs}}$  on factors affecting the rates of thiol–disulfide exchange and conformational (un)folding.

The folding-coupled oxidation step (Figure 1b) of a one-disulfide mutant of RNase T1 provides an example of such an experiment. The  $k_{\text{intra}}$  rate constant of this protein appears to be independent of urea concentration, provided that the fully reduced precursor species remains conformationally unstable (15). If the folded-precursor mechanism were correct, the addition of urea should affect the kinetics of the overall reaction, since urea should alter both the folding rate and the conformational stability of the precursor species. By contrast, the quasi-stochastic mechanism is consistent with the observed urea independence since the absence of a reducing agent (15) should eliminate the back-reaction (the reduction of the native protein) (Figure 3c). Hence, under these conditions, the overall reaction rate predicted by the quasi-stochastic mechanism should be independent of factors affecting conformational folding and stability such as urea (see Appendices A and B of the Supporting Information), as observed (15).

#### *Estimated Rates for the Quasi-Stochastic and Folded-Precursor Mechanisms in RNase A*

Using the measured rate constants for reshuffling and folding in RNase A (7), the quasi-stochastic mechanism predicts a reasonable equilibration rate  $k_{\text{obs}}$  between the unstructured 3S ensemble and the folded des species (Figures 2 and 3a). If it is assumed that all unstructured precursors are equally represented in the 3S ensemble and that all reshuffling reactions are equally probable (which are rough approximations), the estimated  $k_{\text{obs}}$  equals  $4.6 \times 10^{-2} \text{ min}^{-1}$  (see Appendix A of the Supporting Information), which is

consistent with the experimental value of  $1.4 \times 10^{-2} \text{ min}^{-1}$  for formation of des[40–95] from 3S (7). [The relatively high predicted value may reflect an unequal distribution of disulfide species in the unstructured ensembles, as has been observed experimentally (5, 6).] This calculation demonstrates that the quasi-stochastic mechanism may contribute significantly to this folding-coupled regeneration step in RNase A.

By contrast, the folded-precursor mechanism does not seem likely to contribute significantly to this reshuffling step in RNase A, by the following reasoning. In addition to its one nonnative disulfide bond, the three-disulfide precursor species must have two native disulfide bonds. The two native disulfide bonds that contribute most to the stability of wild-type RNase A are the fully buried disulfide bonds [between residues 26 and 84 and residues 58 and 110 (16)]. However, NMR experiments indicate that the native 2S species (disulfide bonds between residues 26 and 84 and residues 58 and 110) is conformationally unstable by at least 3 kcal/mol, since it appears to have no more stable tertiary structure than other 2S species (17). Any three-disulfide precursor species should be less stable than this (disulfide bonds between residues 26 and 84 and residues 58 and 110) 2S species, since it differs by the addition of a nonnative disulfide bond and, possibly, the replacement of the 26–84 and/or 58–110 disulfide bond by a less stabilizing native disulfide bond. Moreover, no structured disulfide species with nonnative disulfide bonds have been observed, even under the most stabilizing conditions (18, 19). Taken together, these arguments suggest that the folded state  $\text{Pre}_N$  of the precursor species is unstable (relative to  $\text{Pre}_U$ ) by at least 4 kcal/mol. Using this estimate of the conformational stability and the fastest reshuffling rate observed in RNase A, the folded-precursor mechanism predicts a maximum observed rate constant of  $0.47 \times 10^{-2} \text{ min}^{-1}$  (see Appendix A of the Supporting Information), roughly 3-fold smaller than the experimental value of  $1.4 \times 10^{-2} \text{ min}^{-1}$  for des[40–95] (7). Since the  $k_{\text{intra}}$  rate constants in RNase A are similar in magnitude to those observed in other proteins (11), and if it is assumed that the conformationally unstable precursors are likewise unstable by at least 4 kcal/mol, these estimates suggest that the folded-precursor mechanism does not contribute significantly to most folding-coupled regeneration steps.

#### *Earlier Studies of the Coupling of Folding and Disulfide-Bond Reactions*

It has long been recognized that stable tertiary structure can alter the rates of disulfide-bond reactions in proteins significantly through its effects on the proximity (i.e., the effective local concentration), accessibility, and reactivity of the relevant disulfide bonds and thiol groups (20–22). Experimental evidence for such effects has been obtained in several proteins by examining how factors (such as mutations, denaturants, and salts) that influence conformational stability affect the rate constants of overall oxidative folding ( $R \rightarrow N$ ) and of interconversions between ensembles of disulfide species (e.g.,  $1S \rightarrow 2S$ ), as well as the distributions of disulfide species within each disulfide ensemble (23). However, these studies are not directly relevant to this article, which is concerned only with the effects of such conformational-stability factors on the observed rate constants

of a single disulfide-bond reaction between two chemically defined species, namely, a conformationally unstable precursor species and a conformationally stable successor species.

In a few cases, the effects of such conformational-stability factors have been measured for single disulfide-bond reactions, e.g., in the work of Frech and Schmid cited above (15) or that of Zhang and Goldenberg (24). However, to our knowledge, such studies have not addressed the question posed in this article, namely, the relative sequence of conformational folding and the disulfide-bond reaction in folding-coupled regeneration steps or, more specifically, the state of the conformational ensemble at the instant when a conformationally unstable precursor species is converted into a conformationally stable successor species. Moreover, these earlier studies have generally not discriminated between folding-coupled regeneration steps and single disulfide-bond reactions of the postfolding stage, in which structured successor species are produced from structured precursors (possibly through a locally or globally unfolded transition state, as noted above). Indeed, specific folding-coupled regeneration steps have been identified for relatively few multi-disulfide-bond proteins [e.g., RNase A and bovine pancreatic trypsin inhibitor (BPTI)] since such identification requires that the presence or absence of stable tertiary structure be determined for the individual precursor and successor species. Accordingly, the oxidative folding of BPTI is more complex than that of RNase A in that it appears to have two independently folding domains (25, 26). The earliest structured intermediates seem to be the one-disulfide intermediates (bond between residues 30 and 51 and, under some conditions, between residues 5 and 55), although the former appears to be structured only in one of the two folding domains. The corresponding folding-coupled regeneration steps would be the formation of these species either by oxidation of fully reduced BPTI or by reshuffling of other one-disulfide species. To our knowledge, the relative order of conformational folding and the chemical reaction has not been determined for these steps.

A thermodynamic cycle of conformational folding and disulfide-bond regeneration similar to the kinetic diagrams in panels b and c of Figure 3 has also been presented in earlier publications (15, 24, 27, 28). However, to our knowledge, these thermodynamic cycles have been employed only as a method for computing the free-energy linkage between conformational stability and disulfide-bond reactions, being considered “mechanistically unrealistic” (24). In the work presented here, this “cycle” is merely a compact representation of two separate limiting-case mechanisms.

Among the models of oxidative folding that allow for structural interpretation, the folded-precursor mechanism seems to be favored over the quasi-stochastic mechanism (27, 29–35). For example, some publications appear to suggest that oxidative folding proceeds through a folded-precursor mechanism in which local regions of the protein adopt their native structure, which is then stabilized by forming nearby disulfide bonds (27, 31); in this scenario, global folding results from the piecemeal accumulation of local “microfolding” events. Other articles appear to suggest that the oxidative folding of some proteins occurs by a folded-precursor mechanism in which global conformational folding and the oxidation (not reshuffling) of disulfide bonds are coupled (29, 30). In both scenarios, a locally or globally

folded conformation is “locked in” (i.e., stabilized) by disulfide-bond formation, whereas the converse happens in quasi-stochastic mechanisms: a set of disulfide bonds is locked in (i.e., protected from further rearrangements) by local or global conformational folding. In the general model presented here, the oxidative folding of single protein domains is characterized by pre- and postfolding stages separated by regeneration steps in which global conformational folding follows the quasi-stochastic formation of a set of disulfide bonds (by either oxidation or reshuffling) that render the final disulfide species conformationally stable. Similarly, two recent articles appear to suggest that local conformational folding occurs in the vicinity of the first native disulfide bonds following their formation from the fully reduced protein (36, 37). Although this suggestion appears to hold for the 30–51 disulfide bond species and (under some conditions) the 5–55 disulfide bond species of BPTI, it does not appear to hold for the 14–38 disulfide bond species of BPTI (38) or, more generally, for the native 1S species of other multi-disulfide-bond proteins (2).

### Conclusions

This article defines the concept of folding-coupled regeneration steps, as well as two limiting-case mechanisms. In the folded-precursor mechanism, conformational folding precedes the forward reaction, which then competes with conformational unfolding in the (transiently folded) precursor species. By contrast, in the quasi-stochastic mechanism, the forward chemical reactions precede folding, which then competes with the backward chemical reactions in the successor species. The kinetics of these two mechanisms exhibit different dependencies on the reaction rates of thiol–disulfide exchange and conformational folding, allowing them to be distinguished experimentally.

The quasi-stochastic and folded-precursor mechanisms describe the conformational ensemble at the moment of the forward chemical reaction. Some proteins may adopt a mechanism that lies between these two extremes; i.e., the transition-state ensemble could be partially structured. Nevertheless, experimental data and structural and biochemical considerations generally favor the quasi-stochastic mechanism over the folded-precursor mechanism, especially for folding-coupled reshuffling steps such as those observed in RNase A.

The quasi-stochastic mechanism suggests an explanation for how the cis–trans isomerization of prolines may be coupled to the oxidative folding of proteins. A nonnative isomer of an essential proline can alter the folding rate nearly 500-fold (8, 9), drastically altering the relative rates of conformational folding and the forward chemical reaction and slowing the effective rate of the folding-coupled regeneration step. Thus, prolyl peptidyl cis–trans isomerases (PPIases) can be coupled to oxidative folding indirectly by promoting the conformational folding of such subpopulations, rendering it more competitive with the back-reactions.

### SUPPORTING INFORMATION AVAILABLE

Appendices A and B on the kinetics of the folded-precursor and quasi-stochastic mechanisms and the mathematical solution of the corresponding kinetic matrix equations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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