VIP Force-Dependent Kinetics

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Kinetics of Thiol/Disulfide Exchange Correlate Weakly with the Restoring Force in the Disulfide Moiety**

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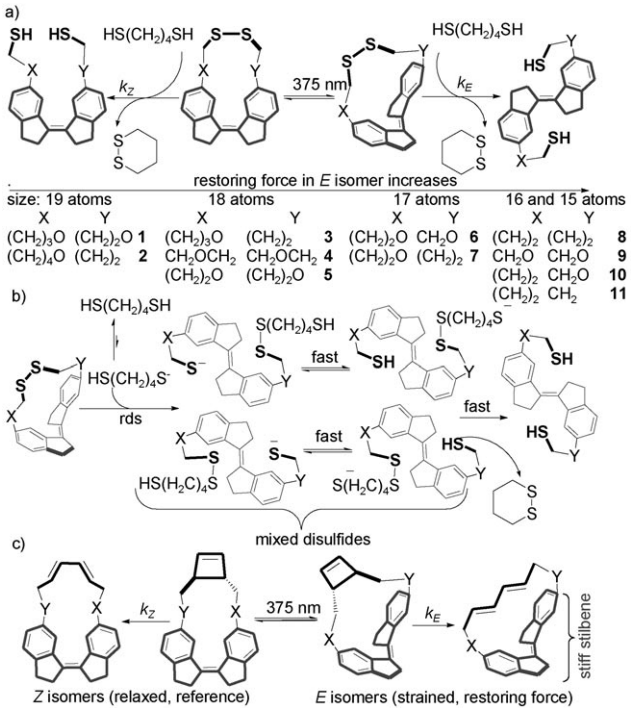
A series of macrocycles **1**–**11** (Scheme 1 a) revealed that stretching the disulfide moiety up to the restoring force of 350 pN along the S–S bond negligibly accelerates its reduction by thiols (Scheme 1 b). The measured rates of thiol/disulfide exchange in the increasingly strained *E* macrocycles were within a factor of two of those in strain-free Z analogues, with the activation enthalpies differing by less than 1 kcal mol^{-1} (Figure 1). This finding contrasts with the greater than



Scheme 1. a) The series of macrocycles for measuring the kinetics of thiol/disulfide exchange as a function of the restoring force of the stretched disulfide moiety. In the inert linkers X and Y, the atom on the right was bound to stiff stilbene. b) A plausible mechanism of thiol/disulfide exchange in **1**–**11**; rds = rate-determining step. The mechanism is assumed to be identical in *E* and *Z* isomers. c) A reference series of cyclobutene-containing analogues of **1**–**11**. The reactive moieties (CH_2SSCH_2 and cyclobutene) are highlighted in bold; stiff stilbene is gray.

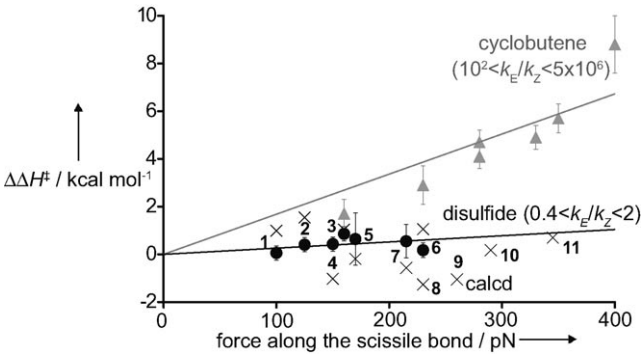


Figure 1. Difference in the activation enthalpies (300 K) of thiol/disulfide exchange (● measured; × B3LYP/6-311G** (CPCM-UA0)) and electrocyclic C–C bond homolysis (▲) between the *Z* and *E* isomers of the same macrocycle ($\Delta\Delta H^{\ddagger} = \Delta H^{\ddagger}_{Z} - \Delta H^{\ddagger}_{E}$) as a function of the calculated restoring force along the scissile S–S or C–C bond. $\Delta\Delta S^{\ddagger}$ values were small in both series (Supporting Information, Table S3). The error bars define the 95% probability intervals; solid lines are linear least-squares regression fits to the experimental data. The regressions are forced to pass through the origin, as by definition the rate must be unperturbed in the absence of restoring force. In the cyclobutene series, the deviation of several experimental $\Delta\Delta H^{\ddagger}$ values from direct proportionality reflects deficiencies of the scissile bond as the approximate reaction coordinate.[1] The more appropriate approximation of the reaction coordinate (C1···C2, Figure 2) yields direct proportionality of $\Delta\Delta H^{\ddagger}$ and force (Supporting Information, Figure S14).

10^6-fold acceleration of a reference reaction, electrocyclic C–C bond dissociation, across a structurally equivalent series (Scheme 1 c).[1] Quantifying the strain in the *E* isomers of **1**–**11** as restoring force offers insights into the mechanism of acceleration of disulfide bond reduction observed when certain proteins are stretched[2–4] and more broadly into factors that determine the changes in reactivity of molecules that have been stretched or compressed.

Such accelerations have profound implications for the stability of proteins to mechanical stresses and biological mechanotransduction.[3] Single-molecule force experiments that measured kinetics of thiol/disulfide exchange as a function of the total restoring force of polyprotein assemblies are broadly consistent with at least three mechanisms: 1) partial unfolding of the protein increases the access of

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water-soluble thiols to the disulfide bond; 2) distortion of the disulfide moiety increases its reactivity; and 3) stabilization of the transition state by partial conformational relaxation of the rest of the molecule results from the disulfide moiety being longer in the transition state than the ground state. The relative importance of each mechanism remains controversial.

Mechanism 1 can only occur in proteins. Mechanisms 2 and 3, however, reflect the intrinsic properties of the disulfide moiety (stiffness and difference in dimensions between the ground and transition state) and are testable using more tractable small molecules in which the disulfide moiety is stretched. Although the potential of small molecules to yield quantitative insights into the mechanism of acceleration of localized reactions in stretched polymers has long been recognized,[5,6] its realization was until recently precluded by the lack of molecular architectures that allowed restoring forces of diverse functional groups to be systematically varied. Unlike strain energy, its gradient (molecular restoring force) is a size-invariant measure of molecular strain.[7] As such, it provides a unifying conceptual framework for interpreting the effect of molecular strain on the kinetics of localized reactions, regardless of the overall size of the reactant. We recently reported[1,8] that incorporating a reactive moiety of interest (substrate) into an inert linker that constrains the C6,C6′ atoms of *E* stiff stilbene (Scheme 1, gray moiety) to a below-equilibrium distance and systematically varying the length and conformational flexibility of the linker creates a series of strained macrocycles. Changes in the kinetics of the substrate reaction across such series are amenable to interpretation within the restoring-force formalism.

Herein, we use this strategy to measure the kinetics of thiol/disulfide exchange as a function of the restoring force of the disulfide moiety under tensile strain. Although the effect of compressive strain on the reactivity of the disulfide moiety has been extensively studied in dithiacyclopropane, -butane, and -pentane,[9,10] such is not the case for tensile strains. The known larger aliphatic cyclic disulfides are either strain-free or their modest ring strains do not correlate with the kinetics of thiol/disulfide exchange.[11,12]

We synthesized strain-free *Z* isomers of **1**–**11** in four to six steps and greater than 10% overall yields (Supporting Information, Figure S1). The strained *E* isomers of **1**–**7** were obtained by irradiating dilute solutions of the *Z* analogues in CH_3CN at 375 nm until the photostationary state was reached. Irradiation of the *Z* isomers of smallest macrocycles **8**–**11** resulted in homolysis of the S–S bond.[13] The activation parameters of the thiol/disulfide exchange in the *E* isomers of **8**–**11** were obtained computationally (see below).

We studied the kinetics of S–S bond reduction with 1,4-butanedithiol (BDT, Scheme 1) in 1:1.4 (mol) H_2O/CH_3CN mixtures using HPLC to monitor the reaction progress. All rates were first-order in both BDT and the macrocycle. We measured the ratios k_E/k_Z (Scheme 1a) by competition experiments in which both isomers of each macrocycle reacted with excess BDT in the same solution. Unlike individual rates, the ratios were independent of the solvent composition and the concentration of BDT. We obtained the differential activation parameters $\Delta\Delta H^{\ddagger} = \Delta H^{\ddagger}_Z - \Delta H^{\ddagger}_E$ and $\Delta\Delta S^{\ddagger}$ from the slopes and the intercepts, respectively, of the linear Eyring plots of k_E/k_Z at 295–313 K (Figure 1 and Supporting Information, Figure S10 and Table S4). The rate constants of thiol/disulfide exchange in *Z* isomers derived from competition experiments were within the experimental uncertainty of those measured directly, ruling out cross-reaction between the two isomers in competition experiments.

The mechanism of disulfide reduction by BDT has been extensively studied:[14,15] the reaction proceeds by a rate-determining nucleophilic attack of the thiolate anion $HS(CH_2)_4S^-$ on a sulfur atom of the disulfide, with subsequent rapid intramolecular formation of 1,2-dithiacyclohexane and release of reduced dithiol. Both steps are thought to be simple S_N2 reactions, with protonated thiol being inert. In agreement with this mechanism, we did not observe the mixed disulfides (Scheme 1b) in any of our reactions either by ^{1}H NMR spectroscopy or HPLC. Consequently, the k_E/k_Z ratios reflect the difference of the free energy of activation of the initial rate-determining step.

To obtain convenient pseudo-first-order kinetics in $HS(CH_2)_4S^-$ without additional buffers, the reductions were carried out with an approximately 250-fold excess of BDT. The use of mixed solvents was dictated by the limited aqueous solubility of BDT. The protonation equilibrium in the H_2O/CH_3CN solvent mixture was established at least 100-fold faster ($\tau_{1/2} < 10$ s) than thiol/disulfide exchange, inferred from the rate of H/D exchange between BDT and D_2O in D_2O/CD_3CN, as measured by ^{1}H NMR spectroscopy.

To interpret the kinetic data within the restoring-force formalism, we optimized the geometry of multiple conformers of each ground and transition state in both isomers of **1**–**11** at the B3LYP/6-311+G** level. For computational efficiency, we modeled the solvent as a conducting polarized continuum with the united-atom topological model cavities (CPCM-UA)[16] and replaced $HS(CH_2)_4S^-$ with $CH_3CH_2S^-$. The calculated activation enthalpies (Supporting Information, Table S7) included the difference in the enthalpies of the solvated solute and the temperature-independent part of the solute/solvent interaction energies at the ground and transition states. The enthalpies calculated with the H_2O-parameterized CPCM and UFF-radii cavities were closest to the experiment (mean absolute deviation MAD ≈ 2 kcal mol^{-1}). The MADs for the differential enthalpies $\Delta\Delta H^{\ddagger}$ were approximately 1 kcal mol^{-1} (Figure 1 and Supporting Information, Table S7).

The optimized geometries of the *Z* isomers were largely strain-free. In the *E* analogues, distortions of stiff stilbene were significantly more pronounced than those of the much stiffer disulfide moiety (Supporting Information, Figure S12). In the ground-state conformers, the difference of the S–S bond lengths between the *Z* and *E* isomers of the same macrocycle was limited to ± 0.006 Å, but the softer C-S-S-C dihedrals increased from approximately 90° in largest macrocycles **1**–**3** to a Boltzmann-weighted average of 97° in the smallest congeners *E*-**10** and *E*-**11**. The calculated transition states were typical of an S_N2 reaction,[17] with the forming and dissociating S–S bonds of comparable length (average of **1**–**11**: (2.51 ± 0.03) Å and (2.53 ± 0.03) Å, respectively, Support-

ing Information, Table S8) and a single imaginary frequency of greater than 100i cm^{-1} (Supporting Information, Table S9), corresponding primarily to the oscillation of the central sulfur atom long the S-S-S axis. These data suggest that the chosen level of theory describes thiol/disulfide exchange in macrocycles **1**–**11** with useful accuracy, lending credence to the calculated restoring forces.

Restoring forces were obtained as previously described.[1,8] Briefly, in macrocycles, the restoring force of the disulfide moiety is exactly balanced by that of the rest of the macrocycle. Excising the former and adding H atoms to satisfy the valencies of the newly terminal methylene carbon atoms reveals uncompensated forces on each atom of the resulting fragments arising from the molecular strain (Supporting Information, Figure S13). These forces were calculated as analytical gradients of the electronic energy. A vectoral sum of atomic forces in each indanyl moiety and its linker gave the total restoring force. As was the case for the cyclobutene-containing macrocycles,[1] these forces in **1**–**11** were nearly parallel to the axis defined by the terminal methyl groups of the fragments. The restoring forces projected on the S–S bond were approximately 0.8 of the total. Ensemble-average forces along the S–S bond were obtained by Boltzmann-weighted averaging over all conformers [Supporting Information, Eq. (S6)]. The total forces in each ground state and the corresponding transition states were comparable and were averaged.

The restoring force along the S–S bond varied with the macrocycle size from 100 pN in *E*-**1** to 350 pN in *E*-**11**. This range is comparable to that in the cyclobutene-containing reference series (Figure 1). The very different rate-force dependences of these two reactions have, however, the same molecular basis. Theoretical considerations suggest[21] that the height of "sharp" activation barriers decreases as the product of the difference between the ground and transition states of the internuclear distance that best approximates the full reaction coordinate and the restoring force along it. In both cases, the slopes of the $\Delta\Delta H^{\ddagger}$ versus force plots corresponded to the change in the separation of the CH_2 groups closest to the scissile bond (C1···C2, Figure 2) between the corresponding ground and transition states (0.08 and 0.8 Å for the disulfide and cyclobutene-containing macrocycles, respectively; Supporting Information, Figure S14 and Table S9). That the C1···C2 distance better approximates the full reaction coordinate than the scissile bond is fully consistent with the established mechanisms of these reactions. The formation of the pseudo-trigonal-bipyramidal S_N2 transition state in thiol/disulfide exchange requires contraction of the angle between the scissile and the spectator bond at the central sulfur atom.[18] The contribution of this bond angle to the full reaction coordinate[19] is not captured by the elongation of the scissile bond in the transition state but is reflected in the change in the C1···C2 separation. Had the scissile bond dominated the full reaction coordinate, its elongation would have yielded 10-fold acceleration of thiol/disulfide exchange per approximately 240 pN of restoring force along the S–S axis by mechanism 3.

Our data suggest that pulling on the disulfide moiety with up to approximately 350 pN of force along the S–S bond does not accelerate its reduction by thiolates. The significance of this finding is two-fold. First, it calls into question large contributions of mechanisms 2 and 3 to the acceleration of thiol/disulfide exchange in stretched proteins.[4] For example, although the S–S bond elongates by approximately 0.4 Å in the transition state, the distance between the CH_2 groups attached to the sulfur atoms does not change. This arrangement precludes relaxation of the nonreactive parts of the stretched molecule, which is required for mechanism 3. Under these circumstances, such relaxation may still occur if different stereoelectronic and solvation preferences of the disulfide moiety in its ground and transition states change the minimum-energy tertiary structure of the protein. The importance of such an effect cannot be evaluated using disulfides **1**–**11** but may be probed in single-molecule force experiments using polymers with simpler tertiary structures than proteins studied to date, for example poly(ethylene glycol) derivatives containing S–S bonds. Until such experiments are performed, the acceleration of thiol/disulfide exchange in stretched proteins should probably be assumed to reflect predominantly the increased accessibility of the disulfide moiety to water-soluble reductants.

More broadly, our finding challenges the conventional notion that stretching a molecule necessarily accelerates its fragmentation.[4] This assumption is based on macroscopic experience (e.g., a stretched rubber band breaks faster than an unstretched one), which, however, maps poorly onto the molecular world. Even in simple bond-dissociation reactions, the formation of the transition states requires structural changes that are not limited to elongation of the scissile bond.

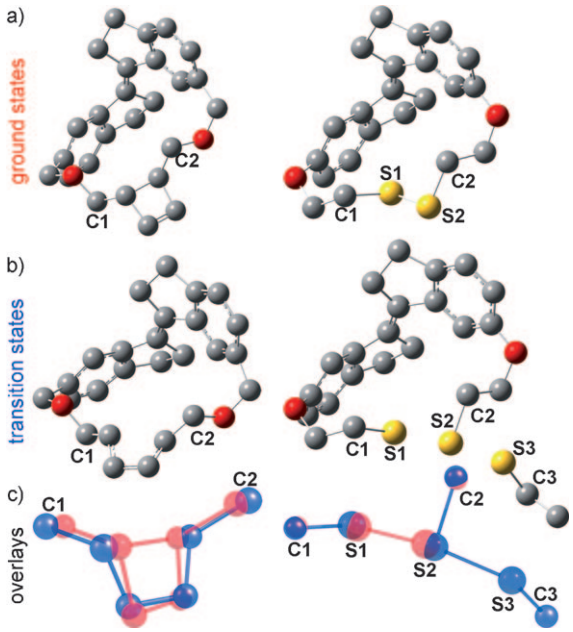

Figure 2. Minimum-energy conformers of the *E* isomers of a 16-atom cyclobutene-containing macrocycle (left, O3LYP/6-311G(2d,p)) and **9** in the a) ground state and b) transition state. C gray, O red, S yellow; H atoms are omitted. c) The minimum root-mean-squared deviation overlays of the reacting moieties in the ground (red) and transition (blue) states. Note the nearly constant relative position of the CH_2 groups (C1 and C2) in the disulfides.

Consequently, the length of the scissile bond is not a suitable state variable, and the assumption that the dissociation rate of a covalent bond under tensile strain is proportional to the product of the restoring force and the difference in the scissile bond length between the ground and transition state may rarely if ever be true. Our data show that the kinetically important structural changes in a reacting molecule can be captured using a single (nonbonding) internuclear distance, the choice of which can be guided by the molecular mechanism of the reaction. This unexpected fact preserves the intuitively appealing "single-coordinate" model of chemomechanical kinetics.[20,21]

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