Free Energy Relationships for Thiol-Disulfide Interchange Reactions between Charged Molecules in 50% Methanol*

(Received for publication, December 12, 1983)

Grayson H. Snyder

From the Department of Biological Sciences, State University of New York, Buffalo, New York 14260

Acid dissociation equilibrium constants and rate constants for disulfide interchange reactions have been measured in 50% methanol at low ionic strength for peptides containing cysteines with local ionic neighboring groups. These physical constants may be correlated by separation of free energy contributions into solvent-independent and solvent-dependent factors. The former represent inductive effects which may be evaluated by extrapolation of pK values to the limit of infinite ionic strength. These solvent-independent contributions give Bronsted coefficients consistent with previously reported values for disulfides with neutral constituents. The solvent-dependent contributions represent thru-solvent electrostatic effects and are consistent with the form of the Bjerrum relation.

Disulfide interchange reactions may be represented by the second order nucleophilic substitution reaction given below where nue is the constituent (R) on the attacking nucleophile, cen is the group attached to the central sulfur in the transition state complex, and lge is the constituent attached to the leaving group sulfur.

\[
\text{Reaction } 1: \quad \text{Rate constant } k = \frac{K_{\text{nue}} K_{\text{cen}}}{K_{\text{lge}}} \]
charged cysteine neighbors, enhance formation of the 2–4 disulfide, and promote the globular topology. In aqueous solutions at low ionic strength, we have succeeded in using coulombic interactions to enhance formation of a mixed disulfide between a cysteine with one positive neighbor and a cysteine with one negative neighbor (5). Since the observed enhancement was small in magnitude, we have extended these studies to reactions in 50% methanol where the lower dielectric constant is expected to amplify electrostatic effects. The results reported below include examples of repulsion and attraction between cysteine-containing peptides exhibiting disulfide interchange rate constants whose values span almost 6 orders of magnitude.

The simple Brønsted relationship above which is useful for correlating rate constants and pKₐ values for reactions involving neutral constituents is not adequate for treating reactions involving charged constituents. Equation 2 lacks terms describing physical interactions between pairs of constituent groups. In H₂O at an ionic strength of 1 M, charged molecules exhibit deviations from predictions based on Brønsted coefficients obtained with neutral molecules (6). One expects greater deviations when deliberately seeking to amplify electrostatic effects by working at low ionic strength in 50% methanol. Thus a different quantitative treatment is required to correlate the data reported below. The simplest approach is to begin with the assumption that the dominant contributions to ΔG and ΔG‡ in small peptides are solvent-independent inductive effects and solvent-dependent electrostatic effects. The former may be treated by the Brønsted relationship, and the latter by the Bjerrum relationship

\[
\Delta G = \frac{Z_i Z_j}{r} \epsilon
\]

(5)

where \( Z_i \) is the charge of molecule \( i \), \( \epsilon \) is the dielectric constant of the solvent, \( r \) is the distance of closest approach of charged groups on colliding molecules \( i \) and \( j \), and \( c \) is a constant at a given temperature.

Data correlation begins by estimating pKₐ values in the limit of infinitely high ionic strength in H₂O, conditions where electrostatic effects are minimal. In this limit, pKₐ values yield estimates of the relative values of inductive effects for different neutral or charged constituent groups. Then in 50% methanol and low ionic strength where both inductive and thio-solvent electrostatic effects contribute significantly, the solvent-independent values of inductive effects estimated above may be subtracted from total observed effects to give values of the electrostatic contributions. The validity of this treatment will be evaluated by determining whether these calculated thio-solvent effects vary with \( c \), \( r \), and \( Z \) values in a manner consistent with the Bjerrum relationship.

**EXPERIMENTAL PROCEDURES**

Experiments described below employ reduced monomers R-SH and oxidized disulfides R-SS-R of acetylcysteine (A), acetylcysteine methyl ester (C), cysteine methyl ester (E), and β-mercaptoethanol (D). A-SH, D-SH, E-SH, and E-S-S-E were obtained from Sigma. C-SS-C and D-SS-D were obtained from Serva Fine Chemicals and Aldrich, respectively. A-SS-A was prepared by air oxidation of A-SH as described previously (5). C-SH was prepared by reducing C-SS-C with thiopropyl-Sepharose 6B, followed by filtration to remove soluble C-SH from insoluble Sepharose. High pressure liquid chromatography indicated that the reduction was 98% complete. Thio-Propyl-Sepharose 6B is provided by Pharmacia Fine Chemicals. Concentrations of sulfhydryl groups were assayed with Ellman’s reagent, and concentrations of disulfides were measured by absorbance at 254 nm or molar extinction coefficients obtained by air oxidation of solutions of known thiol concentration.

pKₐ values of thiol groups were determined by spectrophotometric titration at 245 nm. Values of pH in 50% (w/w) methanol were obtained by subtracting 0.13 from the pH reading of the glass electrode calibrated in H₃O (8). Carboxyl and amine pKₐ values were determined by potentiometric titration using a Metrohm 636 Titroprocessor.

To determine rate constants for thiol disulfide interchange, stock solutions of R-SH and R'-SS-R' were degassed and saturated with low oxygen N₂ (Airco, Inc.) in vials sealed with Teflon/silicone septums (Pierce). Thiol was injected into the disulfide solution with gas-tight Hamilton syringes to initiate the reaction. At subsequent times, aliquots were withdrawn and interchange was quenched by lowering the pH to 3 with glacial acetic acid followed by freezing at −80 °C. Later, aliquots were thawed and separated into components by reverse phase chromatography at pH 3 on a Waters Associates gradient high pressure liquid chromatographic system using a C₁₈-μBondapak column and H₂O/acetonitrile gradients containing 0.1% trifluoroacetic acid. Components were detected at 214 nm using a fixed wavelength detector and a zinc lamp. Peak areas were integrated with an Apple II Plus computer equipped with an analog-to-digital converter and software provided by Interactive Microwave, Inc. L-Tryptophan was used as an internal standard present in the original reaction mixture. Reactions were performed under pseudo-first order conditions where one species (either R-SH or R'-SS-R') was in excess. pH values were chosen to ensure complete ionization of amine and carboxyl groups in thiolate and disulfide-containing molecules. Concentrations were adjusted to give 20% reaction in the first hour, during which net oxidation of thiol by residual oxygen never exceeded 2% of the total thiol concentration. Observed rate constants were calculated from the initial instantaneous velocity for loss of the species not present in excess. These values were corrected for statistical factors by division by 2, recognizing that R-S⁺ can attack either of the 2 eq sulfurs in R'-SS-R'. They then were converted to bimolecular rate constants corresponding to having 100% of the molecules in the reactive R-S⁺ form by using the relationship

\[
\Delta h_{\text{molecule}} = \Delta h_{\text{am}}(1 + \frac{1}{pK_a + pK_b})
\]

(6)

The rate constants are estimated to be accurate within 15% of correct values.

**RESULTS AND DISCUSSION**

**Determination of pKₐ Values**—Table I presents results of pKₐ determinations for thiol and constituent groups in several small molecules. Sulphydryl data for acetylcysteine, acetylcysteine methyl ester, and β-mercaptoethanol were obtained by spectrophotometric titration at 245 nm and analyzed using the Hill equation (9). The Hill coefficients \( n = 1.0 \) as expected for simple titration curves. Sulphydryl data for cysteine methyl ester gave \( n < 1 \) and therefore were treated by a model for two cotitrating groups (7) using the following equation:

**Table I**

<table>
<thead>
<tr>
<th>Temperature = 23 °C.</th>
<th>H₂O with ionic strength of 1 M</th>
<th>MeOH with ionic strength of 20 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>SH</td>
<td>9.51 ± .02</td>
</tr>
<tr>
<td>A</td>
<td>COOH</td>
<td>3.22</td>
</tr>
<tr>
<td>C</td>
<td>SH</td>
<td>8.67 ± .01</td>
</tr>
<tr>
<td>D</td>
<td>SH</td>
<td>9.59 ± .02</td>
</tr>
<tr>
<td>E</td>
<td>SH (near NH₂)</td>
<td>9.06 ± .02</td>
</tr>
<tr>
<td>E</td>
<td>SH (near NH₂+)</td>
<td>7.52 ± .05</td>
</tr>
<tr>
<td>E</td>
<td>NH₃⁺ (near SH)</td>
<td>6.75</td>
</tr>
<tr>
<td>AA</td>
<td>COOH groups</td>
<td>3.77</td>
</tr>
<tr>
<td>E</td>
<td>NH₃⁺ groups</td>
<td>4.66</td>
</tr>
</tbody>
</table>

* A, acetylcysteine; C, acetylcysteine methyl ester; D, β-mercaptoethanol; E, cysteine methyl ester; AA and EE are oxidized dimers of A and E, respectively.
Estimates of \( \Delta pK_a \) are given in the first column of Table III. For C, D, and the NH\(_2\) form of E, \( \Delta pK_a \) is estimated to be zero since these molecules exhibit a negligible ionic strength dependence between ionic strength of 1 M and 20 mM (see Table I). For A, pK\(_a\) values were measured at 13 different ionic strengths between 5 mM and 1 M. The pK\(_a\) values exhibit a monotonically decreasing value as ionic strength is increased and were fit to the equation

\[
pK_a = a + \frac{b pM/c}{1 + c pM/c}
\]

to obtain values for the empirical constants \( a, b, \) and \( c \). Using these values and setting \( c = \infty \), one obtains pK\(_a^\infty\) = 9.24 and \( \Delta pK_a^\infty = -0.27 \). A similar set of extensive experiments was not performed for E since data analysis is complicated by cotitration of the constituent NH\(_2\) and sulfhydryl groups in this molecule. For E, \( \Delta pK_a \) was estimated as follows. The amino group in E is separated from the sulfhydryl group by 2 carbon atoms, the same distance separating the carboxyl and sulfhydryl groups in A. Thus it is reasonable to assume that the thur-space electrostatic effect in E should be equal in magnitude but opposite in sign to effects in A, giving \( \Delta pK_a = +0.27 \) for E. This assumption will be supported below when considering data at ionic strength of 20 mM in H\(_2\)O and 50% methanol.

**Calculated Electrostatic Contributions to \( \Delta G \) for Acid Dissociation Equilibria Are Consistent with the Bjerrum Relationship**—Since inductive effects are independent of solvent, solvent-dependent changes in pK\(_a\) values correspond to thur-solvent electrostatic effects and satisfy the relationship

\[
\Delta G_{total\, eff} = 2.3RT\Delta pK_a
\]

Table IV summarizes values of \( \Delta pK_a \) and total \( \Delta G \) values for changing from the complete absence of thur-solvent electrostatic effects (given by pK\(_a\) at \( \mu = \infty \)) to the expression of electrostatic effects in H\(_2\)O or 50% MeOH (approximately by

**Table III**

**Estimates of pK\(_a\) at \( \mu = \infty \)**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Estimated ( \Delta pK_a^\infty )</th>
<th>Estimated pK(_a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.01</td>
<td>8.67</td>
</tr>
<tr>
<td>D</td>
<td>0.06</td>
<td>9.59</td>
</tr>
<tr>
<td>E (NH(_2) form)</td>
<td>-0.27</td>
<td>9.24</td>
</tr>
<tr>
<td>E (NH(_2)(^+) form)</td>
<td>0.27</td>
<td>7.79</td>
</tr>
</tbody>
</table>

\( \Delta pK_a \) in H\(_2\)O and \( \mu = \infty \) - pK\(_a\) in H\(_2\)O and \( \mu = 1 \) M.

**Table IV**

**Electrostatic free energy contributions to pK\(_a\) values**

<table>
<thead>
<tr>
<th>Solvent parameter</th>
<th>Molecule</th>
<th>Neutral R</th>
<th>A (COO(^-))</th>
<th>E (NH(_2)(^+))</th>
<th>Average*</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(_2)O</td>
<td>( \Delta pK_a^* )</td>
<td>0.01</td>
<td>0.63</td>
<td>-0.42</td>
<td></td>
</tr>
<tr>
<td>( \Delta G ) (kcal/mol)</td>
<td>Total</td>
<td>0.01</td>
<td>0.86</td>
<td>-0.57</td>
<td></td>
</tr>
<tr>
<td>S/( \text{H}_2\text{O}^+ )</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R/( \text{H}_2\text{O}^+ )</td>
<td>0.85</td>
<td>0.58</td>
<td>0.72 ± 0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% MeOH</td>
<td>( \Delta pK_a^* )</td>
<td>0.74</td>
<td>1.57</td>
<td>-0.17</td>
<td></td>
</tr>
<tr>
<td>( \Delta G ) (kcal/mol)</td>
<td>Total</td>
<td>1.01</td>
<td>2.14</td>
<td>-0.23</td>
<td></td>
</tr>
<tr>
<td>S/( \text{H}_2\text{O}^+ )</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R/( \text{H}_2\text{O}^+ )</td>
<td>1.13</td>
<td>-1.24</td>
<td>1.19 ± 0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Average absolute value for molecules A and E.

\( ^a \) pK\(_a\) in H\(_2\)O with \( \mu = 20 \) mM - pK\(_a\) in H\(_2\)O with \( \mu = \infty \).

\( ^b \) pK\(_a\) in 50% MeOH with \( \mu = 20 \) mM - pK\(_a\) in H\(_2\)O with \( \mu = \infty \).
values at $\mu = 20$ mM). The total electrostatic effect corresponds to the energy required to bring $\text{H}_2\text{O}^+$ from some location far from the peptide to a location adjacent to $\text{S}^-$ and a distance $r$ from the constituent $\text{R}$ group (Fig. 2A).

\[ \Delta G_{\text{total-eff}} = \Delta G_{\text{S-H}_2\text{O}}^\text{r} + \Delta G_{\text{TH}_2\text{O}}^\text{r} \]  

(11)

For molecules with neutral $\text{R}$ groups, representing the first column in Table IV, the thru-solvent electrostatic $\Delta G_{\text{rTH}_2\text{O}}^\text{r}$ is independent of the constituent group as discussed above, the values for $\Delta G_{\text{S-H}_2\text{O}}^\text{r}$ obtained in the first column in Table IV are carried over into the second and third columns representing molecules with charged constituents. Using Equation 11, it then is possible to calculate $\Delta G_{\text{TH}_2\text{O}}$ for molecules $\text{A}$ and $\text{E}$ in $\text{H}_2\text{O}$ and 50% methanol.

In each solvent, both molecules exhibit approximately identical absolute values for $\Delta G_{\text{TH}_2\text{O}}$ (0.72 ± 0.14 kcal/mol in $\text{H}_2\text{O}$, 1.19 ± 0.06 kcal/mol in 50% methanol). This supports the assumption applied to calculate the $\text{pK}_A$ of $\text{E}$ at infinite ionic strength. The electrostatic energy in $\text{H}_2\text{O}$ is a factor of 0.61 (equal to 0.72/1.19) smaller in $\text{H}_2\text{O}$ than in 50% methanol. This factor is approximately equal to the value of the inverse ratio of the dielectric constants for the pure solvents (equal to 57/79 for 50% MeOH/$\text{H}_2\text{O}$) (15). Finally setting $Z_2Z_1 = \pm 1$ and substituting the aqueous values of $\Delta G_{\text{TH}_2\text{O}}$ and $\epsilon$ into Equation 5, one calculates that $r = 4.3\, \text{Å}$. This is a reasonable value for the four bonds separating the COO$^-$ group in $\text{A}$ (or the NH$_3^+$ group in $\text{E}$) from the $\text{H}_2\text{O}^+$ ion adjacent to the sulfur (Fig. 2A). Thus the thru-solvent electrostatic $\Delta G_{\text{TH}_2\text{O}}$ calculated by the above procedure appears to be entirely consistent with the Bjerrum relationship.

**Correlation of Rate Constants for Those Disulfide Interchange Reactions Which Are Governed Solely by Inductive Effects—**Table V includes seven examples of reactions of small thiolate molecules with small neutral disulfides. These reactions are governed solely by inductive effects since there can be no electrostatic repulsion or attraction contributing to the frequency of collisions of the thiol- and disulfide-containing molecules. Thus it should be possible to correlate the rate constants using Brønsted relationships and the values of $\text{pK}_A$ at infinite ionic strength (Table III). As discussed previously, the $\text{pK}_A$ values at infinite ionic strength are indicators for relative sizes of inductive effects of constituent groups. For reactions between a common R$^-$ and different R$^-\cdot$SS-R$, a plot of $\log k$ versus $\text{pK}_A^R$ gives a slope equal to the sum ($\beta_{\text{pK}} + \beta_g$). For reactions between different R$^-$ with a common R$^-\cdot$SS-R$, a plot of $\log k$ versus $\text{pK}_A^R$ yields a slope equal to $\beta_{\text{pK}}$. Previously reported values of the Brønsted coefficients for reactions of small molecules at high ionic strength (1-4) are ($\beta_{\text{pK}} + \beta_g$) $\approx -1.0$ and $\beta_{\text{pK}} \approx +0.5$.

The data reported in Table V give coefficients consistent with these previous observations. The two rate constants for reaction of A-S$^-$ with C-SS-C and D-SS-D in 50% methanol give ($\beta_{\text{pK}} + \beta_g$) $\approx -0.88$. The six rate constants for reaction of A-S$^-$, D-S$^-$, and E-S$^-$ with C-SS-C in $\text{H}_2\text{O}$ and 50% methanol give $\beta_{\text{pK}}$ $\approx +0.49 \pm 0.13$ when allowing the constant term in Equation 2 to have different values in the two solvents. Rate constants in 50% methanol are smaller than corresponding values in $\text{H}_2\text{O}$, reflecting differences in solvation of sulfur atoms in the S$^-$, SS, and transition state groups. If these latter six rate constants are correlated incorrectly, using the electrostatically influenced $\text{pK}_A$ values observed at 20 mM ionic strength in $\text{H}_2\text{O}$ or 50% methanol, the fitted value of $\beta_{\text{pK}}$ is 0.34 ± 0.03.

**Calculated Electrostatic Contributions to $\Delta G$ for Disulfide Interchange Reactions between Two Charged Molecules Are Consistent with the Bjerrum Relationship—**Table V includes two examples of disulfide interchange between charged R-S$^-$ and R$^-\cdot$SS-R$^-$ molecules. These are 1) the reaction between ‘A-S$^-$’ and ‘E-SS-E$^+$’ and 2) the reaction between ‘E-S$^-$’ and ‘A-SS-A$^-$‘, both in 50% methanol at low ionic strength. Each of these reactions may be compared with the analogous reaction employing the neutral C-SS-C disulfide. The comparative $\Delta G$ values are given by

\[ \Delta \log k_{\text{observed}} = \log k_{\text{R-S}^-/\text{R}-\text{SS-R}^-} - \log k_{\text{R-S}^-/\text{C}-\text{SS-C}} \]  

(12)

and

\[ \Delta(\Delta G)^{\text{total}} = 2.3RT \Delta \log k_{\text{observed}} \]  

(13)

Results are summarized at the top of Table VI. Each $\Delta(\Delta G)$ represents the sum of differences in inductive effects of the R$^-$ versus C groups and electrostatic effects representing repulsion or attraction between R$^-$ and R$^-\cdot$SS-R$^-$. $\Delta(\Delta G)^{\text{total}} = \Delta(\Delta G)^{\text{inductive}} + \Delta(\Delta G)^{\text{electrostatic}}$  

(14)

If Brønsted relationships are used to predict rate constants, the expected contribution to $\Delta \log k$ from inductive effects is

**Table VI**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\text{E-S}^-/\text{C-SS-C}$</th>
<th>$\text{A-S}^-/\text{C-SS-C}$</th>
<th>$\text{E-S}^-/\text{R-S}^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta \log k$</td>
<td>-1.56</td>
<td>+3.09</td>
<td>+3.09</td>
</tr>
<tr>
<td>$\Delta(\Delta G)^{\text{total}}$ (kcal/mol)</td>
<td>-2.12</td>
<td>+4.23</td>
<td>+4.23</td>
</tr>
<tr>
<td>$\text{pK}_A^R - \text{pK}_A^C$</td>
<td>+0.57</td>
<td>-0.88</td>
<td>-0.88</td>
</tr>
<tr>
<td>$\Delta(\Delta G)^{\text{inductive}}$ (kcal/mol)</td>
<td>-0.78</td>
<td>+1.20</td>
<td>+1.20</td>
</tr>
<tr>
<td>$\Delta(\Delta G)^{\text{electrostatic}}$ (kcal/mol)</td>
<td>-1.34</td>
<td>+3.01</td>
<td>+3.01</td>
</tr>
<tr>
<td>Effective $Z_{R^-}$</td>
<td>-0.96</td>
<td>-0.96</td>
<td>-0.96</td>
</tr>
<tr>
<td>Effective $Z_{E^-}$</td>
<td>+0.44</td>
<td>+0.44</td>
<td>+0.44</td>
</tr>
<tr>
<td>Calculated</td>
<td>+0.5</td>
<td>-0.27</td>
<td>-0.27</td>
</tr>
<tr>
<td>Expected</td>
<td>+0.5</td>
<td>-0.5</td>
<td>-0.5</td>
</tr>
<tr>
<td>R</td>
<td>NH$_3^+$</td>
<td>COO$^-$</td>
<td>COO$^-$</td>
</tr>
</tbody>
</table>

* R-S$^-$ with R$^-\cdot$SS-R$^-$. 

**Fig. 2. Geometries for evaluating thru-solvent electrostatic contributions.** A, reaction of acetylcysteine with $\text{H}_2\text{O}^+$; B, reaction of diacetylcysteine with cysteine methyl ester.
Disulfide Exchange between Charged Molecules in 50% Methanol

given by

\[ \Delta \log K_{\text{inductive}} = (\beta_{\text{ion}} + \beta_q)(pK_{\text{charge}} - pK_{c}) \]  

Then using pK values at infinite ionic strength from Table III and using the previously reported value \((\beta_{\text{ion}} + \beta_q) = -1.0\), one obtains

\[ \Delta (\Delta G^\dagger)_{\text{inductive}} = +2.3RT(pK_{\text{charge}} - pK_{c}) \]  

Subtraction of this term from \(\Delta (\Delta G^\ddagger)_{\text{total}}\) gives \(\Delta (\Delta G^\ddagger)_{\text{electrostatic}}\). Results in Table VI indicate that \(A^-S^-\) and \(E^-SS-E^+\) experience a mutual electrostatic attraction of 3.01 kcal/mol, whereas \(E^-S^-\) and \(A^-SS-A^+\) experience a mutual repulsion of -1.34 kcal/mol.

Consider the two reactions represented in Fig. 2. The upper reaction corresponds to bringing an \(H_2O^+\) group up to a distance \(r\) away from one carboxyl group in acetylcysteine. The average \(\Delta G_{\text{electrostatic}}\) in 50% methanol for this geometry was shown in Table IV to equal 1.19 kcal/mol. The lower reaction \((\Delta G^\ddagger) = -1.34\) kcal/mol corresponds to bringing an \(E^-S^-\) molecule of effective charge \(Z_{R^-S^-}\) up to a distance \(r\) away from the two carboxyl groups in A-SS-A. Comparing these free energies with the Bjerrum relationship gives

\[ \frac{\Delta (\Delta G^\ddagger)_{\text{inductive}}}{\Delta (\Delta G^\ddagger)_{\text{total}}} = \frac{2Z_{R^-S^-}Z_{\text{H}^+}}{\epsilon r} \]  

For this example, \(2Z_{R^-S^-} = -1.34/1.19\), so the effective charge of \(E^-S^-\) is -0.56. Since the thiolate sulfur contributes \(-1.00\) to the effective charge, the amine group on \(E^-S^-\) behaves equivalently to a charge of \(+0.44\) unit located at the sulfur position. Since the distance between the amine and \(R^-\) groups is approximately twice the size of the distance between the \(S^-\) and \(R^-\) groups (Fig. 2), the Bjerrum relationship would require that the amino group exhibit an effective charge of \(+0.50\) unit. This agrees closely with the calculated value of +0.44. Similar calculations for the \(A^-S^-/E^-SS-E^+\) reaction are given in Table VI and yield the result that the carboxyl group of acetylcysteine behaves like an effective charge of \(-0.27\) unit, close to the expected value of \(-0.50\) unit. Thus calculated values of \(\Delta (\Delta G^\ddagger)_{\text{electrostatic}}\) are reasonably consistent with the Bjerrum relationship.

CONCLUSIONS

Strategies for directing disulfide pairing in synthetic proteins may include electrostatic factors which stabilize or destabilize juxtaposition of cysteine-containing regions having particular local ionic charges. Coulombic interactions are enhanced in 50% methanol at low ionic strength. In the examples reported in Table V, the fastest and slowest disulfide interchange rate constants differed by a factor of \(6 \times 10^5\) s\(^{-1}\). This total difference includes a 2 \times 10^3-fold contribution from electrostatic effects representing a free energy difference of 4.35 kcal/mol (+3.01 attraction versus -1.34 repulsion). The pK values and rate constants in 50% methanol may be correlated by separation of free energy contributions into solvent-independent and solvent-dependent factors. The former represent inductive effects and give Brønsted coefficients consistent with previously reported values for neutral molecules. The latter represent thru-solvent electrostatic effects and are consistent with the form of the Bjerrum relationship.

Acknowledgments—I wish to thank Jennifer Cadiz for preliminary kinetic measurements in 50% MeOH, Yiping Sun for technical assistance, and Michael Cennerazzo for helpful discussions and potential titration data.

REFERENCES