and S_{NH_2}) were calculated by evaluating the partition functions from the eigenstates determined in this study. S_{mix}^r is based on 78.4% inner and 21.6% outer rotamers.

It is seen that the spectroscopic entropy is 1.44 eu greater than the heat capacity value. This excess may interpreted as evidence for residual entropy in methylhydrazine at 0°K. In particular, if it is assumed that S_{mix}° ($= R \ln 2$), the entropy of mixing of optical isomers, is present at 0°K, then the spectroscopic entropy agrees with the heat capacity value to within experimental error.

Conclusion

In this paper we have utilized an approximate quantum mechanical method to determine the shape of the barrier hindering the torsion about the N–N bond in methylhydrazine. We have determined the first six Fourier coefficients which may be used to describe this barrier and have presented a reasonable explanation for the far-infrared torsional spectrum of the molecule. Using our barrier description we have reexamined the entropy of methylhydrazine and have found our results to be consistent with the entropy determined experimentally from heat capacity measurements.

Currently in our laboratory we are reexamining the torsional barrier in the parent hydrazine molecule. Kasuya[16] attempted to determine this barrier, but the model used was an incorrect one which assumed the cis and trans barriers to be equal. We are attempting to obtain a more satisfactory barrier description by (1) utilizing additional Fourier terms in the potential energy expression for the barrier, and (2) combining the results of the microwave[16] and far infrared[25] studies of the molecule.

Acknowledgments. We are grateful to the National Science Foundation for support of this research through Grant GP-15127, and one of us (R. P. L.) has appreciated the support from an NSF traineeship. Facilities and computer time made available to us by the University of Kansas Computation Center are greatly appreciated.

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(25) A. Yamaguchi, I. Ichishima, T. Shimanouchi, and S. Mizushima, *Spectrochim. Acta*, **16**, 1471 (1966).

Relaxation Amplitudes for Systems of Two Coupled Equilibria

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Abstract: Equations are developed allowing one to set down, merely by inspection of the stoichiometry, the relation between the overall change in signal following the perturbation of a system of two coupled equilibria and the thermodynamic functions associated with the elementary steps. It is shown that expressions for individual relaxation amplitudes can also be calculated by inspection. The treatment includes the case where a strong kinetic coupling exists between the two equilibria. Applications are cited involving the determination of enthalpies for the fixation of the competitive inhibitors proflavin and benzamidine to trypsin.

In transient chemical relaxation techniques a reaction solution at equilibrium is perturbed by a rapid change in some external variable, such as temperature or pressure.[1] If the perturbation is sufficiently small, the time dependence of the reactant concentrations is characterized by a series of exponential decays. Relaxation amplitudes, or the net signal changes associated with the observed exponentials, are measures of equilibrium shifts and can be used to determine thermodynamic functions (ΔH, ΔV, etc.) for elementary reaction steps. Amplitudes are also related to the question of whether a relaxation process is observable and can serve as a check on a mechanism suggested by kinetic information.

The general thermodynamic relations governing chemical relaxation have been developed by Eigen and DeMaeyer,[1] who discuss a "normal mode" analysis of relaxation amplitudes. Czerlinski,[2] in a different approach, has derived amplitude expressions for quite a number of mechanisms in which the elementary steps equilibrate at very different rates.

For multistep mechanisms the equations relating thermodynamic parameters and relaxation amplitudes are often numerically complex, and their derivations can be tedious. In the present paper such relations are considered for systems of two coupled equilibria. Equations are developed allowing one to set down, merely by inspection of the stoichiometry, the relation between the *overall* change in signal induced by a physical perturbation, and the thermodynamic parameters associated with the elementary steps. It is shown that expressions for *individual* relaxation amplitudes can also be calculated by inspection. The treatment includes the case where a strong kinetic coupling exists between the two equilibria. Practical applications are cited involving the determination of enthalpies for the fixation of the competitive inhibitors proflavin and benzamidine to trypsin.

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(1) M. Eigen and L. DeMaeyer, "Techniques of Organic Chemistry," Vol. VIII, 2nd ed, Part 2, S. L. Friess, E. S. Lewis, and A. Weissberger, Ed., Wiley, New York, N. Y., 1963, p 895.

(2) G. H. Czerlinski, "Chemical Relaxation," Marcel Dekker, New York, N. Y., 1966.

Relaxation Amplitude Expressions for One-Step Reactions

The "instantaneous" perturbation of a one-step equilibrium system is followed by an exponential change in reactant concentrations.[1] If P is a physical property linearly proportional to the concentration of each chemical component (*e.g.*, conductance, absorbance, optical rotation) the time dependence of the observed relaxation effect is

$$\delta P_{(t)} = \delta P^0 \exp(-t/\tau) \tag{1}$$

where τ is the relaxation time, $\delta P_{(t)}$ is the instantaneous deviation of P from its (final) equilibrium value, and δP^0, the relaxation amplitude, is the net change in P due to the chemical relaxation process. It can be shown from mass conservation considerations that the relaxation amplitude is related to the overall shift in the equilibrium concentration of the ith component by eq 2.

$$\delta P^0 = \Delta\phi(\delta\bar{C}_i/\nu_i) \tag{2}$$

$$\Delta\phi = \sum \nu_i\phi_i;\ \ \phi_i = \partial P_i/\partial \bar{C}_i$$

The ϕ_i's are proportional to specific conductivities, extinction coefficients, specific optical rotations, etc. The ν_i's are stoichiometric coefficients, defined as negative for participants on the left side of the reaction equation and positive for those on the right side.[3] Although the sum of stoichiometric coefficients appearing on either side of the reaction equation will rarely exceed a value of 2 for elementary processes in solution, higher order reactions are found if the overall transformation proceeds through intermediates present in undetectable amounts (*e.g.*, (6) of Table II.1, ref 1). Eigen and DeMaeyer have shown that $\delta\bar{C}_i$ for a one-step equilibrium is related to equilibrium concentrations and the forced change in the equilibrium constant by a rather simple expression[4]

$$\delta\bar{C}_i = \nu_i\Gamma\delta \ln K \tag{3}$$

where

$$K = \Pi\bar{C}_i^{\nu_i} \tag{4}$$

$$\Gamma = 1/\sum \nu_i^2/\bar{C}_i \tag{5}$$

$\delta \ln K$ is proportional to the relevant thermodynamic function. For example, in the case of a temperature jump[3]

$$\delta \ln K = (\Delta H/RT^2)\delta T \tag{6}$$

$$\Delta H = \sum \nu_i\bar{H}_i \tag{7}$$

where $\bar{H}_i$ is the partial molar enthalpy of species i, and

$$\delta T = (T_\infty - T_0) = \text{temperature change} \tag{8}$$

The following useful relation is obtained from eq 2 and 3.

$$\delta P^0 = \Delta\phi\Gamma\delta \ln K \tag{9}$$

If the equilibrium constant and $\Delta\phi$ are known, $\delta \ln K$ (and therefore the thermodynamic function) can be conveniently evaluated by plotting δP^0 *vs.* Γ.

It is evident from eq 5 that the appropriate Γ function can be written by simply examining the reaction stoichiometry. Thus, for the temperature perturbation of a dimerization equilibrium

$$2\text{A} \rightleftharpoons \text{A}_2 \tag{10}$$

the relaxation amplitude is

$$\delta P^0 = \Delta\phi[(4/\bar{A}) + (1/\bar{A}_2)]^{-1}(\Delta H/RT^2)\delta T \tag{11}$$

$$\Delta\phi = (\phi_{\text{A}_2} - 2\phi_\text{A});\ \ \Delta H = (\bar{H}_{\text{A}_2} - 2\bar{H}_\text{A}) \tag{12}$$

Table I lists some Γ functions for other one-step equilibria. Examples of practical applications are found in ref 5–7.

Table I. Γ Functions for Some One-Step Reaction Systems

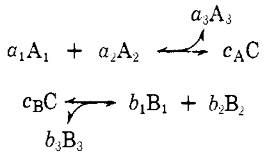
System	Γ^a
$\text{A} \leftrightarrow \text{A}'$	$\bar{A}/(1 + 1/K)$ $KA^0/(1 + K)^2$ $A^0/(1 + K)[1 + (1/K)]$
$\text{A} + \text{B} \leftrightarrow \text{AB}$	$\bar{A}\bar{B}/[\bar{A} + \bar{B} + (1/K)]$ $\overline{AB}/[K(\bar{A} + \bar{B}) + 1]$ $[(1 - 4P/S^2)^{-1/2} - 1]/2K$ $S = A^0 + B^0 + 1/K$ $P = A^0B^0$
Limiting case $B^0, 1/K \gg A^0$	$KA^0B^0/(1 + B^0K)^2$ $A^0/(1 + B^0K)[1 + (B^0/K)]$
$2\text{A} \leftrightarrow \text{A}_2$	$\bar{A}^2/[4\bar{A} + (1/K)]$ $1 + 4KA^0 - (1 + 8KA^0)^{1/2}/$ $8K(1 + 8A^0K)^{1/2}$

[a] The superscript (0) indicates analytical concentrations.

Thermodynamic Relations for Two Coupled Equilibria

Now consider Scheme I for two consecutive equilibria

Scheme I



$$a_1\text{A}_1 + a_2\text{A}_2 \rightleftharpoons c_\text{A}\text{C} + a_3\text{A}_3$$

$$c_\text{B}\text{C} + b_3\text{B}_3 \rightleftharpoons b_1\text{B}_1 + b_2\text{B}_2$$

coupled *via* a common intermediate, C, where C, the A$_i$'s, and the B$_i$'s are all different chemical species. The equilibrium constants are here defined as

$$\begin{aligned} K_\text{A} &= \bar{A}_3{}^{a_3}\bar{C}^{c_\text{A}}/\bar{A}_1{}^{a_1}\bar{A}_2{}^{a_2} \\ K_\text{B} &= \bar{B}_3{}^{b_3}\bar{C}^{c_\text{B}}/\bar{B}_1{}^{b_1}\bar{B}_2{}^{b_2} \end{aligned} \tag{13}$$

and the two microscopic enthalpies are given by

$$\begin{aligned} \Delta H_\text{A} &= a_3\bar{H}_{\text{A}_3} + c_\text{A}\bar{H}_\text{C} - a_1\bar{H}_{\text{A}_1} - a_2\bar{H}_{\text{A}_2} \\ \Delta H_\text{B} &= b_3\bar{H}_{\text{B}_3} + c_\text{B}\bar{H}_\text{C} - b_1\bar{H}_{\text{B}_1} - b_2\bar{H}_{\text{B}_2} \end{aligned} \tag{14}$$

Taking logarithms and then differentials of the K_A and K_B expressions (eq 13) yields

$$-\delta \ln K_\text{A} = a_1\frac{\delta\bar{A}_1}{\bar{A}_1} + a_2\frac{\delta\bar{A}_2}{\bar{A}_2} - a_3\frac{\delta\bar{A}_3}{\bar{A}_3} - c_\text{A}\frac{\delta\bar{C}}{\bar{C}} \tag{15}$$

$$-\delta \ln K_\text{B} = b_1\frac{\delta\bar{B}_1}{\bar{B}_1} + b_2\frac{\delta\bar{B}_2}{\bar{B}_2} - b_3\frac{\delta\bar{B}_3}{\bar{B}_3} - c_\text{B}\frac{\delta\bar{C}}{\bar{C}} \tag{16}$$

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(3) Reference 1, p 928.

(4) We neglect here considerations of activity coefficients and volume changes. Concentration units are assumed to be moles per liter. See ref 1, eq II.3.18 and II.3.24.

(5) G. G. Hammes and W. Knoche, *J. Chem. Phys.*, **45**, 4041 (1966).

(6) R. Winkler, Doctoral Thesis, Göttingen, 1969.

(7) F. Guillain and D. Thusius, *J. Amer. Chem. Soc.*, **92**, 5534 (1970).

Scheme I is characterized by the following mass conservation restrictions

$$\delta\bar{A}_1/a_1 = \delta\bar{A}_2/a_2 = -\delta\bar{A}_3/a_3 \qquad (17)$$

$$\delta\bar{B}_1/b_1 = \delta\bar{B}_2/b_2 = -\delta\bar{B}_3/b_3 \qquad (18)$$

$$-\delta\bar{C} = (c_A/a_1)\delta\bar{A}_1 + (c_B/b_1)\delta\bar{B}_1 \qquad (19)$$

Equations 15 and 16 can be expressed in terms of any two concentration variables, $\delta\bar{A}_i$ and $\delta\bar{C}$, $\delta\bar{B}_i$ and $\delta\bar{C}$, or $\delta\bar{A}_i$ and $\delta\bar{B}_i$. It will be seen below that the latter pair leads to particularly useful expressions. If $\delta\bar{A}_1$ and $\delta\bar{B}_1$ are chosen as variables, eq 15 and 16 reduce to

$$-\delta \ln K_A = \left[\frac{a_1^2}{\bar{A}_1} + \frac{a_2^2}{\bar{A}_2} + \frac{a_3^2}{\bar{A}_3} + \frac{c_A^2}{\bar{C}}\right]\frac{\delta\bar{A}_1}{a_1} + \frac{c_A c_B}{\bar{C}}\frac{\delta\bar{B}_1}{b_1} \qquad (20)$$

$$-\delta \ln K_B = \frac{c_A c_B}{\bar{C}}\frac{\delta\bar{A}_1}{a_1} + \left[\frac{b_1^2}{\bar{B}_1} + \frac{b_2^2}{\bar{B}_2} + \frac{b_3^2}{\bar{B}_3} + \frac{c_B^2}{\bar{C}}\right]\frac{\delta\bar{B}_1}{b_1} \qquad (21)$$

The sums of concentration terms multiplied by $\delta\bar{A}_1/a^1$ in eq 20 and by $\delta\bar{B}_1/b_1$ in eq 21 are simply the reciprocal Γ functions for equilibrium A and B, respectively. These equations are conveniently expressed in matrix form as

$$-\begin{vmatrix}\delta \ln K_A \\ \delta \ln K_B\end{vmatrix} = \begin{vmatrix}\Gamma_A^{-1} & c_A c_B/\bar{C} \\ c_A c_B/\bar{C} & \Gamma_B^{-1}\end{vmatrix}\begin{vmatrix}\delta\bar{A}_1/a_1 \\ \delta\bar{B}_1/b_1\end{vmatrix} \qquad (22)$$

$\delta\bar{A}_1$ and $\delta\bar{B}_1$ can be expressed as linear functions of the $\delta \ln K$'s by taking the inverse of the above matrix. Multiplying numerator and denominator of the resulting equations by $\Gamma_A\Gamma_B$ yields the symmetric relations

$$\delta\bar{A}_1/a_1 = -\frac{\Gamma_A}{1 - \Gamma_A\Gamma_B f^2}[\delta \ln K_A - (\Gamma_B f)\delta \ln K_B] \qquad (23)$$

$$\delta\bar{B}_1/b_1 = -\frac{\Gamma_B}{1 - \Gamma_A\Gamma_B f^2}[\delta \ln K_B - (\Gamma_A f)\delta \ln K_A] \qquad (24)$$

$$f = c_A c_B/\bar{C} \qquad (25)$$

Equations 23 and 24, together with the mass conservation restrictions of eq 17–19, give a complete description of the *total* changes in equilibrium concentrations brought about by small changes in the values of ln K_A and ln K_B. A more general derivation of eq 23 and 24 is found in Appendix I.

Even when all stoichiometric coefficients are equal to unity, Scheme I breaks down into ten different subschemes.[8] The advantage of formulating $\delta\bar{A}_1$ and $\delta\bar{B}_1$ in terms of Γ's and $c_A c_B/\bar{C}$ is that expressions for overall changes in equilibrium concentrations can be set down merely by an examination of the mechanism. In addition, the formalism of eq 23–24 allows one to readily note conditions leading to maximum and minimum thermodynamic coupling between the two steps. The dimensionless terms $c_A c_B\Gamma_A/\bar{C}$ and $c_A c_B\Gamma_B/\bar{C}$ may be considered "coupling constants." These terms approach the following limiting values at relatively low and high concentrations of the coupling species ($\bar{A}_{\min}$ ($\bar{B}_{\min}$) and $\bar{A}_{\max}$ ($\bar{B}_{\max}$) represent respectively the least and most highly populated A_i (B_i) participant).

Case I

$$\bar{C} \ll \bar{A}_{\min} \qquad\qquad \bar{C} \ll \bar{B}_{\min}$$

$$c_A c_B\Gamma_A/\bar{C} \longrightarrow c_B/c_A \qquad\qquad c_A c_B\Gamma_B/\bar{C} \longrightarrow c_A/c_B$$

Case II

$$\bar{C} \gg \bar{A}_{\max} \qquad\qquad \bar{C} \gg \bar{B}_{\max}$$

$$c_A c_B\Gamma_A/\bar{C} \longrightarrow 0 \qquad\qquad c_A c_B\Gamma_B/\bar{C} \longrightarrow 0$$

It will be noted that when both limiting conditions of case I are satisfied, component C will exist in a "steady state" and Scheme I should be formally treated as a one-step reaction. When the coupling is at a minimum (C "buffering"), the elementary steps equilibrate independently and eq 23–24 reduce to the amplitude expressions expected for the isolated reactions

Case II

$$\delta\bar{A}_1/a_1 \longrightarrow -\Gamma_A\delta \ln K_A \qquad (26)$$

$$\delta\bar{B}_1/b_1 \longrightarrow -\Gamma_B\delta \ln K_B \qquad (27)$$

An interesting family of reactions (Scheme II) involving two coupling components is derived from Scheme I by setting C $\equiv$ C_1 and $A_3 \equiv B_3 \equiv C_2$. Using the approach already described, it can be shown that $\delta\bar{A}_1$ and $\delta\bar{B}_1$ are again given by eq 23–24, but the term $c_A c_B/\bar{C}$ is replaced by the sum

Scheme II

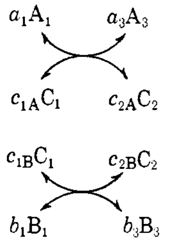
$$a_1A_1 + a_2A_2 \longleftrightarrow c_{1A}C_1 + c_{2A}C_2$$

$$c_{1B}C_1 + c_{2B}C_2 \longleftrightarrow b_1B_1 + b_2B_2$$

$$f = c_{1A}c_{1B}/\bar{C}_1 + c_{2A}c_{2B}/\bar{C}_2 \qquad (28)$$

Equation 28 also applies to the following cyclic system, which is derived from Scheme I by setting C $\equiv$ C_2 and $A_2 \equiv B_2 \equiv C_1$. Scheme III and its submechanisms are particularly interesting with respect to enzyme redox reactions.[8]

Scheme III



$$K_A = \frac{\bar{A}_3^{a_3}\bar{C}_2^{c_{2A}}}{\bar{A}_1^{a_1}\bar{C}_1^{c_{1A}}}$$

$$K_B = \frac{\bar{B}_3^{b_3}\bar{C}_2^{c_{2B}}}{\bar{B}_1^{b_1}\bar{C}_1^{c_{1B}}}$$

Some representative calculations of coupling constants are given in Table II. The examples are typical enzyme–substrate systems. It will be noted that the coupling constants are rather simple functions of equilibrium concentrations and equilibrium constants.

Overall Relaxation Amplitudes. An Application Involving the Determination of Reaction Enthalpies for Two Coupled Second-Order Reactions

It can be shown that for the systems under consideration the induced *overall* change in an observed physical property is[9]

$$-\delta P_{\text{tot}}^0 = \Delta\phi_A(\delta\bar{A}_1/a_1) + \Delta\phi_B(\delta\bar{B}_1/b_1) \qquad (29)$$

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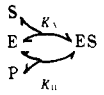
[8] G. H. Czerlinski, *J. Theor. Biol.*, 7, 463 (1964).

[9] If changes in $\Delta\phi_{A,B}$ or volume are significant, additional terms must be added to eq 29.

Table II. Examples of Coupling Constants for Some Typical Enzyme–Substrate Interactions

Example	Reaction system	Coupling constants[a] $\Gamma_A f$	$\Gamma_B f$
Scheme I	$E + S \xrightleftharpoons{K_A} ES \xrightleftharpoons{K_B} (ES)'$	$\dfrac{1}{1 + K_A(\bar{E} + \bar{S})}$	$\dfrac{1}{1 + K_B}$
Scheme I	$E_2 \xrightleftharpoons{K_A} 2E$ $E + S \xrightleftharpoons{K_B} ES$	$\dfrac{2\bar{E}}{4\bar{E} + K_A}$	$\dfrac{2\bar{S}}{\bar{E} + \bar{S} + K_B}$
Scheme II	$ES_2 \xrightleftharpoons{K_A} E + 2S$ $E + S \xrightleftharpoons{K_B} ES$	$\dfrac{2\bar{E} + \bar{S}}{4(\bar{E})(\bar{S}) + \bar{S}^2 + K_A}$	$\dfrac{2\bar{E} + \bar{S}}{\bar{E} + \bar{S} + K_B}$
Scheme III		$\dfrac{1 + K_A\bar{S}}{1 + K_A(\bar{E} + \bar{S})}$	$\dfrac{1 + K_B\bar{P}}{1 + K_B(\bar{E} + \bar{P})}$

[a] $f = \Sigma c_{iA}c_{iB}/\bar{C}_i$.

$$\Delta\phi_A = a\phi_{3A_3} + c_A\phi_C - a_1\phi_{A_1} - a_2\phi_{A_2} \qquad (30)$$

$$\Delta\phi_B = b_3\phi_{B_3} - c_B\phi_C + b_1\phi_{B_1} - b_2\phi_{B_2} \qquad (31)$$

Since $\delta\bar{A}_1$ and $\delta\bar{B}_1$ are linear functions of δ ln K_A and δ ln K_B, $\delta P_{tot}{}^0$ is also a linear function of these parameters, or, from eq 23, 24, and 29

$$\delta P_{tot}{}^0 = \alpha_1 X_1 + \alpha_2 X_2 \qquad (32)$$

$$\alpha_1 = \delta \ln K_A;\ \alpha_2 = \delta \ln K_B \qquad (33)$$

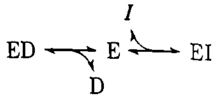
$$X_1 = \frac{\Gamma_A}{1 - \Gamma_A\Gamma_B f^2}(\Delta\phi_A - \Gamma_B f \Delta\phi_B) \qquad (34)$$

$$X_2 = \frac{\Gamma_B}{1 - \Gamma_A\Gamma_B f^2}(\Delta\phi_B - \Gamma_A f \Delta\phi_A) \qquad (35)$$

If the $\Delta\phi$'s and equilibrium constants are known with sufficient precision, α_1 and α_2 (and therefore the relevant thermodynamic functions) can be evaluated by measuring $\delta P_{tot}{}^0$ at various values of $\{X_1, X_2\}$ (where $\{X_1, X_2\}$ is varied by changing analytical concentrations) and fitting the results to eq 32 using a multilinear least-squares procedure.[10]

As an example, consider the case of two inhibitors (I, D) competing for the active site of enzyme E.[11] Assume that E, I, and EI do not absorb ($\Delta\phi_B = 0$) and that D is a dye which undergoes a significant change in absorbance upon binding to enzyme. Scheme IV

Scheme IV



$K_A = \bar{E}\cdot\bar{D}/\overline{ED}$

$K_B = \bar{E}\cdot\bar{I}/\overline{EI}$

is a submechanism of Scheme I, and therefore $f = c_Ac_B/\bar{C} = 1/\bar{E}$. It follows immediately from eq 32–35 that the overall amplitude may be formulated as[12]

$$-\delta I_{tot}{}^0/I^0 = \alpha_1{}'X_1 + \alpha_2{}'X_2 \qquad (36)$$

where I^0 is the intensity of transmitted light at $t = 0$, $\delta I_{tot}{}^0$ is the overall deviation from I^0, the regression coefficients to be determined, $\alpha_1{}'$ and $\alpha_2{}'$, are $2.3(\epsilon_D - \epsilon_{ED})/\delta$ ln K_A and $2.3(\epsilon_D - \epsilon_{ED})/\delta$ ln K_B, respectively, and the independent variables are

$$X_1 = \frac{\Gamma_A}{1 - \Gamma_A\Gamma_B/\bar{E}^2};\ X_2 = -\frac{\Gamma_B}{\bar{E}}X_1 \qquad (37)$$

The Γ's are readily given as explicit functions of equilibrium concentrations (Table I). For example

$$\Gamma_A = \frac{\bar{E}\cdot\bar{D}}{\bar{E} + \bar{D} + K_A};\ \Gamma_B = \frac{\bar{E}\cdot\bar{I}}{\bar{E} + \bar{I} + K_B} \qquad (38)$$

In practice, it would be convenient to keep total concentrations of E and D constant, and "titrate" the E–D mixture by measuring the overall amplitude after successive additions of component I.

In this way the author has determined, using the temperature-jump technique, reaction enthalpies for the fixation of the competitive inhibitors benzamidine (nonchromophoric) and proflavin (chromophoric) to the enzyme trypsin. The proflavin equilibrium constant (K_A) and the parameter $(\epsilon_D - \epsilon_{ED})_{469}$ were determined by a classical spectrophotometric titration.[7] The benzamidine equilibrium constant (K_B) was determined by adding benzamidine to a trypsin–proflavin solution and measuring the decrease in OD_{469}.[7] A method of successive approximations was used to calculate the equilibrium concentrations $\bar{E}$, $\bar{D}$, and $\bar{I}$ in the temperature-jump experiments, which in turn were used to calculate X_1 and X_2.[13] The results of the amplitude titration are presented in Figure 1. Since the experimental error was due mostly to a constant uncertainty in $\delta I_{tot}{}^0$ (background noise), a weighting factor of unity was assumed in the least-squares analysis.[10] All calculations were programmed for a Wang desk-top calculator.

It is interesting that if one treats the overall amplitude expression as a multiple-regression problem, both reaction enthalpies can be evaluated from a single titration. Also to be noted is the ease with which rather small reaction enthalpies can be determined using the temperature-jump technique in comparison with the usual determination of K at different temperatures.[14]

Although the application cited here involved an independent determination of K_B, prior knowledge of this parameter is not necessary if a rough estimate is available. Then the least-squares program for the titration can be modified to calculate the nonlinear coefficient K_B, in addition to the linear terms δ ln K_A and δ ln K_B, by an iterative procedure.[10] However, to obtain values for the thermodynamic parameters, the constant $(\epsilon_D - \epsilon_{ED})$ must be known. If this quantity is not known, an analysis of the overall amplitudes yields values for $\Delta\phi_A\delta$ ln K_A and $\Delta\phi_A\delta$ ln K_B, which is of interest in that the standard errors associated with these parameters are a measure of how well the amplitude data fit the assumed stoichiometry.

The mechanisms under consideration are in general defined by two relaxation times and their corresponding

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(10) For a pertinent review of statistical methods, see W. W. Cleland, *Advan. Enzymol. Relat. Subj. Biochem.*, **29**, 1 (1967).

(11) The kinetics and thermodynamics of this type of reaction system have also recently been discussed elsewhere: (a) G. H. Czerlinski in "Theoretical and Experimental Biophysics," Vol. 2, A. Cole, Ed., Marcel Dekker, New York, N. Y., 1969, p 106, (b) ref 6; (c) ref 7.

(12) Usually differences in transmission are measured. If $\delta I/I^0 \ll 0.1$, the change in OD is approximated by $\delta OD = -\delta I/(2.3)I^0$. Detailed discussions of the relationships between measured signal changes and optical properties are found in ref 1 (pp 973–976) and 2 (Chapter 7).

(13) In the present work the factor $\Gamma_A\Gamma_B/\bar{E}^2$ was always rather small, and no loss in precision was introduced by taking the difference in eq 37.

(14) It is emphasized that the present results have been interpreted in terms of the simplest mechanism consistent with the data available to date. It is possible, for example, that relaxation phenomena exist outside the time range of our temperature-jump instrument.

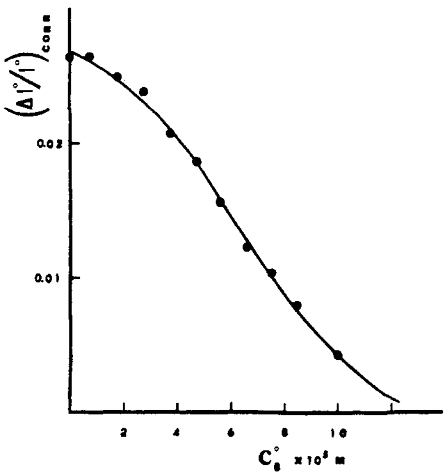

Figure 1. Overall relaxation amplitudes as a function of total benzamidine concentration in the temperature jump titration of the trypsin–proflavin complex. The measured amplitudes have been corrected for a small transmission change observed with proflavin alone $[(\delta I^0/I^0)_{C_E{}^0=0} = -0.0036]$. The latter relaxation is proportional to $C_D{}^0$ and probably represents the direct effect of temperature on ϵ_D. Ordinate values were calculated from the relation $(\delta I^0/I^0)_{corr} = (\delta I^0/I^0)_{obsd} - (\overline{C}_D/C_D{}^0)(\delta I^0/I^0)_{C_E{}^0=0}$. Conditions: $C_D{}^0 = 2.24 \times 10^{-5}$ M, $C_E{}^0 = 9.1 \times 10^{-5}$ M, pH 8.1, 0.14 M tris-Cl$^-$, 0.020 M, CaCl$_2$, 469 nm, 1-cm path length, temperature = 14°, δT = 4.4°. The quantities X_1 and X_2 (eq 38) were calculated for each experimental point from analytical concentrations and the two equilibrium constants. The data were fit to eq 36 with a bilinear regression program. The regression coefficients were $\alpha_1' = (4.94 \pm 0.04)10^3$ and $\alpha_2' = (7.0 \pm 0.2)10^3$ OD M^{-1}. Together with $\Delta\epsilon_{469}$ and δT, these figures yield $\Delta H_A = 3.1 \pm 0.3$ and $\Delta H_B = 4.4 \pm 0.6$ kcal/mol. The line in the figure was calculated from the experimental values of α_1' and α_2'.

individual relaxation amplitudes. The latter are evaluated experimentally by extrapolating the appropriate log plots to $t = 0$, and may be considered by-products obtained in any kinetic study. Although ΔH_A and ΔH_B can in principle also be obtained from the individual amplitudes, it seems worthwhile to note that the overall amplitude offers certain advantages as an experimental parameter.[15] (1) The overall signal change is read directly from the oscilloscope trace, which involves considerably less labor than the graphical evaluation of individual amplitudes. (2) Overall amplitudes can be determined with higher precision than individual amplitudes, owing to the smaller bandwidths which may be employed in the former measurements. For example, the data of Figure 1 were obtained at rather low proflavin concentrations (to prevent aggregation), where meaningful values for relaxation times or individual amplitudes could not always be determined. (3) Rate constants can occur in the expressions for individual amplitudes (see below), in which case both kinetic and amplitude analyses are necessary to estimate thermodynamic parameters. Whenever possible, of course, both overall and individual amplitudes should be evaluated and shown to be consistent with the same mechanism.

Calculating Individual Relaxation Amplitudes by Examination of the Reaction Stoichiometry. The Two Steps Equilibrate at Very Different Rates. The expressions of eq 23 and 24 may also be used to calculate amplitudes of individual relaxation effects. Consider the limiting case where step A is fast enough to equilibrate independently of step B. Then, to a first approximation, changes in the concentration of B_1 can be ignored in the time range of τ_I, and the amplitude of the fast effect will be

$$\delta P_I{}^0 \simeq \Delta\phi_A\Gamma_A\delta \ln K_A \qquad (39)$$

At long times, changes in the concentration of B_1 become important. Owing to the large difference in equilibration rates, the second relaxation effect is associated with the *overall* change in $\bar{B}_1$

$$(\delta B_1/b_1)_t \simeq (\delta\bar{B}_1/b_1) \exp(-t/\tau_{II}) \qquad (40)$$

where $\delta\bar{B}_1/b_1$ can be immediately obtained from eq 24. In the time range of τ_{II}, the signal change is not simply proportional to $\Delta\phi_B$, but will include a contribution from $\Delta\phi_A$. The amplitude of the slow process is the difference

$$\delta P_{II}{}^0 = \delta P_{tot}{}^0 - \delta P_I{}^0 \qquad (41)$$

or, from eq 32 and 39[16]

$$\delta P_{II}{}^0 \simeq \Delta\phi_{II}[\Gamma_B/(1 - \Gamma_A\Gamma_Bf^2)]\delta \ln K_{II} \qquad (42)$$

where

$$\Delta\phi_{II} = \Delta\phi_B - \Gamma_Af\Delta\phi_A \qquad (43)$$

$$\delta \ln K_{II} = \delta \ln K_B - \Gamma_Af\delta \ln K_A \qquad (44)$$

As an example, consider the rapid binding of a substrate to an enzyme followed by a slow isomerization of the ES complex (Scheme V). From eq 39 the amplitude of the fast relaxation phenomenon is simply (Table I)

Scheme V

$$E + S \underset{\text{very fast}}{\rightleftharpoons} ES \underset{\text{slow}}{\rightleftharpoons} (ES)'$$

$$K_A = \overline{ES}/\overline{E}\cdot\overline{S} \qquad K_B = \overline{ES}/\overline{ES'}$$

$$\delta P_I{}^0 = \Delta\phi_A\left[\frac{\overline{ES}}{K_A(\bar{E} + \bar{S}) + 1}\right]\delta \ln K_A$$
$$\Delta\phi_A = (\phi_{ES} - \phi_E - \phi_S) \qquad (45)$$

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(15) A *disadvantage* of overall amplitude measurements is that correction terms may be necessary if volume changes are important or if the coefficients $(\delta\phi_i/\delta T)$ cannot be neglected.

(16) Equation 42 is of interest with respect to the "normal-mode" analysis of relaxation phenomena,[1] where the relaxation times are defined by two completely decoupled "normal concentration variables," Y

$$Y_i = \bar{Y}_i \exp(-t/\tau_i) \qquad (i)$$

The observed amplitudes, $\delta P_i{}^0$, may be expressed in terms of normal-mode extinction coefficients (specific conductivities, etc.), Γ functions, and thermodynamic parameters.

$$\delta P_I{}^0 = \Delta\phi_I\Gamma_I\delta \ln K_I;\ \delta P_{II}{}^0 = \Delta\phi_{II}\Gamma_{II}\delta \ln K_{II} \qquad (ii)$$

If the forcing parameter is temperature, $\delta \ln K_I$ and $\delta \ln K_{II}$ are defined by eq 6, where ΔH is replaced by the normal enthalpies ΔH_I and ΔH_{II}, respectively, which are linear combinations of the enthalpies of the elementary steps. When the two steps equilibrate at very different rates, the procedure described in the present paper leads to a direct calculation of the normal extinction coefficients, Γ functions, and thermodynamic parameters. For example, when step A is decoupled from step B, $Y_I = \delta A_I$, $Y_{II} = \delta B_1$, and therefore $\Delta\phi_I$, Γ_I, and ΔH_I may be identified with $\Delta\phi_A$, Γ_A, and ΔH_A. It follows from eq 42 that $\Delta\phi_{II}$ is given by eq 43, and

$$\Gamma_{II} = \Gamma_B/(1 - \Gamma_A\Gamma_Bf^2) \qquad (iii)$$

$$\Delta H_{II} = \Delta H_B - \Gamma_Af\Delta H_A \qquad (iv)$$

It will be noted that the normal enthalpies and $\Delta\phi$'s are the same linear combinations (compare eq iv above with eq 43 in the text) and that the coupling constants introduced in this paper are equivalent to weighting factors in a normal mode notation.

It is obvious that $f = 1/\overline{ES}$ for Scheme V. In this case it is convenient to express the Γ functions as (Table I)

$$\Gamma_A = \frac{\overline{ES}}{K_A(\bar{E} + \bar{S}) + 1};\ \Gamma_B = \frac{\overline{ES}}{K_B + 1} \qquad (46)$$

The coupling constants are then $\Gamma_A f = (K_A(\bar{E} + \bar{S}) + 1)^{-1}$, $\Gamma_B f = (K_B + 1)^{-1}$, and one sees immediately that the bracketed term in eq 42 is

$$\Gamma_B/(1 - \Gamma_A\Gamma_B/\overline{ES}^2) = \frac{\overline{ES}}{(K_B + 1)\{1 - 1/(K_B + 1)(K_A(\bar{E} + \bar{S}) + 1)\}} \qquad (47)$$

or, upon rearrangement

$$\Gamma_B/(1 - \Gamma_A\Gamma_B/\overline{ES}^2) = \frac{\overline{ES}}{K_B + \dfrac{K_A(\bar{E} + \bar{S})}{K_A(\bar{E} + \bar{S}) + 1}} \qquad (48)$$

Substitution of eq 48 and the coupling constant $\Gamma_A/\overline{ES}$ into eq 42 yields

$$\delta P_{II}{}^0 = \left(\Delta\phi_B - \frac{\Delta\phi_A}{K_A(\bar{E} + \bar{S}) + 1}\right) \times \left[\frac{\overline{ES}}{K_B + \dfrac{K_A(\bar{E} + \bar{S})}{K_A(\bar{E} + \bar{S}) + 1}}\right]\left(\delta \ln K_B - \frac{\delta \ln K_A}{K_A(\bar{E} + \bar{S}) + 1}\right) \qquad (49)$$

$$\Delta\phi_B = (\phi_{ES} - \phi_{ES'})$$

Czerlinski[8] and Eigen and DeMaeyer[3] have also developed amplitude expressions for Scheme V. It is of interest to compare eq 49 with these earlier results.

Czerlinski[8] has expressed equilibrium concentration changes in terms of analytical concentrations and the equilibrium concentration of a single component. It is readily verified that dividing both sides of eq 49 by $\Delta\phi_{II}$, making the substitutions $\bar{E} + \bar{S} = [E^0 + S^0 - 2\overline{ES}'(1 + K_B)]$, $\overline{ES} = K_B\overline{ES}'$, and rearranging gives

$$\frac{\delta\overline{ES}'}{\overline{ES}} = \frac{\delta \ln K_B\{K_A{}^{-1} + E^0 + S^0 - 2\overline{ES}'(1 + K_B)\} - K_A{}^{-1}\delta \ln K_A}{K_A{}^{-1} + (1 + K_B{}^{-1})\{E^0 + S^0 - 2\overline{ES}'(1 + K_B)\}} \qquad (50)$$

which is equivalent to Czerlinski's eq 8.7.

Eigen and DeMaeyer[3] employed a normal-mode notation in their amplitude analysis of Scheme V. These authors considered the limiting case $\bar{E} = \bar{S}$, and introduced in their equations an "overall degree of dissociation," $\alpha = \bar{E}/E^0$. If activity coefficients and volume changes are ignored and if molalities are replaced by molarities, eq II.3.37 of ref 1 may be written as

$$\bar{Y}_{II}/E^0 = \Gamma_{II}(\Delta H_{II}/RT)(\delta T/T) \qquad (51)$$

where $\bar{Y}_{II}$ is the normal concentration variable related to τ_{II}. In the present notation Γ_{II} is given in eq 48.[16] The latter may be rearranged to a form more suitable for comparisons with the derivation of Eigen and DeMaeyer

$$\Gamma_{II} = \left(\frac{1 + K_A(\bar{E} + \bar{S})}{1 + K_B}\right)\left(\frac{\bar{E}(\overline{ES} + \overline{ES}')}{\bar{E} + 2(\overline{ES} + \overline{ES}')}\right) \qquad (52)$$

From the above definition of α it follows that $(\overline{ES} + \overline{ES}') = E^0(1 - \alpha)$ and $[\bar{E} + 2(\overline{ES} + \overline{ES}')] = E^0(2 - \alpha)$. Equation 52 is then

$$\Gamma_{II} = E^0\left(\frac{1 + K_A(\bar{E} + \bar{S})}{1 + K_B}\right)\left(\frac{\alpha(1 - \alpha)}{(2 - \alpha)}\right) \qquad (53)$$

which, when normalized with respect to E^0, is identical with the expression given in eq II.3.38 of ref 1.[17] It is obvious from eq iv of ref 16 that the normal enthalpy associated with τ_{II} is

$$\Delta H_{II} = (\Delta H_A - (\Gamma_A/\overline{ES})\Delta H_B) = \left(\Delta H_A - \frac{\Delta H_B}{K_A(\bar{E} + \bar{S}) + 1}\right) \qquad (54)$$

Equation 54 is equivalent to eq II.3.39 of Eigen and DeMaeyer.

Calculating Individual Relaxation Amplitudes. A Strong Coupling Exists between the Elementary Steps. Now we consider the analysis of individual amplitudes when steps A and B equilibrate at similar rates. When the relaxation times are widely separated, the amplitude expressions are functions of equilibrium parameters only. In strongly coupled systems, however, kinetic parameters must also be taken into account. Even in the former case the results of an amplitude analysis should be substituted into the general solution to verify that the assumption of negligible kinetic coupling is valid.

The pertinent rate equations for the systems discussed in this paper are (Appendix II)

$$\frac{\delta\dot{A}_1}{a_1} = b_{11}\frac{\delta A_{1(t)}}{a_1} + b_{12}\frac{\delta B_{1(t)}}{b_1} \qquad (55)$$

$$\frac{\delta\dot{B}_1}{b_1} = b_{21}\frac{\delta A_{1(t)}}{a_1} + b_{22}\frac{\delta B_{1(t)}}{b_1} \qquad (56)$$

where the b_{ik}'s are functions of specific rate constants and equilibrium concentrations. The solution to eq 55 and 56 is of the form

$$\delta A_{1(t)}/a_1 = A_{11}\exp(-\lambda_1 t) + A_{12}\exp(-\lambda_2 t) \qquad (57)$$

$$\delta B_{1(t)}/b_1 = A_{21}\exp(-\lambda_1 t) + A_{22}\exp(-\lambda_2 t) \qquad (58)$$

where the exponential terms (reciprocal relaxation times) are the roots of the second-order secular equation

$$\lambda_{1,2} = S/2(1 \pm (1 - 4P/S^2)^{1/2}) \qquad (59)$$

$$P = b_{11}b_{22} - b_{12}b_{21};\ S = b_{11} + b_{22} \qquad (60)$$

Although the preexponential terms, A_{ij}, are not usually developed in a kinetic analysis, they must be known if thermodynamic functions are to be evaluated from relaxation amplitudes. By established mathematical procedures one can express the A_{ij} in terms of the b_{ij}'s and λ's of eq 59 and the overall concentration changes, $\delta\bar{A}_1$ and $\delta\bar{B}_1$. The result is[18]

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[17] Since the normal Γ functions are defined in terms of molar concentrations here and in terms of an overall degree of dissociation in the application of ref 1, the relation $\Gamma_{II} = E^0\Gamma_{II\alpha}$ must be borne in mind when comparing eq 53 to eq II.3.38 of ref 1.

[18] The author is grateful to Dr. T. Jovin for discussions concerning derivations of the preexponential terms. In the present work the A_{ij}'s were obtained using eq II.2.9 of ref 1.

$$\begin{aligned} A_{11} &= \frac{(\lambda_2 - b_{11})\delta\bar{A}_1 - b_{12}\delta\bar{B}_1}{\lambda_2 - \lambda_1} \\ A_{12} &= \frac{(b_{11} - \lambda_1)\delta\bar{A}_1 + b_{12}\delta\bar{B}_1}{\lambda_2 - \lambda_1} \\ A_{21} &= \frac{-b_{21}\delta\bar{A}_1 + (b_{11} - \lambda_1)\delta\bar{B}_1}{\lambda_2 - \lambda_1} \\ A_{22} &= \frac{b_{21}\delta\bar{A}_1 + (\lambda_2 - b_{11})\delta\bar{B}_1}{\lambda_2 - \lambda_1} \end{aligned} \tag{61}$$

It follows from the relation $\delta P_{(t)} = (\Delta\phi_A\delta A_{1(t)}/a_1 + \Delta\phi_B\delta B_{1(t)}/b_1)$ that the time-dependent signal change for the reactions considered here is

$$\delta P_{(t)} = \delta P_{\mathrm{I}}^0 \exp(-\lambda_1 t) + \delta P_{\mathrm{II}}^0 \exp(-\lambda_2 t) \tag{62}$$

where

$$\begin{aligned} \delta P_{\mathrm{I}}^0 &= (\Delta\phi_A A_{11} + \Delta\phi_B A_{21}) \\ \delta P_{\mathrm{II}}^0 &= (\Delta\phi_A A_{12} + \Delta\phi_B A_{22}) \end{aligned} \tag{63}$$

The amplitudes for coupled equilibria can be given in terms of equilibrium and kinetic parameters by substituting eq 23, 24, and 61 into the relations of eq 63. In addition, it can be shown that (Appendix II)

$$b_{12} = b_{11}\Gamma_A f; \quad b_{21} = b_{22}\Gamma_B f \tag{64}$$

and therefore the only elements of the secular equation which need be considered explicitly are b_{11} and b_{22}.[19] In this way the following expression for δP_{I}^0 was derived

$$\delta P_{\mathrm{I}}^0 = \Delta\phi_{A_{\mathrm{I}}}\left[\frac{\Gamma_A}{1 - \Gamma_A\Gamma_B f^2}\right]\delta \ln K_A + \Delta\phi_{B_{\mathrm{I}}}\left[\frac{\Gamma_B}{1 - \Gamma_A\Gamma_B f^2}\right]\delta \ln K_B \tag{65}$$

where $\Delta\phi_{A_{\mathrm{I}}}$ and $\Delta\phi_{B_{\mathrm{I}}}$ are the following linear combinations of $\Delta\phi_A$ and ϕ_B.

$$\Delta\phi_{A_{\mathrm{I}}} = \frac{\lambda_2 - b_{11}(1 - \Gamma_A\Gamma_B f^2)}{\lambda_2 - \lambda_1}\Delta\phi_A - \frac{\Gamma_B f(b_{1} + b_{22} - \lambda_1)}{\lambda_2 - \lambda_1}\Delta\phi_B \tag{66}$$

$$\Delta\phi_{B_{\mathrm{I}}} = -\frac{\lambda_2\Gamma_A f}{\lambda_2 - \lambda_1}\Delta\phi_A + \frac{b_{11} + b_{22}\Gamma_A\Gamma_B f^2 - \lambda_1}{\lambda_2 - \lambda_1}\Delta\phi_B \tag{67}$$

The second amplitude is given by

$$\delta P_{\mathrm{II}}^0 = \Delta\phi_{A_{\mathrm{II}}}\left[\frac{\Gamma_A}{1 - \Gamma_A\Gamma_B f^2}\right]\delta \ln K_A + \Delta\phi_{B_{\mathrm{II}}}\left[\frac{\Gamma_B}{1 - \Gamma_A\Gamma_B f^2}\right]\delta \ln K_B \tag{68}$$

where

$$\Delta\phi_{A_{\mathrm{II}}} = \frac{-\lambda_1 + b_{11}(1 - \Gamma_A\Gamma_B f^2)}{\lambda_2 - \lambda_1}\Delta\phi_A + \frac{\Gamma_B f(b_{11} + b_{22} - \lambda_2)}{\lambda_2 - \lambda_1}\Delta\phi_B \tag{69}$$

$$\Delta\phi_{B_{\mathrm{II}}} = \frac{\lambda_1\Gamma_A f}{\lambda_2 - \lambda_1}\Delta\phi_A - \frac{b_{11} + b_{22}\Gamma_A\Gamma_B f^2 - \lambda_2}{\lambda_2 - \lambda_1}\Delta\phi_B \tag{70}$$

Since expressions for Γ_A, Γ_B, f, b_{11}, and b_{22} can be written by an examination of the mechanism, eq 65 and 68 enable one to readily calculate strongly coupled amplitudes in terms of equilibrium and kinetic parameters.

It is interesting to consider the limiting forms of eq 65 and 68 when the two relaxation times are widely separated on the time axis. One can readily verify that the conditions $b_{11} \rightarrow \lambda_1$ and $\lambda_1/\lambda_2 \rightarrow \infty$ yield

$$\Delta\phi_{A_{\mathrm{I}}} \longrightarrow (1 - \Gamma_A\Gamma_B f^2)\Delta\phi_A; \quad \Delta\phi_{B_{\mathrm{I}}} \longrightarrow 0 \tag{71}$$

and thus eq 65 simplifies to eq 39. Regarding the slow process, we have the relations

$$\begin{aligned} \Delta\phi_{A_{\mathrm{II}}} &\longrightarrow \Gamma_B f(\Delta\phi_B - \Gamma_A f\Delta\phi_A) \\ \Delta\phi_{B_{\mathrm{II}}} &\longrightarrow (\Delta\phi_B - \Gamma_A f\Delta\phi_A) \end{aligned} \tag{72}$$

which reduce the general δP_{II}^0 expression to the limiting form of eq 42.

A temperature-jump study of the kinetics and thermodynamics of the trypsin-proflavin-benzamidine reaction system (Scheme IV) was recently reported.[7] The amplitude results suggested that coupling between the two steps was significant, and general amplitude expressions were used to evaluate the enthalpy of the trypsin-benzamidine reaction. The required relations (eq 6 and 7, ref 7) were derived immediately from eq 65 and 68 by assuming that $\Delta\phi_B = 0$.

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Appendix I. The Relationships between Overall Changes in Equilibrium Concentrations and Thermodynamic Functions for a General Reaction Mechanism

From the theoretical treatment of Castellan,[20] it follows that the overall change in the equilibrium concentration of a participant in the αth step of a general reaction mechanism is related to the total change in the advancements of all equilibria by

$$\Delta\bar{C}_\alpha = \left(\frac{1}{V}\right)\sum_{\beta=1}^{R}\nu_{\alpha\beta}\Delta\bar{\xi}_\beta \tag{A-1}$$

or, in matrix notation

$$\Delta\bar{\mathbf{C}} = \left(\frac{1}{V}\right)\boldsymbol{\nu}\Delta\bar{\boldsymbol{\xi}} \tag{A-2}$$

R is the number of elementary chemical reactions and the $\nu_{\alpha\beta}$'s are stoichiometric coefficients. It also follows from Castellan's treatment that the overall free energy changes of the elementary steps can be

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[19] The terms b_{11} and b_{22} are the reciprocal relaxation time expressions for the completely decoupled equilibria A and B, respectively, and can be calculated by an examination of the reaction stoichiometries.[20]

[20] G. W. Castellan, *Ber. Bunsenges. Phys. Chem.*, 67, 898 (1963).

expressed as linear combinations of the overall advancements.

$$\mathbf{\Delta \bar{G}} = (RT/V)\mathbf{g}\mathbf{\Delta \xi} \qquad \text{(A-3)}$$

In those cases where the activity coefficients are independent of the concentrations of the reactants, the elements of the **g** matrix are

$$g_{\alpha\beta} = g_{\beta\alpha} = \sum_{i=1}^{N} \frac{\nu_{i\alpha}\nu_{i\beta}}{\bar{C}_i} \qquad \text{(A-4)}$$

where N is the total number of different chemical species. It is interesting to note that the diagonal element $g_{\alpha\alpha}$ is simply the inverse of the Γ function of the αth elementary step (Γ_α^{-1}). For small perturbations we may write $\Delta \bar{G}_\alpha = -RT\Delta \ln K_\alpha$. The ΔC_α can therefore be expressed as linear combinations of the $\Delta \ln K_\alpha$'s by taking the inverse of eq A-3 and substituting the resulting expression for $\mathbf{\Delta\xi}$ into eq A-2.

$$\mathbf{\Delta \bar{C}} = -\boldsymbol{\nu}\mathbf{g}^{-1}\mathbf{\Delta} \ln \mathbf{K} \qquad \text{(A-5)}$$

Since the matrices $\boldsymbol{\nu}$ and $\mathbf{g}^{-1}$ can be written by inspection, eq A-5 provides a convenient means of expressing overall concentration changes in terms of thermodynamic functions for a completely general reaction mechanism.

In the systems of two coupled equilibria discussed in the present paper, the components A_1 and B_1 participate only in steps "A" and "B," respectively. The matrix $\boldsymbol{\nu}$ is therefore diagonal with $\nu_{11} = a_1$ and $\nu_{22} = b_1$. The elements of g are: $g_{11} = \Gamma_A^{-1}$, $g_{22} = \Gamma_B^{-1}$, $g_{12} = g_{21} = \Sigma_i(c_{iA}c_{iB}/\bar{C}_i) = f$. It follows from the equation $\boldsymbol{\nu}^{-1}\mathbf{\Delta \bar{C}} = -\mathbf{g}^{-1}\mathbf{\Delta} \ln \mathbf{K}$ that

$$\Delta A_1/a_1 = -(\Gamma_A^{-1}\Gamma_B^{-1} - f^2)^{-1}(\Gamma_B^{-1}\Delta \ln K_A - f\Delta \ln K_B) \qquad \text{(A-6)}$$

$$\Delta \bar{B}_1/b_1 = -(\Gamma_A^{-1}\Gamma_B^{-1} - f^2)^{-1}(\Gamma_A^{-1}\Delta \ln K_B - f\Delta \ln K_A) \qquad \text{(A-7)}$$

Equations A-6 and A-7 are equivalent to eq 23 and 24 in the text.

Appendix II. On the Relationships between the Elements of the Secular Determinant and the Coupling Constants for Two-Step Equilibria

Castellan[20] has shown that the relaxation times for any chemical reaction mechanism are the eigenvalues of the matrix **b**, which is defined by

$$-\mathrm{d}\mathbf{\Delta\xi}/\mathrm{d}t = \mathbf{b}\mathbf{\Delta\xi} \qquad \text{(A-8)}$$

The matrix **b** may be written as a product of two matrices **r** and **g**, where the latter is defined in Appendix I and **r** is a diagonal matrix whose typical element r_α is the exchange rate of the αth elementary step. In the systems of two coupled equilibria considered in the present paper, we have $\delta\xi_A = \delta A_1/a_1$, $\delta\xi_B = \delta B_1/b_1$, and therefore the linearized rate equations corresponding to eq A-8 become eq 55 and 56 of the text. The elements of the secular determinant are $b_{11} = r_A\Gamma_A^{-1}$, $b_{12} = r_Af$, $b_{22} = r_B\Gamma_B^{-1}$, and $b_{21} = r_Bf$. It is evident that the ratios b_{12}/b_{11} and b_{21}/b_{22} are equivalent to the coupling constants Γ_Af and Γ_Bf.

Very Low Pressure Pyrolysis. V. Benzylamine, *N*-Methylbenzylamine, and *N,N*-Dimethylbenzylamine and the Heat of Formation of the Amino, Methylamino, and Dimethylamino Radicals[1a]

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Abstract: VLPP studies of the benzyl–amino bond homolysis in benzylamine, *N*-methylbenzylamine, and *N,N*-dimethylbenzylamine yield heats of formation of the amino, methylamino, and dimethylamino radicals, respectively, if RRKM or RRK theory is used to relate the low-pressure rate constants measured to the high-pressure Arrhenius parameters. Values of the high-pressure A factors were obtained by analogy with the relevant alkylbenzenes. Heats of formation for the three radicals are 47.2, 45.2, and 38.2 kcal/mol, respectively. These lead to $DH°(NH_2\text{–}H) = 110$, $DH°(CH_3NH\text{–}H) = 103$, and $DH°((CH_3)_2N\text{–}H) = 95$ kcal/mol, all considerably higher than previously reported.

The thermochemical properties and reactivities of simple carbon-centered free radicals have been measured often and are reasonably well known.[2a] There is considerably less information available as to the properties of nitrogen-centered free radicals, and what there is, is subject to a good deal of uncertainty.[2b]

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(2) (a) S. W. Benson, "Thermochemical Kinetics," Wiley, New York, N. Y., 1968; (b) S. W. Benson and H. E. O'Neal, "Kinetic Data on Gas-Phase Unimolecular Reactions," NSRDS-NBS 21, National Bureau of Standards Reference Data System, U. S. Department of Commerce, 1970; H. E. O'Neal and S. W. Benson, *Int. J. Chem. Kinet.*, 1, 221 (1969).