BIOPOLYMERS VOL. 12, 1161-1169 (1973)

Electrophoretic Light Scattering as a Probe of Reaction Kinetics*

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Synopsis

A new method is proposed for determining chemical rate constants in dimerization reactions of globular proteins. Light scattering from a solution of charged macromolecules in an applied electric field gives a series of bands whose widths can be used to deduce the reaction rate constants. This method should be applicable to other types of reactions. First order reactions are also considered.

INTRODUCTION

In this note we explore the possibility of measuring chemical reaction rates of association reactions of biologically significant molecules by laser light scattering.

The Rayleigh spectrum, $S(k\omega)$, of a dilute non-reactive solution of charged macromolecules consists of a superposition of Lorentzian bands,[1]

$$S(k\omega) = \pi^{-1} \sum_v C_v S_v(\mathbf{k}) \left\{ \frac{k^2 D_v}{\omega^2 + [k^2 D_v]^2} \right\} \tag{1.1}$$

where C_v, D_v, and $S_v(k)$ are, respectively, the concentration, self diffusion coefficient, and polarizability structure factor of macromolecular component v. ω is the frequency shift of the scattered light and k is the scattering vector

$$k = 2k_0 \sin \theta/2$$

where k_0 is the wave number of the incident light and θ is the scattering angle. In general it is difficult to determine the diffusion constants from this complicated superposition.

When an applied electric field E is switched on, the macromolecules are accelerated to different terminal velocities, $\mathbf{V}_v$, depending on their respective mobilities, μ_v,

$$\mathbf{V}_v = \mu_v \mathbf{E} \tag{1.2}$$

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* Supported by NSF GP 23727.

† Alfred P. Sloan Foundation Fellow.

The Rayleigh contribution from each charged component v will then be Doppler-shifted by frequency

$$\omega_v(\mathbf{k}) = \mu_v(\mathbf{k}\cdot\mathbf{E}) \tag{1.3}$$

Thus in an applied field, the Rayleigh spectrum of the solution then becomes[2]

$$S(k\omega) = \frac{1}{\pi}\sum_v C_v S_v(\mathbf{k})\left[\frac{k^2D_v}{[\omega - \omega_v(k)]^2 + [k^2D_v]^2}\right] \tag{1.4}$$

If the difference in the Doppler shifts, $\{\omega_v(k)\}$, is larger than the diffusion widths it is possible to resolve the Lorentzians into separate components, and it is thereby possible to measure concentrations, diffusion coefficients, and mobilities of the macromolecular components of the mixture. This is best accomplished at small scattering angles because the widths k^2D_v diminish faster as a function of k than do the shifts $\omega_v(k)$.

So far we have been discussing non-reactive mixtures. The question immediately arises as to what happens if some of the components can react to form other of the components. It is intuitively clear that in this event Eq. (1.3) should be modified to something like

$$S(k\omega) = \frac{1}{\pi}\sum_v C_v S_v(\mathbf{k})\left[\frac{k_v + k^2D_v}{[\omega - \omega_v(k)]^2 + [k_v + k^2D_v]^2}\right] \tag{1.5}$$

where k_v is the chemical rate at which molecules leave component v through all possible chemical channels. Thus, on intuitive grounds we expect that the separate components will be broadened by the reaction. If the relative Doppler shifts are still larger than the breadths it will be possible to measure the rates k_v.

Our purpose here is to compute the spectrum of a reaction in the presence of an applied electric field and to explore whether "electrophoretic light scattering" could be useful for determination of reaction rates in biologically significant reactions. This method is different in principle from previous suggestions for using light scattering as a probe of reaction kinetics.[3] It is analogous to the study of exchange rates[4] in NMR.

In the present note we limit ourselves for simplicity to dimerization reactions, although it is not difficult to apply the same treatment to other types of reactions.[5] We expect that the dimerization reactions of globular proteins should lend themselves to this kind of study. The theory presented here gives us hope that several interesting classes of biological reactions may one day be studied by this method.

THEORY

In this section we consider the dimerization reaction

$$2A_1 \underset{k_b}{\overset{R_f}{\rightleftarrows}} A_2 \tag{2.1}$$

where k_f' and k_b are the dimerization and dissociation rate constants.

Light is scattered because of thermal fluctuations which give rise to fluctuations in the dielectric constant. In macromolecular solutions the dielectric fluctuation $\delta\epsilon(\mathbf{r},t)$ at the point r and time t is primarily due to the concentration fluctuations $\delta c_1(\mathbf{r},t)$, $\delta c_2(\mathbf{r},t)$ of monomer and dimer around their respective equilibrium concentrations c_1^0 and c_2^0, respectively so that

$$\delta\epsilon(\mathbf{r},t) = \left(\frac{\partial\epsilon}{\partial c_1}\right)_{c_2} \delta c_1(\mathbf{r},t) + \left(\frac{\partial\epsilon}{\partial c_2}\right)_{c_1} \delta c_2(\mathbf{r},t) \tag{2.2}$$

Actually, the heterodyne light-scattering spectrum is given by

$$\left\{\begin{aligned} S(k\omega) &= \frac{2}{\pi} Re \int_0^\infty dt\, e^{-i\omega t} S(k,t) \\ S(k,t) &\equiv Re\, \langle \delta\epsilon^*(k,0)\delta\epsilon(k,t)\rangle \end{aligned}\right\} \tag{2.3}$$

where $\delta\epsilon(\mathbf{k},t)$ is the spatial Fourier transform of $\delta\epsilon(\mathbf{r},t)$, and $\mathbf{k}$ is the scattering vector defined previously.

Combining Eqs. (2.2) and (2.3) shows that

$$S(k,t) = Re \sum_{i,j=1}^{2} \alpha_i\alpha_j F_{ij}(k,t) \tag{2.4}$$

where $\alpha_i = (\partial\epsilon/\partial c_i)c_j$ and

$$F_{ij}(k,t) \equiv \langle \delta C_i(\mathbf{k},0)\delta C_j(\mathbf{k},t)\rangle \tag{2.5}$$

In order to predict the spectrum $S(k,\omega)$ it is necessary to derive equations which describe the rate of change of the concentration fluctuations. It is easy to derive such equations on the basis of mass conservation which gives*

$$\begin{aligned} \frac{\partial C_1}{\partial t} + \nabla\cdot\mathbf{J}_1 &= 2k_bC_2 - 2k_f'C_1^2 \\ \frac{\partial C_2}{\partial t} + \nabla\cdot\mathbf{J}_2 &= k_f'C_1^2 - k_bC_2 \end{aligned} \tag{2.6}$$

where $\mathbf{J}_1$ and $\mathbf{J}_2$ are the fluxes of monomer and dimer, respectively, which in the presence of an electric field are

$$\begin{aligned} \mathbf{J}_1 &= \mu_1\mathbf{E}C_1 - D_1\nabla C_1 \\ \mathbf{J}_2 &= \mu_2\mathbf{E}C_2 - D_2\nabla C_2 \end{aligned} \tag{2.7}$$

where μ_1, μ_2, D_1, and D_2 are, respectively, electrical mobilities and diffusion coefficients of components 1 and 2. In zero field $\mathbf{J}_1$, $\mathbf{J}_2$ reduce to the usual Fick's Law result.

Only small concentration fluctuations δC_1, δC_2 around the equilibrium concentrations C_1^0 and C_2^0 are expected to occur.[6] The very smallness of these fluctuations enables us to linearize Eq. (2.6). $\delta C_1(\mathbf{k},t)$, $\delta C_2(\mathbf{k},t)$, the

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* The concentration and fluxes are functions of position $\mathbf{r}$ and time t.

spatial Fourier transforms of $\delta C_1(\mathbf{r},t)$ and $\delta C_2(\mathbf{r},t)$, satisfy the linearized equations

$$\begin{aligned}\frac{\partial}{\partial t}\,\delta\hat{C}_1 &= i\omega_1(\mathbf{k})\delta\hat{C}_1 + 2k_b\delta\hat{C}_2 - 2k_f\delta\hat{C}_1 - k^2D_1\delta\hat{C}_1\\ \frac{\partial}{\partial t}\,\delta\hat{C}_2 &= i\omega_2(\mathbf{k})\delta\hat{C}_2 + k_f\delta\hat{C}_1 - k_b\delta\hat{C}_2 - k^2D_2\delta\hat{C}_2\end{aligned} \tag{2.8}$$

where $k_f = 2C,^0k'_f$ is the linearized dimerization rate constant, and

$$\omega_j(\mathbf{k}) \equiv \mu_j(\mathbf{k}\cdot\mathbf{E}) \tag{2.9}$$

is the Doppler shift of component j. In deriving these equations we have assumed that the applied field is homogeneous. There are some interesting effects due to inhomogeneous fields which will not be exploited here.

Eq. (2.8) can be solved by Laplace transformation to give

$$\begin{aligned}\delta\tilde{C}_1(k,s) &= \frac{(s+\lambda_2)\delta\hat{C}_1(k,0) + 2k_b\delta\hat{C}_2(k,0)}{\Delta(s)}\\ \delta\tilde{C}_2(k,s) &= \frac{(s+\lambda_1)\delta\hat{C}_2(k,0) + k_f\delta\hat{C}_1(k,0)}{\Delta(s)}\end{aligned} \tag{2.10}$$

where the tilde indicates a Laplace transform with respect to time, s is the Laplace variable, $\lambda_1 = 2k_f + k^2D_1 - i\omega_1$, and $\lambda_2 \equiv k_b + k^2D_2 - i\omega_2$ and

$$\Delta(s) = (s+\lambda_1)(s+\lambda_2) - 2k_fk_b \tag{2.11}$$

In dilute polymer solutions we can make use of the approximation* $F_{12}(k,0) = F_{21}(k,0) = 0$, in which case

$$\begin{aligned}\tilde{F}_{11}(\mathbf{k},s) &= \langle|\delta\hat{C}_1(k,0)|^2\rangle\,\frac{(s+\lambda_2)}{\Delta(s)}\\ \tilde{F}_{12}(k,s) &= \langle|\delta\hat{C}_1(k,0)|^2\rangle\,\frac{k_f}{\Delta(s)}\\ \tilde{F}_{21}(k,s) &= \langle\delta\hat{C}_2(k,0)|^2\rangle\,\frac{2k_b}{\Delta(s)}\\ \tilde{F}_{22}(k,s) &= \langle|\delta\hat{C}_2(k,0)|^2\rangle\,\frac{(s+\lambda_1)}{\Delta(s)}\end{aligned} \tag{2.12}$$

where $\tilde{F}_{ij}(k,s)$ is the Laplace transform of $F_{ij}(k,t)$.

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* The ensuing analysis can be carried out without this approximation, for example:

$$F_{11}(k,s) = \langle|\delta\hat{C}_1(k,0)|^2\rangle\,\frac{(s+\lambda_2)}{\Delta(s)} + \langle\delta\hat{C}_1(k,0)\hat{C}_2(k,0)\rangle\,\frac{2k_b}{\Delta(s)}$$

but the results are less transparent. Nevertheless our conclusions are still valid.

Suppose the electric field was in the opposite direction. Then the only change that would occur would be the replacement of $\omega_j(k)$ by $-\omega_j(k)$ in Eq. (2.12). Let us denote by $\tilde{F}_{ij}^{(+)}(k,s)$ the functions calculated above and by $\tilde{F}_{ij}^{(-)}(k,s)$ the functions with the reversed field. From Eqs. (2.3), (2.4), and (2.12) it then follows that

$$S(k\omega) = \pi^{-1} Re \sum_{i,j=1}^{2} \alpha_i \alpha_j \{\tilde{F}_{ij}^{(+)}(k,s = i\omega) + F_{ij}^{(-)}(k,s = i\omega)\} \tag{2.13}$$

The explicit form of the spectrum, $S(k\omega)$, is given in the appendix.

In the simple case that the splitting $|\omega_2 - \omega_1|$ is large compared with the relaxation rates $\gamma_1 = k^2 D_1 + k_f$, $\gamma_2 = k^2 D_2 + k_b$ or that $k_f \ll k^2 D_1$ and $k_b \ll k^2 D_2$, the spectrum simplifies considerably to the "slow exchange" limit

$$\begin{aligned} \frac{\pi}{2} S(k,\omega) = {} & A_1 \left[\frac{\gamma_1}{(\omega - \omega_1)^2 + \gamma_1^2} + \frac{\gamma_1}{(\omega + \omega_1)^2 + \gamma_1^2}\right] \\ & + A_2 \left[\frac{\gamma_2}{(\omega - \omega_2)^2 + \gamma_2^2} + \frac{\gamma_2}{(\omega + \omega_2)^2 + \gamma_2^2}\right] \\ & + B_1 \left[\frac{(\omega + \omega_1)}{(\omega + \omega_1)^2 + \gamma_1^2} - \frac{(\omega - \omega_1)}{(\omega - \omega_1)^2 + \gamma_1^2}\right] \\ & + B_2 \left[\frac{(\omega + \omega_2)}{(\omega + \omega_2)^2 + \gamma_2^2} - \frac{(\omega - \omega_2)}{(\omega - \omega_1)^2 + \gamma_2^2}\right] \end{aligned} \tag{2.14}$$

where

$$A_1 = \alpha_1^2 f_{11} + (\gamma_2 - \gamma_1) G \alpha_1 \alpha_2$$

$$A_2 = \alpha_2^2 f_{22} - (\gamma_2 - \gamma_1) G \alpha_1 \alpha_2$$

$$B_1 = -(\omega_2 - \omega_1) G \alpha_1 \alpha_2$$

$$B_2 = (\omega_2 - \omega_1) G \alpha_1 \alpha_2$$

$$G = (k_f f_{11} + 2 k_b f_{22}) / [(\gamma_2 - \gamma_1)^2 + (\omega_2 - \omega_1)^2]$$

and where $f_{ij} = \langle \delta C_i^*(k) \delta C_j(k) \rangle$.

From Eq. (2.14) we see that in the absence of reaction ($k_f = k_b = 0$), the spectrum reduces to Lorentzians centered on $\pm\omega_1$ and $\pm\omega_2$ with diffusion widths. When reactions are present the pair of Lorentzians at ω_1 and ω_2 are slightly shifted towards each other and become slightly skewed (due to terms multiplied by B_1 and B_2). It is interesting to note that in these eventualities the rate constants k_f and k_b separately contribute to the widths γ_1 and γ_2, and can thereby be separately determined, along with the coefficients D_1 and D_2. This could be accomplished by determining γ_1 and γ_2 as a function of k^2 (or equivalently $\sin^2 \theta/2$). The slopes of γ_1 and γ_2 versus k^2 give the diffusion constants D_1 and D_2 whereas the intercept gives the corresponding rate constants (k_f for γ_1 and k_b for γ_2).

In the opposite extreme when the reaction rates are much larger than either the splitting or the diffusion widths, the spectrum consists mainly of a single line at the average frequency with a width determined by an average diffusion coefficient. This is the fast "exchange limit" (see Appendix III).

COMPUTATIONS

As a theoretical model, the dimerization of a globular protein in an electrophoresis cell is chosen. Proteins tend to dimerize in (water) solutions with different rate constants depending on pH conditions. The pH conditions are rather critical since they affect the extent of ionization of each species in water (i.e., the mobilities). [Proteins have acidic and basic groups which will dissociate in water depending on the pH conditions.]

We chose for our reaction model the following conditions. The reaction takes place in water (viscosity = 0.01 poise) at 283°K. The (reaction) electrophoresis cell is subject to an external electric field (E = 500 volts/cm). The reacting monomer is a globular protein of molecular weight 25,000. The corresponding "sphere" radius is a = 20 Å. The monomer effective charge* is $q = 5$. The monomer's concentration in the solution at equilibrium is 10^{-6} mole/liter. The light source for light scattering is an Ar^+ laser, and its wavelength is about 5000 Å. The detector is placed at a scattering angle $\theta = 3°$.

The light scattering spectrum (Eq. (2.13)) was calculated for several different rate constants (of different pH's) assuming that the dimerization rate is diffusion limited. The results of these calculations are shown in Figure 1. It is found that the reaction mode should be readily discernable. It should be possible to determine from the spectrum the dimerization and dissociation rate constants of globular proteins. It is our hope in the near future to perform a measurement of dimerization rates using electrophoretic light scattering.

APPENDIX I

Equation (2.13) can be written as

$$S(k,\omega) = \frac{1}{\pi}\,[S^{(+)}(k,\omega) + S^{(-)}(k,\omega)] \tag{A1}$$

where the quantities

$$\begin{aligned} S^{(+)}(k,\omega) &\equiv Re \sum_{ij} \alpha_i\alpha_j F_{ij}{}^{(+)}(k,s = i\omega) \\ S^{(-)}(k,\omega) &\equiv Re \sum_{ij} \alpha_i\alpha_j F_{ij}{}^{(-)}(k,s = i\omega) \end{aligned} \tag{A2}$$

have the explicit forms

$$S^{(\pm)}(k,\omega) = \frac{N^{(\pm)}(k,\omega)}{D^{(\pm)}(k,\omega)} \tag{A3}$$

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* Although the effective charge is not always known we assume that it is $q = 5$.

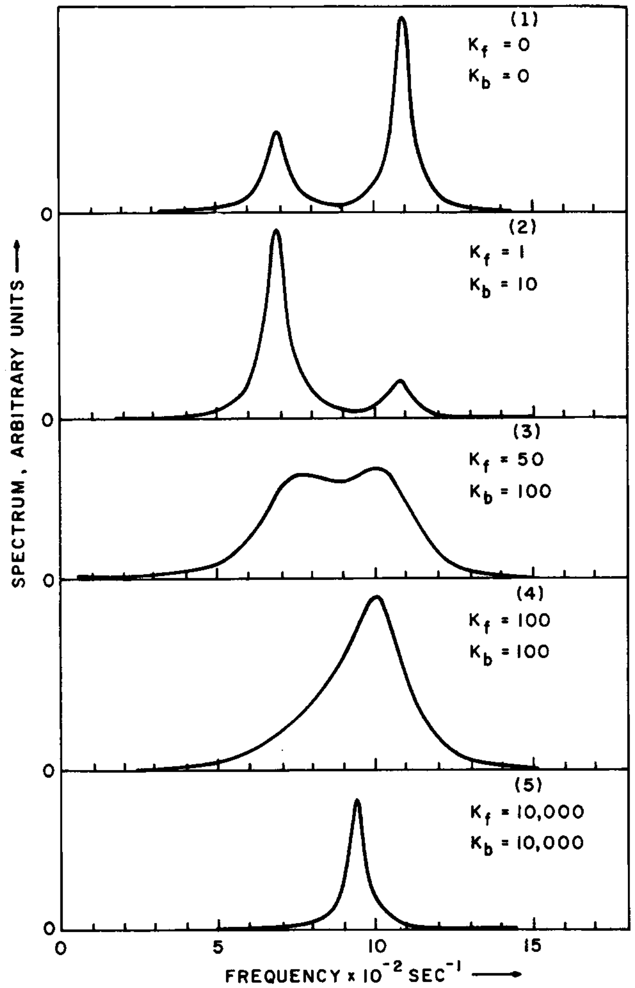

Fig. 1. The computed electrophoresis spectrum for the dimerization of a typical globular protein for different values of the rate constants. The parameters in this computation are ($T = 283$°K, $\eta = 0.01$ poise, $E = 500$ V/cm, $q = 5$, $a = 20$ Å, $K_{eq} = 10^4$, ${}_f = 3^\circ$, $\lambda = 5000$ Å.

where

$$\begin{aligned} N^{(\pm)}(k,\omega) \equiv {} & \alpha_1{}^2 f_{11}[\gamma_2 + i(\omega \mp \omega_2)] \\ & + \alpha_2{}^2 f_{22}[\gamma_1 + i(\omega \mp \omega_1)] \\ & + \alpha_1\alpha_2[k_f f_{11} + 2k_b f_{22}] \end{aligned} \tag{A4}$$

and

$$\begin{aligned} D^{(\pm)}(k,\omega) \equiv {} & [\gamma_1\gamma_2 - 2k_f k_b - (\omega \mp \omega_1)(\omega \mp \omega_2)] \\ & + i[\gamma_2(\omega \mp \omega_1) + (\gamma_1(\omega \mp \omega_2)] \end{aligned} \tag{A5}$$

The quantities in these expressions have been defined in the text.

APPENDIX II

$$A_1 \underset{k_b}{\overset{k_f}{\rightleftarrows}} A_2$$

In this case Eq. (2.10) becomes

$$\delta\tilde{c}_1(k,s) = \frac{(s + \lambda_2)\delta\tilde{c}_1(k,0) + k_b\delta\tilde{c}_2(k,0)}{\Delta(s)} \tag{B1}$$

$$\delta\tilde{c}_2(k,s) = \frac{(s + \lambda_1)\delta\tilde{c}_2(k,0) + k_f\delta\tilde{c}_1(k,0)}{\Delta(s)} \tag{B2}$$

where $\lambda_1 = k_f + k^2D_1 - i\omega_1$ and $\lambda_2 = k_b + k^2D_2 - i\omega_2$ and

$$\Delta(s) = (s + \lambda_1)(s + \lambda_2) - k_fk_b$$

Eqs. (A2) and (A3) are still valid, but now

$$\begin{aligned} N^{\pm}(k,\omega) = a_1{}^2f_{11}[\gamma_2 + i(\omega \mp \omega_2)] + a_2{}^2f_{22}[\pi_1 + i(\omega \mp \omega_1)] \\ + a_1a_2[k_ff_{11} + k_bf_{22}] \end{aligned} \tag{B3}$$

and

$$D^{\pm}(k,\omega) = [\gamma_1\gamma_2 - k_fk_b - (\omega \mp \omega_1)(\omega \mp \omega_2)] + i[\gamma_2(\omega + \omega_1) + \gamma_1(\omega + \omega_2)] \tag{B4}$$

APPENDIX III

FAST EXCHANGE LIMIT

In the limit that k_b, $k_f \gg k^2D_1$, k^2D_2, ω_1, ω_2 the spectrum consists of a sharp band centered at an average frequency

$$\bar{\omega} \equiv \chi_2\omega_2 + \chi_1\omega_1$$

with a width $k^2\bar{D}$ where $\bar{D}$ is an average diffusion coefficient

$$\bar{D} \equiv \chi_2D_2 + \chi_1D_1$$

and of a broad band at the frequency

$$\Delta\omega = \bar{\omega} - (\omega_1 + \omega_2)$$

with a width $\Delta \equiv (2k_f + k_b) + k^2\Delta D$ where

$$\Delta D = \bar{D} - (D_1 + D_2)$$

In these expressions

$$\chi_1 \equiv k_b/2k_f + k_b; \quad \chi_2 \equiv 2k_f/2k_f + k_b$$

can be interpreted as the fraction of time spent by a molecule in monomeric and dimeric states, respectively.

In the fast exchange limit the spectrum is approximately given by

$$\begin{aligned} \frac{\pi}{2}\, S(k,\omega) = A \left\{ \frac{k^2\bar{D}}{(\omega - \bar{\omega})^2 + [k^2\bar{D}]^2} + \frac{k^2\bar{D}}{[\omega + \bar{\omega}]^2 + [k^2\bar{D}]^2} \right\} \\ + B \left\{ \frac{(2k_f + k_b - k^2\Delta D)}{[\omega - \Delta\omega]^2 + [2k_f + k_b - k^2\Delta D]^2} \right. \\ \left. + \frac{(2k_f + k_b - k^2\Delta D)}{[\omega + \Delta\omega]^2 + [2k_f + k_b - k^2\Delta D]^2} \right\} \end{aligned}$$

where

$$A = \chi_1\alpha_1^2 f_{11} + \frac{\alpha_1\alpha_2}{2}\{\chi_2 f_{11} + 4\chi_1 f_{22}\} + \chi_2\alpha_2^2 f_{22}$$

and

$$B = \chi_2\alpha_1^2 f_{11} + \frac{\alpha_1\alpha_2}{2}\{\chi_2 f_{11} + 4\chi_1 f_{22}\} + \chi_1\alpha_2^2 f_{22}$$

The second band is much broader than the first. The spectrum should therefore reduce to a single narrow band at an average Doppler frequency $\bar{\omega}$ with an average diffusion width $k^2\bar{D}$.

One of us (B. J. B.) did part of this work while on a John Simon Guggenheim Foundation fellowship at the University of Tel Aviv and would like to express his appreciation to Prof. Joshua Jortner for making the facilities readily available to him.

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4. Abragam, *The Principles of Nuclear Magnetism*, Oxford University Press, London (1961).
5. Many other examples of reactions that can be studied by the method discussed here can be found in John R. Cann, *Interacting Macromolecules*, Academic Press, New York (1970).
6. It is simple to extend this theory to isomerization reactions and to higher-order kinetics. This is done in Appendix II of this paper.

Received November 9, 1972