Spectrum of Light Quasielastically Scattered from Coupled Reaction Systems of Macromolecules

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ABSTRACT: This paper deals with the spectrum of light quasielastically scattered from solution of coupled reaction systems of macromolecules. The first reaction is of the type $F_0 \rightleftarrows F_1 \rightleftarrows \cdots\cdots \rightleftarrows F_N$ with arbitrary rate constants A (forward) and B (backward). Rate equations were solved by the method of Ninham, Nossal, and Zwanzig (*J. Chem. Phys.*, **51**, 5028 (1969)). The relaxation times associated with the chemical reaction can be written as: $\tau_0^{-1} = 0$ and $\tau_p^{-1} = [(A)^{1/2} - (B)^{1/2}]^2 + 4(AB)^{1/2} \sin^2 p\pi/2(N+1)$ (p: 1, 2, . . . , N). The correlation function of scattered light can be written as

$$I(K,t) \propto \sum_{p=0}^{N} \alpha_i \alpha_j \gamma^{i+j} A_p^{(i+1,j+1)} \exp[-(DK^2 + \tau_p^{-1})t]$$

where α_i is the polarizability of species i, D is the translational diffusion constant and $\gamma = (A/B)^{1/2}$. $A_p^{(i+1,j+1)}$ can simply be expressed by trigonometric functions. For $\gamma = 1$, $B_p = \Sigma \alpha_i \alpha_j A_p^{(i+1,j+1)}$ was evaluated for an assumed form of α_i and it was found that $B_p \propto p^{-4}$. The second reaction is of the type $F_0 + H \rightleftarrows F_1 + H \rightleftarrows \cdots \rightleftarrows F_N$, where $F_i = FH_i$ and F and H stand for some molecules. Since F is assumed to have N binding sites for H, it holds that $\bar{F}_i/\bar{F}_{i-1}\cdot\bar{H} = [(N+1-i)/i](k_f/k_b)$, where bars mean equilibrium concentrations and k_f and k_b are rate constants. Rate equations were solved analytically for small N's and by a machine computation for large N's. Results suggest that the relaxation times can be written as: $\tau_p^{-1} = (k_f\bar{H} + k_b)p$ (p: 1, 2, ... , N). Intensities associated with the second reaction were evaluated by a machine computation and were found to be very weak.

Quasielastic light scattering has emerged as a significant new technique for studying macromolecules in solution.[1,2] This method can provide information about elastic constants of macromolecules as well as their transport coefficients. Furthermore, this method provides information on the kinetics of fast reaction. This has been the subject of many theoretical[3-9] and a few experimental investigations.[10] The quasielastic light-scattering spectrum associated with the reaction arises because of polarizability differences in reactants and products and also because of the differences in diffusion coefficients of reactants and products. We wish to present here the theoretical results based on simplified models of coupled reactions:[11]

$$F_0 \underset{B}{\overset{A}{\rightleftharpoons}} F_1 \underset{B}{\overset{A}{\rightleftharpoons}} F_2 \rightleftharpoons \cdots \underset{B}{\overset{A}{\rightleftharpoons}} F_N \qquad (1)$$

with arbitrary rate constants A (forward) and B (backward), and

$$F_0 + H \rightleftharpoons F_1 + H \rightleftharpoons F_2 + H \rightleftharpoons \cdots \rightleftharpoons F_N \qquad (2)$$

where F_i stands for FH_i (H is not hydrogen). For reaction 1, rate equations can be solved analytically by the method of Ninham *et al.*,[12] so that the result will have an important role in seeing the general feature of the spectrum associated with a coupled reaction. The reaction 2 was studied in order to interprete experimental spectra from solutions of muscle proteins introduced below. However, the result will have applications to various other systems.

Muscle F-actin is a two-stranded helical polymer.[13] It consists of monomers called G-actin (mol wt $\simeq 5 \times 10^4$). F-actin polymerized *in vitro* under a standard condition (*e.g.*, 100 m*M* KCl, pH 8) is longer than 1 μm. Another muscle protein called myosin interacts with F-actin. Since myosin is insoluble at low ionic strength, *in vitro* experiments are usually carried out with heavy meromyosin (mol wt $\simeq 3 \times 10^5$), a product of limited tryptic digestion of myosin.[13] Heavy meromyosin can bind to F-actin in the absence of adenosine triphosphate (ATP). This has been suggested by measurements of turbidity and viscosity and by ultracentrifugation of the solution. On the addition of ATP, however, heavy meromyosin seems to dissociate from F-actin because of a large decrease of turbidity and viscosity. In the presence of Mg ions, the ATPase activity of heavy meromyosin is very low. However, the presence of F-actin greatly activates the MgATPase activity of heavy meromyosin.[13]

We have studied F-actin and the complexes of F-actin and other muscle proteins by quasielastic light scattering.[2,14-19] A long, semiflexible F-actin molecule is expected to undergo a spontaneous bending motion. In fact, half-widths at half-height of spectra of solution of F-actin can be expressed in a homodyne configuration as

$$\Gamma = 2DK^2 + 1/\tau \qquad (3)$$

$$K = (4\pi/\lambda) \sin(\phi/2) \qquad (4)$$

where D is the apparent diffusion coefficient, λ the wavelength of incident light in a medium, and ϕ the scattering

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(9) V. A. Bloomfield and J. A. Benbasat, *Macromolecules*, **4**, 609 (1971).
(10) (a) Y. Yeh, *J. Chem. Phys.*, **51**, 1120 (1969); (b) Y. Yeh and R. Keeler, *ibid.*, **53**, 4409 (1970).
(11) Because of the simplicity of algebra, rate constants at all steps were assumed to be the same, that is, any cooperative nature of the reaction was not taken into account.
(12) B. Nihham, R. Nossal, and R. Zwanzig, *J. Chem. Phys.*, **51**, 5028 (1969).
(13) For example, Y. Tonomura, and F. Oosawa, *Annu. Rev. Biophys. Bioeng.*, **1**, 159 (1972).
(14) S. Fujime, *J. Phys. Soc. Jap.*, **29**, 751 (1970).
(15) S. Ishiwata and S. Fujime, *J. Phys. Soc. Jap.*, **31**, 1601 (1971).
(16) S. Fujime and S. Ishiwata, *J. Mol. Biol.*, **62**, 251 (1971).
(17) S. Fujime and S. Hatano, *J. Mechanochem. Cell Motility*, **1**, 81 (1972).
(18) S. Ishiwata and S. Fujime, *J. Mol. Biol.*, **68**, 511 (1972).
(19) S. Fujime, S. Ishiwata, and T. Maeda, *Biochim. Biophys. Acta*, **283**, 351 (1972).

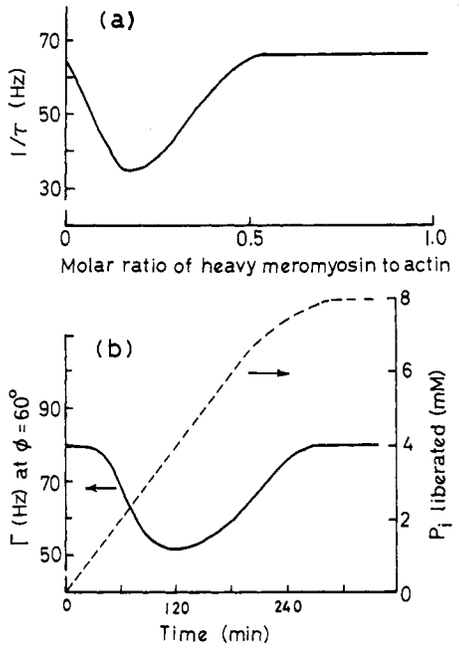

Figure 1. Schematic representation of experimental results of muscle proteins. (a) The $1/\tau$ of the complex of F-actin and heavy meromyosin at various molar ratios of heavy meromyosin to actin in the absence of ATP.[16] (b) Γ at $\phi = 60°$ of the complex of F-actin and heavy meromyosin (at a molar ratio of 0.5) at various concentrations of ATP.[22a,b] The concentration of ATP can be estimated from the amounts of liberated inorganic phosphate (P_i), the initial concentration of ATP being 8 mM. It should be noted that the $1/\tau$ of F-actin depends on the concentration of ATP.

angle.[14,20-21] The τ in eq 3 can be interpreted as an average relaxation time of the spontaneous bending motion of F-actin. On the addition of heavy meromyosin in the absence of ATP, the $1/\tau$ of F-actin greatly decreases as shown in Figure 1a.[16] This has been interpreted to be due to the fact that F-actin becomes flexible as a result of the interaction with heavy meromyosin. Recently, it has been found that the $1/\tau$ of F-actin changes on the addition of heavy meromyosin even in the presence of ATP.[22a,b] Moreover, this change in $1/\tau$ depends on the concentration of ATP and this change is observed even at a molar ratio of heavy meromyosin to F-actin monomer of ½ (Figure 1b),[22a] where no change has been observed in the absence of ATP (Figure 1a). Then there arises a question whether or not the change in $1/\tau$ is due to the occurrence of a cyclic chemical reaction such as

$$\begin{array}{ccccc} \text{HMM} + \text{MgATP} & \rightleftharpoons & \text{HMM·MgATP} & & \text{HMM} + \text{ADP} + \text{P}_i \\ & & \downarrow & & \uparrow \\ \text{F} & \xrightarrow[k_f[\text{HMM}]]{} & \text{F·HMM·MgATP} & \xrightarrow[k_b]{} & \text{F} \end{array} \qquad (5)$$

where HMM and F stand for heavy meromyosin and F-actin, respectively, ADP is adenosine diphosphate and P_i is inorganic phosphate. As shown in subsequent sections, the change in $1/\tau$ of F-actin in the presence of heavy meromyosin and ATP can be concluded not to be due to a chemical reaction but due to the intrinsic change in the F-actin flexibility as a result of a cyclic interaction with heavy meromyosin.

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(21) S. Fujime and M. Maruyama, *Macromolecules*, 6, 237 (1973).
(22) (a) F. Oosawa, S. Fujime, S. Ishiwata, and K. Mihashi, *Cold Spring Harbor Symp. Quant. Biol.*, 37, 277 (1972). (b) S. Ishiwata, private communication (details will be published in a near future).

I. The Reaction 1

Relaxation Times.[23] Let us denote by F_i both the species F_i and its number concentration. Then it holds for reaction 1 that

$$\bar{F}_i = (A/B)\bar{F}_{i-1} = (A/B)^i\bar{F}_0 \qquad (6)$$

where $\bar{F}_i$ stands for the equilibrium concentration of F_i. Letting $F_i(\mathbf{r},t) = \bar{F}_i + \delta F_i(\mathbf{r},t)$, where $\mathbf{r}$ is the position vector and t is the time, it holds for reaction 1 that

$$\dot{C}_0 = -(D_0K^2 + A)C_0 + BC_1 \qquad (7a)$$

$$\dot{C}_i = AC_{i-1} - (D_iK^2 + A + B)C_i + BC_{i+1} \qquad (7b)$$

$$C_N = AC_{N-1} - (D_NK^2 + B)C_N \qquad (7c)$$

where D_i is the diffusion coefficient of species i and C_i the Fourier transform of $\delta F_i(\mathbf{r},t)$

$$C_i = C_i(\mathbf{K},t) = \int \delta F_i(\mathbf{r},t)e^{i\mathbf{K}\mathbf{r}}d\mathbf{r} \qquad (8)$$

(To avoid cumbersome algebra, rotational and internal modes of motion were neglected.) If $D_i = D$ is assumed for all i's,[24] eq 7 can be solved analytically for arbitrary values of A, B, and N. Letting

$$C_i(\mathbf{K},t) = \gamma^i \exp[-(A + B + DK^2)t]\sigma_i(\mathbf{K},\tau) \qquad (9a)$$

$$\gamma = (A/B)^{1/2} \text{ and } \tau = 2(AB)^{1/2}t \qquad (9b)$$

eq 7 becomes

$$2\dot{\sigma}_0 = \frac{1}{\gamma}\sigma_0 + \sigma_1 \qquad (10a)$$

$$2\dot{\sigma}_i = \sigma_{i-1} + \sigma_{i+1} \qquad (10b)$$

$$2\dot{\sigma}_N = \sigma_{N-1} + \gamma\sigma_N \qquad (10c)$$

The Laplace transform of eq 10 becomes

$$\mathbf{M}\hat{\underline{g}}(\epsilon) = 2\underline{g}(0) \qquad (11)$$

where

$$\hat{\underline{g}}(\epsilon) = \int_0^\infty \underline{g}(\mathbf{K},\tau)e^{-\epsilon\tau}d\tau \qquad (12)$$

and

$$\underline{g} = \begin{bmatrix} \sigma_0 \\ \sigma_1 \\ \sigma_2 \\ \vdots \\ \sigma_N \end{bmatrix} \quad \mathbf{M} = \begin{bmatrix} 2\epsilon - \frac{1}{\gamma} & -1 & 0 & & \\ -1 & 2\epsilon & -1 & & 0 \\ 0 & -1 & 2\epsilon & & \\ & & & \ddots & \\ & & & 2\epsilon & -1 \\ & & 0 & & \\ & & & -1 & 2\epsilon - \gamma \end{bmatrix}_{N+1} \qquad (13)$$

Equation 11 can formally be solved as

$$\hat{\sigma}_i(\epsilon) = 2\sum_{k=0}^{N}\sigma_k(0)D_{k+1,i+1}(\epsilon)/D_{N+1}(\epsilon) \qquad (14)$$

where $D_{N+1}(\epsilon)$ is the determinant of $\mathbf{M}$ and $D_{k+1,i+1}$ is the cofactor of the $(k + 1, i + 1)$ element of $\mathbf{M}$. When we write

$$D_{N+1}(\epsilon) = 2\prod_{q=0}^{N}(\epsilon - \epsilon_q)$$

the inverse Laplace transform of eq 14 becomes

(23) For the convenience of the later discussion, the method of Ninham *et al.* (see ref 12) is reviewed in this subsection.
(24) This assumption is not generally valid. As will be discussed later, however, this does not impose serious limitations.

$$\sigma(\tau) = \frac{1}{2\pi i}\oint \hat{\sigma}_i(\epsilon)e^{-\epsilon\tau}d\epsilon = \sum_{k=0}^{N}\sigma_k(0)\sum_{p=0}^{N}A_p^{(k+1,i+1)}\exp(\epsilon_p\tau) \quad (15)$$

where

$$A_p^{(k+1,i+1)} = \frac{\overline{D_{k+1,i+1}(\epsilon_p)}}{\left[\frac{d}{d\epsilon}\prod_{q=0}^{N}(\epsilon-\epsilon_q)\right]_{\epsilon=\epsilon_p}} \quad (16)$$

It is necessary, therefore, to find all roots of $D_{N+1}(\epsilon) = 0$. Letting

$$D_j = \det\begin{bmatrix} 2\epsilon & -1 & 0 & & \\ -1 & 2\epsilon & -1 & & 0 \\ 0 & -1 & 2\epsilon & \ddots & \\ & & & 2\epsilon & -1 \\ & 0 & & -1 & 2\epsilon-\gamma \end{bmatrix}_j \quad (17)$$

$$D_0 \equiv 1,\ D_1 = 2\epsilon - \gamma, \text{ and } D_2 = 2\epsilon D_1 - 1 \quad (18)$$

it is evident that

$$D_j = 2\epsilon D_{j-1} - D_{j-2} \quad (j: 3, 4, \ldots, N) \quad (19)$$

$$D_{N+1} = (2\epsilon - \tfrac{1}{\gamma})D_N - D_{N-1} \quad (20)$$

Equation 19 is the recurrence formula for the polynomial defined by

$$U_j(\epsilon) = \sin(j+1)\theta/\sin\theta,\ U_{-j} = 0 \text{ and } \epsilon = \cos\theta \quad (21)$$

The linear combination of U_j defined by

$$D_j = U_j - \gamma U_{j-1} \quad (22)$$

fulfills eq 18 and 19. Then we have

$$D_{N+1}(\epsilon) = (2\epsilon - \gamma - \frac{1}{\gamma})U_N(\epsilon) = 2\prod_{p=0}^{N}(\epsilon-\epsilon_p) \quad (23)$$

where

$$\epsilon_0 = \tfrac{1}{2}(\gamma + \tfrac{1}{\gamma}) \quad (24a)$$

$$\epsilon_p = \cos\frac{p\pi}{N+1} \quad (p: 1, 2, \ldots, N) \quad (24b)$$

From eq 9, 15 and 24, we find

$$C_i(t) = \gamma^i\sum_{k=0}^{N}\gamma^{-k}C_k(0)\sum_{k=0}^{N}A_p^{(k+1,i+1)} \times \exp[-(DK^2 + \tau_p^{-1})t] \quad (25)$$

where

$$\tau_0^{-1} = 0 \text{ and } \tau_p^{-1} = (\sqrt{A} - \sqrt{B})^2 + 4\sqrt{AB}\sin^2\frac{p\pi}{2(N+1)} \quad (26)$$

The Correlation Function of Scattered Light. Let $\epsilon(\mathbf{r},t)$ be the instantaneous dielectric constant of the solution. Then we have

$$\delta\epsilon(\mathbf{K},t) = \sum_{i=0}^{N}\alpha_i C_i(\mathbf{K},t) \quad (27)$$

where

$$\alpha_i = (\partial\epsilon/\partial F_i)_j \quad (28)$$

The correlation function of scattered light may be written as[1]

$$I(\mathbf{K},t) = \langle\delta\epsilon(-\mathbf{K},0)\delta\epsilon(\mathbf{K},t)\rangle = \sum_{i=0}^{N}\sum_{j=0}^{N}\alpha_i\alpha_j\langle C_i(-\mathbf{K},0)C_j(\mathbf{K},t)\rangle \quad (29)$$

Under the assumption of concentration fluctuations in an ideal solution:[25]

$$\langle C_i(-\mathbf{K},0)C_j(\mathbf{K},0)\rangle = \overline{F}_i\delta_{ij} = \overline{F}_0\gamma^{2i}\delta_{ij} \quad (30)$$

where δ_{ij} is the Kronecker delta, eq 29 becomes

$$I(\mathbf{K},t) = P(\mathbf{K})\sum_{p=0}^{N}B_p\exp[-(DK^2 + \tau_p^{-1})t] \quad (31)$$

and

$$B_p = \overline{F}_0\Sigma_i\Sigma_j\alpha_i\alpha_j\gamma^{i+j}A_p^{(i+1,j+1)} \quad (32)$$

The form factor $P(\mathbf{K})$ was multiplied in order to explicitly show the angular dependence of $I(\mathbf{K},t)$. To evaluate the values of B_p, $D_{i+1,j+1}(\epsilon)$ in eq 16 must be known. Letting (*cf.*, D_j in eq 17)

$$D_j^\circ = \det\begin{bmatrix} 2\epsilon - \frac{1}{\gamma} & -1 & 0 & & \\ -1 & 2\epsilon & -1 & & 0 \\ 0 & -1 & 2\epsilon & \ddots & \\ & & & 2\epsilon & -1 \\ & 0 & & -1 & 2\epsilon \end{bmatrix}_j \quad (33)$$

$$D_0^\circ \equiv 1,\ D_1^\circ = 2\epsilon - \frac{1}{\gamma} \text{ and } D_2^\circ = 2\epsilon D_1 - 1 \quad (34)$$

it is evident that

$$D_j^\circ = 2\epsilon D_{j-1}^\circ - D_{j-2}^\circ \quad (j: 3, 4, \ldots, N) \quad (35)$$

Equations 34 and 35 are satisfied by

$$D_j^\circ = U_j - \frac{1}{\gamma}U_{j-1} \quad (36)$$

Using D_j° and D_j, it is easily shown that

$$D_{i+1,j+1} = D_{N-i} \times D_j^\circ \quad (37)$$

Therefore, it we put

$$\gamma = e^\phi,\ \epsilon_0 = \cosh\phi \text{ and } \theta_p = p\pi/(N+1) \quad (38)$$

eq 16 becomes

$$A_p^{(i+1,j+1)} = A_p(i)A_p(j) \quad (39)$$

where

$$A_0(i) = \sqrt{\frac{\sinh\phi}{e^{N\phi}\sinh(N+1)\phi}}e^{i\phi} \quad (40a)$$

$$A_p(i) = \sqrt{\frac{e^\phi}{(N+1)(\cosh\phi - \cos\theta_p)}} \times \{\sin(i+1)\theta_p - e^{-\phi}\sin(i\theta_p)\} \quad (40b)$$

At $\gamma = 1$ (*i.e.*, $\phi = 0$), these are reduced to

$$A_p^{(i+1,j+1)} = \alpha_p(i)\alpha_p(j) \quad (41)$$

where

$$\alpha_0(i) = \sqrt{\frac{1}{N+1}} \text{ and } \alpha_p(i) = \sqrt{\frac{2}{N+1}}\cos(i+\tfrac{1}{2})\theta_p \quad (42)$$

are the eigenvectors of the Rouse matrix[26] as they should be. To avoid unnecessarily cumbersome expression in seeing the qualitative trend of B_p as a function of p, the following evaluation of B_p was made only for $\gamma = 1$. But the evaluation of B_p for an arbitrary value of γ is easy be-

(25) L. D. Landau and E. M. Lifshitz, "Statistical Physics," London, Pergamon Press, 1958, p 361.
(26) See, for example, B. H. Zimm, *J. Chem. Phys.*, 24, 269 (1956).

cause $A_p^{(i+1,j+1)}$ consists of two factors depending only on i and on j. (i) If $\alpha_i = \alpha$ for all i's, it can be shown from eq 41 and 42 that

$$B_p = \bar{F}_t\alpha^2\delta_{p0} \quad (\bar{F}_t = (N+1)\bar{F}_0) \tag{43}$$

This result is self-evident. (ii) If $\alpha_i = \alpha(1 + i\delta)$, it is easily shown that

$$B_0 = \alpha^2[\Sigma_i(1 + i\delta)\alpha_0(i)]^2 = \alpha^2\bar{F}_t(1 + N\delta/2)^2 \tag{44}$$

$$B_p = \alpha^2[\Sigma_i(1 + i\delta)\alpha_p(i)]^2 = \alpha^2\bar{F}_t(N+1)^2\delta^2\frac{8}{p^4\pi^4}(p:\text{odd}) \tag{45}$$

It should be noted that B_p is inversely proportional to p^4. (If $\alpha_i = \alpha[1 + \delta \sin(\pi i/2N)]$ is assumed, it will be found that $B_p \propto (4p^2 - 1)^{-2}$.) This means that only the lowest mode ($p = 1$) is practically important. The ratio B_1/B_0 is equal to $(8N^2\delta^2/\pi^4)/(1 + N\delta/2)^2$ ($= 4 \times 10^{-2}$ for $N\delta = 1$).

II. The Reaction 2

We assume that (i) each F-actin molecule consists of $2N = 400$ monomers (G-actin) and hence the length of F-actin is 1 μm, (ii) each F-actin molecule has N binding sites for heavy meromyosin.[13] Let denote by F_i both the state of F-actin which binds i heavy meromyosin molecules (H) and the number concentration of F-actin in the i state. Then it may hold for reaction 2 that

$$\frac{\bar{F}_i}{\bar{F}_{i-1}\cdot\bar{H}} = \frac{N-(i-1)}{i}\frac{k_f}{k_b} \tag{46}$$

or

$$\bar{F}_i = \bar{F}_0\binom{N}{i}\left(\frac{A}{B}\right)^i,\ A = k_f\cdot\bar{H} \text{ and } B = k_b \tag{47}$$

where $\bar{F}_i$ and $\bar{H}$ stand for the equilibrium concentrations of F_i and H, respectively, and $\binom{N}{i}$ is a binomial coefficient. The total number concentration of F-actin ($\bar{F}_t$) and that of bound heavy meromyosin ($\bar{H}_b$) are, respectively, given by

$$\bar{F}_t = \sum_{i=0}^{N}\bar{F}_i = \bar{F}_0(1 + A/B)^N \tag{48}$$

$$\bar{H}_b = \sum_{i=0}^{N}i\bar{F}_i = \bar{F}_0\left(\frac{A}{B}\right)N(1 + A/B)^{N-1} \tag{49}$$

At a molar ratio of heavy meromyosin to F-actin monomer of ½, it holds from above assumptions that $\bar{H}_b/\bar{H}_t = ½$ (at $A/B = 1$), where $\bar{H}_t$ is the total concentration of heavy meromyosin. At a molar ratio lower than unity, it is experimentally known that $\bar{H} \simeq 0$ in the absence of ATP, and $\bar{H}_b/\bar{H} \leq 1$ or $\bar{H}_b/\bar{H}_t \leq ½$ in the presence of ATP.[27]

The latter means that the ratio A/B is near or a little smaller than unity.

Letting $F_i(\mathbf{r},t) = \bar{F}_i + \delta F_i(\mathbf{r},t)$ and $H(\mathbf{r},t) = \bar{H} + \delta H(\mathbf{r},t)$

$$\partial\delta F_i/\partial t = (N - i + 1)A\,\delta F_{i-1} - [(N-i)A + iB]\delta F_i + (i+1)B\delta F_{i+1} + [(N - i + 1)\bar{F}_{i-1} - (N-i)\bar{F}_i]\frac{A}{\bar{H}}\delta H \tag{50}$$

Putting $A = B$, the order of magnitude of each term in the right-hand side of eq 50 may be estimated as

$$(N-i+1)\left|\frac{\delta F_{i-1}}{\delta F_i}\right| : N : (i+1)\left|\frac{\delta F_{i+1}}{\delta F_i}\right| : \left[(N-i+1)\frac{\bar{F}_{i-1}}{\bar{H}} - (N-i)\frac{\bar{F}_i}{\bar{H}}\right]\left|\frac{\delta H}{\delta F_i}\right| =$$

$$\sqrt{(N-i+1)i} : N : \sqrt{(N-i)(i+1)} : |2i - N|\sqrt{\binom{N}{i}/N2^{N-1}}$$

where use is made of $\langle\delta F_i^2\rangle \propto \bar{F}_i$ (eq 30). For $N = 200$, the last factor in the above ratio is at least 10^{-3} times smaller than the other factors. This estimation allows us to neglect the term proportional to δH. Since free heavy meromyosin molecules do not appreciably contribute to the scattered light from solution, the rate equation for δH can also be neglected. Then the rate equations for reaction 2 can be approximated as

$$\dot{C}_0 = -NAC_0 + BC_1 \tag{51a}[28]$$

$$\dot{C}_i = (N - i + 1)AC_{i-1} - [(N-i)A + iB]C_i + (i+1)BC_{i+1} \tag{51b}$$

$$\dot{C}_N = AC_{N-1} - NBC_N \tag{51c}$$

$$\dot{\mathbf{C}} = B\mathbf{M}\mathbf{C} \tag{52}$$

or in matrix form as

$$C_i(t) = \int\delta F_i(\mathbf{r},t)e^{i\mathbf{K}\mathbf{r}}d\mathbf{r} \tag{53a}$$

$$\mathbf{M} = \begin{bmatrix} -N\gamma^2 & 1 & 0 & & & & \\ N\gamma^2 & -[(N-1)\gamma^2 + 1] & 2 & & & & \\ 0 & (N-1)\gamma^2 & -[(N-2)\gamma^2 + 2] & \cdots & & 0 & \\ & & & [2\gamma^2 + (N-2)] & N-1 & 0 & \\ & 0 & & 2\gamma^2 & [\gamma^2 + (N-1)] & N & \\ & & & 0 & \gamma^2 & -N & \end{bmatrix}_{N+1} \tag{53b}$$

$$\mathbf{C} = (C_0, C_1, \ldots\ldots, C_N)^T \text{ and } \gamma = (A/B)^{1/2} \tag{53c}$$

(T means transposition)

where eq 53a-c holds true. The formal solution of eq 52 may be given by

$$\mathbf{C}(t) = \exp(B\mathbf{M}t)\mathbf{C}(0) = \sum_{p=0}^{N}\exp(B\epsilon_p t)\mathbf{U}_p\mathbf{U}_p^*\mathbf{C}(0) \tag{54}[29]$$

or, in the component representation, by

(27) E. Eisenberg, L. Dobkin and W. W. Kielley, *Proc. Nat. Acad. Sci. U. S.*, **69**, 667 (1972).

(28) Diffusion terms were neglected here. If we assume $D_i = D$ for all i's, they can be taken into account as in the case of reaction 1.

(29) For example, C. A. B. Smith, "Biomathematics," Charles Griffin & Co. Ltd., London, 1969, Vol. 2, p 48.

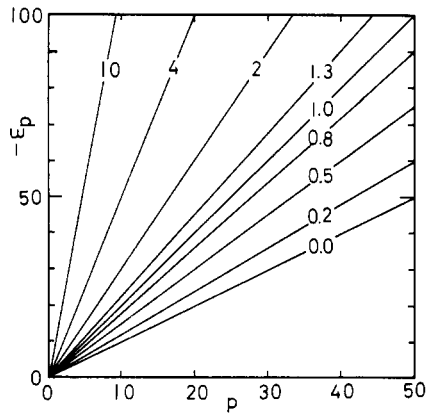

Figure 2. Eigenvalues of **M** at various values of γ^2. Machine computations were made for $N = 50$. The numeral attached to each line indicates the value of γ^2 ($= A/B$). Eigenvalues, ϵ_p, can be expressed by: $\epsilon_p = -(\gamma^2 + 1)p$ (p: 0, 1, . . ., N).

$$C_i(t) = \sum_{k=0}^{N}\sum_{p=0}^{N} \exp(B\epsilon_p t)(\mathbf{U}_p\mathbf{U}_p^*)_{k+1,i+1}C_k(0) \quad (55)$$

$$(\mathbf{U}_p\mathbf{U}_p^*)_{k+1,i+1} = U_p(k)U_p(i) \quad (56a)$$

$$\mathbf{U}_p = (U_p(0), U_p(1), \ldots, U_p(i), \ldots, U_p(N))^{\mathrm{T}} \quad (56b)$$

where ϵ_p and $\mathbf{U}_p$ are the eigenvalue and the normalized eigenvector of **M**, respectively. $(\mathbf{U}_p\mathbf{U}_p^*)_{k+1,i+1}$ just corresponds to $A_p^{(k+1,i+1)}$ in eq 16. Equations 29 and 30 lead to

$$I(\mathbf{K},t) = P(\mathbf{K})\sum_{p=0}^{N} B_p \exp[-(DK^2 + \tau_p^{-1})t] \quad (31)$$

where

$$B_p = \bar{F}_0\Sigma_i\Sigma_j\alpha_i\alpha_j\binom{N}{i}\gamma^{2i}U_p(i)U_p(j) \quad (57)$$

$$\tau_p^{-1} = -\epsilon_p B \quad (58)$$

Trials for small values of N ($N \leq 10$) suggest that

$$D_{N+1}(\epsilon) = |\mathbf{M} - \epsilon\mathbf{I}| = \prod_{p=0}^{N}\{\epsilon + (\gamma^2 + 1)p\} \quad (59)^{30}$$

i.e., eigenvalues are spaced between zero and $-(\gamma^2 + 1)N$;

$$\epsilon_p = -(\gamma^2 + 1)p \quad (p: 0, 1, 2, \ldots, N) \quad (60a)$$

$$\tau_p^{-1} = (A + B)p \quad (60b)$$

Eigenvectors belonging to ϵ_0 and ϵ_N are easily obtained

$$U_0(i) \propto \gamma^{2i}\binom{N}{i}$$
$$U_N(i) \propto (-1)^i\gamma^{2i}\binom{N}{i} \quad (61a)$$

or at $\gamma = 1$

$$U_0(i) = \frac{N!}{\sqrt{(2N)!}}\binom{N}{i}$$
$$U_N(i) = (-1)^i\frac{N!}{\sqrt{(2N)!}}\binom{N}{i} \quad (61b)$$

Using these results and assuming again $\alpha = \alpha(1 + i\delta)$, B_0 and B_N for $\gamma = 1$ are calculated as

$$B_0 = \bar{F}_t\alpha^2(1 + N\delta/2)^2$$
$$B_N = 0 \quad (\bar{F}_t = 2^N F_0) \quad (62)$$

(30) In the limit of $\gamma \to 0$ (or $1/\gamma \to 0$), **M** is a triangular matrix so that eq 59 directly results in. A machine computation supports the validity of eq 59 for $N \leq 50$. However, an algebraic proof of eq 59 has not been obtained for arbitrary values of γ and N.

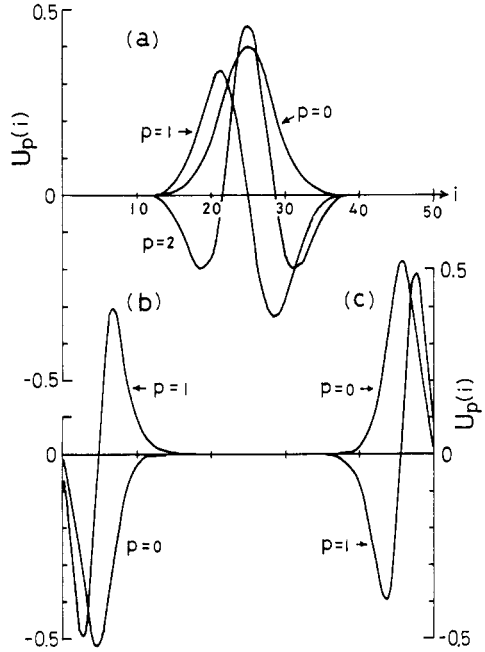

Figure 3. Graphical representation of some of eigenvectors. Computations were made for $N = 50$: (a) $\gamma^2 = 1$, (b) $\gamma^2 = 0.1$, and (c) $\gamma^2 = 10$.

The result for B_0 is self-evident (*cf.* eq 44). The fact that $B_N = 0$ is accidental due to the assumed form of α_i.

Since analytical expressions of eigenvectors are very complicated for large N, a machine computation was carried out. (Because of limited machine time, calculations were made only for $N \leq 50$.) A machine computation confirmed the validity of eq 59 (Figure 2). Figure 3 is the graphical representation of some eigenvectors. The numerical values of B_1/B_0 for $A = B$ are shown in Figure 4, where $\alpha_i = \alpha(1 + i\delta)$ was assumed. The values of B_p ($p \geq 2$) were vanishingly small. When we assumed $\alpha_i = \alpha[1 + \delta \sin(\pi i/N)]$, only the ratio B_2/B_0 was not very small (Figure 4). In any case we have studied, the intensities associated with the chemical relaxation modes were weak. This is due to the strong localization of eigenvectors as a function of i; the larger the value of N, the weaker are the intensities B_p.

III. Concluding Remarks

Generally speaking, a linear macromolecule undergoes conformational fluctuations.[1,2,21] In such a case, the correlation function of scattered light may be written as

$$I(K,t) = P_0(K)e^{-DK^2t} + P_c(K)e^{-(DK^2+\tau_c^{-1})t} + \ldots \quad (63)$$

where τ_c is the longest correlation time of the conformational fluctuation. Under favorable conditions, $P_c(\mathbf{K})/P_0(\mathbf{K})$ may be larger than one-half.[20,21] As shown before, on the other hand, B_p/B_0 is less than 10^{-2}. Therefore, the experimentally observed value of τ of F-actin (Figure 1) might surely come from the conformational fluctuation of F-actin (details will be published elsewhere[22b]).

It should be noted that $P_0 \to 1$ and $P_c \to 0$ as $K \to 0$,[21] whereas B_p is independent of the value of K for $D_i = D$. Even though there are many possible modes in fluctuations, only the concentration fluctuation associated with a chemical reaction is responsible for the broadening of scattered light at very small values of K; $DK^2 \ll \tau_p^{-1}$.

There is yet no experimental evidence suggesting the importance of chemical relaxation modes in the light-

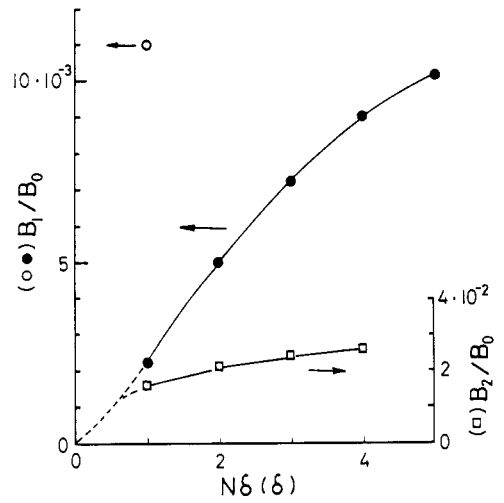

Figure 4. Intensity associated with a chemical relaxation. Computations were made for $N = 50$ (● and ○) and $N = 10$ (○) at $\gamma^2 = 1$. (○) $\alpha_i = \alpha(1 + i\delta)$ with $N\delta = 1$, (●) $\alpha_i = \alpha(1 + i\delta)$ with $1 \leq N\delta \leq 5$ and (□) $\alpha_i = \alpha[1 + \delta \sin(\pi i/N)]$ with $1 \leq \delta \leq 4$.

scattering study. Main reasons for this come from experimental difficulties in separating the weak contributions of chemical relaxation modes from the high intensity associated with the center-of-mass motion. We must therefore work under the condition of $DK^2 \leq \tau_L^{-1} \ll \tau_p^{-1}$ (τ_L: the coherence time of laser light, typically 100 msec.). In that condition, the scattered light associated with the diffusive motion will act as a reference signal in the heterodyne detection and only the broadening associated with chemical relaxation modes will be observed. A depolarized light-mixing technique may also be useful in order to eliminate the strong contribution from the center-of-mass motion,[31] although this technique has inherent difficulties. Because of a collective behavior of a coupled reaction, very fast reaction kinetics may be followed. For example, if $A = B = 10^6$ sec^{-1} is assumed, the τ^{-1} value will become 10^3 sec^{-1} for $N = 100$ (from eq 26). This value of τ_1 is in a favorable range of measurements by the present method.

We are trying to detect the *in vivo* interaction between actin and myosin in the presence of ATP,[32] where both proteins are hard to diffuse randomly.[33]

The following is noteworthy: Throughout this investigation, we assumed $D_i = D$ for all i's. When $D_i \neq D_j$ (and/or $P_i(\mathbf{K}) \neq P_j(\mathbf{K})$), the intensity associated with the chemical reaction, B_P, does not equal zero even for $\alpha_i = \alpha$. As has been pointed out,[9] $D_i \neq D_j$ may be important in detecting a chemical relaxation in the case of small N. However, a possible existence of sample polydispersity might make the analysis difficult for the case of large N.

Acknowledgment. The facilities of the Computing Center of Nagoya University were utilized in this work.

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Viscosity and Normal Stresses of Linear and Star Branched Polystyrene Solutions. I. Application of Corresponding States Principle to Zero-Shear Viscosities[1a]

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ABSTRACT: The zero-shear solution viscosities, η_0, of linear, 4- and 6-star branched, monodispersed polystyrenes in diethylbenzene, $c = 25.5$ g/dl, were measured by a capillary and cone-and-plate method. As for each type of polymer the molecular weights vary by a factor of at least 30, the η_0's varied at least by a factor of 10^3. It was demonstrated that the corresponding states principle (CSP) applied to these systems predicts a superposition of data plotted as $(\eta_0 - \eta_s)/[\eta]_\Theta$ *vs.* $[\eta]_\Theta$. Using experimental $[\eta]_\Theta$'s a remarkable superposition was found. Replacing $[\eta]_\Theta$ by $(M_w g)^{1/2}$, where g has the value computed from Zimm and Stockmayer relation, still a good superposition was achieved. It was also found that the numerical value of the coefficient $(\partial \ln \eta_0/\partial \ln c)_{M,T}$ calculated from the master curve agreed numerically with the experimental value computed from the data published in 1953 by Bueche. For samples with molecular weights larger than the critical entanglement molecular weight the temperature dependence of the viscosity in the range 20–40° can be expressed by an Arrhenius-type equation. The average activation energy of flow E_η equals 5.9 ± 1.1 kcal/mol and is independent of the molecular weight and the structure of the polymer.

In many industrial applications one of the most often discussed parameters is the chain branching of polymer macromolecules. Unfortunately, in spite of numerous papers published on this subject,[2-7] no quantitative prediction can be made in regard to the effect of branching on rheological behavior of polymers. The main reasons for this lie in the diversity of branching and in the heterogeneity of polymer samples as far as branching length, branching density, and molecular weight are concerned. Furthermore, very often the rheological studies are limited to the viscous properties or to the low rate of shear re-

(1) (a) Part of this work was presented at the 55th Annual Meeting of the Chemical Institute of Canada, Quebec, Canada, June 5-7, 1972. (b) Department of Chemical Engineering, McGill University, Montreal, Canada. The shear-dependent properties were measured at the Gulf Oil Canada, Research Center in Ste-Anne-de-Bellevue, Quebec, Canada. (c) Division of Chemistry, National Research Council of Canada, Ottawa KIA OR9, Canada.
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