(65) W. Kauzmann, "Symposium of Sulfur in Proteins", Academic Press, New York, 1959, pp 93–104.
(66) B. Havsteen, B. Labouesse, and G. P. Hess, *J. Am. Chem. Soc.*, **85**, 796 (1963).
(67) F. M. Pohl, *FEBS Lett.*, **3**, 60 (1969).
(68) F. Pohl, Ph.D. Thesis, University of Konstanz, Konstanz, Germany, 1970, cited in Brandts et al.[29]
(69) K. Brew, T. C. Vanaman, and R. L. Hill, *J. Biol. Chem.*, **242**, 3747 (1967).
(70) S. Segawa, Y. Husimi, and A. Wada, *Biopolymers*, **12**, 2521 (1973).
(71) C. Tanford, K. C. Aune, and A. Ikai, *J. Mol. Biol.*, **73**, 185 (1973).
(72) K. Kuwajima, K. Nitta, M. Yoneyama, and S. Sugai, *J. Mol. Biol.*, **106**, 359 (1976).
(73) K. Kuwajima, *J. Mol. Biol.*, **114**, 241 (1977).
(74) R. M. Epand and H. A. Scheraga, *Biochemistry*, **7**, 2864 (1968).
(75) T. E. Creighton, *J. Mol. Biol.*, **113**, 295 (1977).
(76) T. G. Bruckman, Ph.D. Thesis, Cornell University, 1977.
(77) D. H. Sachs, A. N. Schechter, A. Eastlake, and C. B. Anfinsen, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 3790 (1972).
(78) H. Taniuchi, C. B. Anfinsen, and A. Sodjo, *Proc. Natl. Acad. Sci. U.S.A.*, **58**, 1235 (1967).
(79) E. E. Hazen, Jr., D. C. Richardson, J. S. Richardson, and in part A. Yonath, *J. Biol. Chem.*, **246**, 2302 (1971).
(80) D. B. Wetlaufer and S. Ristow, *Annu. Rev. Biochem.*, **42**, 135 (1973).
(81) B. T. Nall, J. R. Garel, and R. L. Baldwin, *J. Mol. Biol.*, **118**, 317 (1978).

Polymer Effects under Pressure. 2. Enzymelike Catalysis in the Hydrolysis of Phenyl Ester by Copolymer Containing Imidazole[1]

Yoshihiro Taniguchi,* Kiyokazu Shimokawa, Hideo Hisatome, Shyoichi Tanamachi, and Keizo Suzuki

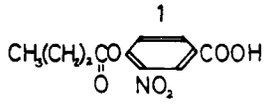
Department of Chemistry, Faculty of Science and Engineering, Ritsumeikan University, Kita-ku, Kyoto, 603, Japan. *Received July 29, 1977*

ABSTRACT: The rates of hydrolysis of 3-nitro-4-butyryloxybenzoic acid (NBBA) and 3-nitro-4-pentanyloxybenzoic acid (NPeBA) catalyzed by the copolymer of 1-vinyl-2-methylimidazole with 1-vinylpyrrolidone (MI-VP) were measured at pressures up to 1471 bar at 30 °C pH 8.0 in 0.068 M in Veronal buffer solution. The reaction was found to follow Michaelis–Menten kinetics. The Michaelis constant K_m was estimated to vary from 6.0 to 10 mM and $k_{cat.}$ from 0.024 to 0.059 min^{-1} between 1 and 1373 bar for NBBA, and K_m from 5.4 to 12 mM and $k_{cat.}$ from 0.022 to 0.077 min^{-1} between 1 and 1471 bar for NPeBA. From the pressure dependence of K_m and $k_{cat.}$, the volume changes accompanying the dissociation of the polymer–substrate complex and the activation volume of the process of the product formation were calculated to be -8.4 ± 2 and -20 ± 2 cm^3/mol for NBBA and -12 ± 2 and -20 ± 2 cm^3/mol for NPeBA, respectively. These negative values for the dissociation of the polymer–substrate complex show that hydrophobic interactions stabilize the complex. The negative activation volumes for the process of product formation may be attributed either to polarity increases in the transition state of the acylation or to bimolecular attack of water on the alkylimidazolium in the deacylation step.

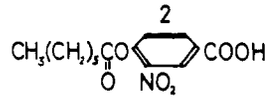
In studying the pressure effect on enzyme reactions, it is desirable for the experiments to be carried out over a range of substrate concentration in order to separate the effects of pressure on the dissociation constants of the Michaelis complex, K_m, and the rate constants of the product formation, $k_{cat.}$. In 1955 the effects of pressure on the kinetics of the myosin-catalyzed hydrolysis of adenosine triphosphate were studied by Laidler and Beardell.[2] Later, similar studies were carried out by Andersen and Broe[3] (interconversion of fumarate to L-malate by fumarase), Mohankumar and Berger[4] (hydrolysis of *p*-nitrophenyl phosphate catalyzed by bovine alkali phosphotase), Williams and Shen[5] (hydrolysis of cytidine 2′,3′-phosphate catalyzed by ribonuclease), and Neville and Eyring[6] (dried *Micrococcus luteus* cell catalyzed by lysozyme).

Kunitake et al.[7] have studied the hydrolysis of phenyl esters catalyzed by a copolymer in which 1-vinyl-2-methylimidazole (MI) residues play the role of the catalytic function and 1-vinylpyrrolidone (VP) residues the role of a hydrophobic binding function. According to them, the rate of the catalytic hydrolysis of the polymer containing low imidazole residues below 20 mol % is simply described by Michaelis–Menten kinetics. The use of such a copolymer for the study of the reaction rates under high pressure is advantageous since the enzymelike catalyst is free of the pressure inactivation found in enzymatically active proteins.[8]

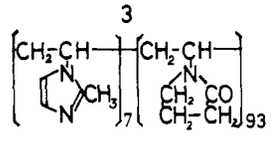
In the present paper, the hydrolysis of NBBA **1** and NPeBA **2** catalyzed by the MI-VP **3** copolymer has been measured at 30 °C and pressures up to 1471 bar in order to obtain the pressure dependences of K_m and $k_{cat.}$. The reaction mechanism is discussed in terms of the volume change accompanying Michaelis–Menten kinetics.



NBBA 1



NPeBA 2



MI-VP 3

Experimental Section

Copolymer. 1-Vinylpyrrolidone, commercial product, was purified by distillation before use, bp 75.5–76.5 °C (6 mm). 1-Vinyl-2-methylimidazole was provided by Shikoku Kasei Co., Japan, and distilled twice before use, bp 69.5–72.5 °C (9 mm). The copolymers of 1-vinyl-2-methylimidazole and 1-vinylpyrrolidone were obtained by radical polymerization of bulk monomer mixtures at 70 °C for 30 min using azobis(isobutyronitrile) as an initiator. The reaction mixture was diluted with methanol and the copolymer was precipitated by excess ethyl ether. The copolymer was reprecipitated twice then dried in vacuo. The molecular weight was measured with a vapor pressure osmometer (Hitachi Perkin-Elmer type 115) to be 10500. The copolymer composition, determined by the titration of the imidazole (Im) groups, contained 7.2 mol % Im.

Substrates. 3-Nitro-4-hydroxybenzoic acid (NHBA), mp 182–184 °C, which was obtained by nitration of *p*-hydroxybenzoic acid, was

0024-9297/78/2211-0829$01.00/0

converted to the butyl ester in *n*-butyric anhydride–pyridine overnight at room temperature. The pale yellow crystals were recrystallized three times from benzene and once from isopropyl ether with charcoal: mp 127–128 °C. Anal. Jasco mass spectrum (JMS-01S), 200 μA, 75 eV. Calcd for $C_{11}H_{11}NO_6$, 253.05864. Found: 253.05855. NPeBA was obtained from the pentanylation in *n*-pentanylic anhydride–pyridine overnight at 17 °C. The crude product was recrystallized four times from benzene with charcoal: mp 117–117.5 °C. Anal. Calcd for $C_{12}H_{13}NO_6$, 267.07430. Found: 267.07456.

Other Materials. *p*-Nitrophenyl acetate (PNPA) and Im (Aldrich Chemical Co. Inc.) recrystallized twice from *n*-hexane and benzene with charcoal had mp 79–80 °C (lit.[9] mp 79.5–80 °C) and mp 89.5–90.0 °C (lit.[10] mp 90 °C), respectively. Acetonitrile (Wako Pure Chemical Ind. Ltd., Japan) was distilled after the treatment with calcium oxide and phosphorus pentoxide. Other compounds used were special grade chemicals (Wako Pure Chemical Ind. Ltd., Japan).

Apparatus and Procedure. The Drickamer type vessel used for the optical measurements to follow the reaction has been described in detail elsewhere.[11] The vessel, which was aligned with the light beam, was placed in a water jacket which was maintained at 30 ± 0.1 °C by circulating water. The optical density at λ_{max} 400 nm of *p*-nitrophenol ion or 410 nm of the dianion of NHBA of the reaction products was determined with a Hitachi Perkin-Elmer EP 139 type spectrophotometer while under pressure. It took about 3 min from the mixing of the catalyst and substrates to the attainment of the desired pressure. Each solution and glass cell were kept at 30 °C in a constant temperature bath prior to mixing.

Results and Discussion

The substrate is hydrolyzed spontaneously in the absence of the catalyst. The rate of the spontaneous hydrolysis was proportional to the substrate concentration at pH 8.0. Therefore, the rate of hydrolysis in the presence of the catalyst V_{app} is separated into two terms, spontaneous rate V_w and catalytic rate $V_{cat.}$

$$V_{app} = V_{cat.} + V_w \tag{1}$$

At each pressure, the hydrolysis rate, $V_{cat.}$, increases with the substrate concentration and then levels off at higher concentrations. This kinetic behavior is similar to that of an enzyme-catalyzed reaction as shown in eq 2

$$C + S \underset{K_m}{\rightleftharpoons} CS \xrightarrow{k_{cat.}} C + P \tag{2}$$

In the case of [C] ≪ [S], the rate of the product formation is expressed as

$$V_{cat.} = \frac{k_{cat.} \cdot [C] \cdot [S]}{K_m + [S]} \tag{3}$$

where C and S denote catalyst and substrate, respectively. K_m and $k_{cat.}$ are determined by the least-squares method from Lineweaver–Burk plots of $1/V_{cat.}$ against 1/[S] in eq 3 as shown in Figures 1 (NBBA) and 2 (NPeBA),

$$1/V_{cat.} = \frac{K_m}{k_{cat.} \cdot [C]} \cdot 1/[S] + \frac{1}{k_{cat.} \cdot [C]} \tag{4}$$

These values are shown in Table I. From the effect of the pressure on the Michaelis–Menten parameters, the volume change ΔV accompanying the dissociation of the Michaelis complex and the activation volume ΔV^* accompanying product formation are given by

$$(\partial \ln K_m/\partial P)_T = -\Delta V/RT \tag{5}$$

$$(\partial \ln k_{cat.}/\partial P)_T = -\Delta V^*/RT \tag{6}$$

where P is the pressure, T the absolute temperature, and R the gas constant. The linear relationships of the ln K_m and ln $k_{cat.}$ vs. pressure are established in Figures 3 and 4. The estimated values of ΔV and ΔV^* were −8.4 ± 2 and −20 ± 2 cm³/mol for NBBA and for NPeBA −12 ± 2 and −20 ± 2 cm³/mol, respectively.

In the present study, the process involves either general-base or nucleophilic catalysis. The $k_{cat.}$ term represents the true turnover constant in the case of general-base catalysis,

Table I
Kinetic Parameters for the Ester Hydrolysis of NBBA and NPeBA Catalyzed by MI-VP at Various Pressures and at 30 °C[a]

Press, bar	K_m, mM		$k_{cat.}$, min^{-1} × 10²	
	NBBA	NPeBA	NBBA	NPeBA
1	6.0	5.4	2.4	2.2
245		5.8		2.8
588	6.6		3.4	
686		8.1		4.2
980	7.6		4.2	
1078		10		5.4
1373	10		5.9	
1471		12		7.7

[a] Reaction conditions: pH 8.0, 0.068 M veronal–HCl buffer, 2% CH_3CN, 0.5 M KCl. Total Im concentration of MI-VP (7.2 mol % Im content) 2.09 × 10⁻⁴ M for NBBA and 1.85 × 10⁻⁴ M for NPeBA.

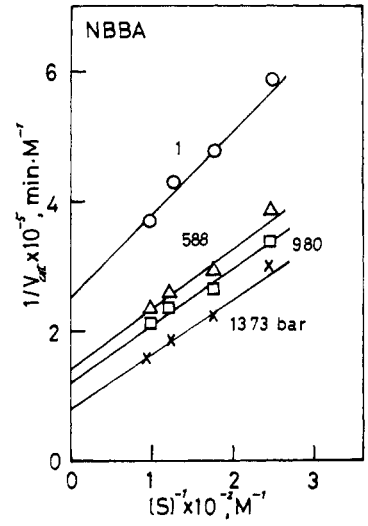


Figure 1. Lineweaver–Burk plots for NBBA under four pressures: pH 8.0 (0.068 M Veronal–HCl buffer), 30 °C, 0.5 M KCl, catalyst (7.2% Im content), total Im concentration 2.09 × 10⁻⁴ M, 2% CH_3CN content.

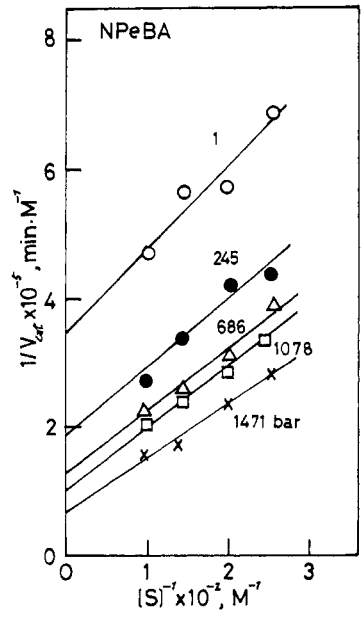


Figure 2. Lineweaver–Burk plots for NPeBA under five pressures: pH 8.0 (0.068 M Veronal–HCl buffer), 30 °C, 0.5 M KCl, catalyst (7.2% Im content), total Im concentration 1.85 × 10⁻⁴ M, 2% CH_3CN content.

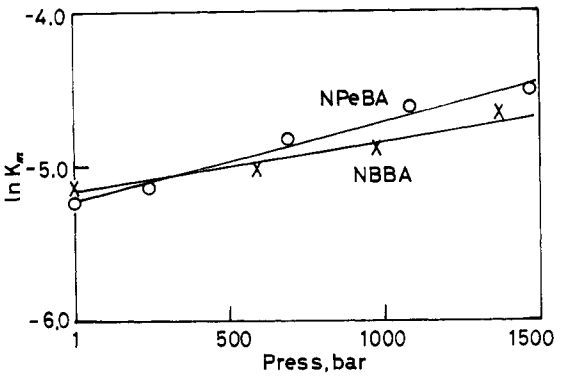


Figure 3. Pressure vs. ln K_m.

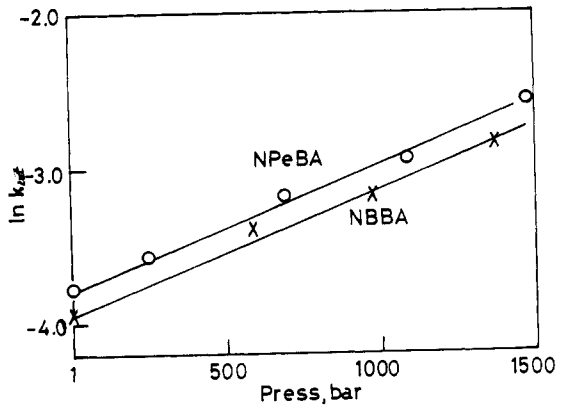


Figure 4. Pressure vs. ln $k_{cat.}$

whereas it is composed of the rates of acylation and deacylation followed by eq 7 in the case of nucleophilic catalysis.

$$C + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CS \xrightarrow{k_{acyl}} \text{acyl catalyst} + HO\text{-}C_6H_3(O_2N)\text{-}COOH \xrightarrow{k_{deacyl}} C + R_3COOH \quad (7)$$

The kinetic parameters will then be expressed by

$$K_m = K_s \cdot k_{deacyl}/(k_{acyl} + k_{deacyl}) \quad (8)$$

$$k_{cat.} = k_{acyl} \cdot k_{deacyl}/(k_{acyl} + k_{deacyl}) \quad (9)$$

where

$$K_s = (k_{-1} + k_{acyl})/k_1 \quad (10)$$

Then

$$K_m = K_s \cdot k_{cat.}/k_{acyl} \quad (11)$$

From the pressure dependences of K_m and $k_{cat.}$, ΔV and ΔV^* are given by the following equations

$$\Delta V = \Delta V_s + \Delta V^* - \Delta V^*_{acyl} \quad (12)$$

$$\Delta V^* = \Delta V^*_{acyl} + \Delta V^*_{deacyl} + RT\, \partial \ln (k_{acyl} + k_{deacyl})/\partial P \quad (13)$$

Substrate Binding. In the present system, there are two possible contributions for substrate binding. One is the electrostatic interactions between the partially protonated Im group in the polymer and the anionic substrate. From the titration of the Im unit in the copolymer, the n' value of the modified Henderson–Hasselbach equation was 1.16. This value is close to that of the Im content of 8.2 mol % of copolymer MI-VP by Kunitake et al.[7] Thus the electrostatic effect of this polymer is small. The copolymer with 7.2 mol % Im content has 11% of its Im groups protonated at pH 8.0, so that it contains 0.9 mol % of protonated Im units.

The other contribution to substrate binding is due to hydrophobic interactions between pyrrolidone groups in the copolymer and substrates. From the investigation of the interaction of polyvinylpyrrolidone (PVP) with aromatic compounds in aqueous solution,[12] it was reported that the binding constant for PVP–aromatic cosolutes at 30 °C was 62 M^{-1} for *p*-hydroxybenzoic acid and 80 M^{-1} for nitrobenzene, respectively. That is, the dissociation constant of the former is 16.1 mM and the latter 12.7 mM. These values are two or three times greater than K_m values of 6.0 mM for NBBA and 5.4 mM for NPeBA in Table I, where K_m values are due to the hydrophobicity of substrates and decrease with an increasing length of the alkyl groups of the substrates. The entropy change ΔS_u[13] for the process of the substrate binding of *p*-acetoxybenzoic acid (PABA) with the polymer catalyst containing a phenylimidazole unit and vinylpyrrolidone (PI-VP) is 19.8 eu.[14] Both results suggest that hydrophobic interactions may contribute to substrate binding.

The volume changes accompanying the rupture of the hydrophobic interaction, −1, −5, and −8 cm^3/mol for CH_3, C_2H_5, and C_3H_7, respectively, obtained by us[15] are consistent with −1 and −7.5 cm^3/mol for CH_3 and C_3H_7 obtained by Gekko and Noguchi.[16] From the density data for several alcohols in nonpolar and water media, the volume changes accompanying the rupture of the hydrophobic interaction are estimated to be −1.2, −2.1, −3.1, and −0.2 cm^3/mol per CH_2, C_2H_4, C_3H_6, and C_6H_5 by Friedman and Scheraga.[17] The benzyl group shows almost no contribution to the volume change.

Therefore, the ΔV values of −8.4 cm^3/mol for NBBA and −12 cm^3/mol for NPeBA may be explained as due to the volume change accompanying the rupture of the hydrophobic interaction between pyrrolidone residues of MI-VP and *n*-alkyl groups (C_3H_7 and C_4H_9) of the substrates. The difference between the volume changes for NBBA and NPeBA, −3.8 cm^3/mol, corresponds to the volume change accompanying the rupture of hydrophobic interactions with a C_2H_5 group. From the above results, it seems that K_m values show a good correlation with the equilibrium constant between MI-VP polymer and each substrate, the true dissociation constants of the Michaelis complexes.

Intracomplex Process. According to the study of the imidazole catalysis of the acyl transfer reactions of PNPA, Brouwer et al.[18] have found that the formation of acetyl imidazole and the release of *p*-nitrophenol proceed at the same rate. The intermediate obtained from *N*-methylimidazole catalyst (a model compound of MI-VP copolymer), *N*-acetyl-*N'*-methylimidazolium ion, is much less stable than *N*-acetylimidazole,[19] because the former cannot stabilize itself by loss of a proton while the latter can. Therefore, at least the deacylation would not be rate limiting in the *N*-methylimidazole reaction. The entropy of activation accompanying the Im hydrolysis reaction was −29.9 eu[11] compared to a value of −27.2 eu reported by Bruice and Schmir,[20] which correlates with the activation volume of −16 cm^3/mol as shown in Table II. For PABA, these values are −48.5 eu for Im and −44.0 eu for phenylimidazole.[14] On the intracomplex process of the

Table II
Activation Volumes Accompanying the Product Formation Process at 30 °C

Sub.	Cat.	ΔV^*, cm^3/mol
NBBA	MI-VP	−20 ± 2
NPeBA	MI-VP	−20 ± 2
PNPA	MI-VP	−19[a]
PNPA	Im	−16[b]

[a] [PNPA] = 3×10^{-4} M, [Im] = 10^{-3} M, 4% CH_3CN, pH 8.0, 0.1 M KCl. [b] [PNPA] = 10^{-4} to 5×10^{-4} M, [Im] = 10^{-4} M, 28.5% EtOH, pH 7.2.

copolymer of PI-VP, ΔS^* is characterized by large negative values of −58.0 eu. For MI-VP polymer, ΔV^* values are −20 cm^3/mol for NBBA and NPeBA, irrespective of the alkyl chain length of the substrates, and −19 cm^3/mol for PNPA as shown in Table II. If the rate-limiting step were the acylation of the polymer catalyst, the observed activation parameters would correspond to the acylation process in eq 13 since $k_{acyl} \ll k_{deacyl}$.

Kunitake and Shinkai[14] concluded that the large negative ΔS^* of −58 eu was caused primarily by the rupture of the hydrophobic interaction in the transition state for the PI-VP system. However, it is expected from the negative ΔV^* that the intracomplex process of MI-VP is predominantly caused by the increasing polarity in the transition state. The ΔV^* of −16 cm^3/mol accompanying the hydrolysis of PNPA catalyzed by Im free from the hydrophobic interaction is due to the polarity increase in the transition state and only the difference of −4 cm^3/mol ($=\Delta V^*_{MI\text{-}VP} - \Delta V^*_{Im}$) would be expected to be caused by the rupture of the hydrophobic interaction in the transition state. As this amount is between a half and one third of the contribution to the hydrophobic interaction of the substrate binding, the hydrophobic interaction still partially exists in the transition state of the MI-VP system. It is concluded that these negative values of ΔV^* may be attributed mainly to polarity increase and partly to the breaking of the hydrophobic interaction.

The entropy of the activation accompanying the deacylation process from the study of the hydrolysis of acetylimidazole is −30.2 eu.[21] This result strongly favors a rate-limiting bimolecular attack of water on the acetylimidazolium cation rather than a monomolecular formation of acetylium cation. The activation volume of −16.0 cm^3/mol for the Im-catalyzed reaction also seems to correspond to the deacylation process, because of the same rate both of acylation and deacylation steps and the large negative entropy of activation. If the rate-limiting step were the deacylation process in the MI-VP systems, the activation volume of −20 cm^3/mol, irrespective of the alkyl chain length of the substrates, would be explained to be the bimolecular attack of water on the alkylimidazolium. On the other hand, the ΔV^* accompanying the deacylation process of acyl-α-chymotripsin[22] depends on the kinds of phenyl esters of aliphatic acid, which shows −6 cm^3/mol for acetic, −3 cm^3/mol for dimethylacetic, −2 cm^3/mol for trimethylacetic acids, respectively. Catalysis by α-chymotripsin belongs to the general acid–base catalysis with Im (His-57) inducing a proton transfer from the hydroxy group (Ser-195) to the carboxylic acid (Asp-102). Therefore, the large difference between the ΔV^* values of the hydrolysis of phenyl esters catalyzed by synthetic polymer containing Im and by α-chymotripsin is due to the difference of the reaction mechanism. Further study is required to probe the meaning of the difference of ΔV^*.

Acknowledgments. We thank Professor T. Kunitake, Kyushu University, Japan and Professor R. K. Williams, University of Guelph, Canada for helpful discussions and advice. We are also grateful to Miss K. Saeki, Kobe Women's College of Pharmacy, Japan for the measurement of the molecular weights of NBBA and NPeBA using the mass spectrometer, and Mr. M. Kawamura, Ritsumeikan University, for the synthesis of the polymer. One of the authors (Y.T.) is grateful for the financial support from the Matsunaga Memorial Science Foundation.

References and Notes

(1) Presented in part at the 4th International Conference on High Pressure, Kyoto, Japan, Nov. 25–29, 1974.
(2) K. J. Laidler and A. J. Beardell, *Arch. Biochim. Biophys.*, **55**, 138 (1955).
(3) B. Andersen and P. E. Broe, *Acta Chem. Scand.*, **26**, 3691 (1972).
(4) K. C. Mohankumar and L. R. Berger, *Anal. Biochem.*, **49**, 336 (1972).
(5) R. K. Williams and C. Shen, *Arch. Biochem. Biophys.*, **152**, 606 (1972).
(6) W. M. Neville and H. Eyring, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2417 (1972).
(7) T. Kunitake, F. Shimada, and C. Aso, *J. Am. Chem. Soc.*, **91**, 2716 (1969).
(8) K. Suzuki and Y. Taniguchi, *Symp. Soc. Exp. Biol.*, **26**, 103 (1972).
(9) F. J. Kezdy and M. L. Bender, *Biochemistry*, **1**, 1097, (1962).
(10) F. Takahashi and S. Otuka, *Nippon Kagaku Zasshi*, **91**, 815 (1970).
(11) K. Suzuki, Y. Taniguchi, and K. Shimokawa, The 14th Symposium on High Pressure, Japan Chemical Society, Osaka, 1972, p 140.
(12) P. Molyneux and H. P. Frank, *J. Am. Chem. Soc.*, **83**, 3169 (1961).
(13) ΔS_u is the unitary entropy given by the equation of $\Delta S_u = \Delta S + 7.98$.
(14) T. Kunitake and S. Shinkai, *J. Am. Chem. Soc.*, **93**, 4256 (1971).
(15) K. Suzuki, Y. Taniguchi, and Y. Watanabe, *J. Phys. Chem.*, **77**, 1918 (1973).
(16) K. Gekko and Y. Noguchi, *Macromolecules*, **6**, 438 (1973).
(17) M. E. Friedman and H. A. Scheraga, *J. Phys. Chem.*, **69**, 3795 (1965).
(18) D. M. Bouwer, M. J. Vlugt, and E. Havinga, *Proc. K. Ned. Akad. Wet., Ser. B*, **60**, 275 (1957).
(19) M. L. Bender and B. Turnquest, *J. Am. Chem. Soc.*, **79**, 1656 (1957).
(20) T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.*, **79**, 1663 (1957).
(21) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272 (1957).
(22) R. C. Neuman, Jr., D. Owen, and G. D. Lockyer, Jr., *J. Am. Chem. Soc.*, **98**, 2982 (1976).

Photon-Count Autocorrelation Spectroscopy. Data Analysis in the Case of Monoexponential Spectra

P. Nieuwenhuysen

Department of Cell Biology, University of Antwerp, 2610 Wilrijk, Belgium.
Received April 6, 1978

ABSTRACT: A series of monoexponentially decaying single clipped photon autocorrelation spectra of the laser light quasielastically scattered by monodisperse solutions of macromolecules was analyzed in different ways to find out how the relaxation times, which are related to the diffusion coefficient of the macromolecules, can be determined accurately in spite of distortions of the spectra. Following various computer fitting procedures different values for relaxation times and quality parameters were obtained from the individual spectra; the best estimate for the relaxation time was then found from a plot which combined all the results.

Diffusion coefficients of macromolecules can be determined accurately by intensity fluctuation spectroscopy.[1] Laser light scattered by a dilute, monodisperse solution of macromolecules which are small compared to the wavelength of light yields the intensity autocorrelation function

$$G^{(2)}(iT) = A' \exp(-\Gamma iT) + C$$

with A' the amplitude, which depends on the geometry of the experimental setup,[2] i a number varying from 1 to the total number of channels of the correlator, T the sample time, C a

0024-9297/78/2211-0832$01.00/0