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## PHYSICAL AND INORGANIC CHEMISTRY

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF CLARK UNIVERSITY, WORCESTER, MASSACHUSETTS]

### The Diffusion of Beta-Alanine in Water at 25°<sup>1</sup>

BY HAIG C. DONOIAN AND GERSON KEGELES

RECEIVED JULY 14, 1960

The differential diffusion coefficient of beta-alanine in water at 25° has been measured over the concentration range 0.25 to 42% by the Gouy method. Through measurements of the interference patterns, the refractive index increments of beta-alanine have been obtained over the concentration range studied. The concentration dependency of the diffusion coefficient has been compared with that predicted by the Gordon relation. Attempts also have been made to correlate the diffusion data with the volume fraction of beta-alanine. Comparison of the data obtained with those for alpha-alanine indicated that despite a polarity of beta-alanine greater than that of alpha-alanine, diffusion coefficients of the beta-isomer show higher absolute values and a lesser dependency on concentration.

#### Introduction

Recently, Longworth<sup>2,3</sup> has reported diffusion coefficients in approximately 0.3% aqueous solutions at 25 and 1° of several peptides, sugars and amino acids in order to study the effects of such factors as chain length, branching and polarity. The work of Lyons and Thomas<sup>4</sup> on glycine and of Dunlop and Gosting<sup>5</sup> on its uncharged isomer glycolamide also have shown that polarity plays an important role in determining the effective diffusing entity. Gosting and Akeley<sup>6</sup> have compared the diffusion in water of urea, sucrose and glycine by applying the Gordon relation,<sup>7</sup> which reflects the concentration dependence of the diffusion coefficients as a function of a thermodynamic factor and a relative viscosity factor. These investigators have found that the Gordon relation describes the concentration dependence of the diffusion coefficient more accurately for the less polar solutes. On the other hand, the diffusion behavior of the more polar compounds may be described with greater accuracy by a relation which includes only the thermodynamic factor.

As Gutter and Kegeles<sup>8</sup> have reported recently the concentration dependency of the diffusion of

D,L- $\alpha$ -alanine in water at 25°, it is the aim of this investigation to provide diffusion data for its more polar isomer beta-alanine for comparison.

#### Experimental

The measurements of diffusion coefficients were made by the Gouy interference method.<sup>9-12</sup> The apparatus, described recently by Biancheria and Kegeles,<sup>13</sup> consists of a convergent light system with a 170 cm. EFL achromatic lens and a resulting optical lever arm of 314.014 cm. A Wratten No. 77A filter was used to isolate green light of wave length *in vacuo* of 5462.2 Å. from a General Electric H100A-4 mercury vapor lamp. The cell employed, which is of 6.0035 cm. thickness, has been described previously.<sup>8</sup>

Reagent grade Eastman Chemicals beta-alanine was recrystallized three times for diffusion measurements. The first two recrystallizations were made by the addition of about four volumes of absolute ethyl alcohol to one volume of filtered hot saturated aqueous solution. This mixture subsequently was cooled in an ice-bath. The third recrystallization was accomplished by pouring a warm saturated solution, 50% in alcohol by volume, into four volumes of absolute ethyl alcohol cooled in an ice bath. This procedure produced very fine crystals of beta-alanine which required no mechanical grinding. The first mother liquor was straw colored, but subsequent ones were colorless. After the last recrystallization, the beta-alanine was dried in a vacuum-desiccator over phosphorus pentoxide. The diffusion coefficient of this material at a mean concentration of 0.25% agreed to within 0.1% with that of a small portion recrystallized a fourth time directly from a 20% by volume alcoholic solution. As both experiments yielded interference

(1) Presented by H. C. D. to the faculty of Clark University, 1958, in partial fulfillment of the requirements for the Ph.D. degree.

(2) L. G. Longworth, *THIS JOURNAL*, **74**, 4155 (1952).

(3) L. G. Longworth, *ibid.*, **75**, 5705 (1953).

(4) M. S. Lyons and J. V. Thomas, *ibid.*, **72**, 4506 (1950).

(5) P. J. Dunlop and L. J. Gosting, *ibid.*, **75**, 5073 (1953).

(6) L. J. Gosting and D. F. Akeley, *ibid.*, **74**, 2058 (1952).

(7) A. R. Gordon, *J. Chem. Phys.*, **5**, 522 (1937).

(8) F. J. Gutter and G. Kegeles, *THIS JOURNAL*, **75**, 3893 (1953).

(9) L. G. Longworth, *ibid.*, **69**, 2510 (1947).

(10) G. Kegeles and L. J. Gosting, *ibid.*, **69**, 2516 (1947).

(11) C. A. Coulson, J. T. Cox, A. G. Ogston and J. St. L. Philpot, *Proc. Roy. Soc. (London)*, **A192**, 382 (1948).

(12) L. J. Gosting, E. M. Hanson, G. Kegeles and M. S. Morris, *Rev. Sci. Instr.*, **20**, 209 (1949).

(13) A. Biancheria and G. Kegeles, *THIS JOURNAL*, **79**, 5908 (1957).

Solution.  $\bar{m}$ 

TABLE I

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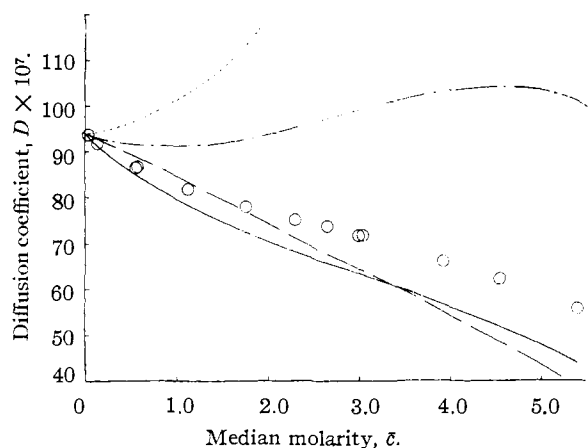


Fig. 1.—Concentration dependence of the diffusion coefficient: O, experimentally observed values; —,  $D_0(1 + \bar{c}\delta \ln y/\delta c)(\eta^0/\eta)$ ; ····,  $D_0(1 + \bar{c}\delta \ln y/\delta c)$ ; ---,  $D_0(1 - 1.66\phi)$ ; - · - ·,  $D_0(1 + \bar{c}\delta \ln y/\delta c)/(1 - 1.66\phi)$ ;  $D_0 = 93.92 \times 10^{-7} \text{ cm.}^2/\text{sec.}$  (extrapolated).

Stokes<sup>28</sup> have suggested that the hydrodynamic portion of the concentration dependence of diffusion which has been considered as a question of solution viscosity<sup>7,29</sup> be reinterpreted as a solute self-obstruction effect, volume fraction values of beta-alanine were calculated by use of available partial molal volume data.<sup>23</sup> In Table III, column  $\delta$ , are shown values of the factor  $(1 - 1.66\phi)$  for beta-alanine at the experimental concentrations. Included in the last column are the values of  $(D_0)_\phi$ , the diffusion coefficient at infinite dilution, obtained by dividing the experimentally observed diffusion coefficients by the factor  $(1 - 1.66\phi)$ . A comparison of these  $(D_0)_\phi$  values with the  $(D_0)_G$  values in column 5 indicates that the solute self-obstruction concept has about the same experimental validity for this system as does the Gordon relation.

Plots of experimental and theoretical values of the diffusion coefficient  $D$  against the median molarity  $\bar{c}$  of beta-alanine are reproduced in Fig. 1. Besides the curves constructed by application of equation 1 and equation 2, also shown are curves obtained from the thermodynamic factor alone as well as one obtained by replacing the relative viscosity factor in the Gordon relation by the volume fraction factor of equation 2, as previously suggested.<sup>13</sup> In Fig. 1 the same value of  $D_0$  was used for all calculated diffusion coefficients, namely,  $93.92 \times 10^{-7} \text{ cm.}^2/\text{sec.}$  This value was derived by extrapolation to  $\bar{c} = 0$  of the experimentally determined points.

### Discussion

It can be seen in Fig. 1 that the experimental points are more closely described by the Gordon relation, equation 1, than by the thermodynamic factor alone, the position of the experimental values with respect to these two theoretical curves being similar to that for alpha-alanine.<sup>8</sup> The greater polarity of the beta-isomer therefore does not apparently contribute additional non-ideality to the diffusion process, a result which is unexpected

(28) R. H. Stokes, *Austral. J. Science*, **19**, 35 (1957).

(29) C. L. Sandquist and P. A. Lyons, *THIS JOURNAL*, **76**, 4641 (1954).

in view of the findings of Gosting and co-workers<sup>5,6,30</sup> on the effect of polarity on diffusion coefficients. In addition, the diffusion of beta-alanine exhibits less dependency on concentration than that of alpha-alanine in solutions up to 2 molar, indicating a more ideal diffusion for the beta-compound.

The solute self-obstruction curve, resulting from equation 2, is essentially a straight line which fits the experimental points better at lower concentrations than at higher concentrations. Since the observed data follow a non-linear path, it is not expected that these could be fitted merely by changing the value, 1.66, of the coefficient of  $\phi$ . The diffusion coefficients obtained by the combination of the volume-fraction factor  $(1 - 1.66\phi)$ , with the thermodynamic factor  $(1 + \bar{c}\delta \ln y/\delta c)$ , are much greater than the observed values but less than those obtained from the thermodynamic factor alone. It appears that the viscosity factor over-corrects and the solute volume-fraction factor under-corrects the thermodynamic factor and that the concentration dependence of diffusion may be considered to be bound by the Gordon relation as a lower limit and by an expression utilizing both the thermodynamic and solute volume-fraction factors as an upper limit.

A point of interest to be noted is the definite inflection in the experimental points in the vicinity of 2.5 to 3 molar, which is at about the half-saturation point for beta-alanine in water. It may be conjectured that there exists a concentration at which significant solute-solute interaction commences producing a new diffusing entity. Inflections of this kind are also observed for electrolytes, such as potassium chloride<sup>31</sup> and calcium chloride.<sup>32</sup> Due to this inflection, attempts to calculate an empirical equation for  $D$  by least-squaring the data as a quadratic or even a cubic function of the concentration were not successful. The theoretical curves, which employ a single value of  $D_0$ , together with thermodynamic and viscosity data, also show the inflection, indicating that the effect is real.

The dipole moments<sup>33</sup> for alpha- and beta-alanine are 15 and 19.4 Debye units, respectively. The higher dipole moment value for beta-alanine is believed to be responsible for the greater densities<sup>23</sup> and electrostriction<sup>33</sup> and the consequent lower adiabatic compressibilities<sup>34</sup> and apparent molal heat capacities<sup>23</sup> of aqueous solutions of beta-alanine in comparison with the corresponding properties for alpha-alanine solutions. These physical measurements all suggest a closer spatial association of the molecules in an aqueous system and, therefore, a probable lower diffusion coefficient and a greater viscosity. This view is supported

(30) R. Wendt and L. J. Gosting, *J. Phys. Chem.*, **63**, 1287 (1959). The diffusion coefficient of lactamide in this work falls below that of beta-alanine at concentrations above 1.65 molar. Polarity and refractive increment do correlate completely for the three compounds, however.

(31) L. J. Gosting, *THIS JOURNAL*, **72**, 4418 (1950).

(32) J. R. Hall, B. F. Wishaw and R. H. Stokes, *ibid.*, **75**, 1556 (1953).

(33) E. J. Coln and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, pp. 233, 290, 158, 159.

(34) F. T. Gucker, Jr., F. W. Lamb, G. A. Marsh and R. M. Haag, *THIS JOURNAL*, **72**, 310 (1950).

TABLE II  
 VALUES OF RELATIVE FRINGE DEVIATIONS  
 $10^4 \Omega_j = 10^4 (e^{-2z_j} - Y_j/C_j)$ 

$\bar{w}$	$f(z) \rightarrow 0.01$	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90
0.2509	1	0	1	1	2	1	1	2	2	1
.2569	-1	0	0	1	0	0	0	0	0	0
.2670	2	-2	-3	-1	-1	-2	-2	-2	-1	-1
1.2547	1	-2	-3	-2	-1	-1	-2	-1	-2	1
4.9309	2	1	2	2	2	3	4	4	5	4
5.1746	4	0	2	3	6	8	10	10	11	12
9.7230	1	-1	0	0	1	0	1	0	1	1
15.0288	1	1	1	1	2	2	3	4	4	5
19.6030	2	1	1	3	4	5	6	7	8	10
22.1338	4	5	8	10	12	14	15	16	16	15
24.7623	4	8	11	14	15	17	17	17	18	
25.1698	5	13	18	22	26	28	30	30	32	33
31.6218	3	-5	-6	-5	-5	-4	-4	-4	-4	-3
36.0352	4	5	6	8	9	11	11	12	14	15
42.2611	6	8	11	14	15	17	18	21	24	

graphs at even fractions of  $f(z_j)$ . Up to 20% by weight the experiments seemed completely satisfactory. At higher concentrations it seemed impossible, after repeated trials, many of which are not indicated in Table II, to obtain results which showed ideal behavior according to this criterion. A completely satisfactory explanation of this difficulty has not been discovered. It is probable that both the diffusion coefficients and the refractive increment values at concentrations above 20% alanine by weight are considerably less accurate than at the lower concentrations, in spite of the self-consistency of the  $D$ -values within each experiment. It should be noted that besides non-ideality of the boundary, small errors in the " $\delta$ " correction<sup>12</sup> and in  $j_m$  can also lead to abnormal relative fringe deviation values.<sup>20-22</sup>

The Gordon relation, mentioned above, is

$$D = D_0(1 + c \partial \ln y / \partial c)(\eta_0/\eta) \quad (1)$$

where  $D$  is the diffusion coefficient at molarity  $c$ ;  $y$ , the activity coefficient of the solute on the molarity scale;  $D_0$  the limiting value of  $D$  at infinite dilution; and  $\eta_0/\eta$ , the reciprocal of the macroscopic relative viscosity. This equation is a modification of the Onsager-Fuoss theory<sup>24</sup> for the variation of  $D$  with concentration for strong electrolytes wherein a mobility factor has been replaced by the viscosity factor. The values of the thermodynamic factor  $(1 + c \partial \ln y / \partial c)$  as well as the reciprocal values of the relative viscosity at the experimental concentrations are given in columns 3 and 4 of Table III. The data of Smith and Smith<sup>25</sup> on the molal activity coefficients of beta-alanine in water at 25.00° were used in computing the thermodynamic factor, and the relative viscosities were taken from the data of Mason, Kampmeyer and Robinson.<sup>14</sup> Also listed in columns 2 and 5 of Table III are the observed diffusion coefficients  $D$ , rounded to 25.000°, and the computed diffusion coefficients  $(D_0)_G$  at infinite dilution resulting from the Gordon relation. If the Gordon relation were to hold, these values should be constant, but it can be seen that this is not the case at high concentrations. This is to

(24) L. Onsager and R. M. Fuoss, *J. Phys. Chem.*, **36**, 2689 (1932).  
 (25) E. R. Smith and P. K. Smith, *J. Biol. Chem.*, **132**, 47 (1940).

be expected, for the expression is meant to hold only for dilute solutions.<sup>7,26</sup>

TABLE III

DIFFUSION COEFFICIENTS, THERMODYNAMIC, VISCOSITY AND VOLUME FRACTION FACTORS FOR BETA-ALANINE AT 25°

Median con. $\bar{c}_j$ , mole/l.	$D \times 10^7$ , cm. <sup>2</sup> /sec.	Thermodyn. factor $(1 + c \partial \ln y / \partial c)$	Reciprocal rel. viscosity	$(D_0)_G \times 10^7$ , cm. <sup>2</sup> /sec.	$1 - 1.66\phi$	$(D_0)\phi \times 10^7$
0.02813	93.58	1.0009	0.9936	94.10	0.9973	93.84
.02879	93.45	1.0009	.9935	93.97	.9972	93.71
.02993	93.38	1.0009	.9932	93.93	.9971	93.65
.03442 <sup>a</sup>	93.27	1.0011	.9923	93.90	.9967	93.58
.14099	91.75	1.0052	.9684	94.25	.9862	93.03
.56049	86.68	1.0322	.8771	95.74	.9446	91.77
.58863	86.83	1.0346	.8712	96.34	.9417	92.20
1.1225	81.82	1.0958	.7616	98.05	.8875	92.19
1.7660	77.93	1.2071	.6398	100.91	.8205	94.98
2.3397	75.21	1.3410	.5415	103.58	.7595	99.02
2.6648	73.98	1.4314	.4902	105.43	.7245	102.12
3.0083	71.98	1.5383	.4396	106.45	.6871	104.76
3.0620	72.02	1.5561	.4320	107.14	.6812	105.72
3.9313	66.31	1.8837	.3212	109.59	.5848	113.38
4.5431	62.49	2.1592	.2562	112.96	.5155	121.22
5.4194	56.04	2.6185	.1797	119.11	.4134	135.56

<sup>a</sup> Data of Longworth (ref. 3).

According to Wang<sup>27</sup> the self-diffusion coefficient  $D$  of water related to the volume fraction  $\phi$  of large unhydrated obstructing molecules is expressed by the equation

$$D = D_0(1 - \alpha\phi) \quad (2)$$

where  $D_0$  is the self-diffusion coefficient in the absence of the obstructing particles, and  $\alpha$  is a constant which for spherical unsolvated obstructing particles has the value of 1.5. Biancheria and Kegeles<sup>13</sup> have shown that the measurements of diffusion coefficients of glycolamide and of acetamide in dilute aqueous solutions of different viscosities containing a third component can be expressed by equation 2, wherein  $\bar{D}$  is the diffusion coefficient of the solute;  $\phi$ , the volume fraction of the viscosity-increasing third component;  $D_0$ , the diffusion coefficient in the absence of the third component; and  $\alpha$  assumes the constant value of 1.66. As Biancheria and Kegeles<sup>13</sup> and R. H.

(26) A. R. Gordon, *THIS JOURNAL*, **72**, 4840 (1950).  
 (27) J. H. Wang, *ibid.*, **76**, 4755 (1954).

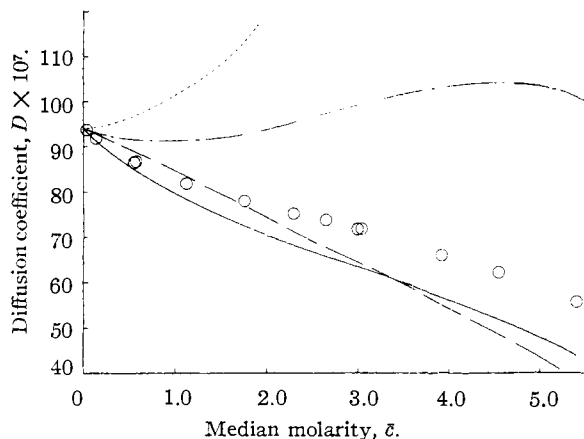


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(34) F. T. Gucker, Jr., F. W. Lamb, G. A. Marsh and R. M. Haag, *THIS JOURNAL*, **72**, 310 (1950).

by the fact that glycolamide has a greater compressibility<sup>34</sup> and diffusion rate<sup>4,5</sup> than glycine in water. On the other hand, the behavior of the alkali metal halides is contrary; for example, the more compressible aqueous solutions of lithium chloride show a lower diffusion rate than those of sodium chloride.<sup>35,36</sup> The results of this investigation indicate a higher limiting diffusion coefficient  $D_0$  of  $93.92 \times 10^{-7}$  cm.<sup>2</sup>/sec. for the most polar isomer, beta-alanine, in water at 25.00° than the corresponding value of  $91.46 \times 10^{-7}$  cm.<sup>2</sup>/sec. for alpha-alanine,<sup>8</sup> a difference of over 2.5%. On the other hand the value of  $D_0$  for lactamide,<sup>30</sup> the least polar isomer, is  $99.11(10)^{-7}$  cm.<sup>2</sup>/sec.

(35) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," Reinhold Publ. Corp., New York, N. Y., 1950 pp. 180, 181.

(36) J. W. Williams and L. C. Cady, *Chem. Revs.*, **14**, 171 (1934).

Furthermore, the work of Mason, Kampmeyer and Robinson<sup>14</sup> shows that solutions of beta-alanine in water exhibit lower relative viscosities and lower activation energies for viscous flow than do solutions of the alpha-isomer. These comparisons of the properties of the alanines lead to the conjecture that a macroscopic physical measurement such as electrostriction, compressibility and heat capacity, indicating closeness of nearest-neighbor molecules in a liquid, is not necessarily a precise measure of the effective size of the kinetically independent particles in a flow process.

**Acknowledgment.**—This study was made possible through support by U. S. Public Health Service Research Grant RG-3449, entitled "Sedimentation and Diffusion of Low Molecular Weight Substances."

[CONTRIBUTION FROM THE GIBBS CHEMICAL LABORATORY OF HARVARD UNIVERSITY, AND THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINNESOTA]

## Degradation of Poly- $\alpha$ ,L-glutamic Acid. I. Degradation of High Molecular Weight PGA by Papain

BY WILMER G. MILLER<sup>1</sup>

RECEIVED MAY 13, 1960

Long chain poly- $\alpha$ ,L-glutamic acid under suitable conditions is shown to be an excellent substrate for papain. The maximum velocity ( $V_m$ ) is extremely pH dependent and in the region of the helix-coil transition changes with pH faster than the change in hydrogen ion concentration. Chromatographic analysis of the products of degradation shows that the enzyme attacks bonds randomly except for discrimination near the ends of the chain. The interpretation of the data seems most consistent with the assumption that enzymic hydrolysis occurs almost exclusively at helix-coil junctions in the polypeptide. The importance of electrostatic charge in influencing enzymic attack could not be determined explicitly.

### Introduction

The degradation of proteins by proteolytic enzymes has been investigated many times. The configurational state of the protein often plays an important role but interpretation is generally handicapped by a lack of knowledge of the conformation of the protein. The increasing availability of high molecular weight synthetic polypeptides of known conformation permits a study of the effect of conformation on degradation. Waley and Watson<sup>2</sup> made an extensive study of the hydrolysis by trypsin of poly-L-lysine in the random coil conformation. Other<sup>3</sup> less extensive studies of the hydrolysis of synthetic polypeptides have been made but there appears to be no investigation of the effect of changing conformation.

The present study was undertaken in an attempt to determine the effect of conformation, in particular the effect of going from an  $\alpha$ -helix to a random coil. Although the simple synthetic polypeptides can hardly be called synthetic proteins, they nevertheless should serve as useful model compounds. The enzyme papain seemed attractive to study since the kinetics of its action on small substrates has been thoroughly studied<sup>4</sup> and since it had been reported<sup>5</sup> to attack poly- $\alpha$ -L-glutamic acid (PGA).

The water-soluble PGA has been shown to exist in solution both as an  $\alpha$ -helix and as a random coil<sup>6</sup> and the helix-coil transition occurs at a pH where the activity of papain is nearly maximum. Unfortunately the use of PGA is complicated by the fact that an ionization of the polypeptide accompanies the helix-coil transition.

### Experimental

Poly- $\alpha$ ,L-glutamic acid was purchased from Pilot Chemicals, Inc. The procedure used for synthesis is such that the PGA has an unblocked C-terminal carboxyl, as far as is known.<sup>7</sup> The PGA was received as the sodium salt monohydrate. Titration of the carboxyls gave a residue weight of 170, the expected result for the sodium salt monohydrate. PGA with a weight average molecular weight ( $MW_w$ ) of 58000 in the acid form as determined by viscosity measurements<sup>8</sup> was used during most of the kinetic runs. In all, four preparations were used with weight average weights of 50000-60000. Papain (crystalline 2X) was obtained from Nutritional Biochemical Corporation and was used without further recrystallization. Other chemicals were of analytical grade.

Unless stated, all measurements were made at 25°, controlled to  $\pm 0.02^\circ$  for viscosity-kinetic runs and to  $\pm 0.5^\circ$  for runs lasting several days or longer. Optical rotations were measured with a Rudolph model 200 high precision polarimeter, using a mercury lamp as the light source and a 40 cm. cell. Concentrations were 0.2-1.0% and the ro-

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(3) M. Sela and E. Katchalski, *Adv. Protein Chem.*, **14**, 391 (1959).

(4) See J. R. Kimmel and E. L. Smith, *Adv. Enzymology*, **19**, 267 (1957), for an excellent review.

(5) M. Green and M. A. Stahmann, *J. Biol. Chem.*, **197**, 771 (1952).

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(7) Mark Hyman, Jr., Pilot Chemicals, Inc., private communication.

(8) We are indebted to Drs. Paul Doty and A. Wada for making available the calibration of the molecular weight-viscosity scale for L-PGA (A. Wada and P. Doty, to be published).