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The Conversion of Fibrinogen to Fibrin. XVI. Electrical Birefringence of Fibrinogen and Activated Fibrinogen[1]

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The electrical birefringence of fibrinogen and activated fibrinogen has been studied in the *p*H range from 6 to 10 in a urea–water–glycerol solvent using a single electrical rectangular pulse method developed by Benoit. The birefringence was displayed as a photomultiplier signal on an oscilloscope screen. From the measured Kerr coefficients and from the shapes of the curves for rise and decay of birefringence, dipole moments and rotary diffusion coefficients for the molecules were determined. The average rotary diffusion coefficient was 36,000 sec.$^{-1}$, in satisfactory agreement with the results of flow birefringence measurements. The dipole moment of fibrinogen was calculated by Benoit's theory to be approximately zero from *p*H 6 to 7, to rise to a maximum of about 500 *D* at *p*H 8.5, then to decrease again with increasing *p*H. The Kerr coefficients and therefore the dipole moments were sensitive to small amounts of ions left in the protein solution after dialysis; an increase in electrolyte concentration caused a decrease in the calculated dipole moment. The dipole moment of activated fibrinogen was greater than that of fibrinogen by an amount which varied with the *p*H. A maximum increase in the absolute value of the dipole moment on activation of 110 *D* was found. This difference, together with the fact that about 10 charges are lost on activation, provides evidence that the activation of fibrinogen occurs at a site near the center of symmetry of the molecule.

Introduction

When thrombin acts on fibrinogen, two peptides are split off,[3] with a loss of 10 to 14 negative charges[4]; since only one collision appears to be involved,[5] it may be inferred that the alteration of charge density is confined to a relatively small site on the fibrinogen molecule. The new electrostatic configuration of the activated fibrinogen probably guides the subsequent polymerization, in which the rod-like fibrinogen units are believed[6] to undergo lateral dimerization with partial overlapping. Such a pattern can be readily visualized following loss of a group of negative charges either from one end or from the middle of one side of the fibrinogen rod,[7] but there has been no evidence concerning the actual location of the site of attack by thrombin.

It should be possible to draw some conclusions about the latter by determining the change in dipole moment accompanying activation. The classical method of dielectric constant measurements is difficult to apply for this purpose; although fibrinogen can be dissolved in moderately concentrated urea solution with no added electrolyte, it would not be easy to reduce the conductivity enough to control electrode polarization.[8] Moreover, recent interpretations of the dielectric increment in protein solutions[9,10] have cast doubt on the usual methods for calculating dipole moments. However, electrical birefringence provides an alternative method for measuring dipole moment. From measurements of electrical birefringence in response to square pulses, the dipole moment can be determined as well as the rotary diffusion coefficient and additional optical information, according to the theory elaborated by Benoit.[11] Fibrinogen is particularly suited for these measurements because it is a well characterized, monodisperse protein. Such experiments are described in the present paper; they provide evidence concerning the site of attack by thrombin on fibrinogen, as briefly reported in a recent note.[12]

Materials

The fibrinogen was refractionated from Armour bovine Fraction I, preparation L-210, by ammonium sulfate, to give the fraction designated I-L in Paper X of this series.[13] Fibrinogen assays were made by the method of Morrison.[14] The thrombin used was a bovine preparation (No. 51341) containing 32 units/mg., furnished us through the kindness of Dr. E. C. Loomis of Parke, Davis and Company. The urea, a Mallinckrodt Analytical Reagent, was used without further purification. Fibrinogen solutions in the urea–water–glycerol solvents were made by mixing stock solutions all containing 3 *M* urea. The concentration of glycerol was determined by a density measurement of the aqueous glycerol used in making the stock solution.

The comparison of the fibrinogen and activated fibrinogen solutions was made as follows. A volume of thrombin solution was added to half of the fibrinogen stock solution, *p*H about 6.5, and an equal volume of water was added to the other half. Both solutions were set to dialyze at room temperature against 0.15 *M* sodium chloride. About an hour after a firm clot had been formed,[15] the dialysis bath was changed to 3 *M* urea and the dialysis was continued for at least 24 hours at 5°. The fibrin dissolved in the urea to give a solution of activated fibrinogen. Although these solutions polymerized to form soluble polymers in the *p*H range from 8 to 9.5, Kerr effect measurements could still be made in this *p*H range because the presence of glycerol, a clotting retarder,[17] kept any appreciable amounts of polymer from forming. Any polymer with at least 3 times the length of fibrinogen would not be detected in the Kerr effect measurements as it would not have time to rotate perceptibly in the 1 millisecond pulse time used.

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(2) Procter and Gamble Fellow in Chemistry, 1953–1954.

(3) F. R. Bettelheim and K. Bailey, *Biochim. Biophys. Acta*, **9**, 578 (1952).

(4) E. Mihalyi, *J. Biol. Chem.*, **209**, 723 (1954); D. R. Kominz and K. Laki, presented at the 126th National ACS Meeting, September, 1954.

(5) D. F. Waugh and B. J. Livingstone, *J. Phys. Colloid Chem.*, **55**, 1206 (1951).

(6) J. D. Ferry, S. Shulman, K. Gutfreund and S. Katz, THIS JOURNAL, **74**, 5709 (1952).

(7) J. D. Ferry, S. Katz and I. Tinoco, Jr., *J. Polymer Sci.*, **12**, 509 (1954).

(8) J. D. Ferry and J. L. Oncley, THIS JOURNAL, **63**, 272 (1941).

(9) J. G. Kirkwood and J. B. Shumaker, *Proc. Natl. Acad. Sci. U. S.* **38**, 855 (1952).

(10) B. Jacobson and M. Wenner, *Biochim. Biophys. Acta*, **13**, 577 (1954).

(11) H. Benoit, *Ann. Phys.*, **6**, 561 (1951).

(12) I. Tinoco, Jr., and J. D. Ferry, THIS JOURNAL, **76**, 5573 (1954).

(13) S. Katz, K. Gutfreund, S. Shulman and J. D. Ferry, *ibid.*, **74**, 5706 (1952).

(14) P. R. Morrison, *ibid.*, **69**, 2723 (1947).

(15) The formation of fibrin (and therefore the activation of fibrinogen) is substantially complete under these conditions.[16]

(16) J. D. Ferry and P. R. Morrison, THIS JOURNAL, **69**, 388 (1947).

(17) S. Shulman, *Arch. Biochem.*, **30**, 353 (1951).

Traces of ions left in the solutions after dialysis caused irreproducibility in the Kerr effect measurements. Therefore in one experiment all solvents were ion exchanged using Amberlite IR-120 cation-exchange resin and IRA-400 anion-exchange resin (Rohm and Haas).

An intrinsic viscosity measurement and fibrinogen assays made a week apart on the same solution indicated that no appreciable denaturation occurred in salt-free 3 *M* urea.

Method

The apparatus is patterned after that of Benoit.[11] A rectangular electrical pulse is applied to the solution illuminated between crossed nicols. The resulting light transmitted by the analyzing nicol is displayed as a photomultiplier signal on an oscilloscope screen.

Optical.—The optical bench is a polarimeter on which the position of the analyzer can be read to 0.01 degree. The light source is a 6–8 volt automobile headlight bulb connected to an 8 volt storage battery. The Kerr cell has microscope cover glass windows to avoid depolarization of the light and 18K gold–silver alloy electrodes, 8.73 cm. in length and spaced 0.202 cm. apart. An RCA 1P21 photomultiplier tube detects the light signals. The oscilloscope screen is photographed by a Voigtlander 35 mm. camera with an f/1.5 lens using Kodak Linagraph Pan film.

Electrical.—The pulse generator[19] produces a 0.5 to 1.5 millisecond rectangular pulse of 250–350 volt amplitude across a 1000 ohm load. The circuit uses a 12AT7 twin triode as a univibrator whose output pulse is applied to the grids of two 6Y6G tubes. The amplified pulse is connected to the electrodes in the Kerr cell through a 525 microfarad photoflash capacitor. The rise and decay times of the pulse are each 4 microseconds or less.

The output of the photomultiplier cannot be connected directly to the oscilloscope because at the desired sensitivity an excessively long response time is obtained. Therefore the photomultiplier output is connected to the oscilloscope through a 6C4 triode used as a cathode follower. This combination gives a 10% to 90% response time of 25 microseconds with a 1 megohm load resistor across the photomultiplier output.

The oscilloscope is a Du Mont 304A Model whose sweep is synchronized with the pulse. A calibrated time base is provided by brightening the oscilloscope trace with an audio oscillator, a Berkshire Labmarker and an amplifier.

Calculations.—Enlarged tracings were made of the photographs of the oscilloscope screen. The Kerr coefficient was calculated from the birefringence curve, using Benoit's[11] method of comparing light intensities. The electric field strength was determined by comparing the height of the cell voltage pulse with a 100 volt calibrating voltage present on the oscilloscope panel. The rotary diffusion coefficient (θ) was always obtained from a picture showing only the decay of the birefringence, using an expanded sweep. Since the equation of the decay curve is $y = e^{-12\theta t}$ the slope of a plot of relative height *versus* time on semi-logarithmic paper gave θ. To correct the value to that for water at 20°, the viscosity of the solvent was determined with a capillary viscometer, the density having been measured by a Westphal balance. The value of α, a measure of the orienting torque, was obtained by comparing the experimental curve of the rise of the birefringence with theoretical curves.[11]

The Kerr coefficient, $B = \Delta n/\lambda E^2$, is related to molecular parameters by the following equation [18]

$$B = (2\pi V/15\lambda n)(g_1 - g_2)(P + Q)$$

V = vol. fraction of protein in soln. calcd. using the partial specific vol. ($\bar{v} = 0.71$) of fibrinogen

λ = wave length of light. A value of 5,110 Å. was taken as the average for the white light used

n = refractive index of soln. measured with an Abbe refractometer

$g_1 - g_2$ = an optical factor which will be discussed in a following section

$P = (\mu/kT)^2$

μ = dipole moment, assumed parallel to the long axis of the molecule

k = Boltzmann constant

T = absolute temp. All experiments were made at room temp., an av. value of 298°A. was used

$Q = v(g_1^0 - g_2^0)/kT$

v = vol. of protein molecule = 38.7×10^{-20} cm.^{3} for fibrinogen[20]

$g_1^\circ - g_2^\circ$ = a factor involving the principal dielectric constants of the protein molecule. If the assumption is made that these dielectric constants are small compared to that of the solvent, the factor reduces to $0.0775\epsilon_0$

ϵ_0 = dielectric constant of solvent calculated from the table for aqueous glycerol solutions in the ACS Monograph, "Glycerol,"[21] and Wyman's dielectric increment of 2.69 for urea in water[22]

Results

The Kerr coefficients[23] obtained are given in Tables I and II. The specific conductance of the solutions is also included, as the measured Kerr coefficient depends on the excess electrolyte pres-

TABLE I

KERR COEFFICIENT AND DIPOLE MOMENT OF FIBRINOGEN AS A FUNCTION OF *p*H

Fibrinogen (7 g./l.) in 3 *M* urea in a solvent of 64.0 weight per cent. glycerol in water.

Experiment	*p*H	$\kappa \times 10^5$ (mho/cm.)	$B \times 10^6$ (cm./volt^2)	μ (*D*)
K 14	7.09	4.6	0.12	(0)
	7.63	5.9	.28	84
	8.11	5.7	.44	250
	8.38	6.0	.42	230
	8.60	6.1	.40	220
	8.88	6.2	.42	230
	9.37[a]	7.0	.31	140
	9.52	7.4	.33	150
K 13	8.70	3.6	0.91	470
	9.13	3.8	.86	450
	9.62[b]	5.1	.76	410
K 12	6.69	..	0.29	(0)
	6.73	..	.25	(0)
	7.62[b]	..	.43	240
	7.95	..	.72	390
	9.17	..	.76	410
K 11	6.18	4.3	0.32	(0)
	7.00	..	.37	(0)
	7.78	6.4	.50	290
	8.50	5.7	.42	230
	8.78	6.8	.58	330
	9.17	7.2	.49	280
	9.63	7.8	.36	180
K 10	6.70[c]	..	0.22	(0)
	7.34[d]	..	.25	..
	7.41	..	.33	150
	7.48[e]	..	.30	120
	7.48[e,f]	..	.25	..
K 9	6.02[g]	..	0.43	(0)
	7.50[h]	..	0.24	81

[a] Cell voltage not measured. [b] Fibrinogen 6.96 g./l., 63.7 weight per cent. glycerol. [c] Fibrinogen 7.1 g./l. [d] 5.0×10^{-4} *M* NaCl added. [e] Electrodialyzed fibrinogen. [f] 6.67×10^{-4} *M* NaCl added. [g] Fibrinogen 6 g./l., 64.3 weight per cent. glycerol. [h] Fibrinogen 6 g./l.

(18) According to the theory of Benoit (ref. 11).

(19) The pulse generator was designed and tested by Mr. M. Murray of the University of Wisconsin Physics Department.

(20) S. Shulman, THIS JOURNAL, **75**, 5846 (1953).

(21) C. S. Miner and N. N. Dalton, Editors, "Glycerol," Reinhold Publ. Corp., New York, N. Y., 1953.

(22) J. Wyman, Jr., THIS JOURNAL, **55**, 4116 (1933).

(23) These coefficients are assumed to be positive. In one experiment at *p*H 7.62 in 2 *M* urea, this assumption was verified by use of a quarter wave plate.

TABLE II

KERR COEFFICIENTS AND DIPOLE MOMENTS OF FIBRINOGEN IN 2 *M* UREA IN WATER

Experiment	*p*H	Fibrinogen (g./l.)	$B \times 10^4$ (cm./volt^2)	μ (*D*)
IB	6.77	8.4	0.70	(0)
	7.62	8.4	1.04	210
	7.88	3.26	0.45	250
	8.06	4.06	0.65	300
49	7.8	8.7	3.18	580
	9.2	4.4	2.18	700
	9.8	8.0	1.04	220
K 3	6.71	13	1.16	(0)
	8.20	12.6	2.94	420
	9.00	12.6	2.44	360
	9.85	11.7	1.41	200
	10.21	11.3	1.77	290

ent. This salt concentration was not calculated because of lack of knowledge about the conductances of the hydrogen and hydroxyl ions and protein and counter ions in the mixed solvent of glycerol and water. Kerr coefficients in some other aqueous glycerol solvents were obtained, but they are not included because sufficient information to calculate dipole moments was not available for these solvents. Such data are listed in a thesis[24] which also contains further details of all this work.

The rotary diffusion coefficients, corrected to the viscosity of water at 20°, are given in Table III; they are in satisfactory agreement with the flow birefringence value[25] of 39,400 sec.$^{-1}$. The discrepancy is partly due to the effect of the 25 microsecond response time of the photomultiplier which will tend to give rotary diffusion coefficients a few per cent. lower than the actual value.

TABLE III

ROTARY DIFFUSION COEFFICIENTS OF FIBRINOGEN

Fibrinogen (7 g./l.) in 3 *M* urea in a solvent of 64.0 weight per cent. glycerol in water. Viscosity = 0.1145 poise, temperature = 25°.

Experiment	*p*H	$\theta_{20,w}$ (sec.$^{-1}$)	Experiment	*p*H	$\theta_{20,w}$ (sec.$^{-1}$)
K 9[a]	6.02	39,400	K 13[c]	8.70	34,700
K 10	7.41	33,600	K 12	8.76	38,100
K 9[b]	7.70	29,500	K 11	8.78	44,800
K 12[c]	7.89	33,600	K 13[c]	9.00	33,900
K 12	7.95	35,800	K 13	9.13	39,200
K 12[c]	8.11	33,600	K 12[c]	9.16	35,800
K 12[c]	8.28	34,200	K 12	9.17	33,600
K 11	8.50	33,600	K 13[d]	9.52	44,000
K 12[c]	8.71	32,500	K 13[c,d]	9.68	44,500
				Av.	36,000

[a] Fibrinogen 6 g./l. in 64.3 weight per cent. glycerol (η = 0.1152 poise). [b] Fibrinogen 6.95 g./l. in 60.4 weight per cent. glycerol (η = 0.0893 poise). [c] Activated fibrinogen. [d] Fibrinogen 6.96 g./l. in 63.7 weight per cent. glycerol (η = 0.1138 poise).

The values of α calculated from the shape of the appearance of the birefringence curve are given in Table IV; these quantities indicate the mechanism responsible for the orientation of the protein. For $\alpha = 0$ the orientation is entirely due to an induced dipole, while for $\alpha = \infty$ only a permanent dipole mechanism is important.

TABLE IV

ORIENTATION MECHANISM OF FIBRINOGEN AS A FUNCTION OF *p*H

Fibrinogen (7 g./l.) in 3 *M* urea in a solvent of 64.0 weight per cent. glycerol in water.

Experiment	*p*H	α	*P*/*Q*
K 11	6.18	0	..
K 13	6.69	0	..
K 12	6.73	0	..
K 11	7.00	0	..
K 10	7.41	2	1.3
K 12	7.62[b]	5	0.67
K 12	7.89[a]	5	3.0
K 12	7.95	>5	1.8
K 13	8.11	>5	2.7
K 11	8.50	1–5	0.61
K 13	8.70	1–2	2.6
K 13	8.70[a]	5	3.5
K 11	8.78	>5	1.2
K 13	9.00[a]	5	3.6
K 13	9.13	2–5	2.4
K 12	9.16[a]	2–5	2.4
K 12	9.17	2–5	1.9
K 13	9.62[b]	5	1.9
K 13	9.68[a,b]	2–5	2.1

[a] Activated fibrinogen. [b] Fibrinogen 6.96 g./l., 63.7 weight per cent. glycerol.

Discussion

The fact that $\alpha = P/Q = 0$ at low *p*H suggests a method for obtaining the optical factor $(g_1 - g_2)$ necessary for the calculation of the dipole moment. If *P* is set equal to zero in the *p*H range 6 to 7.1, then $g_1 - g_2 = (15\lambda nB)/(2\pi VQ)$. Values of $(g_1 - g_2)$ obtained in this manner are: 1.8×10^{-2} for the 3 *M* urea in a 64.0 weight per cent. glycerol in water solvent ($n_0 = 1.43$) and 3.3×10^{-2} for the 2 *M* urea in water solvent ($n_0 = 1.35$). The values of $(g_1 - g_2)$ are of similar magnitude to those obtained by flow birefringence measurements[25] and measurements of depolarization of scattered light.[26]

If the optical factor is considered independent of *p*H in the same solvent, *P* and therefore μ can be calculated at other values of *p*H. This value of *P* calculated from the Kerr coefficient can be divided by *Q* to give a value of α to be compared with that obtained from the shape of the appearance of the birefringence curve. Table IV shows this comparison between α, dependent only on the shape of the birefringence curve, and *P*/*Q* calculated from the height of the curve, the electric field strength, the composition of the solvent, etc. These values are mostly in reasonable agreement and lend confidence to Benoit's method.

The calculated dipole moments listed in Table I are shown in Fig. 1. It is evident that increasing salt concentration decreases the apparent dipole moment; the highest values are obtained for the ion-exchanged solutions. Whether this effect is due to an actual change in the charge configuration of the protein by ion binding, a phenomenon associated with the ion atmosphere, or an artifact caused by electrode polarization cannot be resolved

(24) I. Tinoco, Jr., Ph.D. Thesis, University of Wisconsin, 1954.

(25) C. S. Hocking, M. Laskowski, Jr., and H. A. Scheraga, THIS JOURNAL, **74**, 775 (1952).

(26) E. F. Casassa, private communication.

from these data. Oncley, *et al.*,[27] found that preparations of mercaptalbumin which had been treated with ion-exchange resins gave much higher dielectric increments than had previously been reported, and that addition of various ions reduced the dielectric increment; this would seem to indicate that the effect is at least partly due to ion binding. However, the role of the ion atmosphere is still quite uncertain (C. T. O'Konski, personal communication).

A recent extension of Benoit's theory[28] shows that, if a small component of dipole moment parallel to the short axis exists, the values of μ calculated here are slightly smaller than the true components parallel to the long axis. A large component parallel to the short axis would lead to very different values of α and P/Q as compared in Table IV and is therefore unlikely.

It should be pointed out that at *p*H values other than the isoelectric *p*H only effective dipole moments can be calculated.[29] As Mysels[30] has shown, a symmetrical ion whose center of charge is not at the center of rotation of the ion will have an apparent dipole moment equal to that calculated if a neutralizing charge were placed at the center of rotation.

The effective dipole moments of fibrinogen are surprisingly small and indicate a very high degree of electrical symmetry. It is difficult to try to determine what dissociable groups cause the variation in dipole moment with *p*H. Groups which have a *pK* in the *p*H dispersion range and could be responsible are: imidazole of histidine (5.6–7.0), α-amino (7.6–8.4), ϵ-amino of lysine (9.4–10.6), and the phenolic hydroxyl of tyrosine (9.8–10.4).[31]

TABLE V

INCREASE OF KERR COEFFICIENT AND DIPOLE MOMENT OF FIBRINOGEN ON ACTIVATION

Fibrinogen (7 g./l.) in 3 *M* urea in a solvent of 64.0 weight per cent. glycerol in water.

Experiment	*p*H	$B \times 10^4$ (cm./volt²)	$\lvert\mu_a\rvert - \lvert\mu_u\rvert$ (*D*)	$\lvert\mu_a\rvert + \lvert\mu_u\rvert$ (*D*)
K 12	6.73	0.24	(0)	(0)
K 12-A	6.66	.27		
K 9	7.50[a]	.24	80	240
K 9-A	7.50[a]	.29		
K 12	7.62[b]	.43	4	485
K 12-A	7.65[b]	.44		
K 12	7.95	.72	110	890
K 12-A	7.89	1.03		
K 13	8.70	0.91	81	1020
K 13-A	8.70	1.16		
K 13	9.13	0.86	100	1000
K 13-A	9.00	1.18		
K 12	9.17	0.76	40	860
K 12-A	9.16	.87		
K 13	9.62[b]	.76	20	840
K 13-A	9.68[b]	.82		

[a] Fibrinogen 6 g./l. [b] Fibrinogen 6.95 g./l., 63.6 weight per cent. glycerol.

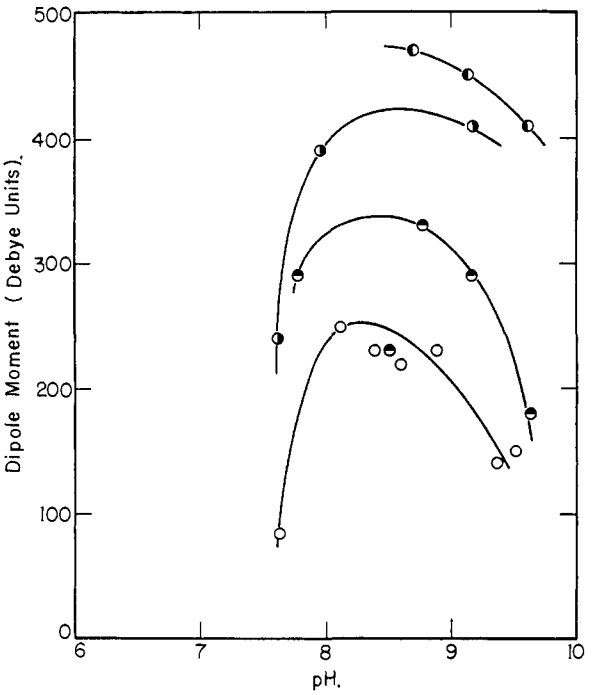

Fig. 1.—Dipole moment of fibrinogen plotted against *p*H: circles left black, experiment K 13; right black, K 12; top black, K 11; open, K 14.

The change of dipole moment on activation is shown in Table V. Both solutions in each experiment were prepared in exactly the same way, but in the absence of buffer the *p*H values still differed slightly in some instances; A indicates the activated fibrinogen solution.

The change in dipole moment, parallel to the long axis of the molecule, on activation may be written as

$$\mu_a - \mu_u = \sum_i \epsilon_i x_i$$

where ϵ_i = electronic charge (4.8×10^{-10} e.s.u.), which can be positive or negative; x_i = distance from center of symmetry[32] of molecule to the location of a charge. The sum is taken over all charges lost or gained on activation.

On activation by thrombin, fibrinogen loses two peptides[3]: peptide A contains 12 dicarboxylic acid groups of which 4 are in the amide form, 1 lysine and 2 arginine; peptide B contains 10 dicarboxylic acid groups of which 3 are in the amide form, 1 lysine, 2 arginine and 1 tyrosine.[4] Moreover, 1 α-amino group is lost and 3 α-amino groups are gained.[33] The net change in charge, Δz, is therefore about ten[34] in the *p*H range from 7 to 10, but will depend slightly on *p*H because of the α-amino groups. If it is assumed[5] that the alteration of charge all takes place in a relatively small area on the fibrinogen molecule at a distance d from the center of symmetry, this distance can be calculated

(27) H. Neurath and K. Bailey, Editors, "The Proteins," Academic Press, Inc., New York, N. Y., 1953, p. 708.

(28) I. Tinoco, Jr., THIS JOURNAL, in press.

(29) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 552.

(30) K. J. Mysels, *J. Chem. Phys.*, **21**, 201 (1953).

(31) Neurath and Bailey, ref. 27, p. 477.

(32) The center of symmetry is chosen as the origin so that the neutralizing charges which must be added at this point[30] need not be explicitly considered.

(33) L. Lorand and W. R. Middlebrook, *Science*, **118**, 595 (1953).

(34) Mihalyi[4] has calculated a net change in charge of 14 from electrophoresis measurements. The slight discrepancy in these values does not affect the conclusions drawn.

using the equation

$$d = \frac{|\mu_a| \pm |\mu_v|}{\epsilon \Delta z}$$

Both signs must be included as only the absolute magnitude of the dipole moment can be measured. Since the maximum change in dipole moment observed was either 110 or 1020 D, the value of d is found to be $\leqslant$ 2.5 Å. or $\leqslant$ 25 Å. The result thus indicates that activation takes place near the center of the fibrinogen molecule.

A potential source of doubt regarding the interpretation of the low $\Delta\mu$ is the possibility that the charge pattern may readjust, through proton migration, to minimize $\Delta\mu$ regardless of the site of loss of the negative charges.[35] However, it seems unlikely that the result would be so closely similar at pH 7, where the migrating protons will be exchanging on histidine residue, and at pH 9–10 where they will be exchanging on tyrosine and lysine residues.

The two peptides have nearly equal charges; therefore it could also be possible that the peptides are released equidistant from the center of symmetry of fibrinogen so that the net effect, assuming proper positions for the α-amino groups, would be the change of only one charge. Using this hypothesis one cannot exclude the possibility that activation takes place at each end of the fibrinogen molecule.[36]

While the unknown effects of the ionic atmosphere[28] leave the magnitudes of the calculated dipole moments somewhat in doubt, it would take a very large error to invalidate the conclusion that if activation occurs at only one site, this site is near the center of the molecule.

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(35) C. Tanford, private communication.

(36) We are indebted to Dr. F. R. Bettelheim for his comments and suggestions on the preceding interpretation.

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NOTES

Thermodynamic Properties of the Ammonium Ion

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Structural and spectroscopic data on the ammonium ion recently have been available which make possible the statistical thermodynamic calculation of the thermodynamic functions C_p°, $(H^\circ - H_0^\circ)/T$, $-(F^\circ - H_0^\circ)/T$ and S° for $NH_4^+(g)$. The N–H internuclear distance in NH_4^+ has been obtained from both neutron diffraction[1-3] and nuclear magnetic resonance measurements.[4,5] The most accurate determination of the N–H distance[5] gives a value of 1.032 ± 0.005 Å. Thus the moment of inertia, I, has the value (4.75 ± 0.05) × 10^{-40} g. cm.^{2}. The vibrational frequencies of the spherical top NH_4^+ (T_d symmetry is assumed for NH_4^+ in the gaseous state) have been found to be $\nu_1(1)$ = 3041 cm.$^{-1}$; $\nu_2(2)$ = 1682 cm.$^{-1}$; $\nu_3(3)$ = 3090 cm.$^{-1}$ and $\nu_4(3)$ = 1403 cm.$^{-1}$ from infrared measurements on films of NH_4Cl.[6]

The values of the thermodynamic functions C_p°, $(H^\circ - H_0^\circ)/T$, $-(F^\circ - H_0^\circ)$ and S° for NH_4^+ (g) from 200 to 1000°K. as calculated by the rigid rotator-harmonic oscillator approximation are listed in Table I for the ideal gas state at one atmosphere pressure.

TABLE I

THERMODYNAMIC FUNCTIONS FOR THE AMMONIUM ION IN THE IDEAL GAS STATE IN CAL./DEG./MOLE

T, °K.	C_p°	$(H^\circ - H_0^\circ)/T$	$-(F^\circ - H_0^\circ)/T$	S°
200	7.98	7.98	33.25	41.23
298.16	8.34	8.04	36.43	44.47
400	9.30	8.23	38.81	47.04
500	10.56	8.58	40.67	49.25
600	11.91	9.03	42.27	51.29
700	13.23	9.54	43.69	53.23
800	14.47	10.08	45.00	55.08
900	15.61	10.64	46.21	56.85
1000	16.65	11.19	47.36	58.55

The values of the thermodynamic functions probably are accurate to ±0.1 cal./deg./mole. However, at the temperatures near 1000°K. neglect of anharmonicity and other effects may increase the uncertainties in the thermodynamic functions.

The entropy of the reaction

$$NH_3(g) + H^+(g) \longrightarrow NH_4^+(g)$$

at 298.16°K. may now be calculated. The entropy of $NH_3(g)$ is 46.01 e.u.[7] The entropy of $H^+(g)$ is 26.01 e.u. from the Sackur–Tetrode equation and from Table I the entropy of $NH_4^+(g)$ at 298.16°K. is 44.47 e.u. Thus the entropy of the protonation reaction is −27.55 e.u.

Combining the entropy of the gaseous ammonium ion with its partial molal entropy in water,[8] 26.8 ±

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