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Proof of the Charging of Polypeptides and Polyamides in Organic Acids

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Synopsis

The electrophoresis of poly- γ -benzyl-L-glutamate (PBLG), poly-L-alanine (PLA) and nylon dissolved in various solvents was studied in a glass cell containing three sintered glass partitions. After the passage of a measured amount of charge the concentration of PBLG remained constant in all four chambers when the helicogenic solvents dimethylformamide and ethylene dichloride were used, but in mixtures of ethylene dichloride and dichloroacetic acid and in trifluoroacetic acid, polypeptide migrated to the cathode. Electrophoresis also occurred with PLA in trifluoroacetic acid and with nylon in formic acid. Although the total charge on the polyion could not be determined, the results show beyond reasonable doubt that polypeptides and polyamides are protonated in the presence of moderately strong organic acids.

The proposal by Klotz and co-workers¹⁻³ that the peptide groups of polypeptides are protonated in strong organic acids (dichloroacetic acid (DCA) and trifluoroacetic acid (TFA)) has resulted in considerable controversy (summarized in Refs. 4 and 5) in spite of the fact that earlier work,^{6,7} had shown that polyamides are protonated in the much weaker organic acid, formic acid. The criterion used to show charging in the latter case was the expansion of the polyion which occurs on progressive reduction of the ionic strength of the solution on dilution and results in a large increase in viscosity; the so-called polyelectrolyte effect. We have also observed this effect with a wide range of polypeptides in DCA, TFA, and mixed solvents⁸⁻¹⁰ and emphasize that the occurrence of a polyelectrolyte effect is *diagnostic* of the presence of polyions.¹¹

However many chemists are unfamiliar with the technique of viscometry and are therefore not likely to be persuaded by arguments based thereon, particularly when several spectroscopic techniques such as optical rotatory dispersion, circular dichroism, and ultraviolet absorption¹²⁻¹⁵ purport to show the absence of charging. Infrared and nuclear magnetic resonance spectroscopic measurements have been interpreted either to indicate charging^{1-5,16,17} or the lack of it.¹⁸⁻²⁰ Clearly, a simple unequivocal test is required to resolve the controversy. We have now made such a test by measuring the migration in a potential gradient of the positively charged polyion dissolved in an organic acid, using an electrolysis cell with porous glass partitions.

Samples of poly-L-alanine (PLA) (Pilot sample, estimated degree of polymerization $DP_w \sim 800^8$), poly- γ -benzyl-L-glutamate (PBLG) (Pilot sample, G51, $DP_w 1233^8$), and nylon 6:6 (I.C.I., molecular weight of 35,000, kindly supplied by Dr. I. C. Watt) were used. The solvents were all of analytical reagent-grade quality and were used without further purification, except for ethylene dichloride which was washed thoroughly with water and dried over a molecular sieve before use.

The electrolysis cell consisted of a Pyrex glass cylinder 5 cm in diameter, and 9 cm long closed at both ends and containing three sintered glass partitions spaced along it. The four compartments thus produced could be filled separately if necessary via ground glass openings which also served as points of entry in the end compartments for the bright platinum electrodes.

A constant volume of 50.0 ml of liquid was used in each experiment. Electrolysis was usually carried out using a d.c. voltage of 300 volts (3,000 volts in two cases) which produced a small current of 6–1,000 μ A, and did not raise the temperature of the solution above room temperature. The amount of charge passed was determined by means of a recorder. The amount of charge passed on electrolysis of the solvents in the absence of solute (q_s) was measured in control experiments. The polypeptide was dissolved in the solvent (which normally required about 15 min), and was then electrolyzed under the same conditions as the pure solvent. In those cases where migration of polyion occurred (see Table I) the current increased by amounts ranging from 10% to twentyfold due to the presence of solute, as would be expected if protonation of the polypeptide occurred, in qualitative agreement with Stake and Klotz.²¹ At the end of the electrolysis an aliquot of solution was withdrawn from each of the four compartments, and the polypeptide was either precipitated by pouring into a non-solvent, filtered, and dried to constant weight, or else (for PLA and nylon samples) the solvent was removed by lyophilization and the residue weighed. The concentration of polypeptide in the four compartments labelled (1) cathode, (2), (3), (4) anode, respectively, was thus readily calculated. The identity of the recovered polypeptide was checked by NMR spectroscopy. The spectra of the original material and that recovered by precipitation was found to be identical, but there was some broadening of the resonances of PLA after lyophilization.

The decomposition of the benzyl ester of PBLG in TFA and DCA was monitored by separate NMR experiments, in which two small peaks due to the phenyl and benzyl CH_2 resonances of the decomposition product occurred downfield from the corresponding resonances of PBLG. These were first observed after 3.25 hr immersion in TFA at 25°, but were not seen even after 5 days immersion in DCA. The possibility of peptide bond hydrolysis of PLA in TFA was checked by measuring rapidly the intrinsic viscosity in TFA of a sample of PLA which had been immersed in TFA at 25° for increasing lengths of time (0.33, 2, 6, and 24 hr). All the η_{sp}/c data obtained over the first 6 hr of measurements fitted the same straight line with $[\eta] = 0.79$ dl/g, whereas that obtained after 24 hr was appreciably lower with $[\eta] = 0.74$ dl/g.

The total charge q which is transported through the solution in Faradays is given by the following equation

$$q = zM_p + M_A + q_s \quad (1)$$

where M_p represents the number of moles of polyion transported into the cathode compartment, M_A the number of moles of univalent acid anion which have migrated into the anode compartment, z is the total charge on the polyion, and q_s is the amount of charge transported by the solvent under the same experimental conditions, but in the absence of the polyion. Rearrangement of eq. (1) gives

$$z = \frac{q - M_A - q_s}{M_p} \quad (2)$$

The value of M_A is unknown, since it would be difficult to determine experimentally and can be positive or more likely negative with a polyion, because the bulk of the counterions move with the polyion.²² However we can calculate a quantity z' which is defined by the equation

$$z' = \frac{q - q_s}{M_p} \quad (3)$$

which on combination with equation (2) gives

$$z = z' - \frac{M_A}{M_p} \quad (4)$$

The experimental results and the calculations are given in Table I. It is seen that in every case where the acids formic acid, DCA, or TFA are used the polypeptide and polyamide concentrations become higher in the cathode compartment and lower in the anode compartment after electrolysis. For comparison purposes it is noted that there is no increase in current on the addition of PBLG to the solvents DMF or EDC, neither is there any transport of the polypeptide in the potential gradient. These results show conclusively that the polypeptides in TFA and DCA solutions and the polyamide in formic acid are charged, but as expected PBLG is not charged in DMF or EDC.

Furthermore the charging cannot be the result of peptide bond fission, since the viscosity results on PLA in TFA show that this would be negligible in the short time for which the PLA was exposed to TFA in the transport experiments (maximum of 6 hr). The fission of the ester side chain of PBLG was found by NMR measurements to be negligible in extent, during the times of exposure to TFA and DCA given in Table I.

We are unable to evaluate the *total* charge z on the polyion,^{3,4} because of an inability to determine the fractions of the total charge carried by the polyion and the anion and the degree of specific ion binding of the anion to the polyion. The latter is known to be very considerable for polyions with closely spaced charges in aqueous media²² and ion pair formation would probably be accentuated further in our case by the low dielectric constants of the solvents.

TABLE I

Polymer	Solvent ^a	Concen- tration of solution, mg/ml	Concentration (mg/ml) of solution after electrolysis, in compartments ^b				Time of electrolysis, hr	q , Faraday $\times 10^6$	$(q - q_0)$, Faraday $\times 10^6$	$M_p \times$ 10^6	z' (eq. (3))
			(1) Cathode	(2)	(3)	(4) Anode					
PBLG ^c	EDC	2.1	2.1	2.1	2.0	2.1	20	0.31	—	—	—
PBLG	DMF	2.6	2.4	2.7	2.6	2.7	42	110	—	—	—
PBLG	2% DCA	3.0	6.7	3.0	2.5	0.04	7.5	5.8	5.5	0.112	50
PBLG	98% EDC										
PBLG	20% DCA	2.7	8.4	2.9	1.2	0.00	24	15.5	13.5	0.162	80
PBLG	80% EDC										
PBLG ^c	DCA	3.0	5.4	3.5	2.7	0.24	6	15.5	8.5	0.07	122
PBLG	DCA	3.4	4.4	not meas.	3.4	1.9	24	8.9	5.3	0.035	152
PBLG	TFA	3.8	5.4	3.6	3.6	2.8	0.5	4.2	2.4	0.048	50
PLA	TFA	2.2	2.3	2.2	2.2	2.1	0.042	1.1	0.92	0.022	42
PLA	TFA	2.3	3.1	2.2	2.3	1.7	0.30	8.3	7.1	0.11	65
PLA	TFA	0.68	1.7	0.66	0.46	0.15	1.1	13.5	8.2	0.15	55
PLA	TFA	3.6	11.3	4.2	1.0	0.34	6.0	135	107	1.08	99
Nylon	FA	4.2	11.4	4.1	3.0	0.20	5.0	785	486	1.63	300

^aSolvents used are ethylene dichloride (EDC), dimethylformamide (DMF), dichloroacetic acid (DCA), trifluoroacetic acid (TFA), and formic acid (FA). Mixed solvents are made up on a volume basis.

^bThe volumes of each compartment are as follows: (1) cathode 8.0 ml, (2) 16.9 ml, (3) 16.9 ml, (4) 8.2 ml.

^cPotential difference 3000 volt in these cases.

Values of z' are given in Table I, which are related to z by equation (4). The total charge on the polyion will be lower than z' if M_A is positive and higher than z' if M_A is negative, due to a large amount of counterion binding. As stated above, the latter result is much more likely than the former, hence it is probable that $z > z'$. The values of z' vary from about 50 to 100 in TFA and are apparently independent of the nature of the polyion, its concentration, and the time of electrolysis. For PBLG, z' increases from 50 in 2% DCA-98% EDC ($\leq 4\%$ of peptide groups charged) to about 135 ($\leq 11\%$ of peptide groups charged) in 100% DCA. For 6:6 nylon of molecular weight 35,000 there are 450 amide residues per molecule of which at least 300 appear to be charged. The much larger value in the latter case may be due to the greater distance between the amide groups of the polyamide than of the polypeptides and/or to less ion pair formation in formic acid due to its much higher dielectric constant than DCA and TFA. Further work is in progress on the determination of z , but irrespective of this it is believed that these experiments show beyond reasonable doubt the reality of the charging of peptide groups in polypeptides and amide groups in polyamides, in the presence of moderately strong organic acids.

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