FREE ENERGY OF DISSOCIATION OF AMINE HYDROCHLORIDES AT 25° FROM CELL MEASUREMENTS[1]

Sir:

The standard free energy of the reaction

$$\mathrm{RNH_3Cl(s) = RNH_2(g) + HCl(g)}$$

is a criterion of base strength. It is given by

$$-\Delta F^{\circ} = RT \ln P_{\mathrm{RNH_2}} P_{\mathrm{HCl}} \quad (1)$$

The two pressures can be obtained from the electromotive force of the cell

$$\mathrm{H_2(g),\ RNH_2}(m = x)\mathrm{,\ RNH_3Cl(s),\ AgCl,\ Ag} \quad \text{(A)}$$

With water as solvent the silver chloride electrode is attacked, but if alcohol be used this difficulty is avoided. The value of $E^0{}_{\mathrm{g}}$, for the cell

$$\mathrm{H_{2(g)},\ HCl(g),\ AgCl(s)\ Ag} \quad \text{(B)}$$

is $E^0 = -0.1509$.[2] From this and the electromotive force of cell A the fugacity of the hydrogen chloride can be calculated.

The partial pressure of the amine can be obtained by passing the exit hydrogen through dilute sulfuric acid, then through activated alumina. The total increase in weight together with the amount of amine (as determined by microtitration) carried over by a measured volume of hydrogen, allow the calculation of the necessary mole fractions to calculate the partial pressure (fugacity) of amine. The methylammonium chloride system has been studied.

In the first measurements, in which the alcohol was purified by distilling 95% alcohol over lime followed by final drying with magnesium and iodine and storing under nitrogen, the electromotive force stayed constant for about one hour and then started to fall. Seven values of ΔF^0 at 298.16°K., with a variation of the amine molality from 0.17 to 0.45, varied between 23,204 and 23,229 cal. The most probable value, calculated using the value of $\Delta H^0{}_{298.16}$,[3] together with the entropies of methylammonium chloride[3] and methylamine calculated from the spectroscopic data[4] is 22,867 ± 54 cal. The difference is well outside any reasonable error and was at first taken to indicate a possible zero point entropy of $R \ln 2$ in methylammonium chloride.[5] However, as a test of thermodynamic consistency, measurements were made of cell A at several temperatures between 0 and 40°, and it was found that the discrepancy was traceable to aldehydes (or other reducible material) in the alcohol. These reduce silver chloride and produce a local excess of chloride ion, thereby making the HCl partial pressure and hence ΔF^0 appear higher. Finally all alcohol was distilled from ethyl phthalate, sodium and silver nitrate and stored under nitrogen. Excess silver chloride was always placed on the silver side of the cell and the cells were made to come to equilibrium from the high side, as well as the low, by electrolysis. The value of ΔF^0 at 298.16°K. was finally found to be 22,831 ± 12 cal. The value of $\Delta H^0{}_{298.16}$ obtained from the temperature coefficient agreed with the measured ΔH^0[13] within 30 cal. The close agreement with the calorimetric free energy indicates no randomness in methylammonium chloride.

As a matter of interest the corresponding ΔF^0 at 298.16 for the dissociation of ammonium chloride is 21,860 cal.,[6] which is 1000 cal. lower than that for the methyl substituted derivative. The effect of the methyl group in increasing the basicity of the amine is thus not as great by this criterion as from base strength measurements in aqueous solution which correspond to a 1800 cal. difference.

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(1) Contract N60nr-269 T.O. III of the Office of Naval Research.

(2) J. G. Aston and F. L. Gittler, to be published.

(3) John G. Aston and Charles W. Ziemer, *ibid.*, **68**, 1405 (1946).

(4) J. G. Aston and Paul J. Doty, *J. Chem. Phys.*, **8**, 743 (1940).

(5) J. G. Aston, *Faraday Society Discussions*, No. 10, 119 (1951).

(6) C. C. Stephenson, *J. Chem. Phys.*, **12**, 319 (1944).

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POLYPEPTIDES. I. THE SYNTHESIS AND THE MOLECULAR WEIGHT OF HIGH MOLECULAR WEIGHT POLYGLUTAMIC ACIDS AND ESTERS[1]

Sir:

We wish to report the synthesis of a number of polypeptides of very high molecular weight (>100,000) and the determination of their molecular weights.[2] γ-Benzyl-L-glutamate is treated with an excess of phosgene in tetrahydrofuran solution at 60° to yield γ-benzyl-N-carboxy-L-glutamate anhydride, which is crystallized from ethyl acetate until the melting point is 93–94° (dec.) and the chloride content is less than 0.02% (Volhard method). γ-Methyl-N-carboxy-L-glutamate anhydride, m.p. 96–97° (dec.) is also prepared from the methyl ester[3] by treatment with phosgene.

The reproducible attainment of high molecular weight polymers depends upon the purity of the anhydride and particularly upon the elimination of traces of amino acids which may act as initiators, or traces of halogen acids or phosgene which neutralize the added initiator. The polymerization is carried out at 25° by shaking a 3% solution of the anhydride in dry dioxane or a mixture of dioxane–tetrahydrofuran using sufficient initiator to neutralize the halide and to initiate to the desired molecular weight. The preferred initiator is sodium hydroxide in methanol. Polymerizations are essentially complete in four hours, as indicated by titration of the residual anhydride.[4]

Polymers prepared from γ-benzyl-L-glutamate (BG) are particularly interesting because of their relatively high solubility in organic solvents and because removal of the benzyl groups by phosphonium iodide[3] provides polymers of L-glutamic acid (GA) which are water soluble, as the partial sodium salts, at *p*H's >4. We also find that some of the methyl groups of copolymers containing γ-methyl glutamate (MG) are removed by phosphonium io-

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(1) Supported by the Office of the Surgeon General, Department of the Army.

(2) See for example (a) E. Katchalski, in *Advances in Protein Chemistry*, VI, 123 (1951); (b) R. R. Becker and M. A. Stahmann, THIS JOURNAL, **74**, 38 (1952).

(3) W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

(4) A. Berger, M. Sela and E. Katchalski, *Anal. Chem.*, **25**, 1554 (1953).

dide treatment. The properties of some polymers are listed in the table.

TABLE I

Sample	Polymer of	Viscosity (a)	Intrinsic (dl./g.) (b)	Molecular weight	$[\alpha]^{25}_D$
329	BG	1.19	4.10	(225,000)	~14[b]
365	BG	1.42	5.62	262,000[b]	13.0[b]
397	BG	1.87	7.56	336,000[b] 358,000[a]	13.0[b]
245	MG	0.80	Insol.		−33.7[a]
307	BG and MG[d]	0.85	1.90	180,000[b]	−22.5[a] +13.5[b]
361	GA[e]	0.54[c]	Insol.	62,000[c]	−86.5[c]
318	BG, MG and GA[f]	0.42[c]	Insol.	61,000[c]	−54[c]

[a] Dichloroacetic acid. [b] Chloroform–formamide. [c] 0.2 M Na_2SO_4, pH ≅ 7.3. [d] Calcd. for ratio 60% BG, 40% MG: C, 59.5; H 6.1; N, 7.7; benzyl, 24.6. Found: C, 59.6; H, 6.1; N, 7.5; benzyl, 25 (by ultraviolet absorption). [e] Prepared from 329: calcd. for polyglutamic acid: C, 46.5; H, 5.5; N, 10.8. Found: C, 46.6; H, 5.8; N, 10.5. [f] Prepared from 307: calcd. for ratio 16% BG, 28% MG, 56% GA: C, 50.7; H, 5.8; N, 9.8; benzyl, 6.5. Found: C, 50.6; H, 5.9; N, 9.5; benzyl, 6.4.

The molecular weights were obtained by light scattering measurements employing Zimm plots over the angular range 30 to 135°. The absence of association is demonstrated by the agreement obtained in dichloroacetic acid and chloroform–formamide solutions as well as the linearity of the concentration dependence down to the lowest measurable concentrations. The viscosities in chloroform–formamide solutions were found to depend upon the gradient; the intrinsic viscosities listed in the table are values obtained by extrapolation to zero gradient. The level of molecular weights obtained shows that in this system termination reactions which in other cases[5] may limit molecular weights do not do so here. The molecular weight of the water-soluble derivatives indicates that only about one or two bonds in the backbone of the molecules are broken during de-esterification. The action of the water-soluble polypeptides in biological systems is now being investigated.

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(5) M. Sela and A. Berger, THIS JOURNAL, **75**, 6350 (1953).

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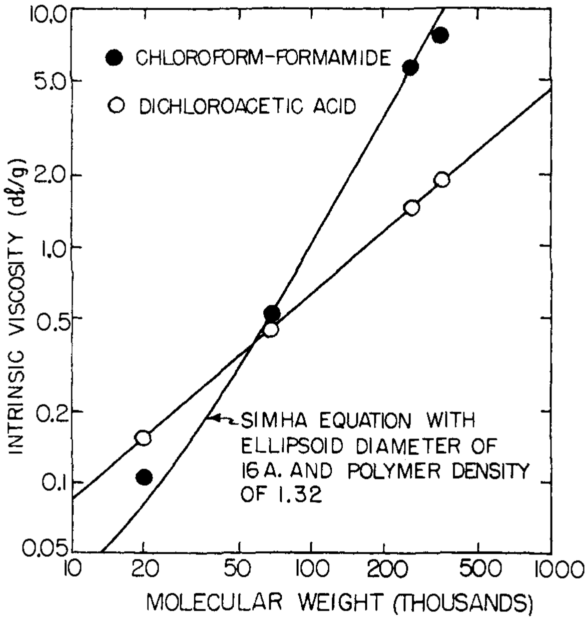
POLYPEPTIDES. II. THE CONFIGURATION OF POLYMERS OF γ-BENZYL-L-GLUTAMATE IN SOLUTION[1]

Sir:

The existence of two configurations, intramolecularly-bonded helical (α) and intermolecularly-bonded extended (β), for polypeptides in the solid state is well established.[2,3] We wish to report the existence of the counterpart of these two forms in solution as well as a solvated, randomly coiled form.

High molecular weight samples of poly-γ-benzyl-L-glutamate (PBG) were prepared[4] covering the molecular weight range of 20,000 to 350,000. In the solid state and in solutions such as chloroform and dioxane only the characteristic 1650 ± 2 cm.$^{-1}$ band is observed in the carbonyl amide region.[5] The specific rotation is +13.0° ($CHCl_3$), −14° (dichloroacetic acid, DCA) and independent of concentration. The molecular weight–intrinsic viscosity relation is shown as a log–log plot in the figure. It is seen that in chloroform–formamide (CF) solution the slope has the unusually high value of 1.7. Moreover, it is found that these data can be fitted with the theoretical relation for ellipsoids of revolution.[6] The line drawn in the figure is based on lengths equal to 1.5 Å. times the degree of polymerization (DP) and a (solvated helix) diameter of 16 Å. Furthermore, the scattering envelopes suggest a rod-like shape for the molecules of high molecular weight with lengths equal to 1.5 Å. times DP. The behavior in DCA is quite different: the slope in the figure is 0.87, typical of coiled molecules, and the size determined from light scattering for the higher DP samples is much smaller and consistent with that required of a randomly coiled polymer exhibiting the observed intrinsic viscosities.



Low molecular weight polypeptides which show

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(1) Supported by Office of Naval Research and Office of the Surgeon General, Department of the Army.

(2) (a) E. J. Ambrose and A. Elliott, *Proc. Roy. Soc. (London)*, **205A**, 47 (1951); (b) C. H. Bamford, L. Brown, A. Elliott, W. E. Hanby and I. F. Trotter, *Nature*, **169**, 357 (1953); (c) A. Elliott, *Proc. Roy. Soc. (London)*, **221A**, 104 (1953); (d) C. H. Bamford, W. E. Hanby and F. Happey, *ibid.*, **206A**, 407 (1951).

(3) L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci.*, **37**, 241 (1951)

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(5) The existence of an intramolecularly bonded form in solution has been suggested previously on the basis of infrared evidence (references 2a and 2c) and on the basis of a correlation between the optical rotation in cresol solution and the fraction of this form present in the solid state (C. Robinson and M. J. Bott, *Nature*, **168**, 325 (1951)).

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