Equation (14) is as follows

$$\log K''_p = \log \frac{P^2_{\mathrm{DCl}}}{P_{\mathrm{D_2}}} = -\frac{3200}{T} + 4.57 \qquad (14)$$

and was found from the graph of the relation between log K''_p and $1/T$ (see diagram 3) plotted from our data.

Summing up equations (1) and (13) we find

$$\mathrm{H_2 + 2DCl = D_2 + 2HCl} \qquad (15)$$

It is evident that

$$K = \frac{P^2_{\mathrm{HCl}} P_{\mathrm{D_2}}}{P_{\mathrm{H_2}} P^2_{\mathrm{DCl}}} = \frac{K^1_p}{K''_p} \qquad (16)$$

For the two temperatures, 690 and 658°K, we find that K is equal to 0.6 and 0.5, respectively, while Urey and Rittenberg[12] basing their computations of the free energies of deuterium and its compounds on the statistics of Fermi and of Bose–Einstein, find that at the same temperatures K is equal to 0.9 and 0.8, respectively. Thus theory and experiment result in values of the same order of magnitude, although the theoretical figures are higher than those found experimentally.

The author wishes to express his gratitude to Professor G. N. Lewis for suggesting this field of research, and for his valuable advice and helpful criticism during the course of the investigation.

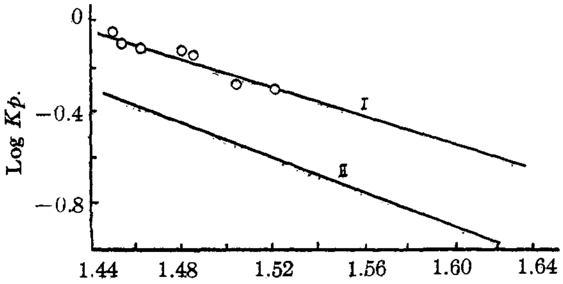

Fig. 3.—Dependence of equilibria $\mathrm{D_2 + CuCl}$ and $\mathrm{H_2 + CuCl}$ upon temperature: I, $\mathrm{H_2 + CuCl \rightleftarrows 2HCl + Cu}$; II, $\mathrm{D_2 + CuCl = 2DCl + Cu}$.

Summary

1. The equilibrium between the light and heavy isotopes of hydrogen and cuprous chloride was studied in the range of temperatures from 320 to 400°.

2. The entropy, free energy and the heat of formation of cuprous chloride were calculated from the experimental data. The constant of equilibrium for the reduction of hydrogen chloride by deuterium was also calculated.

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[12] Urey and Rittenberg, *J. Chem. Phys.*, **1**, 142 (1933).

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY IN THE LABORATORIES OF PHYSIOLOGY, HARVARD MEDICAL SCHOOL, AND THE BIOLOGICAL LABORATORIES OF HARVARD UNIVERSITY]

Further Studies on Dielectric Constant and Electrostriction of Amino Acids and Peptides

BY JESSE P. GREENSTEIN AND JEFFRIES WYMAN, JR.

In a recent communication[1] an account was given of studies on the electrostriction and dielectric constant of aqueous solutions of multivalent peptides. In the present paper further studies of this sort on other peptides and amino acids are reported.

I. **Preparation of Materials.**—*l*-Asparagine and *l*-glutamine were provided by Dr. E. J. Cohn of the Harvard Medical School. Their purification has been reported previously.[2] The 4,4′-diphenylbetaine was prepared and sent to us by Dr. Devoto of the University of Milan.

The peptides were prepared according to the methods of Emil Fischer[3] and were purified by repeated crystallization. Acetylhistidine was synthesized according to Bergmann and Zervas.[4] Creatine and β-indolepropionic acid were Hoffman–LaRoche products. The glycocyamine was prepared according to the usual procedure of treating sodium glycinate with an equivalent amount of S-methyl isothiourea sulfate in aqueous solution, finally crystallizing the product twice from hot water.

ϵ,ϵ-Diguanido-di(α-thio-n-caproic acid) was part of the original material reported by Greenstein[5] but purified by final crystallization from hot water rather than from an alcohol–water mixture. Under these conditions the m. p. of the diguanido acid was 205°; N calcd., 20.6; N found, 20.6.

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[1] Greenstein, Wyman and Cohn, THIS JOURNAL, **57**, 637 (1935).

[2] McMeekin, Cohn and Weare, *ibid.*, **57**, 626 (1935).

[3] Fischer, "Untersuchungen über Aminosäuren, Polypeptide, und Proteine," Vols. I and II, Verlag Julius Springer, Berlin, 1906.

[4] Bergmann and Zervas, *Biochem. Z.*, **203**, 280 (1928).

[5] Greenstein, *J. Biol. Chem.*, **109**, 529 (1935).

II. Experimental Results

The experimental results are contained in Table I. The partial molal volumes (V) were calculated from density measurements made with a pycnometer. The dielectric constant was measured as heretofore by the method developed by Wyman.[6] In the case of glutamine and *l*-asparagine the frequencies used were close to 81 megacycles, corresponding to a wave length of 3.7 meters; in all other cases the frequencies were in the neighborhood of 46 megacycles, corresponding to a wave length of 6.5 meters. The results are given in terms of δ, the dielectric increment per mole of solute per liter, the dielectric constant concentration relation being in all cases linear. The range of concentration (moles per liter) over which the dielectric constant was measured is in each case given in the last column.

TABLE I

Substance	V	δ	Range of concn. (moles per liter)
l-Asparagine	78 ±0.5	20.4 ±0.3[a]	0.09 –0.19
l-Glutamine	......	20.8 ±0.3[a]	.10 – .15
Creatine	87.0 ± .5	32.2 ±0.4	.042– .073
Glycocyamine	......	30 ±1.5	.010– .023
Acetylhistidine	134 ± .5	62 ±1.5	.005– .018
β-Indolpropionic acid	......	−8 ±2.[b]	.034
4,4′-Diphenyl betaine	......	155 ±8.0	.002– .0096
Glycyl-leucine	139.8 ±1.4	74.6 ±1.0	.079– .119
Leucyl-glycine	143.2 ±1.4	68.4 ±1.0	.035– .085
N-Methyl-leucyl-glycine	162 ±0.5	67 ±2.0	.016– .081
Glycyl-alanine	94 ±1	71.8 ±1.0	.034– .056
Alanyl-glycine	95.5 ±0.5	71 ±3.0	.031– .121
Glycyl-phenylalanine	155.4 ±1.0	70.4 ±1.5	.009– .034
Phenylalanyl-glycine	160 ±2.0	56.7 ±1.0	.024– .046
Leucyl-glycyl-glycine	178 ±0.5	120.4 ±1.2	.041– .082
ϵ,ϵ'-Diguanido-di-(α-thio-*n*-caproic acid)	......	151 ±9.0	.002– .005

[a] These values are slightly higher than preliminary values previously reported.[2]

[b] In 47.05% ethanol, $\epsilon_0 = 50.70$.

III. Discussion

It has been shown by Cohn and co-workers[7] that peptides and amino acids as zwitterions produce electrostriction of the solvent. The magnitude of the effect is determined by the number of pairs of opposite charges and their relative positions in the molecule. For monoamino monocarboxylic acids the electrostriction increases with the distance between the charges (*i. e.*, the dipole moment) as indicated by the following figures: α acids 13.3; β acids 13.8–14.4; γ acids 15.9; ϵ acids 17.3–18.3; diglycine 16.1; triglycine 16.1. The electrostriction appears to approach an upper limiting value of "20 cc. for one amino plus one carboxyl group at infinite distance and dilution."[7] For molecules containing a number of dipoles sufficiently separated from one another the effects are additive.

In order to reckon the electrostriction for the cases in hand it is necessary to calculate the theoretical volumes for the various molecules in the undissociated (non-zwitterionic) form. This may be done on the basis of the data on the volumes characteristic of specific molecules, groups and radicals given by Cohn.[7] The results, together with the corresponding values of the electrostriction (*i. e.*, the difference between these and the observed values), are given in Table II. Data are not available for the calculation of the volumes of creatine, glycocyamine and the diguanido acid. On the other hand, experimental values for the volumes are lacking for the other compounds not listed in Table II. In the right-hand column of the table the δ values are repeated for convenience of comparison.

TABLE II

	Volume (cal.) cc.	Electrostriction, cc.	δ
l-Asparagine	93.3	15.3	20.4
Acetyl-histidine	145.0	11	62
Glycyl-leucine	158.5	18.7	74.6
Leucyl-glycine	158.5	15.3	68.4
N-Methyl-leucyl-glycine	174.8	12.8	67
Glycyl-alanine	109.6	15.7	71.8
Alanyl-glycine	109.6	14.1	71
Glycyl-phenylalanine	171.3	15.9	70.4
Phenylalanyl-glycine	171.3	11.3	56.7
Leucyl-glycyl-glycine	194.8	16.3	120.4

Although it is impossible to make any exact calculations of electric moments from the dielectric constant data in polar solvents, it is clear that the δ value is very closely correlated with molecular polarization (*i. e.*, the square of the electric moment, neglecting optical polarizations), and indeed the evidence is strong that it is nearly proportional to it. At any rate molecules of roughly the same size having the same δ value may safely be regarded as having very closely the same moment, and moments may be compared as to relative magnitude by comparing the δ values.

If we interpret the data in this way a number of points of interest are brought out. There is a very satisfactory correlation between δ and electrostriction for closely similar molecules. This is apparent from a comparison of glycyl-leucine, leucyl-glycine, and N-methyl-leucyl-glycine; or of glycyl-alanine and alanyl-glycine; or of glycyl-

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(6) Wyman, *Phys. Rev.*, **35**, 623 (1930).

(7) Cohn, McMeekin, Edsall and Blanchard, THIS JOURNAL, **56**, 784 (1934).

phenylalanine and phenylalanyl-glycine. In these cases also it is striking that the electrostrictions and δ values seem to be always less when the more bulky half of the molecule (*e. g.*, phenylalanine in peptides of this compound and glycine) is acting as the cation, that is, when it carries the positive charge of the zwitterion. This would appear to be related to the fact pointed out by Edsall and Wyman[8] that the greater part, *i. e.*, approximately two-thirds at least and quite possibly more, of the total electrostriction of amino acids and related compounds is to be attributed to the amino group bearing the positive charge.

It is clear from the dielectric constant data that asparagine and glutamine both ionize as α-amino acids, since the δ values of both are very like that of glycine (22.5) and other α acids. On the other hand creatine and glycocyamine both have δ values close to those characteristic of β acids (β-alanine and β-aminobutyric acid have δ values of 32.4 and 34.6, respectively). In both these cases therefore it is one of the terminal nitrogens of the guanidine group which bears the charge. As a matter of fact, in all probability there is resonance between these nitrogens so that they are indistinguishable from one another and the positive charge should be regarded as located midway between them. Acetyl-histidine has a δ value close to that of δ-aminovaleric acid, given by Devoto as 63.[9] From this it appears that the positive charge is associated with one or other of the nitrogen atoms of the imidazole ring, but here again there is probably resonance between the two nitrogens so that the center of the charge is located midway between them. This is structurally consistent with a dipole moment close to that of a δ acid. β-Indole-propionic acid was too insoluble and too conducting to permit of measurements in water but in 47% alcohol it has a negative δ value, and we may conclude therefore that, in marked contrast with acetyl-histidine, it is not a zwitterion, that is, the nitrogen contained in the ring, unlike the nitrogens of histidine, does not behave like an amino nitrogen. In this connection it may be mentioned that in regard to their biological activity various indole compounds are closely similar to the corresponding indene compounds in which the nitrogen of the ring is replaced by a carbon atom and in which therefore there is no possibility of zwitterion formation.[10] The diphenyl compound supplied by Devoto is a 4,4′-betaine. It is interesting to note therefore that it gives a δ value 2.1 times as great as *p*-benzbetaine. If both molecules were undistorted we should expect on the basis of interatomic dimensions the dipole distances to be 10.7 and 6.36 Å., respectively.

Apart from what has already been said there is little to remark in regard to the peptides other than the diguanido acid. Their δ values are in general agreement with those of the glycine peptides.[11] The diguanido compound contains two dipoles. It exhibits a somewhat higher δ value than the closely related ε,ε′-diamino-di-(α-thio-*n*-caproic acid). The difference (151 as compared with 131) corresponds to the greater dipole distance between the amino and carboxyl pair in each half of the molecule; for, just as in the case of creatine and glycocyamine, it must be the terminal nitrogen atoms, undergoing resonance, which carry the positive charge.

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[8] Edsall and Wyman, THIS JOURNAL, **57**, 1964 (1935).

[9] Devoto, *Gazz. chim. ital.*, **63**, 50 (1933).

[10] Thimann, *Koninklijke Akad. van Wetenschappen Te Amsterdam*, **38**, 896 (1935).

[11] See Wyman and McMeekin, THIS JOURNAL, **55**, 908 (1933).