formation of a complex between the iron(II)–polyethylenepolyamine complex and oxygen. Admitting the possibility of its formation such a complex would be the cause of the acute maximum noted in the cathodic wave. Since addition of suppressors would displace the oxygen from the complex, it would have to also eliminate the prewave.

The total of four anodic waves present for the pentaethylenehexamine complex indicate contamination by lower polyethylenepolyamines. These have $E_{1/2}$ values of -0.49, -0.27, $+0.06$ and $+0.24$. Inasmuch as iron(II)–pentaethylenehexamine should be a hexaminated complex, there should be no hydroxy complexes other than those brought about by decomposition of the complex by hydroxide ion. This leads to the conclusion that this sample of polyethylenepolyamine was contaminated by lower polyethylenepolyamines. The three more negative waves $(E_{1/2} = -0.49, -0.27$ and $+0.06)$ are similar to those obtained from the triethylenetetramine complex $(E_{1/2} = -0.42, -0.21, +0.06)$ at this pH. The discrepancies in the two more negative waves may be experimental error but more probably resulted from the contributions of other polyamine complexes present (e.g.,

iron(II)–tetraethylenepentamine) distorting the waves.

The polarographic evidence presented supports the conclusions reached previously as to the existence and number of these complexes and can probably be considered as confirming those postulates. Due to the irreversibility of the reductions it was not possible to determine the equilibrium constants between complexes having varying numbers of hydroxyl groups.

It is not implied that a structure corresponds with any one wave. It is sufficient that the polarographic behavior may be explained by the postulates as to structure change with pH for these complexes that seemed necessary to explain data arising from a completely independent source and to that extent makes these postulates more reliable.

Metal–Amine Complexes in Ion Exchange[1]

By Ruth H. Stokes[2] and Harold F. Walton

Received November 23, 1953

We have measured the stability of ammonia and amine complexes of silver and copper in resinous cation exchangers. In sulfonic acid exchangers the ammonia complexes are just as stable as in aqueous solution, but in a carboxylic exchanger they are decidedly less stable. Silver–amine complexes are more or less stable in the resin, depending on the balance between adsorptive and steric effects. An explanation is offered for the special stability of the silver–ethylenediamine complex in the exchanger.

Introduction

Metal–ammonia complex cations enter into ion exchange, as do the hydrated metal cations, and some of these exchanges, such as that of the cupric–ammonia cations, are commercially useful.[3] It has been shown that the metal ion may be coördinated with ammonia in the exchanger, just as it is in water containing ammonia.[3,4] The object of the present investigation was to measure the stability of metal–ammonia and metal–amine complexes in cation exchangers, and to find out whether such complexes were more, or less, stable within the exchanger than they are in aqueous solution.

Most of the work here described was done with the silver ion. A few experiments were made with cupric ions. The behavior of other metal ions is being studied and will be described in a later communication.

Experimental

Materials. Sulfonated Polystyrene Resins.—Two batches of "Permutit Q," both nominally containing 10% divinyl-

benzene, were used. They showed different swelling characteristics. They were washed thoroughly with 2 N hydrochloric acid and air-dried before use. Moisture contents were determined by drying at 110°, and the exchangeable hydrogen ion contents were found by titration with standard base. The swollen volumes (in water) were measured by a microscopic technique, the dry volumes (unswollen) by displacement of hexane. The internal solution volumes in water, found from these data, were: resin A, 0.093 ml./meq.; resin B, 0.150 ml./meq. The exchange capacities per gram of dry H-resin were: resin A, 5.13 meq./g.; resin B, 5.05 meq./g.

Carboxylic Resin.—"Amberlite IRC-50," a cross-linked polyacrylic acid, was washed with 2 N sodium hydroxide and hydrochloric acid, then treated with ammonia, washed and air-dried. The resin then contained both ammonium ions and replaceable hydrogen ions. The latter were determined by titration, as was the total exchange capacity. The swollen volume of the resin was found pycnometrically,[5] the dry, unswollen volume of the H-form by displacement of hexane. The internal solution volume was calculated to be 0.268 ml./meq., the exchange capacity, 8.67 meq./gram dry H-resin.

Equilibration Technique.—Weighed quantities of resin, usually 1 gram of H-resin, were placed in 130-ml. polyethylene bottles with measured volumes of solutions containing metal salt, amine salt and free amine. Commonly, 0.300 meq. of nitric acid and 0.200 meq. of metal nitrate were used, together with enough amine to neutralize all the nitric acid and resin acid and in addition to coördinate with the metal ions, and water to make a total volume of 100 ml.

(1) Presented in part at the 123rd meeting of the American Chemical Society, Los Angeles, California, March 18, 1953.

(2) Taken in part from a thesis presented by Mrs. Ruth H. Stokes in partial fulfillment of requirements for the Master of Science degree.

(3) F. Gerstner, Z. Elektrochem., **57**, 221 (1953).

(4) R. Nelson and H. F. Walton, J. Phys. Chem., **48**, 406 (1944).

(5) H. P. Gregor, K. M. Held and J. Bellin, Anal. Chem., **23**, 620 (1951).

The bottles were stoppered and rotated in a thermostat for at least 8 hours. Tests showed no change in composition of the solutions after five or six hours. The attainment of equilibrium was further checked in some cases by starting with the metal in the resin instead of in solution. In all these cases the final distribution reached was independent of the direction of approach.

The temperature of equilibration was 25.00°, except in a few cases which are noted in the graphs. Temperature had very little effect on the distributions obtained.

Analytical.—Three determinations were made on the solutions after equilibration: pH, metal content and total titratable base. The pH was measured with a Beckman Model G meter in whose salt bridge the potassium chloride was replaced by saturated potassium nitrate for measurements in silver salt solutions. Silver was titrated with thiocyanate, copper with iodide and thiosulfate. The total base (free and combined with metal ions) was found by titration with standard acid, potentiometrically when necessary. The free base concentration was calculated from the pH, the (known) concentration of ammonium or substituted ammonium ions, and the ionization constant of the base. Except in the case of ammonia itself, no attempt was made to correct for activity, since the available ionization constants are not thermodynamic constants and the concentrations were low. The calculated free base concentration was subtracted from the total base concentration to obtain the concentration of base coördinated with the metal. The concentrations of metal ions and coördinated base in the resin were found by difference. No allowance was made for the exclusion of dissolved salts from the resin by the Donnan equilibrium.

Results

(a) **Copper and Ammonia.**—Two series of experiments were made with sulfonated polystyrene resin A (see Experimental section) and one with carboxylic resin. In the first series, half-gram portions of resin A containing about 0.5 meq. of Cu and 1 meq. of NH$_4$ were shaken with 65 ml. of solutions containing 1 meq. of NH$_4$Cl and varying amounts of NH$_3$. No copper could be detected in these solutions after shaking, and the concentration of free ammonia was measured directly by titration with N/100 acid. In the second series, portions of resin A containing only NH$_4$ were shaken with 85 ml. of solutions containing 27 meq. of NH$_4$, 1 meq. of Cu and varying amounts of NH$_3$. These solutions contained significant quantities of copper after shaking; the activity of free ammonia therefore had to be calculated from the pH, the ammonium ion concentration, the activity coefficient of the ammonium ion (taken as 0.60), and the
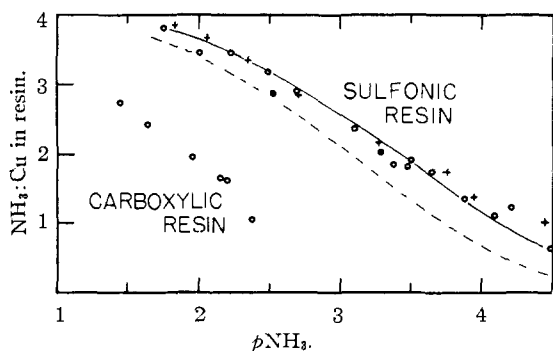

Fig. 1.—Formation curves for cupric–ammonia complex: crosses, NH$_4$-resin initially; open circles, Cu–NH$_4$-resin initially; filled circles, Cu–NH$_4$-resin initially, temp. 35°. Open circles in carboxylic resin graph, NH$_4$-resin initially. Solid line, Bjerrum's formation curve for 5 M NH$_4$NO$_3$; broken line, Bjerrum's curve for 0.5 M NH$_4$NO$_3$.

thermodynamic ionization constant of the ammonium ion (6.04 × 10^{-10}).

The two series of experiments gave similar distributions (see Fig. 1), showing that equilibrium was attained. Checks were also made to see if copper was irreversibly bound to the resin or if the resin suffered any irreversible change. After equilibration, the resin was separated and the copper eluted with sulfuric acid, then titrated; the resin, now in the hydrogen form, was washed and titrated with standard base. Within experimental error, all the copper which had entered the resin was eluted, and the resin had the same exchange capacity after the experiment as before.

In the carboxylic resin series, 0.8-g. portions of resin containing H and NH$_4$ were shaken with 100-ml. portions of solutions containing 2 meq. of Cu and 3 meq. of NH$_4$ together with ammonia. After equilibration, no copper could be detected in the solution, and the resin was a light blue, not the deep purple-blue of the copper–ammonia complexes in aqueous solution. The concentration of free ammonia in solution was found by titration.

Typical data obtained were the following: (i) Sulfonic resin containing 0.568 meq. of Cu and 1.160 meq. of NH$_4$ shaken with 65 ml. of solution containing 1.000 meq. of NH$_4$ and 1.000 meq. of NH$_3$. After shaking, solution contained 0.177 meq. of NH$_3$ and no Cu.

Calcd.: p NH$_3$ by titration, 2.56; NH$_3$:Cu ratio in resin, 2.90.

(ii) Sulfonic resin containing 2.08 meq. of NH$_4$ shaken with 85 ml. of solution containing 27.64 meq. of NH$_4$, 1.041 meq. of Cu and 1.561 meq. of NH$_3$. After shaking, solution contained 0.323 meq. of Cu and 0.527 meq. total of NH$_3$ (free and bound to Cu); pH 7.22.

Calcd.: p NH$_3$, from ionization constant and activity coefficient quoted above, 2.70; NH$_3$:Cu mole ratio in resin, 2.88; in solution, 2.85.

(iii) Carboxylic resin containing 4.13 meq. of NH$_4$ and 0.50 meq. of H shaken with 100 ml. of solution containing 3.000 meq. of NH$_4$, 2.000 meq. of Cu and 3.570 meq. of NH$_3$. After shaking, solution contained 1.09 meq. of NH$_3$ and no Cu.

Calcd.: p NH$_3$, 1.96; NH$_3$:Cu mole ratio in resin, 1.98.

The results are shown graphically in Fig. 1. Our data are shown by points; the lines represent the data of J. Bjerrum[6] for the ammonia–cupric ion association in aqueous ammonium nitrate solutions.

(b) **Silver and Ammonia.**—Three series of experiments were made with sulfonated polystyrene resin A and one with the carboxylic resin in the ammonium form. The three series with resin A started with pure NH$_4$-resin, pure Ag-resin, and resin containing both Ag and NH$_4$. As will be seen from Fig. 2, the three series all gave the same final distribution relationship, showing that true equilibrium was reached. All of the silver in the resin could be eluted afterwards with dilute nitric acid.

The data are plotted in Fig. 2 in the same way as the copper–ammonia data of Fig. 1, the line showing the data of J. Bjerrum for an aqueous ammonium

(6) J. Bjerrum. "Metal–Ammine Formation in Aqueous Solutions," P. Haase, Copenhagen, 1940.
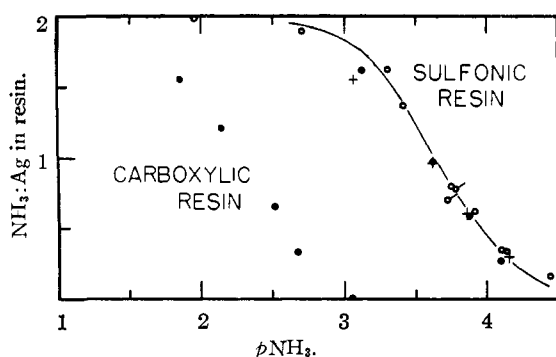
Fig. 2.—Formation curves for silver–ammonia complex: crosses, Ag-resin initially; open circles, Ag–NH₄-resin; tagged open circles, Ag–NH₄-resin at 43; filled circles, NH₄-resin. Solid line, Bjerrum's formation curve for $2M$ NH₄NO₃ at 22°.

Fig. 3.—Ratio bound amine:total Ag in solution *versus* same ratio in sulfonic resins. Broken line represents equal stability in resin and solution. *Benzylamine curves:* "A," resin A, 2 meq. of Ag; "B1", resin B, 2 meq. of Ag; "B2," resin B, 5 meq. of Ag. *Other curves*, resin A.

nitrate solution, and the points our own experimental results for the ion exchangers.

Some tests were also made with a sulfonated phenolic cation exchanger, "Amberlite IR-100." Considerable reduction of silver ions to metallic silver occurred with this resin, and the distribution data obtained have little significance.

(c) **Silver and *n*-Butylamine and Piperidine.**— In these tests, 1-g. portions of sulfonic acid resin A were shaken with 100 ml. of solution containing 2 meq. of AgNO₃, 3 meq. of HNO₃ and varying amounts of amine. In the piperidine series a certain amount of metallic silver was formed, due to reduction by the amine, but this amount was less than 0.01 meq. and the resin was not affected.

In calculating the concentrations of free amine in solution from the pH, the following ionization constants, K_B, were used[7,8]: *n*-butylamine, 4.1 × 10⁻⁴; piperidine, 1.7 × 10⁻³. In Fig. 3 the ratio (bound amine):(total silver) in the resin is plotted against the same ratio in the solution. This method of plotting is preferred to that of Figs. 1 and 2, and it is possible here because the silver concentration in the solution is large enough, and the concentration of free amine small enough, to allow a reasonably accurate calculation of the ratio (bound amine):(total silver) in the solution as well as in the resin. This was not so in the copper–ammonia tests. Our silver–ammonia data of Fig. 2, replotted on the axes of Fig. 3, fall along a straight line of 45° slope.

(d) **Silver and Benzylamine.**—Tests were made with both sulfonic resins, A and B. The proportions of reactants were the same as in (c) above, except that with resin B, a series of tests was run in which the resin was shaken with 5 meq. of AgNO₃ and no HNO₃. This was done to explore the effect of varying the concentration of silver in the resin. The results are plotted in Fig. 3. The ionization constant, K_B, was taken as 2.4 × 10⁻⁵.[9] Several tests were made in which the final distribution was approached from both sides, that is, starting with

(7) I. M. Kolthoff and V. A. Stenger, "Volumetric Analysis," Vol. 1, Interscience Publishers, New York, N. Y., 1942, p. 284.

(8) R. J. Bruehlman and F. H. Verhoek, THIS JOURNAL, **70**, 1401 (1948).

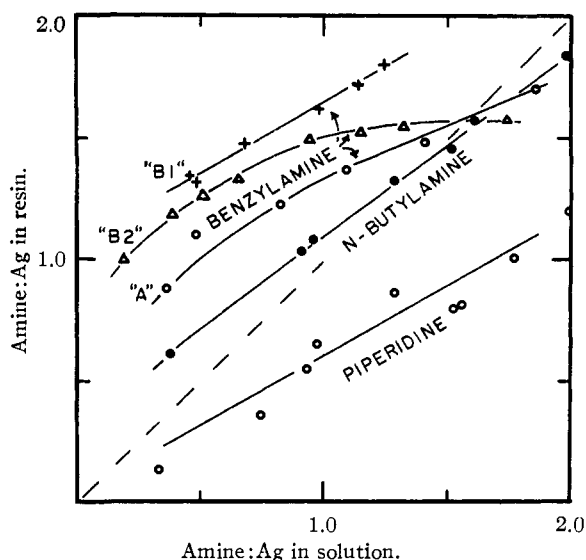(9) W. H. Carothers, C. F. Bickford and C. J. Hurwitz, *ibid.*, **49**, 2908 (1927).

silver in the resin instead of in the solution. These showed satisfactorily that equilibrium was reached.[10]

(e) **Silver and Ethylene Diamine.**[10]—The proportions of silver salt, acid, base and resin were the same as with the other amines, but to interpret the data it was necessary to know the proportions of the two cations, $C_2H_4N_2H_5^+$ and $C_2H_4N_2H_6^{++}$ (called enH⁺ and enH₂⁺⁺), in both solution and resin. Studies to be published separately[11] show that for resin B in the concentration used, the distribution constant

$$\frac{[\text{enH}_2^{++}]_r}{[\text{enH}^+]^2_r} \times \frac{[\text{enH}^+]^2_s}{[\text{enH}_2^{++}]_s} = 5.0$$

(units, millimoles/gram in resin, millimoles/ml. in solution). Because of the dilution of the solution, the resin contained an overwhelming preponderance of enH₂⁺⁺. In solution the ratio of enH₂⁺⁺ to enH⁺ was found from the pH, using graphs obtained by potentiometric titration of ethylene diamine with nitric acid at two different concentrations. At pH 6.0, enH₂⁺⁺:enH⁺ in solution was 1:11.0; if the pH was much higher than this, a small error in pH made a large error in the final result; this limited the validity of the measurements to the lower base:silver ratios.

To illustrate the kind of data obtained and the nature of the calculations, the following set of sample data is presented.

Taken: 4.269 meq. of H-resin; 3.00 meq. of HNO₃, 1.995 meq. of AgNO₃, 11.22 meq. (5.61 mmoles) of ethylene diamine in 100 ml. of solution.

Found after shaking: in solution, pH 6.61; base titrated to pH 4.5, 1.035 meq.; Ag, 0.475 meq.

Calculations: in solution, mole ratio enH⁺: enH₂⁺⁺ = 0.39 at pH 6.61 (from our titration

(10) These measurements were made by Leone Cockerell, of the University of Colorado.

(11) By R. S. Lang, University of Colorado.

curves); meq. (enH$^+$ plus enH$_2$$^{++}$) = 4.52, therefore mmole enH$^+$ = 0.74, mmole enH$_2$$^{++}$ = 1.89; en bound to Ag = 1.035 − 0.74 = 0.295 meq. = 0.148 mmole.

Total en in solution = 2.78 mmoles, whence total en in resin = 5.61 − 2.78 = 2.83 mmoles; meq. (enH$^+$ plus enH$_2$$^{++}$) = 4.269 − meq. Ag = 2.749; mole ratio enH$^+$:enH$_2$$^{++}$ in resin = 1:45 (from equilibrium constant); mmole enH$^+$ = 0.03, mmole enH$_2$$^{++}$ = 1.36, whence en bound to Ag = 2.83 − 1.36 − 0.03 = 1.46 mmole.

Therefore, mole ratio bound en:total Ag = 0.31 in solution, 0.96 in resin.

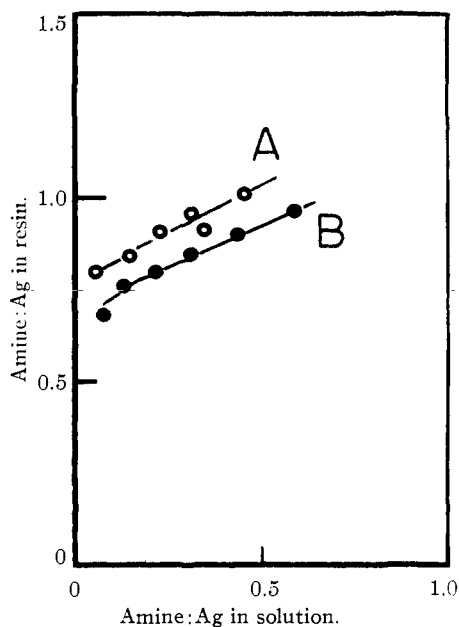The results are shown in Fig. 4.

Fig. 4.—Ratio bound ethylene diamine:total Ag in resin B, calculated according to assumptions explained in text, *versus* the same ratio in solution. Curve A, 2 meq. of Ag; curve B, 5 meq. of Ag.

(f) **Distribution of Silver between Resin and Solution.**—Distribution coefficients, (total Ag/amine cation)$_{resin}$/(total Ag/amine cation)$_{solution}$, were calculated for the experiments with ammonia, butylamine, piperidine and benzylamine. For resin A they averaged 3.75, 4.1, 5.0 and 5.1, respectively. A small effect of the base:silver ratio was seen, but it was significant only with benzylamine, where increasing the ratio of total excess base:silver from 0.75 to 1.35 increased the coefficient from 4.24 to 5.65. These distributions depend on several factors, and we make no attempt to interpret them at present.

Discussion

(a) **Ammonia Complexes.**—In the sulfonic acid resins, the Ag–NH$_3$ and Cu–NH$_3$ complexes are just as stable in the resin as they are in an aqueous solution. Comparing our formation curves with those found by Bjerrum in aqueous ammonium nitrate solutions, our data fit the 5 N NH$_4$NO$_3$ curves better than those for the more dilute solutions, which is expected, since the interior of the

resin resembles a concentrated aqueous salt solution. We may conclude that the Cu and Ag ions are not covalently bound to the sulfonic groups of the resin, or that if they are so bound, the binding energy is far less than the binding energy between the ions and ammonia.

With the carboxylic resin the situation is different. Here the ammonia complexes are definitely less stable than in water; that is, it takes a much higher ammonia concentration to form the complexes. Cupric ions are known to associate with carboxyl groups; the formation constants for the various cupric ion–acetate complexes have been measured by Fronaeus.[12] He finds for the reaction Cu(OH$_2$)$_4$$^{++}$ + Ac$^-$ → Cu(OH$_2$)$_3$Ac$^+$ + OH$_2$, $\Delta F° = -2280$ cal./mole. Combining this value with $\Delta F° = -5700$ cal./mole for Cu(OH$_2$)$_4$$^{++}$ + NH$_3$ → Cu(OH$_2$)$_3$NH$_3$$^{++}$ + OH$_2$ (Bjerrum[6]), we find that to break a Cu–Ac link and form a Cu–NH$_3$ link in its place requires $\Delta F° = -3420$ cal./mole, corresponding to an equilibrium constant of 300. This means that the ratio of free carboxylate ion concentration to free ammonia concentration should be roughly 300 when the copper in the resin is equally loaded with ammonia and carboxyl groups, or, ignoring hydration, when the copper is half saturated with ammonia. From Fig. 1, this occurs at p NH$_3$ = 2.0. In the sample calculation quoted earlier in this paper, 4.6 meq. of carboxylic resin contained 2 meq. of Cu; there was therefore at least 2.6 meq. of free carboxylate ions. The internal solution volume of the NH$_4$-resin was 0.27 ml./meq.; or 1.25 ml. for the sample used. The free carboxylate ion concentration was thus at least 2 N. The ratio [COO$^-$]:[NH$_3$] in the resin was thus at least 200:1, or about the expected value.

(b) **Complexes of *n*-Butylamine and Piperidine.** —With piperidine, the complex is decidedly less stable in the resin than in the solution. This can be attributed to steric hindrance. With *n*-butylamine, two effects are apparent. For large ratios of amine to silver, steric hindrance operates to make the comp. less stable in the resin, but with smaller amine to silver ratios, the complex is more stable in the resin. This must be attributed to non-polar or "van der Waals" adsorption of the hydrocarbon chain by the polystyrene resin matrix. The same effect favors the replacement of small inorganic ions by quaternary ammonium cations in phenolic and polystyrene type exchangers.[13,14]

(c) **The Benzylamine–Silver Complex.**—Except at the highest amine:silver ratios this complex is considerably more stable in the resin than in the solution. The primary effect is thus the "van der Waals" adsorption, which is especially great between the benzene rings of the amine and those of the resin. The same effect is found in the ion exchange distribution of quaternary cations containing the benzene ring.[13,14] The steric effect also operates, however. It is greater, the greater the amine:silver ratio, the greater the proportion of exchange sites occupied by the silver–amine complex, and the less the swollen volume of the resin.

(12) S. Fronaeus, *Acta Chem. Scand.*, **5**, 859 (1951).

(13) T. R. E. Kressman and J. A. Kitchener, *J. Chem. Soc.*, 1190 (1949).

(14) H. P. Gregor and J. I. Bregman, *J. Colloid Sci.*, **6**, 323 (1951).

Again there is a parallelism with exchange equilibria of quaternary ammonium cations.[14]

(d) **The Silver–Ethylene Diamine Complex.**—Here it appears that the complex is much more stable in the resin, the effect being far greater than one would expect from van der Waals attraction (by comparison with *n*-butylamine). As a possible explanation we suggest that in the resin the ion $H_2NCH_2CH_2NH_3^+$ (enH$^+$) coördinates with the silver ion to form a doubly charged complex. In dilute solution such a complex would be very unstable on account of electrostatic repulsions; in the resin, however, there will be a considerable screening effect due to the high concentration of negative charges. (For a 5 M solution of a uni-univalent salt, the Debye–Hückel parameter, $1/\kappa$, is 1.36 Å.; this gives us an order of magnitude for the screening effects.) The concentration of enH$^+$ in the resin, while small, is about ten thousand times as great as the concentration of uncharged en. It is not unreasonable to suppose, therefore, that most of the silver ions in the resin are coördinated with enH$^+$, and some of them

with en as well, giving complexes

$$H_2NCH_2CH_2NH_2 \longrightarrow Ag \longleftarrow H_2NCH_2CH_2NH_3^+$$
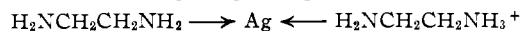
If this is so, there will be more enH$^+$, free and bound, in the resin than we thought there was, and therefore more millimoles of ionic ethylene diamine; the neutral en bound to Ag will then be less than we calculated, and the binding of neutral en to silver may be little, if any, stronger in the resin than in the solution. Unpublished data on coördination of silver with ethanolamine support this interpretation.

That a steric repulsion of en from the resin exists is seen from the effects of increasing en:Ag ratio and of increasing the silver concentration in the resin (Fig. 4).

Acknowledgments.—We thank the Permutit Company and the Rohm and Haas Company for providing the resins used. Thanks are also due to the University of Colorado Council on Research and Creative Work for assistance in starting this research, and to Leone Cockerell and Kaye L. Motz for help with the experimental work.

BOULDER, COLORADO

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY OF IOWA]

Hydrogen Ion Equilibria of Lysozyme[1,2]

BY CHARLES TANFORD AND MYRON L. WAGNER[3]

Hydrogen ion titration curves of crystalline lysozyme have been obtained at two temperatures and three ionic strengths. There are found present 10.5 carboxyl groups, 1 imidazole group, 1 α-amino group, 5 or 6 ϵ-amino groups, 3 phenolic groups and 12 or 11 guanidine groups, per molecule of assumed molecular weight 14,200. The intrinsic pK's of all basic nitrogen groups appear to be normal, the intrinsic pK of the phenolic groups is about 10.8 instead of the expected 9.6, and the titration of the carboxyl groups appears to be complicated and no pK values for these groups could be obtained. The empirical values of the electrostatic factor w obtained in the isoelectric region suggest that the lysozyme molecule is compact and sparingly hydrated.

Introduction

It was first shown in the pioneering study of Cannan, Kibrick and Palmer on ovalbumin[4] that the dissociation of hydrogen ions from proteins can often be explained on the basis of the intrinsic dissociation tendencies of the various types of dissociating groups, coupled with the electrostatic effect due to the charges present on protein molecules at any pH other than the isoelectric pH. The present paper represents a similar study on another protein crystallized from egg white, lysozyme.

The results of such a study serve to reveal abnormalities of any of the dissociating groups, and such are shown to exist in the phenolic and carboxyl groups of lysozyme. The study of hydrogen ion equilibria serves another purpose in that it is an indispensable prerequisite to an understanding of the pH-dependence of all properties of a protein molecule, including, in the case of lysozyme, its

physiological activity. The experimental and computational methods used have been reviewed in another paper[5] and will receive only brief discussion here.

Experimental

The lysozyme used in this work was lot 003L1, obtained from Armour and Co., Chicago, Ill. According to the manufacturer's assay the moisture content was 4.12%, ash (sulfated) 1.52%, nitrogen 18.3%. Electrophoretic measurements performed by Armour and Co., in phosphate buffer at pH 7.7 and ionic strength 0.2, showed two components with mobilities 1.82 (95%) and 0.60 (5%).[6]

Stock solutions were prepared as needed by dissolving the protein in water. To lower the amount of inorganic impurity indicated by the 1.52% ash content each stock solution was dialyzed twice against conductivity water in bags made from 36/32″ Visking dialysis tubing. There was probably some loss of protein in this procedure. The ash

(4) R. K. Cannan, A. C. Kibrick and A. H. Palmer, *Ann. N. Y. Acad. Sci.*, **41**, 243 (1941).

(5) C. Tanford, "Symposium on Electrochemistry in Biology and Medicine," in press.

(6) The occurrence of about 5% electrophoretic impurity in lysozyme is usual.[7] Tallan and Stein[7] suggest that it may be due to hydrolysis of some of the amide groups of lysozyme, in analogy with the two components of insulin, observed by Harfenist and Craig.[8] The mobilities here given are lower than those found at close to the same pH by Tallan and Stein, who, however, used barbiturate buffers instead of phosphate. Abnormally low mobilities in phosphate have been observed by Alderton, Ward and Fevold.[9]

(7) H. H. Tallan and W. H. Stein, *J. Biol. Chem.*, **200**, 507 (1953).

(8) E. J. Harfenist and L. C. Craig, THIS JOURNAL, **74**, 3083 (1952).

(9) G. Alderton, W. H. Ward and H. L. Fevold, *J. Biol. Chem.*, **157**, 43 (1945).