XXXI. THE POTENTIOMETRIC DETERMINATION OF POLYPEPTIDES AND AMINO-ACIDS

III. THE TITRATION OF AMINO-ACIDS AND PEPTIDES IN THE PRESENCE OF SUGARS

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IN a previous communication [Balson & Lawson, 1936] a potentiometric investigation of the action of formaldehyde on amino-acids was outlined. This investigation was continued employing other aldehydes, including glucose and other aldoses, without results of any particular significance being obtained. In view, however, of the recent publication of Frankel & Katchalsky [1937] we feel it desirable to publish some of these results.

The problem of the interaction between amino-acids and sugars has been studied by various workers. Borsook & Wasteneys [1925] found that some reaction occurs leading to a lowering of the NH_2 -nitrogen value as determined by the Van Slyke method. They also found that the diminution in NH_2 -nitrogen was increased with increasing alkalinity of the medium. Neuberg & Kobel [1925; 1926; 1927; 1928] studied the reaction by following the changes in optical rotation on mixing an amino-acid with a sugar, and found that only the reducing sugars were affected. Euler *et al.* [1926; 1928] employed the cryoscopic method of examination and were able to study the reaction quantitatively. They found, with increasing alkalinity, a progressive rise in the extent of combination, in agreement with the above authors, but no optimum at pH 8 as reported for the reaction between glycylglycine and glucose by Waldschmidt-Leitz & Rauchalles [1928]. Frankel & Katchalsky [1937] made a potentiometric investigation of the action of a number of sugars, together with formaldehyde and glyceraldehyde, on various amino-acids and peptides and concluded that there is a pH optimum for the reaction at about pH 7-8, in agreement with the results of Waldschmidt-Leitz & Rauchalles.

The experimental findings of Frankel & Katchalsky confirm our work, but the conclusions at which we arrived are much at variance with those they have published.

The effect of added sugar on the titration curves of amino-acids in general, is to bring these to a more acid level, but they are not of the same form as those for the titration of amino-acids in aqueous solution, and possess little or no inflection at the end-point. It can therefore be concluded that the titration constants which describe these curves are fairly close to one another and thus the pH values in the curves cannot be ascribed to the titration of any one acid group. Hence, in order to determine whether any significant change in the acid function of the amino-acid has occurred it is necessary to construct a titration curve in which the increment due to the acidity of the sugar has been eliminated.

In order to obtain such a curve, the procedure of Michaelis & Rona [1913] with slight modifications was adopted. An aqueous solution of the sugar and amino-acid was titrated with caustic soda and the titration curve plotted. An

equal volume of the same sugar solution together with a volume of water equal to the volume of the amino-acid solution added in the first titration, was then titrated with the alkali and the titration curve again plotted.

Shaffer & Urban [1932] have evaluated the titration constants of various sugars and have shown the presence of two weakly acidic groups with the possibility of a third extremely weak group. The form of our sugar curves is in agreement with these results, it being found impossible to describe the curves by means of a single titration constant. Since the complete analysis of the sugar curves was not required in the theoretical treatment below, the derivation of the titration constants was not further investigated.

In the amino-acid-sugar-alkali titration the electroneutrality equation always holds: i.e.

$$\text{Na}_1^+ = S' + A' + \text{OH}', \quad \text{.....(1)}$$

where S' = anion of sugar; A' = anion of amino-acid.

Similarly in the titration of the sugar alone with alkali,

$$\text{Na}_2^+ = S' + \text{OH}'. \quad \text{.....(2)}$$

Now at the same pH ,

$$\text{Na}_1^+ - \text{Na}_2^+ = \Delta\text{Na} = A',$$

and this is the equation for the titration of the amino-acid alone in the given solution, since if M = amount of amino-acid used, and K_a is its acid dissociation constant, then

$$K_a = \text{H}^+ \cdot A' / \text{HA} = \text{H}^+ \cdot A' / (M - A') = \text{H}^+ \cdot \Delta\text{Na} / (M - \Delta\text{Na}).$$

Accordingly, the differences between the NaOH additions at the same pH were plotted against pH , giving a curve in which the acidity due to the sugar had been eliminated. This was directly compared with the titration curve of the same amount of amino-acid in the same volume of water originally used.

It was found that this difference curve obtained in the above way was of the standard form for the titration of a weak acid by caustic soda, and was in some cases identical with, and never appreciably different from that obtained by direct titration of the amino-acid in water. The observed discrepancies did not exceed about 0.1 pK unit (5 mV.), and the method employed would normally be associated with an experimental error of this order. Moreover, it is unlikely that any change in junction potential resulting from the addition of sugar to the aqueous amino-acids would be greater than this value. Any reaction therefore, taking place between the two components in water produces no significant change in the acid function of the amino-acid. In other words the amino-acid-sugar complex, if formed, has a dissociation constant very nearly equal to that of the amino-acid itself.

The titration curves of Frankel & Katchalsky are therefore, in themselves, of no value for assessing the extent of reaction, since the observed pH changes on addition of sugar to an amino-acid are due solely to the acidic function of the sugar. The same defect is also apparent in the pH optimum curves of the above authors.

Another point in the paper of Frankel & Katchalsky is worthy of notice. A pH optimum curve is described for the interaction of formaldehyde and amino-acids, the curve exhibiting a sharp maximum at pH 7–8. This curve was obtained by plotting the pH change on addition of a standard quantity of formaldehyde to an amino-acid brought to a given initial pH , against this pH .

The form of this curve is directly at variance with the results of previous workers in this field, notably Harris [1924; 1929], Levy [1933], Tomiyama [1935], and Balson & Lawson [1936].

Since the titration curve of an amino-acid in formaldehyde is accurately represented by:

$$pK_a = \log (M - Na^+) / (Na^+) + pH,$$

where pK_a is the apparent dissociation constant of the amino-acid in the given formaldehyde solution, M is the amount of amino-acid employed, Na^+ is the amount of caustic soda added, and since, in water an equation of the same type holds, the two titration curves, in water and in aqueous formaldehyde, will run parallel with each other, the formaldehyde curve being generally at the more acid level. Further, for the same extent of neutralization in both water and formaldehyde, the difference in pH values, i.e. ΔpH , is clearly constant.

Thus for any amino-acid-alkali buffer the pH change on adding formaldehyde should be independent of the initial pH , provided that the latter is in the region of the titration curve covered by the above equations.

EXPERIMENTAL

All titrations were carried out at 25°, using the hydrogen electrode, and technique as described in a previous paper [Balson & Lawson, 1935]. The half cell employed was the normal calomel, E.M.F. 0.2792 V. at 25°. The amino-acids were mostly samples from B.D.H., the sucrose and glycine being of AnalaR purity.

(1) The system glycine-sucrose:

Run I. 1.01 ml. glycine (82.3 mg./10 ml.) and 10 ml. sucrose (42.8 g./100 ml.) titrated with 0.0841 *N* NaOH.

Run II. 10 ml. sucrose (42.8 g./100 ml.) and 1.01 ml. water titrated with 0.0841 *N* NaOH.

Run III. 1.01 ml. glycine (82.3 mg./10 ml.) and 10 ml. H₂O titrated with 0.0841 *N* NaOH.

Fig. 1. Glycine-sucrose.

Run I. —○— Glycine-sucrose mixture. Run II. —△— Sucrose.
 —×— Glycine in sucrose-calculated from I and II.
 Run III. —□— Glycine in water.

(2) The system leucylglycine-maltose:

Run I. 1.01 ml. leucylglycine (95.6 mg./5 ml.) and 10 ml. maltose (24.0 g./100 ml.) titrated with 0.0841 *N* NaOH.

Run II. 10 ml. maltose (24.0 g./100 ml.) and 1.01 ml. H₂O titrated with 0.0841 *N* NaOH.

Run III. 1.01 ml. leucylglycine (95.6 mg./5 ml.) and 10 ml. H₂O titrated with 0.0841 *N* NaOH.

(3) The system leucylglycine-glucose:

Run I. 1.01 ml. leucylglycine (95.6 mg./5 ml.) and 10 ml. glucose (40.9 g./100 ml.) titrated with 0.0841 *N* NaOH.

Run II. 10 ml. leucylglycine (95.6 mg./5 ml.) and 1.01 ml. H₂O titrated with 0.0841 *N* NaOH.

Run III. 1.01 ml. leucylglycine (95.6 mg./5 ml.) and 10 ml. H₂O titrated with 0.0841 *N* NaOH.

The observed values of *pH* and alkali increment are shown in Figs. 1, 2, 3.

Fig. 2.

Fig. 3.

Fig. 2. Leucylglycine-maltose.

Run I. —○— Leucylglycine-maltose mixture. Run II. —△— Maltose.
—×— Leucylglycine in maltose—calculated from I and II.

Run III. —□— Leucylglycine in water.

Fig. 3. Leucylglycine-glucose.

Run I. —○— Leucylglycine-glucose mixture. Run II. —△— Glucose.
—×— Leucylglycine in glucose—calculated from I and II.

Run III. —□— Leucylglycine in water.

SUMMARY

1. The observed changes in *pH* on adding a sugar to an amino-acid buffer are shown to be due to the acidic function of the sugar only. The reaction product must therefore possess an acid dissociation constant not far different from that of the amino-acid itself.

2. Since no significant changes in *pH* arise from the interaction, the electro-metric method cannot be employed as a means of studying the equilibrium.

3. The *pH* optimum curves obtained for the formaldehyde titration by Frankel & Katchalsky are shown to be inconsistent with the accepted theory.

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