118. INTERACTION OF ALDOSES WITH α -AMINO-ACIDS OR PEPTIDES

4. THE PERCENTAGE COMBINATION

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IN the previous papers of this series [Frankel & Katchalsky, 1937; 1938] evidence was put forward for the interaction of amino-acids and peptides with aldoses and for the concept that the pH depressions serve as a measure of the interaction. It has further been shown that at equilibrium the pH depressions are quantitatively related to the equilibrium constants of the reaction [Katchalsky, 1941]. But the pH depressions do not directly express the amount of the reaction product formed.

Previous authors have tried to measure the concentration of the reaction product by various methods, either during the reaction course [Borsook & Wasteneys, 1925; Waldschmidt-Leitz & Rauchalles, 1928; Shiga, 1937] or at equilibrium [Neuberg & Kobel, 1925; 1928; Euler & Brunius, 1928]. Their results are to some extent contradictory and cannot be combined to give a uniform picture.

In the present paper it will be shown that the amount of the reaction product formed can be calculated from the pH depressions during the reaction as well as at equilibrium. Furthermore, it is shown that the experimental data for the amount of combination obtained by the measurement of the diminution of free NH_2-N of the system are in a very satisfactory agreement with the results derived from potentiometric measurements.

Theoretical

Let us consider the reaction from its start to time t . For the initial hydrogen ion concentration the Henderson formula holds:

$$H_0 = K \frac{1-\alpha}{\alpha}, \quad \dots\dots(1)$$

where H_0 denotes the initial hydrogen ion concentration; K denotes the apparent acidic dissociation constant of the amino-acid or peptide, and α denotes the degree of ionization of the amino-component.

At time t the hydrogen ion concentration of the system has increased to H_t and the pH has decreased to the value pH_t , the pH depression being represented by $\Delta pH_t = pH_0 - pH_t$. A fraction of the anions of the amino-acid or peptide (R^-) has evidently combined with the glucose to give the reaction product RG^- . At time t the ratio of the ionized to the unionized part of the amino-acid or peptide is given by the expression

$$\frac{R^- + RG^-}{HR_t} = \frac{\alpha}{1-\alpha}. \quad \dots\dots(2)$$

It can safely be assumed that the reaction between glucose and the non-ionic amino group proceeds much more slowly than the attainment of equilibrium in

the ionic system $H_t^+ + R_t^- = HR_t$. The following equation (3) is therefore valid for any time t

$$\frac{H_t \cdot R_t^-}{HR_t} = K. \quad \text{.....(3)}$$

On introducing the value of HR_t from equation (3) in equation (2) we obtain

$$\frac{R_t^- + RG_t^-}{\frac{H_t \cdot R_t^-}{K}} = \frac{\alpha}{1 - \alpha},$$

$$\text{or} \quad H_t = K \frac{1 - \alpha}{\alpha} \frac{R_t^- + RG_t^-}{R_t^-}. \quad \text{.....(4)}$$

As the alkali concentration of the system does not change during the interaction, the concentration of the anions remains constant. Thus for a given pH , $\frac{1 - \alpha}{\alpha}$ does not change during the interaction and can therefore be introduced into equation (4) from equation (1).

$$H_t = H_0 \frac{R_t^- + RG_t^-}{R_t^-}, \quad \text{.....(5)}$$

$$\therefore \frac{RG_t^-}{R_t^-} = \frac{H_t}{H_0} - 1.$$

$\frac{H_t}{H_0}$ is the antilogarithm of the pH depression at time t , i.e.

$$\frac{H_t}{H_0} = \text{antilog } \Delta pH_t = A_t.^1$$

$$\text{Hence} \quad \frac{RG_t^-}{R_t^-} = A_t - 1 \quad \text{or} \quad R_t^- = \frac{RG_t^-}{A_t - 1}. \quad \text{.....(6)}$$

The known concentration of the amino-acid or peptide (c) is the sum of the concentrations of the undissociated molecules, of the anions and of the compound with glucose, i.e.

$$c = HR_t + R_t^- + RG_t^-. \quad \text{.....(7)}$$

Introducing the value of HR_t from equation (3) and of R_t^- from equation (6) we obtain

$$c = RG_t^- \left[\frac{1}{A_t - 1} \left(1 + \frac{H_t}{K} \right) + 1 \right], \quad \text{.....(8)}$$

$$\text{or} \quad \frac{RG_t^-}{c} = \frac{K(A_t - 1)}{K \cdot A_t + H_t}.$$

As $H_t = H_0 \cdot A_t$, equation (8) can be transformed into

$$\frac{RG_t^-}{c} = \frac{K}{K + H_0} \frac{A_t - 1}{A_t}. \quad \text{.....(9)}$$

$100 \frac{RG_t^-}{c}$ is the percentage (p_t) of the amino-acid or peptide combined with the glucose at time t .

$$100 \frac{RG_t^-}{c} = p_t = 100 \frac{K}{K + H_0} \frac{A_t - 1}{A_t}. \quad \text{.....(10)}$$

From equation (10) it is possible to calculate the amount of the reaction product at any time if the pH depression, the dissociation constant of the amino-acid or peptide and the initial pH are known.

At equilibrium A_t becomes the equilibrium value A which was shown [Katchalsky, 1941] to be $1 + L \cdot G$ (L denoting the equilibrium constant of

¹ $\Delta pH_t = pH_0 - pH_t = -\log H_0 + \log H_t = \log \frac{H_t}{H_0}$.

the reaction in question; G the concentration of glucose). Introducing the last expression into equation (10) we obtain the percentage of the amino-acid or peptide combined with glucose at equilibrium:

$$p = \frac{K}{K + H_0} \frac{L \cdot G}{1 + L \cdot G} 100. \quad \text{.....(11)}$$

The percentage of the reaction product present at equilibrium reaches a maximum at high initial pH when $H_0 \ll K$. The maximum can be deduced from equation (11) on neglecting H_0 in comparison with K

$$p_{\max.} = 100 \frac{L \cdot G}{1 + L \cdot G}. \quad \text{.....(12)}$$

Results

We checked our formulae deriving from the pH depressions the percentage combination of the amino-component with glucose by determining this amount by another independent method, i.e. estimation of the free amino groups by Van Slyke's volumetric method. The results of Shiga [1937] and others show that Van Slyke's method is suitable for our purpose. Previous workers have always worked in the presence of a buffer. Since the percentage combination of the amino-component depends on the pH , the addition of a buffer would affect considerably the results obtained. Accordingly all our experiments were made without the addition of any external buffer.

Fig. 1. Percentage combination at equilibrium of glycine or leucylglycine with glucose for various initial pH values.

- glycine (0.1 M) + glucose (M) calc. from potentiometric data.
- glycine (0.1 M) + glucose (M) measured by NH_2 -N diminution.
- leucylglycine (0.05 M) + glucose (M) calc. from potentiometric data.
- leucylglycine (0.05 M) + glucose (M) measured by NH_2 -N diminution.

Fig. 1 represents the percentage combination of glycine and leucylglycine with glucose at equilibrium for different initial pH . The experimental values of the NH_2 -depressions determined gasometrically were found to be on the curves calculated from the potentiometric data. The curves show that in order to obtain a fixed percentage of combination a higher pH is required in the case of the amino-acid than in that of the peptide. This is due to the difference in the

magnitudes of the apparent acidic dissociation constants (cf. equation (11)) of glycine ($K=2 \times 10^{-10}$) and leucylglycine ($K=6 \times 10^{-9}$). The different 'reactivities' of amino-acids and peptides, discussed already in the earliest papers dealing with the reaction in question, thus appear to be mainly due to the difference in the well-known dissociation constants.

Fig. 2. Course of pH depressions with time in the interaction of glycine (0.1 M) with glucose (M) for different initial pH values.

▽ Initial pH = 8.71.   □ Initial pH = 9.34.   ○ Initial pH = 9.57.

Fig. 3. Increase of percentage combination with time in the interaction of glycine (0.1 M) with glucose (M) for different initial pH values.

Initial pH 8.71; ▽ calc. from potentiometric data; ▽ measured by $\text{NH}_2\text{-N}$ decrease.
Initial pH 9.34; □ calc. from potentiometric data; ■ measured by $\text{NH}_2\text{-N}$ decrease.
Initial pH 9.57; ○ calc. from potentiometric data; ● measured by $\text{NH}_2\text{-N}$ decrease.

As indicated by equation (11) and verified experimentally the percentage combination is small at pH values lower than the isoelectric point of the amino-components, increases with increasing pH and attains maximal values at high alkalinities. These maximal values are independent of the dissociation constants (K) and thus show distinctly the differences in the equilibrium constants (L). The maximal values could not, however, be achieved without introducing large errors due to the decomposition of glucose discussed in previous papers.

In Figs. 2-5 the course of the interaction is followed by both experimental methods. Figs. 2 and 4 show the course of the pH depressions with time for

different initial pH values, while Figs. 3 and 5 show the corresponding increase in the percentage of the reaction product calculated from the potentiometric data and measured by Van Slyke's gasometric method. The agreement between the results of both methods is good throughout.

Fig. 4. Course of pH depressions with time in the interaction of leucylglycine (0.05 M) with glucose (M) for different initial pH values.

▽ Initial $pH=7.66$.   □ Initial $pH=7.99$.   ○ Initial $pH=8.70$.

Fig. 5. Increase of percentage combination with time in the interaction of leucylglycine (0.05 M) with glucose (M) for different initial pH values.

Initial pH 7.66; ▽ calc. from potentiometric data; ▼ measured by NH_2-N decrease.

Initial pH 7.99; □ calc. from potentiometric data; ■ measured by NH_2-N decrease.

Initial pH 8.70; ○ calc. from potentiometric data; ● measured by NH_2-N decrease.

The pH depressions in the reaction of glycine or leucylglycine with glucose reach a constant value dependent only upon the equilibrium constant of the reaction system (cf. Katchalsky [1941], $\Delta pH = \log(1 + L.G)$), although the time necessary for the achievement of equilibrium increases with increasing

initial pH . The percentage-time curves show on the other hand different final values, increasing with increasing initial pH , corresponding to the equilibrium data of Fig. 1.

We hope to discuss the kinetics of the interaction in more detail in a future paper.

Experimental

The potentiometric measurements were carried out exactly as described in the previous paper [Katchalsky, 1941].

The gasometric NH_2-N measurements were carried out in the micro Van Slyke apparatus for volumetric determinations. The reagents were those recommended by Kendrick & Hanke [1937]; Dunn & Porush [1939] have remarked that in the presence of iodine glucose would not interfere with the precision of the results.

All the materials used were twice recrystallized and analysed before use. Sterility was maintained during the preparation of the solutions as well as during the withdrawal of samples for the pH and NH_2-N determinations.

SUMMARY

1. It is shown that the amount of the reaction product formed from glucose and amino-acids or peptides can be calculated from the actual pH depressions.

2. The values calculated from potentiometric measurements were verified by measurements of NH_2-N diminution during the course of the reaction as well as at equilibrium.

3. The percentages of combination at equilibrium are small at pH values lower than the isoelectric point of the amino components, increase with increasing pH and attain maximal values at high alkalinities.

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