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

5. CONTINUOUS BACK TITRATION OF THE SYSTEM TO ITS INITIAL pH

BY MAX FRANKEL AND AARON KATCHALSKY

From the Hebrew University, Jerusalem

(Received 26 August 1941)

SHIGA [1938] re-examined the change in pH resulting from the combination of amino-acids or peptides with glucose by the method reported in the first paper of this series [Frankel & Katchalsky, 1937]. He confirms our experimental results but claims that 'the displacement in pH does not necessarily indicate the amount of acid produced and is greatly influenced by the buffering capacity of the solution'. Shiga comes to the conclusion that it is not the pH depression but the amount of alkali necessary to restore the pH to its initial value that represents the amount of combination. He found that in the back titration of glycylglycine and glucose at various pH values the required additions of alkali reached a maximum near pH 8, while no such maximum was detected in the case of glycine and glucose; he therefore came to the conclusion that the interactions in the two cases are of different types.

In the preceding paper [Frankel & Katchalsky, 1941] the amount of combination was calculated and verified experimentally. In this paper it will be shown that Shiga's interpretation of the results obtained in his back titration method is erroneous. A continuous back titration method has been used, in which the pH of the system is kept constant during the interaction by suitable additions of alkali till equilibrium is attained. The relation between the percentage combination of the NH_2 -bearing component with glucose and the total alkali added at any moment was considered. The amount of alkali added when equilibrium was reached was calculated from the pH of the particular experiment and the constants: K , the apparent acidic dissociation constant of the amino-component, and L , the equilibrium constant of the reaction system, as determined by Katchalsky [1941]. The agreement between the experimental and calculated values was very satisfactory throughout.

This quantitative treatment is supported by new experimental data.

Theoretical

Let us assume that the alkali contained in the reaction mixture of amino-acid or peptide with glucose at the start of the reaction is b , and the concentrations of the amino component and of glucose are c and G respectively. As glucose can be considered a non-electrolyte, the alkali concentration determines the hydrogen ion concentration of the system according to the equation

$$\text{H} = K \frac{c-b}{b}, \quad \dots\dots(1)$$

$$\text{or} \quad b = \frac{K \cdot c}{K + \text{H}}. \quad \dots\dots(2)$$

After a time interval t , anions of the complex RG^- (of conc. RG_t^-) are formed and in order to maintain the $p\text{H}$ at its initial value an amount of alkali Δb_t is to be added, such that the total alkali concentration is then given by $b + \Delta b_t$. The total concentration of the amino-acid or peptide is given by¹

$$\begin{aligned} c &= \text{HR}_t + \text{R}_t^- + \text{RG}_t^-, & \text{.....(3)} \\ \therefore c - \text{RG}_t^- &= \text{HR}_t + \text{R}_t^-. \end{aligned}$$

Assuming that equilibrium between the ions of the amino component is attained instantaneously, i.e. that $\frac{\text{H} \cdot \text{R}_t^-}{\text{HR}_t} = K$ is valid throughout the interaction, we obtain

$$\text{R}_t^- = \frac{K(c - \text{RG}_t^-)}{K + \text{H}}. \quad \text{.....(4)}$$

The condition of electroneutrality requires that

$$b + \Delta b_t = \text{R}_t^- + \text{RG}_t^-. \quad \text{.....(5)}$$

Introducing the value of b from equation (2) and of R_t^- from equation (4) we obtain

$$\frac{K \cdot c}{K + \text{H}} + \Delta b_t = \frac{K(c - \text{RG}_t^-)}{K + \text{H}} + \text{RG}_t^- = \frac{K \cdot c}{K + \text{H}} + \text{RG}_t^- \left(1 - \frac{K}{K + \text{H}}\right), \quad \text{.....(6)}$$

$$\Delta b_t = \text{RG}_t^- \frac{\text{H}}{K + \text{H}}. \quad \text{.....(7)}$$

Equation (7) represents the required expression for the relation between the amount of the complex present and the total alkali addition at any time during the reaction. It shows that the amount of alkali required depends not only on the amount of complex present but also on the hydrogen ion concentration of the system.

In the previous paper the expression $100 \frac{\text{RG}_t^-}{c}$ was defined as the percentage combination of the glucose with the amino-acid or peptide; this percentage is readily derived from the last expression and thus can be measured by the continuous back titration method.

$$p_t = 100 \frac{\text{RG}_t^-}{c} = 100 \frac{\Delta b_t}{c} \frac{K + \text{H}}{\text{H}}. \quad \text{.....(8)}$$

At equilibrium the percentage combination is represented by equation (8) of the previous paper, taking into account that H is the hydrogen ion concentration after equilibrium had been reached (as distinct from the initial hydrogen ion concentration appearing in equations (9) and (10) of that paper), i.e.

$$p = 100 \frac{K(A-1)}{\text{H} + K \cdot A} = 100 \frac{K \cdot L \cdot G}{\text{H} + K(1 + L \cdot G)},$$

comparing the last expression with equation (8) of the present paper we obtain the amount of alkali to be added until equilibrium, expressed by the dissociation constant of the amino-acid or peptide, by the equilibrium constant of the reaction system and the concentration of glucose

$$\frac{\Delta b}{c} = \frac{K \cdot L \cdot G}{\text{H} + K(1 + L \cdot G)} \frac{\text{H}}{K + \text{H}}. \quad \text{.....(9)}$$

¹ HR_t , R_t^- and RG_t^- represent the concentrations of the unionized (or zwitterionic) molecules of the amino-acid or peptide, the free anions of the amino-component and those combined with glucose respectively, at time t .

The curve representing $\frac{\Delta b}{c}$ as a function of pH (equation (9)) has a maximum. The maximum value of the alkali additions is found by differentiation to be given by

$$\left(\frac{\Delta b}{c}\right)_{\max.} = \frac{\sqrt{A}-1}{\sqrt{A}+1}. \quad \text{.....(10)}$$

The corresponding pH being

$$pH_{\max.} = pK - \frac{1}{2} \log A = pK - \frac{1}{2} \log (1 + L.G). \quad \text{.....(11)}$$

Results

The experimental verification of the equations deduced above was carried out as follows: solutions of glucose to which suitable indicators were added, were

Fig. 1. Additions of alkali ($\Delta b_i/c$) necessary to keep the solution mixtures at various initial pH values during the interaction of glycine ($0.1 M$) with glucose (M).

Fig. 2. Increase of percentage combination in the interaction between glycine ($0.1 M$) and glucose (M) for various initial pH values which are kept constant.

brought to pH 7, 8, 8.5, 9, 9.2, and 9.7 by the addition of alkali and diluted until the concentration was $2 M$; similarly solutions of glycine were brought to the

same pH values and diluted until the concentration was $0.2M$. 5 ml. of each glucose solution were withdrawn and mixed with 5 ml. of the glycine solution of equal pH . Thus solutions of different pH but having the same glucose concentration (M) and the same glycine concentration ($0.1M$) were obtained. The pH values, as measured colorimetrically, started to decrease with different velocities and suitable amounts of alkali were added from time to time throughout the course of the reaction to maintain the pH values at their initial levels. The values of $\frac{\Delta b_t}{c}$ obtained from the total amounts of alkali added and the calculated p_t values are represented in Figs. 1 and 2 respectively.

The colorimetric data indicate that the velocity constant was approximately proportional to the concentration of the hydrogen ions in the solution; or that the reaction velocity decreases with increasing pH .

The equilibrium data calculated according to equation (9), as well as the measured values, are represented in Fig. 3. The agreement of the experimental

Fig. 3. Total alkali additions necessary to keep the mixture solutions of glycine ($0.1M$) and glucose (M) at various initial pH values until equilibrium is reached.

values with the calculated curve is very good throughout. At very high alkalinities the quantity of alkali actually added was higher than the calculated value. This fact is explained by the decomposition of glucose already discussed in previous papers [cf. Frankel & Katchalsky, 1937].

A closer examination of Fig. 3 reveals the cause of the apparent difference in the 'reaction types' of the peptides and the amino-acids, described by Shiga [1938]. While the pH of the maximum alkali addition lies in the case of the peptides near pH 7.5–8 (e.g. glycylglycine: $pK=8.2$; $L=20.8$; $G=1$; $pH_{max.}=pK-\frac{1}{2}\log(1+L.G)=8.2-\frac{1}{2}\log 21.8=7.53$) it lies in the case of amino-acids near pH 9 (e.g. glycine: $pK=9.7$; $L=8.6$; $G=1$; $pH_{max.}=9.7-\frac{1}{2}\log(1+8.6)=9.2$). Shiga carried out his experiments only up to pH 9 and while observing the maximum of the $NaOH$ additions in the case of peptides he failed to reveal it in the case of amino-acids and believed the curve to increase steadily with increasing pH . Our measurements were extended over a larger pH range and the maximum appears in the case of glycine as distinctly as in the case of glycylglycine.

SUMMARY

1. It is shown that contrary to the interpretation of Shiga [1938] the alkali additions necessary to keep the pH of a mixture of solutions of glucose and glycine at its initial value, do not express directly the amount of reaction product.

2. The percentage combination was calculated from the total alkali added at any moment during the reaction and the hydrogen ion concentration of the system. The reaction velocity decreases with increasing pH .

3. The total alkali additions for various pH values, when equilibrium is reached, show a maximum.

4. Glycine requires a maximum of alkali additions near pH 9, while glycyglycine shows the maximum at pH 7.5–8. This fact is explained by the differences in the acidic dissociation constants of glycine and glycyglycine and by the different equilibrium constants of their interaction with glucose (cf. equation (11)). Shiga's view that the reactions of glycine and glycyglycine with glucose are of different types is therefore erroneous.

REFERENCES

- Frankel & Katchalsky (1937). *Biochem. J.* **31**, 1595.
— — (1941). *Biochem. J.* **35**, 1028.
Katchalsky (1941). *Biochem. J.* **35**, 1024.
Shiga (1938). *J. Biochem., Tokyo*, **27**, 307.