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

3. DETERMINATION OF EQUILIBRIUM CONSTANTS

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It has been shown in previous investigations [Frankel & Katchalsky, 1937; 1938] that the *p*H depressions occurring in mixtures of an aldose and an amino-acid or peptide in solution can be utilized to investigate the interaction between the amino and aldehyde groups. It is intended in the present paper to calculate the equilibrium constants of the system glucose + amino-acid or peptide from the measured *p*H depressions.

As pointed out previously [Frankel & Katchalsky, 1937], the reaction in question bears a close resemblance to the well investigated reaction between formaldehyde and amino-acids or peptides. It seemed probable that the reaction scheme developed for the formaldehyde titration [Levy, 1933; Balson & Lawson, 1936] would apply also to the reaction of aldoses with amino-acids or peptides. Measurements reported here fully confirmed this consideration.

The measurements were carried out potentiometrically on solution mixtures of different concentrations in the *p*H range 7–9. Within this *p*H range the reaction attains equilibrium in a relatively short time (3–48 hr.) and no interference of side reactions was observed.

The reaction could be described satisfactorily on the assumption that one molecule of glucose reacts with one molecule of amino-acid or peptide. In the range *p*H 7–9 a single constant is therefore sufficient to describe the equilibrium; at higher *p*H values, however, besides decomposition the occurrence of reactions of a higher order is not excluded. As according to the zwitterion theory only the anion of the amino-acid or peptide carries the unionized amino-group it is assumed that the interaction takes place between the anion of the amino-component and the undissociated glucose, and that the reaction product is completely dissociated and exists in solution only in the form of its anions.

Equilibrium constants of the following systems were determined: glucose and one of the following: glycine; glycylglycine; diglycylglycine; glycylalanine; glycylleucine; alanine; alanylglycine; alanylleucylglycine; leucine; leucylglycine; leucylglutamic acid; leucylphenylalanine; leucylglycylglycine.

On comparing the equilibrium constants of the reaction between glucose and various peptides it was found that the magnitude of the constant was mainly determined by the terminal amino-acid bearing the free amino group.

Theoretical

The reaction scheme used is similar to that of Levy [1933] and of Balson & Lawson [1936] for the formaldehyde titration. It is applied to the case of a bimolecular reaction and developed in the following way:

$$\mathrm{HR} \underset{}{\overset{K}{\rightleftharpoons}} \mathrm{R^-} + \mathrm{H^+} \qquad K = \frac{\mathrm{H^+}.\mathrm{R^-}}{\mathrm{HR}}$$

$$\begin{array}{c} + \\ \mathrm{G} \\ \uparrow\downarrow L \\ \mathrm{RG^-} \end{array} \qquad L = \frac{\mathrm{RG^-}}{\mathrm{R^-}.\mathrm{G}}$$

Where HR is the concentration of the zwitterionic form of the amino-acid or peptide ($^-OOC—R—NH_3^+$); R^- is the concentration of the anionic form ($^-OOC—R—NH_2$); G the total concentration of the glucose[1]; H the hydrogen ion concentration of the system; K the apparent acidic dissociation constant of the amino-acid or peptide, and L is the equilibrium constant of the reaction between glucose and the amino-acid or peptide.

Denoting the ionized part of the amino-acid or peptide by α, whether existing in the free state or combined with glucose, and the zwitterionic or undissociated part by $1-\alpha$, we obtain according to the reaction scheme:

$$\frac{\alpha}{1-\alpha}=\frac{R^-+RG^-}{HR}. \qquad \text{.....(1)}$$

Expressing the values of RG and HR by L, K, and R we obtain

$$\frac{\alpha}{1-\alpha}=\frac{R^-+L.R^-.G}{\frac{H}{K}R^-}=\frac{1+L.G}{\frac{H}{K}}, \qquad \text{.....(2)}$$

or

$$H=K\,(1+L.G)\,\frac{1-\alpha}{\alpha} \qquad \text{.....(3)}$$

introducing $pH=-\log H$ and $pK=-\log K$ we obtain

$$pH=pK-\log\,(1+L.G)+\log\frac{\alpha}{1-\alpha}. \qquad \text{.....(4)}$$

The last equation shows that as long as the concentration of the glucose is kept constant the reaction scheme adopted leads to equations similar in form to that of Henderson as modified by Hasselbalch [1916]. The apparent dissociation constant of the system involves the equilibrium constant (L) and the dissociation constant of the amino-acid or peptide (K).

The pH of a solution of the component amino-acid or peptide of equal concentration and containing the same amount of alkali as the reaction mixture will be according to Henderson-Hasselbalch's equation

$$pH'=pK+\log\frac{\alpha}{1-\alpha}. \qquad \text{.....(5)}$$

As the alkali concentrations both in the solution of the amino-acid or peptide and in the solution mixture with glucose are equal, it may be safely assumed that the degrees of ionization (α) in both systems are equal, i.e. $\frac{\alpha}{1-\alpha}$ appearing in equations (4) and (5) has the same value. From equations (4) and (5) we obtain calculated values for the pH depressions which can be verified experimentally.

$$pH'-pH=\Delta pH=\log\,(1+L.G) \qquad \text{.....(6)}$$

denoting the antilogarithm of the pH depression by A we obtain

$$A=\text{antilog}\,\Delta pH=1+L.G. \qquad \text{.....(7)}$$

Equation (7) provides an easy means of evaluating the equilibrium constants L from the known glucose concentrations and the measured pH depressions. The values of L obtained for different concentrations of glucose were sufficient to prove the validity of equation (7).

[1] Two simplifying assumptions, which seemed to be reasonable under the experimental conditions used, were made. (1) The glucose was considered as an ideal non-electrolyte in the pH range of the experiments. (2) Since the concentration of glucose was many times higher than that of the amino-component and RG was negligible in comparison with G, the concentration of *free* glucose (G) was considered equal to the *total* concentration of glucose.

Results

Table 1 illustrates the use of equation (7) for the calculation of the equilibrium constant in the reaction of glucose with diglycylglycine. The calculation for the other cases was carried out in a similar manner.

Table 1. *Interaction of diglycylglycine with glucose*

Initial *p*H 7·09. 23°

G Conc. of glucose *M*	*p*H at equilibrium	Δ*p*H	A Antilog Δ*p*H	*L* Equilibrium const.
0·1	6·61	0·48	3·02	20·2
0·3	6·25	0·84	6·92	19·7
0·5	6·05	1·04	10·96	19·9
0·7	5·90	1·19	15·49	20·7
0·9	5·80	1·29	19·50	20·5

$L = 20{\cdot}2$.

The equilibrium constants of the systems glucose + various amino-acids or peptides are listed in Table 2.

Table 2. *Equilibrium constants of the reaction of glucose with amino-acids or peptides*

Glycine and glycyl-peptides		Alanine and alanyl-peptides		Leucine and leucyl-peptides	
Substance	*L*	Substance	*L*	Substance	*L*
Glycine	8·6	Alanine	6·7	Leucine	4·2
Glycylglycine	20·8				
Glycylalanine	17·3	Alanylglycine	7·4		
Glycylleucine	21·8			Leucylglycine	5·8
Diglycylglycine	20·2	Alanylleucylglycine	8·6	Leucylglycylglycine	5·9

Somewhat remarkable are the high values of the equilibrium constants of glycine and glycyl-peptides. Table 2 indicates that as might be expected in the simple aliphatic peptides the equilibrium constant of the reaction with glucose, and correspondingly the *p*H depressions, are mainly determined by the terminal amino-acid bearing the free amino-group. Thus the differences in the *p*H depressions even measured colorimetrically [Frankel & Katchalsky, 1937] constitute a method of determining the terminal amino-acid in a simple peptide.

Some peptides containing an aromatic group (phenylalanine) or an additional carboxyl group do not conform to the above regularity as shown in Table 3.

Table 3

Substance	*L*
Leucylglycine	5·8
Leucylglutamic acid	7·3
Leucylphenylalanine	8·4

Experimental

The determination of the *p*H depressions as a function of the glucose concentration was carried out as follows. Two sterile stock solutions, one of amino-acid or peptide and one of glucose, considerably more concentrated, were prepared. The stock solution of the amino component was brought to the desired *p*H by suitable additions of NaOH. To the glucose no alkali additions were necessary as glucose is practically a non-electrolyte and therefore has no effect

on the *p*H. The necessary volumes of the stock solutions to give mixtures of the compositions amino-acid or peptide solution to glucose solution of 1 : 9, 3 : 7, 5 : 5, 7 : 3, 9 : 1, were withdrawn and mixed. As the amino-acid and peptide solutions are fairly strong buffers in the *p*H range used, the dilution by mixing had no appreciable effect on the initial *p*H. Thus a series of isohydric mixed solutions of different glucose concentrations was obtained. The mixed solutions were kept in sterile test tubes at 23° for 5–6 days. Samples of 0·5–1 ml. were withdrawn under sterile conditions from each solution and the *p*H determined by means of the hydrogen electrode. The first measurements were performed immediately after mixing and were continued for 3 hr., as during the first hours the *p*H changes were rapid and large. In general the values became constant after 25–48 hr., nevertheless the measurements were continued for 5–6 days.

A constant potential at the hydrogen electrode was achieved after 2–5 min. with 0·5–1 ml. of solution. The measurements made for several days after equilibrium was reached agreed within 1–2 mV. All the calculated constants are therefore liable to deviations within this range of error.

The glucose used throughout was 'glucose purissimum' of Merck. All the amino-acids were products of Hoffman-la-Roche; most peptides were prepared in this laboratory and the remainder obtained from la-Roche. All the products were recrystallized and their purity determined by analysis. The stock solutions were freed from micro-organisms by filtering through a purified Seitz filter and kept in tightly stoppered, sterile test tubes. During the withdrawal of samples precautions were taken to secure sterility.

Summary

1. The equilibrium constant of the interaction of glucose with amino-acids or peptides was calculated. The underlying assumption is that the reaction is bimolecular and proceeds between glucose and the anion of the amino component.

2. The values of the equilibrium constants obtained from potentiometric measurements within the *p*H range 7–9, show a satisfactory agreement for various concentrations.

3. In the case of peptides the magnitude of the equilibrium constants was found to be mainly determined by the terminal amino-acid of the peptide bearing the free amino-group.

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