KENETICS OF ALDOSE-AMINO ACID INTERACTION

by

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INTRODUCTION

The reaction of amino acids, peptides and proteins with aldoses is of interest for the understanding of a number of biological phenomena. It bears a resemblance to enzyme-substrate combination, as pointed out by PIGMAN[1], and may be of importance for the quantitative interpretation of transport phenomena in biological fluids. Its application to technological problems such as the browning reaction[2] should not detract from the insight which the study of this reaction under mild conditions gives into the labile equilibria which exist between cell constituents.

In order to ascertain the mechanism of the interaction, its kinetics under different experimental conditions was studied. These kinetics have not been investigated fully before, although the equilibria between various reactants have been studied extensively and the literature amply reviewed[3]. Some years ago FRANKEL AND KATCHALSKY[4] presented kinetic measurements of the approach to equilibrium. At about the same time KUBOTA[5] made an extensive investigation of this interaction using the disappearance of the amino groups, determined by VAN SLYKE's method, as an indication of the degree of interaction. Since VAN SLYKE's method is, in this case, not entirely reliable for a quantitative kinetic study, his data cannot be used for exact calculations. Before the completion of this investigation HAUGAARD, TUMERMAN AND SILVESTRI[6] advanced a new method for the determination of the kinetics of interaction by studying the rate of solution of a saturated amino acid in the presence of an aldose. Their method is restricted to slightly soluble amino acids and to a narrow range of concentration. Recent studies of glucosyl amines mainly by ISBELL AND FRUSH[7-8] and by PIGMAN *et al.*[9] clarify a number of important details in the mechanism of the reaction between amino and glycosidic groups.

The aim of the present paper is to describe a new kinetic method applicable to any soluble amino acid at a wide range of concentrations and at various pH values. The results obtained by this method are correlated by the theoretical scheme presented in the next paragraph.

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* From a thesis to be submitted to the Hebrew University, Jerusalem, by N. SHARON in partial fulfilment of the requirements for the Ph. D. degree.

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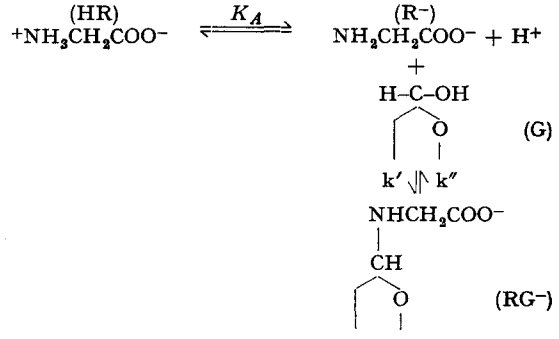
THEORETICAL

1. *Overall reaction*

It has been shown[10] that the reaction occurs between undissociated amino groups and aldoses. If the zwitterionic form of the amino acid or peptide be denoted by HR (*e.g.* $^+NH_3CH_2.COO^-$) it is only the negative ion R^- (*e.g.* $NH_2CH_2COO^-$) which can combine with the aldose G in aqueous solution. As the concentration of the negative ion depends on the equilibrium $HR \rightleftharpoons R^- + H^+$

$$\frac{H^+ \cdot R^-}{HR} = K_A \tag{1}$$

the extent of its reaction with aldoses will depend markedly on the pH. The overall reaction is represented by the scheme:



The overall rate equation is

$$\frac{dRG^-}{dt} = k' \cdot R^- \cdot G - k'' \cdot RG^- \tag{2}$$

and the equilibrium constant L

$$L = \frac{RG^-}{R^- \cdot G} = \frac{k'}{k''} \tag{3}$$

is independent of pH[10], while an apparent equilibrium constant found in the literature[11], based on the assumption of the interaction of all forms of the amino component, leads to a variable "constant", increasing with increasing pH.

This scheme does not represent any details of the reaction mechanism nor does it give any information about the dependence of the kinetic constants k' and k'' on the catalytic effect of the hydrogen and hydroxyl ions present.

2. *Reaction scheme*

Although the *extent* of reaction decreases with increasing hydrogen ion concentration (H^+) the *rate* of reaction, as will be shown, increases and in certain instances is almost linearly dependent on H^+. Various attempts by the authors to describe the reaction by a scheme depending on simple hydrogen ion catalysis failed, and it was found that a satisfactory explanation of all the experimental results could only be obtained by assuming dual catalysis by both hydrogen and hydroxyl ions. We found the scheme of ISBELL AND FRUSH[7] for the hydrolysis of glycosyl amines as determined by polarimetry very helpful. By simplifying part of their scheme and applying it to

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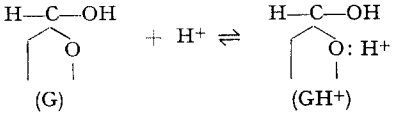
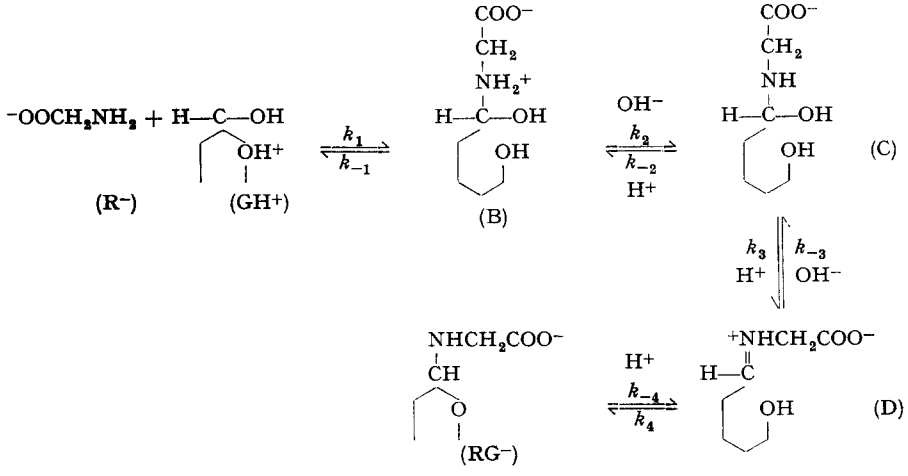
the case of the equilibrium reaction under consideration, we propose the following reaction mechanism:

The reacting species of the aldose is the positive oxonium ion. The concentration of this ion is governed by the equilibrium



$$K_G = \frac{GH^+}{G \cdot H^+} \tag{4}$$

The detailed scheme is:



According to this scheme the rate determining steps are:

a. The nucleophilic attack of the free amino groups of the negative ion R^- on the oxonium ion GH^+ with a subsequent opening of the pyranose ring in reaction 1.

b. The formation of the ion of SCHIFF's base by the ionisation of the amino groups of the reaction product RG^- with the opening of the ring in reaction −4.

The hydrogen ion concentration governs the concentration of the oxonium compound and hence the rate of formation of B, as well as the rate of decomposition of the "crossroad" compound C and of the final product RG^-. The hydroxyl ion, the second factor in the dual catalysis, governs the transition from both intermediate compounds B and D to the "crossroad" compound C.

A steady state is assumed, implying that the concentrations of the intermediate compounds B, C and D are constant during the major part of the process and that they are negligibly small as compared with those of the reactants and products of the reaction.

With these assumptions the reaction scheme leads to the following set of equations:

$$\frac{dB}{dt} = k_1.R^-.(GH^+) + k_{-2}.C.H^+ - k_{-1}.B - k_2.B.OH^- = 0 \tag{5}$$

$$\frac{dC}{dt} = k_2.B.OH^- + k_{-3}D.OH^- - k_{-2}.C.H^+ - k_3.C.H^+ = 0 \tag{6}$$

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$$\frac{dD}{dt} = k_3.\,C.\,H^+ + k_{-4}.\,RG^-.\,H^+ - k^-{}_3.\,D.OH^- - k_4.\,D = O \tag{7}$$

$$\frac{d(RG^-)}{dt} = k_4\,D - k_{-4}.\,RG^-.\,H^+ \tag{8}$$

with the equation $H^+.OH^- = K_W$ (9)

Equation (8) is the equation for the rate of reaction. The value of D can be evaluated from equations (5), (6) and (7). Here R^-, RG^-, B, C, D, H^+ and OH^- denote the concentrations and not the activities of the corresponding species. In a later publication the activity corrections, necessary for the interpretation of the salt effects in this reaction, will be included.

Solving the above kinetic equations we find for the overall rate (*cf.* eq. 2)

$$\frac{d(RG^-)}{dt} = k'.R^-.G - k''.RG^- = \frac{R^-.G}{k_\alpha + k_\beta\,OH^-} - \frac{RG^-}{L(k_\alpha + k_\beta\,OH^-)} \tag{10}$$

were

$$k_\alpha = \frac{k_{-1}.k_4(k_{-2} + k_3)}{k_1k_2k_3k_4.K_G.K_W};\quad k_\beta = \frac{k_{-1}k_{-2}k_{-3} + k_2k_3k_4}{k_1k_2k_3k_4.K_G.K_W} \tag{11}$$

and the equilibrium constant L is represented here by

$$L = \frac{k_1k_2k_3k_4K_G}{k_{-1}k_{-2}k_{-3}k_{-4}} = L_1L_2L_3L_4K_G = \frac{k'}{k''} \tag{12}$$

L_1, L_2, L_3 and L_4 being the equilibrium constants of reactions 1, 2, 3 and 4 respectively.

$$k' = \frac{1}{k_\alpha + k_\beta\,OH^-} \text{ and } k'' = \frac{1}{L}\cdot\frac{1}{k_\alpha + k_\beta\,OH^-} \tag{13}$$

Equation (13) gives the observed, pH dependent, rate constants of the forward and reverse reactions, as defined by equation (2).

3. *Method for the evaluation of rate constants*

The method employed here is based on the observation that the reaction of amino acids or peptides with aldoses is accompanied by an increase in the acidity of the solution. The reaction product is a stronger acid than the amino acid component and hence its formation causes a measurable drop in pH. In order to keep the pH constant alkali must be added continuously. This amount of alkali added is a convenient method for following the course of the reaction. Since the rate of the reaction is dependent on pH only, according to equation (10) constant values for the apparent rate constants k' and k'' should be obtained at a constant pH.

The method of evaluation of the rate constants from the amount of alkali added at a time t to the reaction mixture in order to keep the pH constant is as follows:

The alkali concentration necessary to bring the pH of the reaction mixture at time $t = 0$ to its initial value is denoted by b_0. After a time t alkali is added to keep the pH constant, thereby increasing the total alkali concentration in solution by Δb, so that the alkali concentration at time t is $b = b_0 + \Delta b$. The final concentration when equilibrium is attained at $t = \infty$ is b_∞.

Electroneutrality requires that the total positive charges be equal to the negative, *i.e.*

$$Na^+ + H^+ + GH^+ = R^- + C + RG^- + OH^- \tag{14}$$

Since by assumption the concentration of the intermediate products is very small,

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C and GH^+ may be neglected. The experimental conditions are so chosen as to make both H^+ and OH^- negligible in comparison with b_0 and b, also $b = Na^+$.

Hence, the last equation reduces to

$$b = R^- + RG^- \tag{15}$$

Another experimental simplification is obtained by making the aldose concentration much larger than that of the amino components so that the concentration G appearing in equation (10) may be considered as the total aldose concentration.

By introducing (15) into equation (2) the following is obtained

$$\frac{dRG^-}{dt} = k'.R^-.G - k''.RG^- = L.k''.(b - RG^-)G - k''.RG^- = k''.[L.b - RG^-(1 + L.G)] \tag{16}$$

Denoting the total concentration of the amino acid by c_A and neglecting the concentration of B, C and D as previously

$$c_A = HR + R^- + RG^- = HR + b$$
$$HR = c_A - b \tag{17}$$

As equation (1) holds during the reaction

$$K_A = \frac{H^+.R^-}{HR} = \frac{H^+.R^-}{c_A - b} \qquad R^- = \frac{K_A(c_A - b)}{H^+} \tag{18}$$

we get from equation (15)

$$RG^- = b - \frac{K_A}{H^+}(c_A - b) = b\,\frac{K_A + H^+}{H^+} - \frac{K_A.c_A}{H^+} \tag{19}$$

Now passing from the mixed equation (16) which depends on both RG^- and b to an equation relating only b—the alkali content at time t—with the apparent rate constants, we obtain.

$$\frac{dRG^-}{dt} = \frac{dRG^-}{db} \cdot \frac{db}{dt}. \tag{20}$$

As the process is carried out at constant pH and c_A is constant during each kinetic run, differentiation of (19) gives

$$\left(\frac{dRG^-}{db}\right)_{H^+} = \frac{K_A + H^+}{H^+} \quad \text{or} \quad \frac{dRG^-}{dt} = \frac{K_A + H^+}{H^+}\,\frac{db}{dt} \tag{21}$$

Introducing (19) and (21) into (16) and denoting the constant $1 + L.G$ by A we find,

$$\frac{db}{dt} = \frac{k''}{K_A + H^+}[K_A\,c_A.A - b\,(K_A.A + H^+)] \tag{22}$$

At equilibrium, when $b = b_\infty$, $\dfrac{db}{dt}$ equals zero, hence

$$b_\infty = \frac{K_A.c_A.A}{H^+ + K_A.A} \tag{23}$$

Similarly at the beginning of the reaction when $RG^- = 0$ and $b = b_0$ equations (15) and (18) yield

$$b_0 = \frac{K_A.c_A}{K_A + H^+}$$

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Introducing b_0 and b_∞ into (22) we get the simple expression

$$\frac{db}{dt} = k'' A \frac{b_0}{b_\infty} (b_\infty - b) \tag{24}$$

On integration from b_0 to b and from 0 to t equation (24) gives

$$k'' = \frac{b_\infty}{t.A.b_0} \ln \frac{b_\infty - b_0}{b_\infty - b} \tag{25}$$

and correspondingly

$$k' = \frac{L.b_\infty}{t.A.b_0} \ln \frac{b_\infty - b_0}{b_\infty - b} \tag{26}$$

As will be shown later equation (26) describes the experimental results closely and permits the evaluation of k' and k'' for every pH. After supplying a set of values of k' at different pH values, use is made of equation (13) to obtain the pH independent constants k_α and k_β.

Equation (13) may be written in the form

$$\frac{1}{k'} = k_\alpha + k_\beta \, OH^- \tag{27}$$

hence a plot of $1/k'$ versus OH^- should give a straight line with k_α as intercept and k_β as its slope.

EXPERIMENTAL

1. *Materials and solutions*

The materials investigated were the aldoses: D-glucose, galactose, lactose, D-xylose, D-arabinose and maltose; the amino acids: glycine, D-L-α-alanine, L-leucine, D-L-serine, D-L-valine, D-L-β-phenyl alanine, taurine, L-asparagine, β-alanine and δ-amino valeric acid*. All these substances were of analytical grade from commercial sources. Two peptides, leucylglycine and leucylglycylglycine, prepared in this Laboratory, were included. Stock solutions in twice distilled water were prepared and a trace of thymol added to each to ensure sterility. The concentration of the aldose stock solution was 2 M while the amino acid compounds were prepared in stock solutions of 0.1–0.4 M.

2. *Kinetic measurements*

10–25 ml of the amino acid or peptide stock solution were transferred to the reaction vessel and sufficient standard NaOH added to bring it to the required pH. This amount of alkali determines the initial value b_0. The reaction vessel was then dipped into a thermostat (accurate to $\pm$ 0.1° C). After 10–15 minutes the required amount of aldose solution, kept beforehand in the same thermostat, was added and the volume made up to 50 ml. The pH was measured at once with a Beckmann glass electrode reproducible to within $\pm$ 0.02 pH units. As soon as the pH starts to decrease, alkali is added from a microburette and the time of addition and amount of alkali noted. A series of preliminary experiments proved the measurements to be reproducible within $\pm$ 5 %. The total amount of alkali necessary to achieve equilibrium is b_∞. The alkali concentration was so chosen that the total amount added will not change the volume of the reaction mixture by more than 10 %.

3. *A sample experiment*

12.5 ml of 0.4 M glycine solution are transferred to the reaction vessel, 0.55 ml of 0.093 N NaOH solution added and filled up to 25 ml. After achieving thermal equilibrium 25 ml of 2 M glucose solutions are added. Hence $c_A = 0.1$ M, $G = 1$ M; initial pH was 7.55. The pH started to decrease almost immediately and alkali of the same concentration was added to keep it constant. Fig. 1 represents the plot of $\ln \frac{\Delta b_\infty}{\Delta b_\infty - \Delta b}$ versus time in minutes.

The degree of reproducibility may be deduced from the following Table I.

* δ-amino valeric acid was liberated from its hydrochloride by Ag_2O. From total analysis and NH_2-determination the free acid was analytically pure.

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TABLE I

THE REPRODUCIBILITY OF KINETIC MEASUREMENTS

Glucose 1.0 M + glycine 0.1 M, 25° C

pH	*k' Run 1*	*k' Run 2*
7.11	$6.20 \cdot 10^{-3}$	$6.11 \cdot 10^{-3}$
7.55	$2.37 \cdot 10^{-3}$	$2.42 \cdot 10^{-3}$
8.30	$0.524 \cdot 10^{-3}$	$0.502 \cdot 10^{-3}$

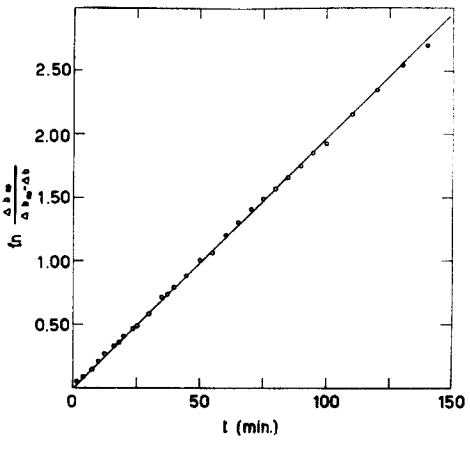

Fig. 1. Interaction of 1.0 M. Glucose with 0.1 M glycine at pH 7.55 ± 0.03 and 25° C. Alkali added at time $t = 0$ is $b_0 = 0.55$ ml of 0.093 N NaOH. Δb-alkali addition in ml (of the same hydroxide) at time t. Total alkali added at equilibrium is $b_\infty = b_0 + \Delta b_\infty = 4.25$ ml. Equilibrium constant of the reaction $L = 14.6$ liter mole^{-1} (Table IV); $A = 1 + L.G = 15.6$.

From the slope of the line $\tan \alpha = k' \frac{A.b_0}{L.b_\infty}$ (equation (26)) the rate constant is $k' = 2.37.10^{-3}$ liter mol^{-1} sec^{-1}.

RESULTS AND DISCUSSION

1. *Independence of rate constants from the concentration of reactant*

The mechanism proposed requires that the rate constants k' and k'' be independent of concentration. The following table proves that within a wide range the kinetic constants are insensitive to changes of concentration.

TABLE II

THE INDEPENDENCE OF k' OF CONCENTRATION (temp. 30°)

pH	*Glucose concn mol liter*$^{-1}$	*Glycine concn mol liter*$^{-1}$	*k', liter mol*$^{-1}$ *sec*$^{-1}$
7.63	1.0	0.10	$2.48 \cdot 10^{-3}$
	1.0	0.05	2.40
	0.50	0.10	2.56
	0.20	0.04	2.52 $\quad \bar{k}' = 2.49 \cdot 10^{-3}$
8.11	0.80	0.10	$1.10 \cdot 10^{-3}$
	0.40	0.04	1.02 $\quad \bar{k}' = 1.06 \cdot 10^{-3}$

2. *Dependence of the apparent rate constants on pH and evaluation of pH independent constants*

Table III represents a series of observed rate constants for the interaction of glucose with glycine at different pH values and at various temperatures.

In Fig. 2 the results of Table III are used for the evaluation of the pH independent constants k_α and k_β according to equation (27). The linearity of the plots of $1/k'$ versus OH^- proves the applicability of the proposed mechanism.

The measurements for all the amino compounds investigated in the interaction with glucose at 30° C are summarised in Fig. 3. In all cases the linearity of the plot was found to be satisfactory.

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TABLE III

OBSERVED RATE CONSTANTS FOR THE REACTION GLUCOSE-GLYCINE AT VARIOUS TEMPREATURES AND pH VALUES (k' in liter mole^{-1} sec^{-1})

		temp. 25° C			
pH	7.11	7.55	8.00	8.30	8.79
k'	$6.16 \cdot 10^{-3}$	$2.39 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$	$0.513 \cdot 10^{-3}$	$0.213 \cdot 10^{-3}$
		temp. 30° C			
pH		7.63	8.11		
k'		$2.49 \cdot 10^{-3}$	$1.06 \cdot 10^{-3}$		
		temp. 35° C			
pH		7.30	7.95	8.52	
k'		$7.09 \cdot 10^{-3}$	$1.78 \cdot 10^{-3}$	$0.64 \cdot 10^{-3}$	
		temp. 45° C			
pH	7.15	7.60	7.70	8.18	8.44
k'	$17.7 \cdot 10^{-3}$	$6.33 \cdot 10^{-3}$	$5.0 \cdot 10^{-3}$	$2.02 \cdot 10^{-3}$	$1.53 \cdot 10^{-3}$

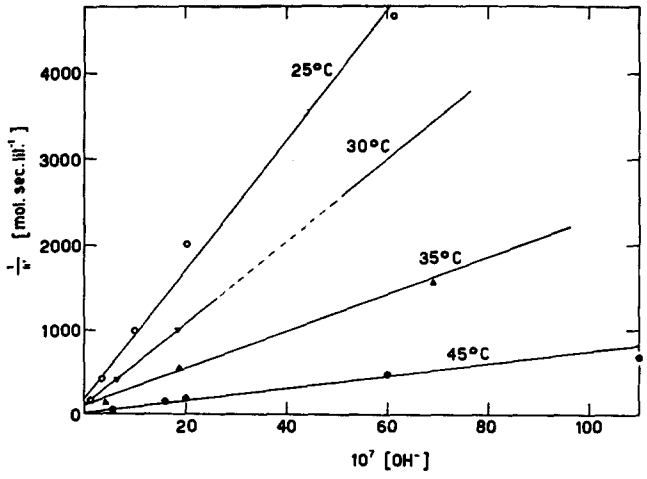

Fig. 2. The dependence of the reciprocal of the observed rate constant (k') on OH^- ion concentration. $1/k' = k_\alpha + k_\beta . OH^-$ for the reaction of glucose and glycine at different temperatures.

The pH independent rate constants k_α and k_β are represented in Table IV, together with the equilibrium constants L calculated from b_∞ according to equation (23). The volume change by alkali addition has been accounted for in calculating L.

The data from which the k_α and k_β in the interaction of glycine with various aldoses have been derived are given in Fig. 4.

Table V gives the pH independent rate constants of the interaction of various aldoses with glycine.

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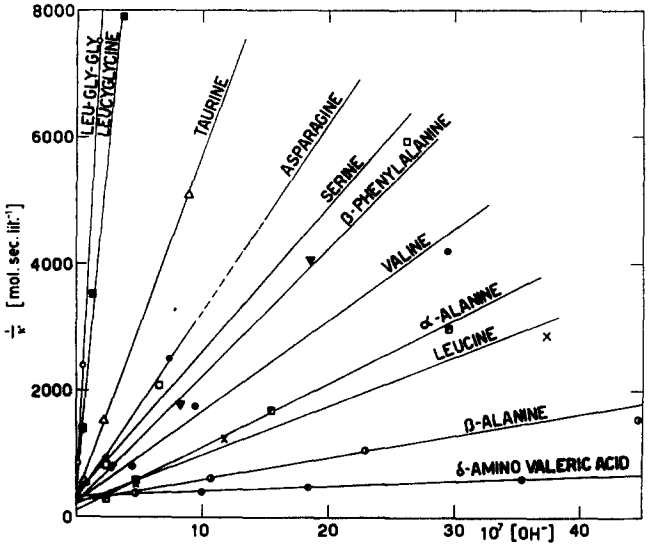

Fig. 3. The dependence of the reciprocal of the observed rate constants (k') on OH^- ion conc. $1/k' = k_\alpha + k_\beta . OH^-$ for the reaction of glucose with the following amino acids and peptides:

Leucylglycylglycine	○	Valine	◎
Leucylglycine	■	α-alanine	◪
Taurine	△	Leucine	×
Asparagine	●	β-alanine	◑
Serine	□	δ-aminovaleric acid	◉
β-Phenylalanine	▼		

temp. 30° C

TABLE IV

THE pH INDEPENDENT RATE CONSTANTS k_α AND k_β AND EQUILIBRIUM CONSTANTS L FOR THE INTERACTION OF GLUCOSE WITH AMINO COMPONENTS AT 30° C

pK_A-NEGATIVE LOGARITHM OF THE ACIDIC DISSOCIATION CONSTANT OF THE AMMONIUM GROUP

	k_α	k_β	pK_A	L
1. leucylglycylglycine	240	390.10^8	7.70	6.4
2. leucylglycine	450	212	7.70 a	3.16
3. taurine	210	55.8	8.62 c	8.9
4. L-asparagine	210	30.6	8.67 c	4.5
5. D-L-serine	390	23.7	9.02 c	5.37
6. DL-β-phenylalanine	240	20.1	9.11 c	6.6
7. DL-valine	300	14.2	9.592 b	5.5
8. DL-α-alanine	120	9.95	9.738 b	6.9
9. L-leucine	210	8.1	9.619 b	7.7
10. glycine	120	4.8	9.651 b	14.6
11. β-alanine	270	3.06	10.06 c	15.1
12. δ-aminovaleric acid	360	0.70	10.64 c	18.0

References (for pK_A)

a. J. TILLMANS *et al.*, *Biochem. Z.*, 199 (1928) 399.
b. E. J. COHN AND J. EDSALL, *Proteins, Amino Acids and Peptides*, ACS monographs 90 (1943) p. 79.
c. E. J. COHN AND J. EDSALL, *ibid.* p. 84 extrapolated from values for 25° C to 30° C.

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TABLE V

THE pH INDEPENDENT RATE CONSTANTS k_α AND k_β AND THE EQUILIBRIUM CONSTANTS L FOR THE INTERACTION OF VARIOUS ALDOSES WITH GLYCINE (temp. 30° C)

	k_α	k_β	L
1. Maltose	36	$75.6 \cdot 10^7$	22
2. D-glucose	120	48	14.6
3. Lactose	72	33.6	23
4. Galactose	30	9.0	17.4
5. D-xylose	24	6.94	22.3
6. D-Arabinose	6.9	4.14	22.3

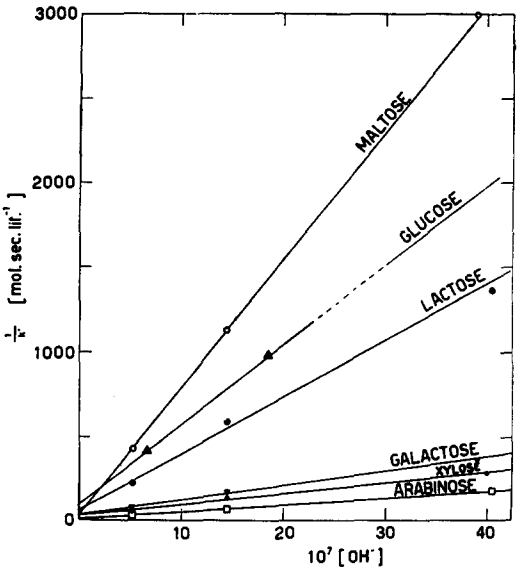

Fig. 4. The dependence of the reciprocal of the observed rate constants (k') on the OH^- ion concentration $1/k' = k_\alpha + k_\beta . OH^-$ for the reaction of glycine with the following aldoses:

Maltose	○	Galactose	▽
Glucose	▲	Xylose	●
Lactose	◉	Arabinose	□

temp. 30° C

3. *Rate constants and constitution of reactants*

The rate constant k_α as obtained from the extrapolation to zero OH^- concentration is not very exact. Without independent data on the constituent rate constants its theoretical interpretation is complicated. On the other hand k_β—the slope of the lines in Figs. 2, 3 and 4 is—more accurate and may be interpretated as follows: From equation (11)

$$k_\beta = \frac{k_{-1}.k_{-2}.k_{-3} + k_2.k_3.k_4}{k_1 k_2 k_3 k_4 . K_G . K_W} = \frac{1}{k_{-4}.L.K_W} + \frac{1}{k_1 K_W} = \frac{1}{K_W}\left(\frac{1}{k_1} + \frac{1}{L k_{-4}}\right) \qquad (28)$$

According to ISBELL AND FRUSH[7] the formation of SCHIFF's base in reaction −4 from the glycosyl amine is rapid. It may be assumed by analogy that the nucleophilic attack of the amino acid on the glucosidic ion in reaction 1 is a slower process than the first step of the hydrolytic reaction −4. *i.e.* $k_1 < k_{-4}$. It is clear from Table IV that L is always larger than 1, and hence it can be assumed that $k_1 \ll L.k_{-4}$.

Therefore in equation (28)

$$\frac{1}{k_1} \gg \frac{1}{L.k_{-4}} \qquad i.e.$$

$$k_\beta \simeq \frac{1}{K_W . k_1}$$

$$\text{or} \quad k_1 = \frac{1}{K_W . k_\beta} \qquad (29)$$

$$\log k_1 = pK_W - \log k_\beta \qquad (30)$$

With these assumptions k_β is directly related to k_1 and gives useful information about the nucleophilic reaction between the amino acid and the glycosidic carbon atom. The approximate value of the true rate constant k_1 of the primary reaction can be correlated with other nucleophilic properties of the nitrogen of the amino components.

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According to HAMMETT[12], a linear dependence should exist between the logarithms of the hydrolytic rate constants and the acidic dissociation constant of the reactants. A plot of the logarithms of k_1 versus pK_A is given in Fig. 5. The figure shows that as expected, the increasing proton affinity is bound with an increasing rate of attack of the amino group on the glycosidic carbon, in accordance with HAMMETT's relation.

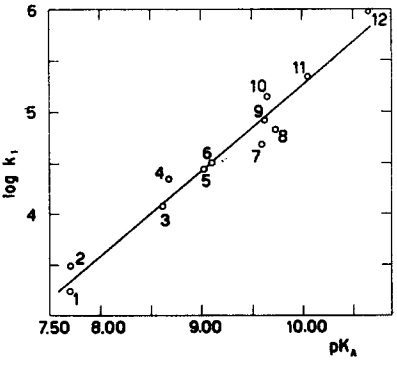

Fig. 5. Comparison of interaction rates (log k_1) of various amino acids and peptides and glucose with ionization constants (pK_A) of their ammonium groups. The enumeration of the amino compounds according to Table IV. Both log k_1 and pK_A are for 30° C. log k_1 calculated by equation (30).

The other contributing factor to the magnitude of k_1 is the stability of the pyranose ring. According to Table V the interaction of glycine with different aldoses shows that k_1 increases in the order: arabinose > xylose > galactose > lactose > glucose > maltose. STEPANENKO AND SERDYUK[13] give the following order for the amount of open aldehydic forms in various aldoses: arabinose > xylose > galactose > glucose > maltose > lactose. These observations indicate that our results may be correlated with the ease of opening of the pyranose ring.

4. *Temperature dependence*

Further information about the nature of the interaction may be derived from the energy of activation. Assuming k_β to be related to k_1 according to equation (30) we obtain from the dependence of k_β on temperature the energy of activation of the primary step of the glucose amino acid interaction. Fig. 6 gives a plot of $\log k_1 = pK_W - \log k_\beta$ versus $1/T$. The values of pK_W at the respective temperatures were taken from HARNED AND OWEN[14] while the k_β's are experimental constants. The energy of activation E as calculated from Fig. 6 and the frequency factors Z, for the ARRHENIUS equation $k_1 = Z \cdot e^{-E/RT}$ are given in the following Table VI.

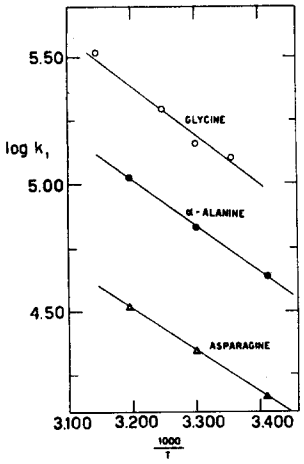

Fig. 6. Dependence of k_1 on temperature in the reaction of glucose with glycine, α-alanine and asparagine.

TABLE VI

ENERGIES OF ACTIVATION E AND FREQUENCY FACTORS Z FOR THE REACTION OF GLUCOSE WITH ASPARAGINE, α-ALANINE AND GLYCINE

	Z lit mol^{-1} sec^{-1}	E cal mol^{-1} deg^{-1}
Glucose-asparagine	$0.083 \cdot 10^{11}$	7700
Glucose-α-alanine	$0.69 \cdot 10^{11}$	8300
Glucose-glycine	$3.3 \cdot 10^{11}$	8800

The calculated energies are seen to vary between 7.7–8.8 kcal mol^{-1} deg^{-1}

The authors wish to thank Dr J. BABAD, Head of the Dairy Research Laboratory, for providing them with part of the facilities for this work.

References p. 301.

SUMMARY

1. The kinetics of the reaction between various aldoses and amino acids and peptides has been investigated.

2. A method for the determination of rate constants, based on the addition of alkali to keep the pH of the reaction mixture constant, has been developed.

3. The velocity of the reaction decreases with increase of pH according to the equation

$$\frac{dRG^-}{dt} = \frac{R^-.G}{k_\alpha + k_\beta.OH^-} - \frac{RG^-}{(k_\alpha + k_\beta.OH^-)L}$$

where RG^- is the concentration of the reaction product, R^- —that of the amino acid anion, G—of the aldose; OH^- —the hydroxyl ion concentration and k_α, k_β pH independent rate constants.

4. The rate constant k_β is inversely proportional to the rate constant of the primary attack of the amino acid on the aldose. The rate of the primary reaction increases with the basicity of the amino component in accordance with the HAMMETT relation.

RÉSUMÉ

1. La cinétique de la réaction entre différentes aldoses, des acides aminés et des peptides a été étudiée.

2. Une méthode basée sur l'addition d'alkali maintenant le pH de la réaction constante, pour la détermination des constantes de vitesse, a été développée.

3. La vitesse de la réaction décroit quand le pH augmente selon l'équation

$$\frac{dRG^-}{dt} = \frac{R^-.G}{k_\alpha + k_\beta.OH^-} - \frac{RG^-}{(k_\alpha + k_\beta.OH^-)L}$$

ou RG^- est la concentration du produit de réaction, R^- —celui de l'anion de l'acide aminé, G—de l'aldose; OH^- —la concentration de l'ion hydroxyl et k_α, k_β des constantes indépendantes du pH

4. La constante k_β est inversement proportionnelle à la constante de l'attaque primaire de l'acide aminé sur l'aldose. La vitesse de la réaction primaire augmente avec la basicité du composé aminé selon la formule de HAMMETT.

ZUSAMMENFASSUNG

1. Die Reaktionskinetik der Einwirkung verschiedener Aldosen auf Aminosäuren und Peptide wurde untersucht.

2. Die in dieser Arbeit entwickelte Methode zur Bestimmung der Reaktionskonstanten beruht auf der Konstanthaltung des pH des Reaktionsgemisches durch Hinzufügung von Alkali.

3. Die Reaktionsgeschwindigkeit fällt mit austeigendem pH gemäss der Beziehung

$$\frac{dRG^-}{dt} = \frac{R^-.G}{k_\alpha + k_\beta.OH^-} - \frac{RG^-}{(k_\alpha + k_\beta.OH^-)L}$$

wobei die folgenden Konzentrationen dargestellt sind: RG^- — Reaktionsprodukt, R^- — Anion der Aminosäure, G — Aldose, OH^- — Hydroxylion, k_α und k_β sind von pH unabhängige Reaktionskonstanten.

4. Die Geschwindigkeitskonstante k_β ist der Konstante der Initialreaktion zwischen der Aminosäure und der Aldose umgekehrt proportional. Die Geschwindigkeit dieser Anfangsreaktion wächst mit der Alkalinität der Aminogruppe in Übereinstimmung mit der HAMMETT'schen Beziehung.

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Received July 2nd, 1952