CC. THE INTERACTION OF α-AMINO-ACIDS AND PEPTIDES WITH SUGARS IN AQUEOUS SOLUTION

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The question of the interaction of sugars with amino-acids and polypeptides is important both to the chemist and physiologist. It has therefore been the subject of several investigations.

On the basis of experiments with protein hydrolysates or amino-acids and glucose, Kostytchew & Brilliant [1923] were the first to conclude that in slightly alkaline aqueous solutions at 55° a reaction occurs which leads to a diminution of the detectable NH_2-nitrogen.

Borsook & Wasteneys [1925], working at 37°, and employing a more exact technique (NH_2-nitrogen determinations according to Van Slyke) confirmed the findings of the above authors. They also found that the reaction, as measured by the NH_2-nitrogen diminution is increased with increasing alkalinity of the medium.

Neuberg & Kobel [1925; 1926; 1927, 1, 2; 1928] were the first to use a static method for investigating the reaction in question. Their method consisted in measuring the changes in the optical rotation. They found that on adding an amino-acid to fructose an immediate change in the optical rotation occurs, whereas with glucose no such change appears.

Euler and co-workers [1926; 1928] used the cryoscopic method and were the first to study the reaction quantitatively. For their discussion of possible errors involved in the technique of the other authors the reader should refer to the 1928 paper. In accordance with previous findings of other investigators they found that, with increasing *p*H, the reaction takes place to an increasing degree. The contrary results reported by Waldschmidt-Leitz & Rauchalles [1928], who found a reaction optimum at *p*H 8 in the case of glycylglycine and glucose, are explained by assuming that *p*H 8 forms a velocity optimum but not an equilibrium optimum of the reaction.

All these findings seem to indicate an additive combination of the two components. There are also references in the literature, mainly of recent date, to a reaction between the two components which seems to be of a quite different type. Borsook & Wasteneys [1925] had observed that mixed solutions of glucose and glycine acquired the property of reducing methylene blue. Watanabe [1932] found that in solution mixtures of glucose and amino-acids at a high alkalinity and a temperature of 100° decomposition takes place leading to the formation of strongly reducing products. These findings were confirmed and extended by Akabori [1933] working at 37° as well as at higher temperatures.

On reviewing these results it becomes clear that there exists evidence of an interaction between amino-acids and aldoses in mixed solution. But fundamental differences exist in the findings of the various authors. Thus most of them are of the opinion that with simple amino-acids and sugars the reaction only

occurs at an alkaline reaction, and that with increasing *p*H it proceeds without showing a *p*H optimum. On the other hand Waldschmidt-Leitz & Rauchalles [1928] found a *p*H optimum in the only case investigated by them, that of glycylglycine and glucose. This discrepancy in the results makes it probable that different types of reaction between the amino-acids and sugars occur. Perhaps under different experimental conditions and a different technique different parts or the totality of the chemical interactions are measured.

The present investigation was undertaken in the hope of clarifying the position. It was to be expected that under mild reaction conditions the interaction between amino-acids and sugars in solution would be confined to a reaction between the amino-group of the acid and the aldehyde group of the sugar, these being the prominently reactive groups of the two components. It was ascertained that only aldoses react with amino-acids under the conditions of our experiments. A decrease in *p*H following the combination of the basic NH_2-group of the amino-acid and the aldehydic group of the sugar was to be expected, and was indeed subsequently found. By the use of this fact it was possible to develop a potentiometric method of measurement, similar to that used by various authors [Harris, 1924; 1929; Levy, 1933; 1934; 1935; Balson & Lawson, 1936; Tomiyama, 1935] in the theoretical investigation of Sørensen's form-aldehyde titration.

Method

The interaction of the amino-group of the α-amino-acid and the aldehyde group of the sugar is marked by a decrease in the *p*H, since in this reaction the basic group of the amino-acid disappears. The extent of the decrease in the *p*H would be expected to constitute a quantitative indicator of the extent of the reaction. This consideration is the common basis for all the forms of the method used.

(1) *Comparative titration of* (*a*) *mixtures of the two components and* (*b*) *the individual amino-acids by NaOH.* The standard curve was obtained by electrometric titration of the amino-acid with NaOH. The second curve was constructed on the basis of a titration of a mixture of sugar and amino-acid, in the same concentration as in the standard solutions, with NaOH. The difference in *p*H between the amino-acid curve and the mixture curve at equal additions of NaOH can be accounted for by the reaction. Special experiments have shown that only negligible amounts of the NaOH reacted with the sugars in question under the experimental conditions maintained (*p*H 5–11) [cf. Urban & Shaffer, 1932].

(2) *Mutual titrations.* Each component was separately dissolved, both were brought to the same *p*H and one component was titrated by the other. This method permits one to follow the *p*H changes within a wide range of concentration starting at the same *p*H.

In both (1) and (2) the potentiometric technique was used.

(3) *Colorimetric method.* In certain cases a colorimetric method for detecting the *p*H changes was used. This method is sometimes advantageous since the interaction between the amino-acids and sugars in aqueous solution proceeds over a relatively long time interval, and it may be more convenient in this method to allow the mixtures and appropriate controls, after adding the indicators, to stand till the reaction is finished.

For exact quantitative determinations potentiometric methods must be relied on.

Results

With all combinations of amino-acids and aldoses tested, we found that under suitable *p*H conditions a reaction takes place. We investigated (1) amino-acids and mono-aldoses, (2) amino-acids and aldo-disaccharides, (3) some peptides and mono-aldoses, (4) some peptides and aldo-disaccharides.

(1) To test the assumption that the interaction between sugars and amino-acids depends upon the presence of an aldehydic group in the sugar molecule, glycine or alanine as representatives of α-amino-acids, and fructose, sucrose or raffinose for non-aldehydic sugars and mannitol were investigated; no indication of a reaction, of the kind characterized above, could be found by colorimetric or potentiometric measurements. The curves obtained from the

Table I. *Potentiometric titration of amino-acids and non-aldehydic sugars in mixed solution and of amino-acids alone at equal concentrations, against* N/*10 NaOH at 17°*

Additions of NaOH in ml.	5 ml. glycine $M/2+5$ ml. H_2O *p*H	5 ml. glycine $M/2+5$ ml. sucrose $M/1$ *p*H	5 ml. glycine $M/2+5$ ml. fructose $M/1$ *p*H	5 ml. alanine $M/2+5$ ml. H_2O *p*H	5 ml. alanine $M/2+5$ ml. sucrose $M/1$ *p*H	5 ml. alanine $M/2+5$ ml. fructose $M/1$ *p*H
0·0	6·29	6·29	6·29	6·29	6·29	6·29
0·5	8·07	8·07	8·07	8·07	8·04	8·04
1·5	8·59	8·58	8·58	8·60	8·56	8·56
3·5	8·98	8·98	8·96	8·98	8·96	8·96
5·5	9·19	9·19	9·19	9·20	9·19	9·19
8·5	9·46	9·46	9·46	9·46	9·46	9·46

data of the titration of the binary mixtures with NaOH were identical with those of the amino-acids themselves. On the other hand, the aldo-disaccharides maltose and lactose gave a definite reaction. It is thus clear that the presence of an aldehydic group in the sugar is essential for the reactions.

(2) The α-amino-acids glycine, *dl*-alanine, *dl*-valine, *dl*-leucine, *dl*-asparagine, *d*-glutamic acid and *l*-aspartic acid were tested in solution with either glucose, mannose or galactose for the appearance of the interaction. In all cases a distinct reaction was observed in aqueous solutions, although quantitative differences exist. In principle the same results were obtained with the dipeptides, glycyl-glycine, glycylleucine and leucylglycine. Here also quantitative differences seem to exist both between the peptides themselves and between peptides and amino-acids. These points are under special investigation as is also the behaviour of the basic amino-acids. Most of the results will be summarized in connexion with other points to be discussed later. Table II, containing the results obtained with *dl*-valine and H_2O, sucrose, glucose and mannose, presents a typical example of the mutual titration technique.

Table II. *Mutual potentiometric titrations of* dl-*valine with* H_2O, d-*glucose*, d-*mannose and sucrose at 24°*

Additions of either H_2O or sugars to 5 ml. *dl*-valine $M/4$ in ml.	$+H_2O$ *p*H	+glucose $M/1$ *p*H	+mannose $M/2$ *p*H	+sucrose $M/1$ *p*H
0·0	7·58	7·58	7·58	7·58
1·0	7·60	7·52	7·48	7·60
2·0	7·60	7·43	7·38	7·60
4·0	7·60	7·31	7·30	7·60
6·0	7·60	7·23	7·24	7·61

(3) In order to compare the reactivity of formaldehyde and different sugars towards amino-acids, the reaction between α-*dl*-alanine and formaldehyde, glyceraldehyde, *l*-arabinose, *d*-glucose, *d*-galactose and lactose respectively was investigated in a series of experiments. The results are presented in Table III

Table III. *Potentiometric titrations of mixture solutions containing* dl-*alanine and either* H_2O, *formaldehyde, glyceraldehyde,* l-*arabinose,* d-*glucose or lactose against* N/*10 NaOH at 24°*

Additions of *N*/10 NaOH in ml.	5 ml. alanine *M*/4 + 5 ml. H_2O *p*H	5 ml. alanine *M*/4 + 5 ml. formaldehyde *M*/4 *p*H	5 ml. alanine *M*/4 + 5 ml. glyceraldehyde *M*/4 *p*H	5 ml. alanine *M*/4 + 5 ml. arabinose *M*/4 *p*H	5 ml. alanine *M*/4 + 5 ml. glucose *M*/4 *p*H	5 ml. alanine *M*/4 + 5 ml. lactose *M*/4 *p*H
0·0	6·26	5·67	5·65	6·17	6·11	6·21
0·5	8·27	7·24	7·61	8·08	8·13	8·21
1·0	8·62	7·63	7·93	8·40	8·48	8·57
2·0	8·96	8·04	8·16	8·77	8·82	8·91
3·0	9·15	8·33	8·57	8·94	8·98	9·10
5·0	9·46	8·74	8·94	9·32	9·32	9·42
7·0	9·73	9·12	9·25	9·61	9·61	9·68
9·0	10·01	9·47	9·52	9·88	9·91	9·95
12·0	10·61	10·23	10·32	10·47	10·47	10·49

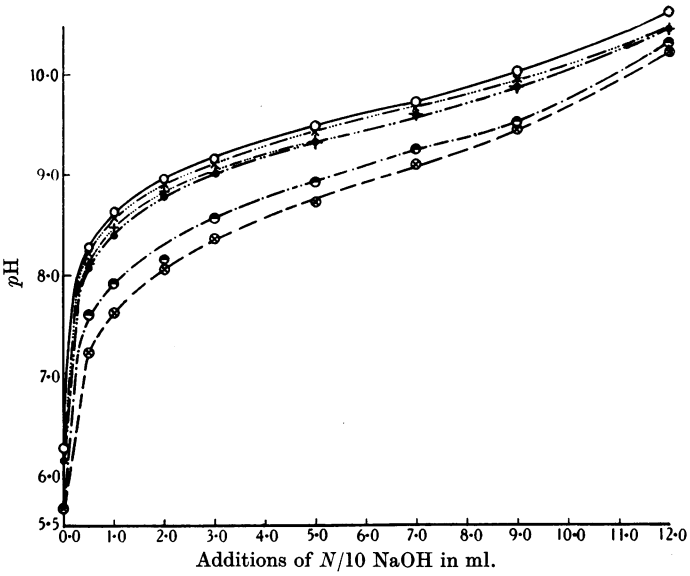

Fig. 1. Comparative potentiometric titrations of mixture solutions containing alanine and either H_2O, formaldehyde, glyceraldehyde, arabinose, glucose or lactose against *N*/10 NaOH.

o 5 ml. alanine *M*/4 + 5 ml. H_2O
× 5 ml. alanine *M*/4 + 5 ml. lactose *M*/4
+ 5 ml. alanine *M*/4 + 5 ml. glucose *M*/4
• 5 ml. alanine *M*/4 + 5 ml. arabinose *M*/4
⊖ 5 ml. alanine *M*/4 + 5 ml. glyceraldehyde *M*/4
⊗ 5 ml. alanine *M*/4 + 5 ml. formaldehyde *M*/4

and in Fig. 1. Under comparable conditions the general shape of the curves is the same; it therefore may be assumed that the reaction is the same in principle throughout, but it appears that the reaction is strongest with formaldehyde and the open-chain sugar (glyceraldehyde), diminishes with lactonic monosaccharides (arabinose, glucose), and is weakest with aldo-disaccharides (lactose).

(4) *The question of the* p*H range and the* p*H-optimum of the reaction.* Investigations were carried out to determine the *p*H range of the interaction in aqueous solution and whether a *p*H optimum is detectable. Starting at different hydrogen ion concentrations and applying the technique described as form (2), it was shown in all investigated cases that the *p*H range within which the reaction occurs varied with the nature of the components involved, but in no case exceeded approximately 4·5–11. Tables IV and V, and Figs. 2 and 3, give some

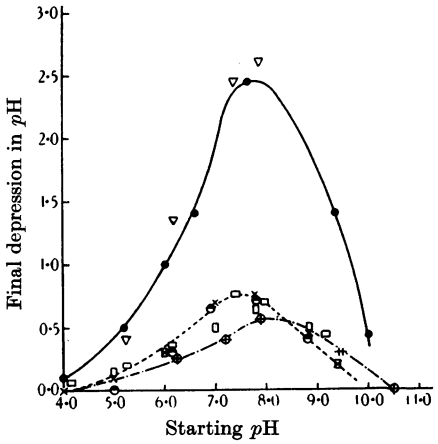

Fig. 2.

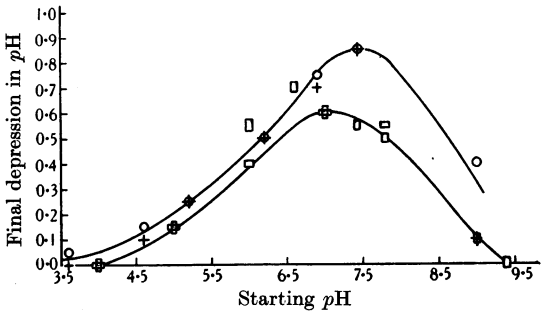

Fig. 3.

Fig. 2. *p*H range and optimum of the reaction between glycine *M*/2 and formaldehyde and various sugars.

——— { • Glycine *M*/2 + formaldehyde *M*/2
{ ▽ Glycine *M*/2 + glyceraldehyde *M*/2
—·— { o Glycine *M*/2 + maltose *M*/2
{ + Glycine *M*/2 + lactose *M*/2
------ { □ Glycine *M*/2 + xylose *M*/2
{ ▯ Glycine *M*/2 + galactose *M*/2
{ × Glycine *M*/2 + mannose *M*/2
{ ⊖ Glycine *M*/2 + glucose *M*/2

Fig. 3. *p*H range and optimum of the reaction between glycylglycine and various sugars.

o Glycylglycine *M*/4 + galactose *M*/2
+ Glycylglycine *M*/4 + glucose *M*/2
▯ Glycylglycine *M*/4 + lactose *M*/2
□ Glycylglycine *M*/4 + maltose *M*/2

examples of the observed *p*H range of the reaction between glycine and formaldehyde, glyceraldehyde, xylose, glucose, mannose, galactose, lactose and maltose respectively, and between glycylglycine and glucose, galactose, lactose and maltose respectively. Within this range, in all cases, the existence of an optimum *p*H zone was clearly revealed. It may be of importance also from the physiological point of view that in all cases investigated the optimum *p*H zone is within the physiological range.

In order to ascertain whether the range and maximum of the *p*H depression are attributable to the chemical reaction only of the mixture components, and not to a variation in the buffer capacity of the amino-acid with varying *p*H, colorimetric experiments were performed with glycine and glucose in varying concentrations.

The results show that neither the shape of the curve nor the optimum of the *p*H depression is influenced by the concentration of the components (cf. Fig. 4).

The results presented in Tables VII and VIII show that, like the α-amino-acids, glycine ethyl ester, the dibasic α-amino-acids (aspartic and glutamic

Table IV. *Colorimetric determination of the reaction range and of the reaction optimum in solution mixtures containing glycine and either formaldehyde, glyceraldehyde, xylose, glucose, mannose, galactose, lactose or maltose*

The amino-acid solution was mixed with an equal volume of the aldose solution at room temperature

	Glycine $M/2$ + formaldehyde $M/2$						
(*a*)	4·0	5·4	6·0	6·6	7·65	9·4	10·0
(*b*)	3·9	4·9	5·0	5·2	5·1	8·2	9·8
(*c*)	3·9	4·9	5·0	5·2	5·1	8·0	9·6
(*d*)	0·1	0·5	1·0	1·4	2·55	1·4	0·4

	Glycine $M/2$ + glyceraldehyde $M/2$					
(*a*)	4·15	5·25	6·2	7·4	7·9	9·5
(*b*)	4·1	5·1	5·6	6·25	6·05	8·6
(*c*)	4·1	4·85	4·85	4·95	5·3	—
(*d*)	0·05	0·4	1·35	2·45	2·6	—

	Glycine $M/2$ + xylose $M/2$					
(*a*)	4·15	5·25	6·2	7·4	7·9	9·2
(*b*)	4·1	5·1	5·9	6·65	7·2	9·2
(*c*)	4·1	5·05	5·85	6·65	7·2	8·75
(*d*)	0·05	0·2	0·35	0·75	0·7	0·45

	Glycine $M/2$ + glucose $M/2$						
(*a*)	4·1	5·0	6·2	6·9	7·8	8·8	9·4
(*b*)	4·05	5·0	5·6	6·4	7·3	8·7	9·4
(*c*)	4·05	5·0	5·9	6·25	7·1	8·4	9·1
(*d*)	0·05	0·0	0·3	0·65	0·7	0·4	0·3

	Glycine $M/2$ + mannose $M/2$						
(*a*)	4·0	5·0	6·0	7·0	7·8	8·85	9·4
(*b*)	4·0	4·9	5·75	6·3	7·1	8·4	9·2
(*c*)	4·0	4·9	5·7	6·3	7·05	8·4	9·2
(*d*)	0·0	0·1	0·3	0·7	0·75	0·45	0·2

	Glycine $M/2$ + galactose $M/2$						
(*a*)	4·0	5·0	6·0	7·0	7·8	8·85	9·4
(*b*)	4·0	4·85	5·75	6·6	7·2	8·35	9·2
(*c*)	4·0	4·85	5·7	6·5	7·15	8·35	9·2
(*d*)	0·0	0·15	0·3	0·5	0·65	0·5	0·2

	Glycine $M/2$ + lactose $M/2$						
(*a*)	5·0	6·25	7·2	7·9	9·45	9·5	10·5
(*b*)	4·95	6·0	6·8	7·35	9·25	9·3	10·5
(*c*)	4·9	6·0	6·8	7·35	9·15	9·2	10·5
(*d*)	0·1	0·25	0·4	0·55	0·3	0·3	0·0

	Glycine $M/2$ + maltose $M/2$					
(*a*)	5·0	6·25	7·2	7·9	9·45	10·5
(*b*)	4·9	6·0	6·8	7·35	9·15	10·5
(*c*)	4·85	6·0	6·8	7·35	8·9	10·5
(*d*)	0·15	0·25	0·4	0·55	0·55	0·0

(*a*) Denotes the starting *p*H of the component solutions.
(*b*) Denotes the *p*H of the mixture solution after standing 2 hr.
(*c*) Denotes the *p*H of the mixture solution after standing 2 days.
(*d*) Denotes the difference in *p*H between the starting- and end-points.

Table V. *Colorimetric determinations of the reaction range and of the reaction optimum in solution mixtures containing glycylglycine or glycylleucine with either glucose, galactose, maltose or lactose*

Equal volumes of the peptide and aldose solutions were mixed at room temperature

	Glycylglycine $M/4$ + glucose $M/2$						
(*a*)	3·6	4·6	5·2	6·2	6·9	7·45	9·0
(*b*)	3·6	4·5	5·15	5·75	6·55	7·1	9·0
(*c*)	3·6	4·5	4·95	5·7	6·2	6·6	8·9
(*d*)	0·0	0·1	0·25	0·5	0·7	0·85	0·1

	Glycylglycine $M/4$ + galactose $M/2$						
(*a*)	3·6	4·6	5·2	6·2	6·9	7·45	9·0
(*b*)	3·55	4·5	5·1	5·7	6·15	6·65	8·65
(*c*)	3·55	4·45	4·95	5·7	6·15	6·6	8·6
(*d*)	0·05	0·15	0·25	0·5	0·75	0·85	0·4

	Glycylglycine $M/4$ + lactose $M/2$								
(*a*)	4·0	5·0	6·0	6·6	7·0	7·45	7·8	8·6	9·4
(*b*)	4·0	4·95	5·45	5·95	6·5	7·4	7·8	8·6	9·4
(*c*)	4·0	4·85	5·45	5·9	6·4	6·9	7·3	8·5	9·4
(*d*)	0·0	0·15	0·55	0·7	0·6	0·55	0·5	0·1	0·0

	Glycylglycine $M/4$ + maltose $M/2$					
(*a*)	4·0	5·0	6·0	7·0	7·8	9·0
(*b*)	4·0	4·9	5·6	6·6	7·65	8·9
(*c*)	4·0	4·85	5·6	6·4	7·45	8·9
(*d*)	0·0	0·15	0·4	0·6	0·35	0·1

	Glycylleucine $M/4$ + glucose $M/2$					
(*a*)	4·1	5·35	6·4	6·9	7·9	9·2
(*b*)	4·1	5·25	5·85	6·35	6·4	9·2
(*c*)	4·1	5·2	5·85	6·35	7·45	9·2
(*d*)	0·0	0·15	0·55	0·55	0·45	0·0

(*a*) Denotes the starting *p*H of the component solutions.
(*b*) Denotes the *p*H of the mixture solution after standing 2 hr.
(*c*) Denotes the *p*H of the mixture solution after standing 24 hr.
(*d*) Denotes the difference in *p*H between the starting- and end-points.

Table VI. *Colorimetric determination of the reaction range and reaction optimum at different concentrations of glycine and glucose*

The amino-acid solution was mixed with an equal volume of the sugar solution at room temperature

	Glycine $M/1$ + glucose $M/1$						Glycine $M/2$ + glucose $M/2$							Glycine $M/8$ + glucose $M/1$					
(*a*)	5·0	6·35	7·04	7·72	8·8	9·4	4·1	5·0	6·2	6·9	7·8	8·8	9·4	5·0	6·35	7·05	7·72	8·8	9·4
(*b*)	4·9	5·87	6·2	6·9	8·18	9·05	4·05	5·0	5·9	6·25	7·1	8·4	9·1	5·0	6·05	6·45	7·02	8·27	9·2
(*c*	0·1	0·48	0·84	0·82	0·62	0·35	0·05	0·0	0·3	0·65	0·7	0·4	0·3	0·0	0·3	0·6	0·7	0·53	0·2

(*a*) Denotes the starting *p*H of the component solutions.
(*b*) Denotes the *p*H of the mixture solution after standing 2 days.
(*c*) Denotes the difference in *p*H between the starting- and end-points.

Table VII. *Colorimetric determination of the reaction range and reaction optimum of solution mixtures containing glucose and either* l-*aspartic acid,* l-*asparagine or* d-*glutamic acid*

The amino-acid solution was mixed with an equal volume of the sugar solution at room temperature

	Aspartic acid $M/10$ + glucose $M/1$						Asparagine $M/10$ + glucose $M/1$						Glutamic acid $M/15$ + glucose $M/1$					
(*a*)	5·4	6·68	7·5	8·0	9·17	9·6	5·35	6·7	7·5	8·0	9·2	9·6	5·2	6·8	7·45	7·9	9·2	9·4
(*b*)	5·3	6·4	7·2	8·0	9·17	9·6	5·25	6·5	7·2	7·9	9·2	9·6	5·2	6·6	7·0	7·6	8·95	9·3
(*c*)	5·3	6·4	7·1	7·8	9·05	9·6	5·2	6·47	7·07	7·6	9·0	9·5	5·2	6·6	7·0	7·55	8·95	9·25
(*d*)	0·1	0·28	0·4	0·2	0·12	0·0	0·15	0·23	0·43	0·4	0·2	0·1	0·0	0·2	0·45	0·35	0·25	0·15

(*a*) Denotes the starting *p*H of the component solutions.
(*b*) Denotes the *p*H of the mixture solution after standing 2 hr.
(*c*) Denotes the *p*H of the mixture solution after standing 2 days.
(*d*) Denotes the difference in *p*H between the starting- and end-points.

Table VIII. *Colorimetric determination of the reaction range and reaction optimum in solution mixtures of* N *glucose and glycine ethyl ester*

1·25 ml. dissolved in 25 ml. of water. The glucose solution was mixed with an equal volume of the glycine ethyl ester solution at room temperature*

Starting *p*H of the component solutions	4·85	7·1	7·93	9·2
*p*H of the mixture solution after standing 15 hr.	4·6	6·3	7·05	8·4
Difference in *p*H between the starting- and end-points	0·25	0·8	0·88	0·35†

* Owing to the known instability of glycine ethyl ester in solution, the experiments were not extended over a longer time interval and the data should be regarded with some reserve.

† Arrived at after deduction of the *p*H decrease of ester solution itself from 9·2 to 8·75 after 15 hr.

acids) and the amide of aspartic acid have approximately the same *p*H range and optimum of reaction. It therefore seems that the *p*H range and optimum of the reaction are largely independent of molecular substituents other than the α-amino-group.

(5) As already mentioned, Borsook and afterwards Euler and his co-workers inferred from their measurements (NH_2-nitrogen decrease; cryoscopic method) that the interaction between amino-acids and aldoses increased continuously with increasing *p*H. We were able to confirm that in alkaline medium a reaction between the two components occurs. In our opinion, however, the reaction occurring at relatively strong alkaline reactions is different from that dealt with previously in this paper, which is marked by a lowering of *p*H and by a *p*H optimum. It is well known that in alkaline solutions sugars, e.g. glucose, easily undergo decomposition. As mentioned above, Watanabe [1932; 1933] and Akabori [1933] found that in the presence of amino-acids the decomposition of glucose in gently heated alkaline solutions is accelerated and that the amino-acids themselves are decomposed, forming aldehydes, ammonia and CO_2. They carried out their experiments in a range of 100° to 37°. Neuberg & Kobel [1927, 2] found that decomposition products of sugars—methyl glyoxal and others—decompose the amino-acids.

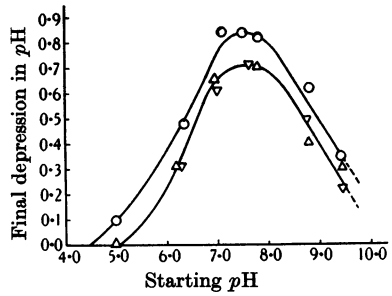

Fig. 4. *p*H optimum of the reaction between glucose and glycine in different concentrations.

o Glycine *M* + glucose *M*
△ Glycine *M*/2 + glucose *M*/2
▽ Glycine *M*/8 + glucose *M*

As we mentioned above, the interaction between amino-acids and sugars takes place in slightly acid, neutral or in slightly alkaline medium and is practically entirely inhibited at about *p*H 11. On the other hand we were able to show that, in alkaline media, a second type of reaction occurs which is favoured by high alkalinity even at room temperature. This second reaction type is distinguished from the first by a much lower velocity and is not accompanied by a marked decrease in *p*H. We sought some preliminary insight into its nature.

No ammonia could be detected even after prolonged standing (about 10 days) of the mixture solutions at different alkaline reactions. This observation was also made by Neuberg and by Kostytchew. A suitably prepared fuchsine-SO_2 reagent, which reacts with aldehydes but not with monosaccharides, gave no reaction with the fresh mixture solution at *p*H 9, 10 or 11. But after standing at room temperature for 2 days or more at the same *p*H, the solution mixture developed an ever deeper red, indicative of the presence of carbonyl groups other than those of the sugars. The colour was obtained only in solutions containing both components; at the same *p*H and at the same time interval no colour was obtained in the control solutions containing one of the two components. The particulars of this process are under investigation.

It is known that the stability of the sugars is strongly reduced with increasing alkalinity. As a working hypothesis we suggest that a decomposition occurs. The reducing decomposition products might react with the amino-group of the amino-acids or destroy the whole amino-acid molecule [Neuberg and Kobel, 1927, 2], causing a diminution in the free NH_2-nitrogen, and possibly by a further reaction a diminution in the number of the molecules, such as has been found by various authors. The progress of this reaction with increasing *p*H would, on these assumptions, be easily comprehensible.

The results of Borsook & Wasteneys and Euler and collaborators in alkaline medium seem to be due therefore to the overlapping in slightly alkaline medium of both reactions and to the dominance of the second, more complicated type of reaction, at the higher *p*H. The interaction of sugars and amino-acids for which the formation of Schiff's bases and the formaldehyde titration are the prototype, plays no role or at least no decisive role in this second reaction.

Experimental

Substances. The amino-acids were pure, prepared and analysed by Hoffman-La Roche. The sugars were chemically pure samples and of different manufacture, mainly Schering-Kahlbaum and B.D.H.

Technique. Potentiometric: the usual platinum blackened electrodes were used in suitable titration vessels. With constant stirring, a stream of purified hydrogen was passed during the whole time through the vessels. Additions to the vessel contents were made through burettes fitted by corks.

Colorimetric technique: Clark and Lubs indicators were used. The solutions were kept in stoppered test-tubes in the dark.

Summary

1. A method is developed whereby the interaction of α-amino-acids and sugars may be followed by the lowering of *p*H, consequent upon the disappearance of the $-NH_2$ groups, during the reaction.

2. The interaction occurs only with aldo-sugars.

3. The interaction of a number of α-amino-acids and peptides with monoaldoses and aldo-disaccharides has been investigated.

4. With various aldo-sugars, differences in the reactivity, dependent upon the constitution, were found; in the amino-acids studied, the α-amino-group alone seems to be the dominating factor.

5. The reaction proceeds only within a *p*H range of about 4·5–11 and shows a *p*H optimum zone.

6. In a strongly alkaline medium (pH > 10) a second reaction, which is clearly distinguished from the first, predominates.

7. An explanation for the two different types of interaction is suggested.

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