CCXLV. THE TIME FACTOR IN THE INTERACTION OF AMINO-ACIDS WITH SUGARS

BY MAX FRANKEL AND AARON KATCHALSKY

From the Hebrew University, Jerusalem

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IN a previous communication we have shown [Frankel & Katchalsky, 1937] that the interaction of α -amino-acids and peptides with sugars in slightly alkaline, neutral and slightly acid solution leads to a decrease in pH owing to the disappearance of basic amino-groups, but no quantitative conclusions were drawn. It was mentioned that the interaction in question "proceeds over a relatively long time interval". In the experiments now reported the time progress of the decrease in pH is described in greater detail.

The study of the time factor throws light also on a recent paper by Balson & Lawson [1938] who, on the other hand, state that the pH decreases shown by amino-acids or peptides in mixture with sugars is solely attributable to the acidic properties of the sugars themselves. The discrepancy between the results of Balson & Lawson and our own is, we believe, due mainly to the fact that, in choosing their experimental technique, these authors failed to take into account the time factor of the interaction. The interaction between the aldoses and amino-acids or peptides becomes noticeable, under certain conditions, only after some time. In the experiments described by us intervals of 20–30 min. were allowed between NaOH additions in the potentiometric technique, and observations in the colorimetric experiments were extended over periods of 24–48 hr. Balson & Lawson, on the other hand, used their special technique [1935] which enables them to shorten even that time interval which is normally required in potentiometric titrations between consecutive additions, and thus created markedly unfavourable conditions for observations of the interaction.

Other minor causes of the discrepancy are dealt with later in this paper.

In order to study the time factor more fully we carried out the present experiments as follows.

I. Mixtures of amino-acid and sugars were titrated by the potentiometric method under different time conditions: in (1) the interval between each addition of NaOH was 10 min., in (2) the interval was 3 hr. Time in each case was counted from the moment at which initial potential was attained.

II. Equal parts of mixtures containing both components of the reaction were brought to desired pH values by addition of NaOH and both the initial pH and the changes of pH were determined electrometrically during a period of several hours.

In all cases controls containing either the amino-acid or the sugar at the pH and concentration of the test mixtures, were run.

The amino-acid was glycine. The sugars were: sucrose and fructose for non-aldehydic, non-reactive sugars; glucose and galactose for the aldehydic sugars.

The experiments were carried out under sterile conditions at 20° throughout.

RESULTS

I. Potentiometric titrations with different time intervals between NaOH additions

(a) Glycine + non-aldehydic sugars

1. Time interval between additions—10 min.

When the slight correction necessitated by the acidity of the sugars is allowed for (0.3 mV. for sucrose, 0.6 mV. for fructose) the curves obtained are identical with that of glycine + water.

2. Time interval—3 hr.

Once the potentials are established no change in pH occurs. The titration curves of the solutions are therefore the same whether the NaOH is added at intervals of 10 min. or 3 hr.

Fig. 1. Titration curves of glycine-sugar mixtures with different time intervals between NaOH additions. ∇ 1 ml. glycine $M/4$ + 1 ml. H_2O . $\circ$ 1 ml. glycine $M/4$ + 1 ml. sucrose $M/1$ (time interval 10 min. or 3 hr.). + 1 ml. glycine $M/4$ + 1 ml. fructose $M/1$ (time interval 10 min. or 3 hr.). $\square$ 1 ml. glycine $M/4$ + 1 ml. glucose $M/1$ (time interval 10 min.). $\bullet$ 1 ml. glycine $M/4$ + 1 ml. glucose $M/1$ (time interval 3 hr.).

Table I. Potentiometric titration of 2 ml. of mixtures containing glycine $M/8$ and glucose $M/2$ by NaOH $N/10$, each addition of alkali being made (1) 10 min. or (2) 3 hr. after the initial potential was attained.

Additions of alkali (ml.)	pH	
	(1)	(2)
0.0	5.61*	5.61*
0.05	7.71	7.03
0.1	8.13	7.34
0.2	8.51	7.89
0.3	8.73	8.11
0.5	9.04	8.61
0.7	9.21	8.91
1.0	9.47	9.21
1.4	9.72	9.41
1.8	9.96	9.90

* The measured pH; a decrease in pH might have occurred here during the mixing and measuring.

(b) *Glycine + aldehydic sugars*1. *Time interval—10 min.*

In this case also the necessary correction for the acidity of the glucose is small (0–4 mV.) within the *pH* range and concentration ratio of the experiments.

Even when the potentiometric titration was conducted at time intervals of 10 min. the potentials initially attained tend to decrease. Yet the decrease under these conditions (Δ *pH* about 0.1), though greater than the range of error, is still rather small (Fig. 1; Table I (1)). Balson & Lawson [1938] correctly concluded that a *pH* lowering of this size is too small for an interaction to be inferred.

2. *Time interval—3 hr.*

The *pH* values found after each addition were lower than the corresponding values obtained with additions at intervals of 10 min. by as much as 0.8 *pH*. (Cf. Fig. 1, Table I (2).)

II. *Electrometric pH measurements during 3 hr. in mixture solutions brought by additions of NaOH to different initial pH values*(a) *Glycine + non-aldehydic sugars*

The sugars were either sucrose or fructose.

All *pH* values remained constant at their initial level for 3 hr. Sterile mixture solutions at *pH* up to 8.8 and sterile acid and neutral controls underwent no change in *pH* within one or more days; both sugar controls and mixtures of higher alkalinity undergo *pH* depressions when kept for 24 hr. or more. This result is to be expected in view of the known instability of sugars in alkaline solution.

(b) *Glycine + aldehydic sugars*

Glycine + glucose. Marked and rapid decreases in the *pH* values were observed (Table II). The velocity of the depression decreased with increasing alkalinity. All *pH* values attained constancy at least 30 min. before the end of the observation period.

Table II. *Potentiometric measurements of pH changes with time in 2 ml. mixtures containing glycine M/8 + glucose M/2 + x ml. NaOH N/10*

Time after mixing min.	<i>x</i> = ml. NaOH N/10					
	0.05	0.1	0.3	0.5	1.0	1.8
	<i>pH</i> values					
25	7.71	8.13	8.73	9.04	9.43	9.95
30	7.63	8.08	8.71	9.02	9.40	9.93
37	7.47	8.01	8.68	9.01	9.40	9.89
45	7.34	7.94	8.65	8.99	9.40	9.89
60	7.23	7.89	8.64	8.99	9.40	9.89
75	7.17	7.78	8.61	8.95	9.38	9.89
150	7.17	7.47	8.47	8.88	9.36	9.87
180	7.17	7.47	8.46	8.88	9.35	9.87

In sterile mixtures and controls of *pH* < 8.3 additional decreases did not occur even after several days. In the alkaline range, above *pH* 8.3, glucose controls prepared and maintained under sterile conditions showed depressions in *pH* after one or more days showing decomposition of the sugar. The mixtures under the same conditions also showed additional depressions. It is obvious that

sugars which are allowed to stand for a longer period in strongly alkaline media undergo chemical changes which, as in certain cases observed by the authors and others, are aided by the presence of amino-acids. As has previously been shown, more strongly alkaline media induce a new type of reaction in the mixtures. It seems, therefore, advisable to reduce the observation time, which in the colorimetric experiments was extended over one or two days, to an interval of e.g. 3 hr. within which no decomposition of the sugars occurs.

It may be noted here that the observations on the diminution of the pH decrease at higher alkalinities were taken at points far enough from the end-point of the titration to be uninfluenced by the final convergence of the titration curves.

Galactose when mixed with glycine behaves in the same way as glucose, except that within a pH range of 7–8 the readings with galactose became constant within 20 min., whereas in the pH range 8–9 constancy is attained after 2 hr.

The molar concentration ratio of amino-acid to sugar in our previous experiments was 1 : 1, and in the mixtures the concentration of each component was $M/4$. The corrections for the acidity of the sugars in the pH range investigated were found to be between 1–2 mV. and were therefore practically negligible.

It may also be remembered that in our previous colorimetric experiments the acidity of the sugars was eliminated by equalizing the pH of both component solutions before mixing (principle of isohydric solutions).

Lastly it may be mentioned, in anticipation of a later paper, that leucyl-glycine, the peptide investigated by Balson & Lawson, is less reactive when mixed with aldoses than glycine or its peptides in which the free NH_2 -group belongs to the glycine moiety.

SUMMARY

1. Mixtures of glycine and either non-aldehydic or aldehydic sugars were potentiometrically titrated under different time conditions.

No pH depression occurred in mixtures containing non-aldehydic sugars and no influence of the length of the intervals between NaOH additions on the titration curve was detectable. In mixtures containing glucose a pH depression appeared which was much more marked when the time interval between the NaOH additions was extended.

2. No change within at least 3 hr. in the pH values occurred in mixtures of glycine and non-aldehydic sugars brought to different initial pH . Under the same conditions marked and rapid decreases in the pH values occurred in mixtures containing glycine and either glucose or galactose.

3. The view of Balson & Lawson, that the pH depressions observed by us are to be attributed solely to the acidic properties of the sugars is shown to be incorrect.

4. Owing to different experimental conditions no direct conclusion in regard to our previous findings can be drawn from the results of Balson & Lawson.

REFERENCES

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