The Thermal Destruction of Vitamin B₁

2. THE INFLUENCE OF BUFFER SALTS ON THE RATE OF DESTRUCTION OF CO-CARBOXYLASE AT 100°C.

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(Received 19 April 1945)

The rate of destruction of aneurin at 100° has been studied in detail (Farrer, 1941*b*; Beadle, Greenwood & Kraybill, 1943; Booth, 1943; Farrer, 1945). However, aneurin also occurs in food products as a pyrophosphoric ester, co-carboxylase. Greenwood, Beadle & Kraybill (1943) have stated that aneurin is slightly less stable than co-carboxylase. Booth (1943), on the other hand, found that co-carboxylase was destroyed more rapidly than aneurin in boiling phosphate-phthalate buffer solutions. Because of these conflicting statements and the characteristic behaviour of aneurin on boiling in various buffer solutions, it was decided to apply the methods used with aneurin (Farrer, 1945) to a similar study of the rate of destruction of co-carboxylase on boiling in different buffer solutions. Lincoln, Hove & Harrel (1944) examined the percentage destruction of aneurin and co-carboxylase from pH 3.5 to 7.0 at 120° in McIlvaine's citric acid-phosphate and Clark and Lubs's phosphate-sodium hydroxide buffers. They found that co-carboxylase was destroyed rather more rapidly than aneurin, though no detailed study was attempted. The work now recorded shows that at 100° co-carboxylase is destroyed more rapidly than aneurin at all pH values from 3.0 to 7.5 (above which destruction is very rapid indeed), and describes quantitatively the rate of destruction.

METHODS

Buffer solutions. The following solutions were prepared with A.R. reagents: (a) 0.1 M-citric acid, (b) 0.2 M-Na₂HPO₄, (c) M/15-NaH₂PO₄, (d) M/15-Na₂HPO₄, (e) a solution 0.2 M with respect to both KH₂PO₄ and K₂HPO₄. The McIlvaine buffers were made from (a) and (b), the Sorensen series from (c) and (d), and (e) was adjusted to pH 5.8 with NaOH (10%).

Co-carboxylase stock solution. A sample of co-carboxylase, supplied gratis by Messrs Merck and Co., Rahway, N.J., U.S.A., was used. 10 mg. were dissolved in water and made to 100 ml.

Estimation of aneurin. The Jansen thiochrome method was used, and the procedure and apparatus employed were the same as previously described (Farrer, 1941*a*, 1945).

Experimental procedure. 5 ml. of co-carboxylase stock solution were made to 200 ml. with buffer solution, and the final pH checked with a Cambridge pH meter, using the glass electrode. The solution was then boiled under

reflux in a 300 ml. Erlenmeyer flask connected to a Davies condenser by means of a ground glass joint. Samples (22–25 ml.) were withdrawn at regular time intervals (e.g. 15 min.) into 75 ml. Erlenmeyer flasks and cooled rapidly under the tap. When cool, 20 ml. were pipetted into 50 ml. volumetric flasks, diluted almost to 50 ml. with acetate buffer of pH 4.0–4.5 (approx. 60 ml. glacial acetic acid and 67 g. anhydrous sodium acetate/l.), and incubated overnight, with the addition of takadiastase, at 37°. The volume was finally adjusted to 50 ml., and 5 ml. samples taken for aneurin determination.

In the case of those solutions from which it was necessary to withdraw samples more frequently than at 15 min. intervals, the above procedure was modified somewhat. The co-carboxylase was added to the already boiling buffer solution, and the time was taken by means of a stop-watch which was started when half the co-carboxylase solution had run in. Samples were taken with the wash bottle arrangement mounted in a cork stopper (Farrer, 1941*b*).

In each case the pH was checked after the solution which remained at the conclusion of the experiment had cooled. Agreement with the original pH was good in all cases.

To ensure that the temperature of boiling was virtually constant throughout, the barometer reading was taken for each solution studied. Deviations from 100° were insignificant.

RESULTS

The galvanometer deflexions were plotted against time for each solution, giving curves of the same type as were obtained for aneurin. The monomolecular reaction law has been applied to each curve by plotting $\log(a-x)$ against time, and straight lines

Table 1. *Rate of destruction of co-carboxylase at different pH values*

(k denotes velocity coefficient.)			
Sorensen phosphate buffer		McIlvaine citric acid-phosphate buffer	
pH	k	pH	k
5.10	0.00322	3.07	0.00152
5.55	0.00374	3.48	0.00216
5.95	0.00553	3.96	0.00321
6.43	0.0316	4.53	0.00560
6.82	0.0392	4.96	0.00955
7.31	0.151	5.67	0.0155
		6.12	0.0277
		6.62	0.0822
		7.15	0.0575
		7.55	0.107

have again been obtained. From these straight lines, k , the velocity coefficient, has been calculated (see Table 1). The progress of the destruction of co-carboxylase with time can be followed readily from

Fig. 1. Log k /pH curves for aneurin and co-carboxylase.

the value of k and the large number of curves already presented for the buffer solutions used (Farrer, 1941b, 1945). Log k has again been plotted against pH (Fig. 1).

DISCUSSION

As with aneurin so also with co-carboxylase, the curves obtained by plotting pH against log k (pH/log k curves—Fig. 1) are similar to those obtained by plotting pH against added acid or alkali for the buffer mixture used (buffer curves), in that the discontinuities on the pH/log k curves occur at approximately the same pH values as the discontinuities on the buffer curves. The latter are sufficiently well known not to need reproduction here. It can be said, therefore, that the destruction of co-carboxylase, too, is conditioned by pH and by the ionic environment.

It is also apparent that the rate of destruction of co-carboxylase is, in most solutions studied, very much greater than the rate of destruction of aneurin. With the Sorensen phosphate buffer the pH/log k curves for aneurin and co-carboxylase appear to be almost parallel in the range investigated (pH 5.0–7.0), but this is not the case with the McIlvaine buffer which was examined over a much greater range (pH 3.0–7.5), as it is seen that the curves diverge as the pH rises to 7.0 and thereafter they tend to converge.

Log k values for aneurin ($\log k_A$) and for co-carboxylase ($\log k_C$) for each solution examined are tabulated, together with the difference, $\log k_C - \log k_A$, which is hereafter referred to as $\Delta \log k$ (see Table 2).

Table 2. Comparison of the rates of destruction of aneurin and co-carboxylase at different pH values

(k_A , velocity coefficient for aneurin destruction; k_C , for co-carboxylase destruction; $\Delta \log k$, denotes difference between $\log k_A$ and $\log k_C$.)

Sorensen phosphate buffer			
pH	$\log k_A$	$\log k_C$	$\Delta \log k$
5.1	3.17	3.51	0.34
5.5	3.17	3.57	0.40
6.0	3.32	3.74	0.42
6.5	3.68	3.21	0.53
6.8	2.00*	2.59	0.59
7.0	2.18	2.82*	0.64
7.3	2.70	1.18	0.48
McIlvaine citric acid-phosphate buffer			
pH	$\log k_A$	$\log k_C$	$\Delta \log k$
3.1	3.11	3.18	0.07
3.5	3.29	3.33	0.04
4.0	3.43	3.51	0.08
4.6	3.58	3.75	0.17
5.0	3.70	3.98	0.28
5.7	3.75	2.19	0.44
6.1	3.97	2.44	0.47
6.6	2.14	2.66	0.52
7.1 (5)	2.40	2.76	0.36
7.5 (5)	2.73	1.03	0.30

* Obtained from the pH/log k curves.

From pH 3.5 to 7.0 a steady increase in $\Delta \log k$ is apparent and has been plotted against pH (Fig. 2).

Fig. 2. $\Delta \log k$ /pH curve for all buffer solutions.

• McIlvaine. ○ Sorensen. × Phosphate-phthalate.

It is seen from the figure that the relation between pH and $\Delta \log k$ is virtually linear and appears at first sight to be independent of the buffer used, i.e. co-carboxylase is destroyed at a faster rate than

aneurin, and it is progressively more vulnerable to rising pH irrespective of the buffer salts present. If, however, the points common to each individual buffer are considered separately, it can be shown that once again discontinuities occur and curves very similar in form to the buffer curves are obtained. Thus, although the rate of increase of $\Delta \log k$ depends almost entirely on the pH it is conditioned to some extent by the buffer solution used. It is worth pointing out that the value of $\Delta \log k$ is of the same order at the same pH in different buffer solutions, but that this is not necessarily so for $\log k_A$ or $\log k_C$, which differ very considerably from buffer to buffer at the same pH value. These considerations apply only between pH 3.5 and 7.0. Above pH 7.0 $\Delta \log k$ decreases but this is almost certainly due to the great speed with which both aneurin and co-carboxylase are destroyed in alkaline solution. The higher value at pH 3 is probably due to experimental errors, as at such low values of $\Delta \log k$ these will be greatly magnified.

Booth (1943), who studied the destruction of aneurin and co-carboxylase in boiling phosphate-phthalate buffer solutions at pH 5.4, 5.8 and 6.4, only recorded the percentage destruction in a given time. Booth's curves have been analyzed as described earlier (Farrer, 1945) and $\log k_A$ and $\log k_C$ calculated for each pH value (Table 3).

Table 3. *Values of velocity coefficients calculated from data of Booth (1943)*

(k_A , velocity coefficient for aneurin; k_C , velocity coefficient for co-carboxylase; $\Delta \log k$ is difference between $\log k_A$ and $\log k_C$.)

pH	$\log k_A$	$\log k_C$	$\Delta \log k$
5.4	3.33	3.72	0.39
5.8	3.49	2.14	0.65
6.4	3.81	2.29	0.48

Log k values for pH 5.4 and 6.4 agree very well with figures expected from the straight line in Fig. 2. However, 0.65 is very much higher than one would expect for pH 5.8, and on referring back to Booth's work it is seen that the curve obtained by plotting his data for destruction against time for this pH value shows serious abnormalities. This curve has, therefore, been repeated using buffer solution (e) at pH 5.83. The result was a smooth logarithmic curve which gave a good straight line when $\log (a-x)$ was plotted against time. The value for $\log k$ was 3.94. This gives $\Delta \log k = 0.45$, a figure which fits much more neatly on the $\Delta \log k/\text{pH}$ curve.

Just why co-carboxylase is more vulnerable to changes in pH than aneurin is not clear. The only difference between the two molecules is the esterification of an alcoholic hydroxyl-group of aneurin with pyrophosphoric acid, so that the presence of the pyrophosphoric group would appear to strain the molecule in some way.

SUMMARY

1. The rate of destruction of co-carboxylase in Sorensen phosphate and McIlvaine buffer solutions at various pH values has been studied.

2. As in the case of aneurin, the rate of destruction of co-carboxylase is conditioned by pH and by the ionic environment.

3. Co-carboxylase is more vulnerable to rising pH than aneurin and, between pH 3.5 and 7.0, this increase in vulnerability can be expressed as a linear relation between pH and the difference between the logarithms of the velocity coefficients for the destruction of co-carboxylase and aneurin at any given pH value.

The author is indebted to the Directors of the Kraft Walker Cheese Co. Proprietary, Ltd., for permission to publish this paper.

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