600. *The Influence of Ions on the Activity of Q-Enzyme.*

By G. A. Gilbert and A. J. Swallow.

The activity of Q-enzyme solutions is shown to be reduced by dialysis against dilute buffer solutions and restored by the addition of salts such as ammonium sulphate and sodium acetate. The dependence of the activity on the pH and concentration of solutions of ammonium sulphate has been determined. Sodium borate inhibits the enzyme even in the presence of activating ions.

The nature of Q-enzyme, an enzyme which catalyses the formation of amylopectin from amylose, has been discussed recently by Peat, Bourne, and Barker (*Nature*, 1948, **161**, 127). A similar or identical enzyme has been reported by Bernfeld and Meutémédian (*Helv. Chim. Acta*, 1948, **31**, 1724). Little is known about the mode or kinetics of reactions of Q-enzyme, but for some time it has been suspected that it is activated by ions (Peat, Bourne, and Rotholz, unpublished work). The same conclusion was drawn from the loss in activity which followed dialysis against sodium borate solution during the preparation of the enzyme for electrophoretic examination (Mr. A. D. Patrick), and it was considered desirable to study the phenomenon in detail.

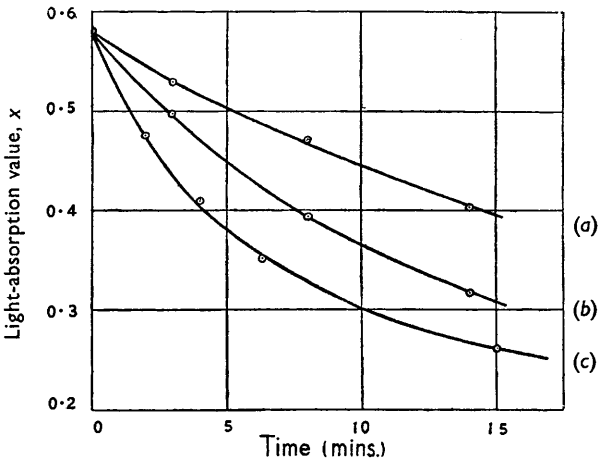
Measurement of Q-Enzyme Activity.—The existing method of measurement by the fall in light-absorption value of an amylose solution involves the use of amylose which contains salt (Bourne, Macey, and Peat, *J.*, 1945, 882). To eliminate this source of possible activators, the method was modified by substituting " AnalaR " soluble starch for amylose since this, unlike amylose, is stable in salt-free solution.

When the rate of loss of blue colour was analysed, it was found that, if allowance were made for the final absorption value to which the solution tended, the reaction obeyed the first order equation $dx/dt = -k(x - x_\infty)$ or, in its integrated form, $2{\cdot}30 \log(x - x_\infty)/(x_0 - x_\infty) = -kt$, where x, x_∞, and x_0 are, respectively, the light absorption after time t, after the reaction is complete, and at zero time.

Thus in a representative example shown in Figs. 1 and 2 the function $\log(x - x_\infty)$ may be seen to depend linearly on t, for the three solutions (*a*), (*b*), and (*c*). The velocity constant k is proportional to the slope of the line and this in turn is approximately proportional to the enzyme concentration as shown below. Within the limits of concentration employed in these experiments the activity of the enzyme can therefore be expressed in terms of the velocity

FIG. 1.

The action of Q-enzyme on starch.



constant. This is conveniently calculated from the time taken to fall under standard conditions to a particular absorption value.

Soln.	Q-Enzyme concn. (mg. of dry powder per ml. of digest).	$100k$ (min.$^{-1}$) at 20°.	$100k$/enzyme concn.
(*a*)	1·4	4·74	3·4
(*b*)	2·9	9·05	3·1
(*c*)	4·8	16·3	3·4

Examination by Dialysis.—The detection of ionic activators was first attempted by complete dialysis against distilled water, but Q-enzyme was found to be insoluble and denatured at the low ionic strengths attained. To avoid this trouble, dilute buffer solutions were substituted for the distilled water, the first buffer chosen being sodium borate, since the electrophoretic experiments had indicated that borate did not activate the enzyme. No success was achieved, however, for, although the activity disappeared, it could not be restored by the addition of the ions present in the original active solution of the enzyme. A separate measurement was made of the activity lost through adsorption on the dialysis membrane. After the addition of concentrated sodium borate to an active solution of enzyme had been shown to cause complete loss of activity, it was clear that sodium borate deactivated the enzyme and was not a suitable medium for dialysis. Phosphate could not be used because the Q-enzyme in these experiments contained traces of P-enzyme, which in the presence of phosphate would have interfered with the activity measurements.

Sodium acetate solution was chosen for the next experiment, and in this no loss in activity was recorded, beyond that shown to be due to a considerable adsorption of the enzyme by the "Cellophane" membrane. It was concluded that either Q-enzyme requires no activator or sodium acetate can function as such. In a further attempt to remove all activators and so differentiate between these possibilities, dialysis was carried out against glycine in the expectation that this would not be an activator, but the enzyme was precipitated. The precipitate was soluble in ammonium sulphate to give an active solution, suggesting that ammonium sulphate is an activator or again that no activator is required.

The next experiment was more conclusive, for, when very dilute ammonium sulphate solution was used as the medium, a considerable loss in activity occurred without precipitation of the enzyme, and on addition of concentrated ammonium sulphate or sodium acetate solution to the dialysed solution the activity increased sharply. The dependence of activity on salt concentration was then studied with the result shown below :

log (ionic strength)	$\bar{1}\cdot60$	$\bar{2}\cdot64$	$\bar{3}\cdot90$
$100k$ (min.$^{-1}$)	5·30	3·50	1·92

This leaves no doubt that ammonium sulphate activates Q-enzyme, provided that the effect is not due to a displacement of the pH–activity curve with change of ionic strength (cf. Sumner and Somers, " Chemistry and Methods of Enzymes," 2nd Edn., 1947, p. 18). pH–activity curves were therefore determined for ammonium sulphate at two ionic strengths, and the results shown in Fig. 3 confirm that true activation is occurring.

FIG. 2.

The dependence of $\log(x - x_\infty)$ *on the time of reaction.*

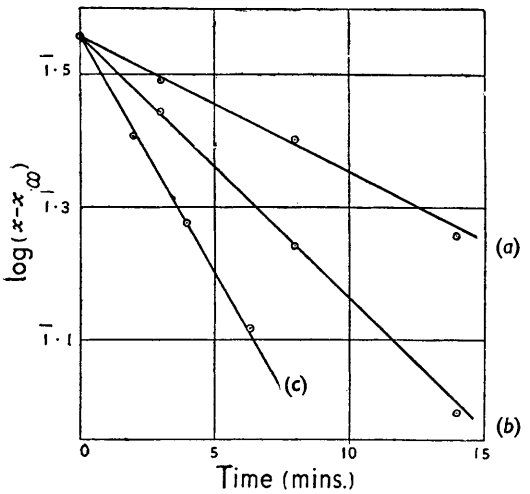
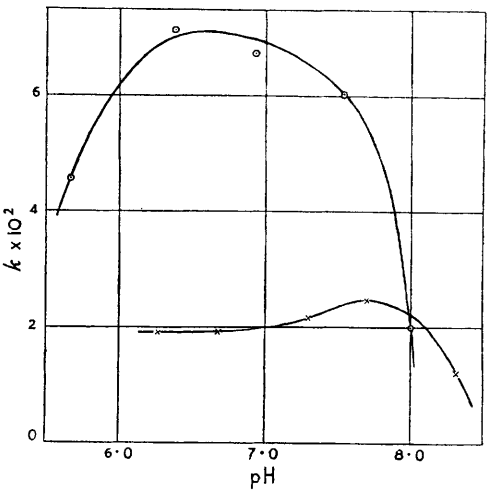


FIG. 3.

Dependence of activity on pH.



⊙ *Ionic strength* 0·4.
× *Ionic strength* 0·008.

Besides sodium acetate and ammonium sulphate, ammonium chloride was shown to be an activator, and further experiments by Barker, Bourne, Peat, and Wilkinson (in the press) prove that sodium citrate and sodium phosphate can function in the same way. Thus it seems that ionic strength, rather than a specific action by the ions present, controls the activity, although the deactivating effect of sodium borate shows that specificity can play some part.

It is evident from these results that for comparing different preparations of Q-enzyme their activity should be measured under conditions such that the salt concentration is high enough for small variations in the salt content of the protein and amylose to be immaterial. Within the concentration limits for which the first order equation holds, the value of the velocity constant for unit concentration of protein (1 mg. per ml. of digest) is then a measure of the purity of the enzyme preparation.

EXPERIMENTAL.

Preparation of Q-Enzyme.—The directions of Barker, Bourne, and Peat (this vol., p. 1705) were followed for the preparation of the dried enzyme.

Estimation of Activity.—Q-Enzyme solution (1 ml.) was mixed with the selected buffer solution (1 ml.) and incubated at 20° with a solution of " AnalaR " soluble starch (0·5 ml.). After suitable intervals, 0·5-ml. samples were withdrawn and added to 50 ml. of a solution containing 1 mg. of iodine and 10 mg. of potassium iodide. The light absorption was measured with a Hilger Spekker Absorptiometer (Ilford Gelatine Spectrum Filter No. 608). Usually the starch concentration was such that the initial reading was about 0·8.

Dialysis Experiments.—Sodium borate. Q-Enzyme (0·3 g.) was dissolved in 0·01M-sodium borate solution, pH 6·74 (25 ml.), and divided into three equal parts. One was dialysed through "Cellophane" against 0·01M-borate solution (4 l.) for 40 hours at 0°, another was left in contact with "Cellophane" of the same dimensions, and the third was retained as a control. The velocity constant for the control was $2·5 \times 10^{-2}$ min.$^{-1}$, that of the second solution was $0·9 \times 10^{-2}$ min.$^{-1}$, and the dialysed enzyme had no detectable activity. No activity could be restored by adding boiled Q-enzyme solution. A further attempt to restore the activity was made by adding the diffusible ions obtained by dialysing Q-enzyme against a small volume of sodium borate. This led to no activation.

Sodium acetate. The above experiment was repeated using 0·01M-sodium acetate solution, pH 7·18. The velocity constant of the control was $6·8 \times 10^{-2}$ min.$^{-1}$ and that of the second solution $1·6 \times 10^{-2}$ min.$^{-1}$, and the dialysed enzyme retained activity equal to $1·6 \times 10^{-2}$ min.$^{-1}$.

Glycine. Q-Enzyme (0·4 g.) was dissolved in 0·05M-glycine solution, pH 7·03 (10 ml.), and dialysed overnight at 0° through "Cellophane" against more of the glycine solution (4 l.), and then for a further 24 hours against fresh glycine solution (4 l.). The enzyme was found to be precipitated in the dialysing bag. It was resuspended by shaking, and the suspension (1 ml.) was dissolved with M-ammonium sulphate, pH 7·05 (1 ml.), and incubated with starch solution (0·5 ml.). Its velocity constant was 7×10^{-2} min.$^{-1}$.

Ammonium sulphate. Q-Enzyme (0·1 g.) was dissolved in 0·01M-ammonium sulphate solution, pH 7·11 (10 ml.), and dialysed overnight at 0° against more ammonium sulphate (4 l.). The dialysate was diluted with 0·01M-ammonium sulphate (20 ml.), and its activity found to be $0·39 \times 10^{-2}$ min.$^{-1}$. The ionic strength of the ammonium sulphate in the digest was 0·012. A further digest was prepared in which the ionic strength of the ammonium sulphate was 1·2. The corresponding activity was $1·4 \times 10^{-2}$ min.$^{-1}$. A third sample of the enzyme in the dilute ammonium sulphate solution was then used to prepare a digest containing sodium acetate of ionic strength 0·4. The activity was $1·1 \times 10^{-2}$ min.$^{-1}$.

Another sample of Q-enzyme was dialysed under similar conditions, and the velocity constant measured in the presence of ammonium sulphate of three concentrations to give the results shown in the second table (p. 2851).

Q-Enzyme (0·4 g.) was dissolved in 0·01M-ammonium sulphate, pH 6·68 (10 ml.), and dialysed against more ammonium sulphate at 0° with two changes of dialysing medium. Activities were then measured in the presence of ammonium sulphate (to which ammonia had been added) of ionic strength 0·4 at five pH values (5·67 to 8·00). The curve is shown in Fig. 3. A similar experiment was carried out at ionic strength 0·008. The results are shown in the same figure.

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