The Influence of Some Cations on the Kinetics
of Ox Liver Arylsulphatase A

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The effects of some cations on the kinetics of sulphatase A are reported. It is shown that barium ions inactivate the enzyme if substrate is present, and a possible reaction mechanism is discussed. Strontium ions have no inhibitory effect on the enzyme, and it is therefore recommended to use strontium instead of barium ions to precipitate sulphate in investigations of this enzyme.

In a previous paper investigations of the kinetics of the enzymic hydrolysis of 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate) were reported [1]. As sulphate ions inhibit the reaction, the sulphate ions liberated in the course of the reaction were precipitated as barium sulphate. It was found nescessary to add barium chloride in such a manner that there would never be excess of barium ions in the reaction mixture, as barium ions have some secondary effects on the reaction. In this paper some investigations of the influence of excess barium ions on the enzymic reaction are reported together with investigations of the influence of some other cations.

EXPERIMENTAL

The progress of the reaction was followed as described previously by continued automatic titration of the liberated acid in a recording pH-stat [1]. The base which was added from a syringe in small portions consisted of a solution of equivalent amounts of KOH, substrate and $BaCl_2$, so that both pH and the substrate concentration were kept constant during the reaction, and the sulphate was precipitated as $BaSO_4$.

The cations investigated have preferably been used as their chlorides, only for Zn^{++} this has not been the case as pure $ZnCl_2$ was not at hand. Instead $Zn(NO_3)_2$ was used as experiments conducted with KCl and KNO_3 did not show any difference in the reaction progress. When the effect of monovalent cations was investigated the concentrations of the salts were 0.10 M and when divalent cations were used the concentrations were 0.05 M, so the concentration of chloride ions was the same in all the experiments. Only $CuCl_2$ made an exception in this respect as it is not sufficiently soluble at pH 5. A saturated solution was therefore used.

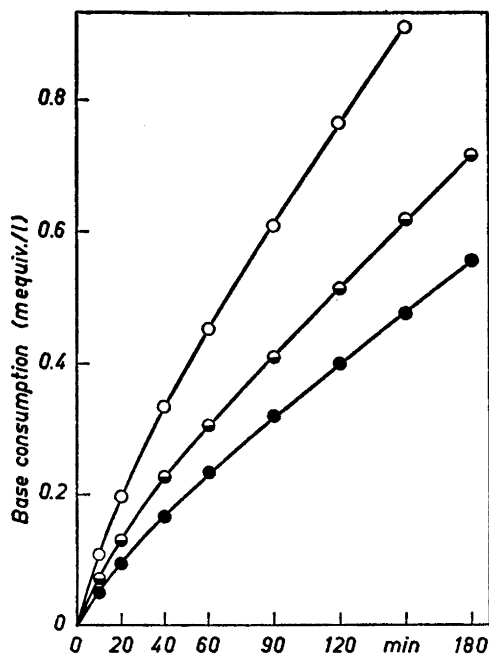* Present address: Universitetets zoofysiologiske laboratorium B, Copenhagen, Denmark.

Fig. 1. Effect of cations on the activity of sulphatase A at 20°C and pH 5. The substrate concentration was 3 mM. O—O—O: 0.10 M KCl or NaCl, ◐—◐—◐: 0.05 M CoCl₂, ●—●—●: 0.05 M CdCl₂.
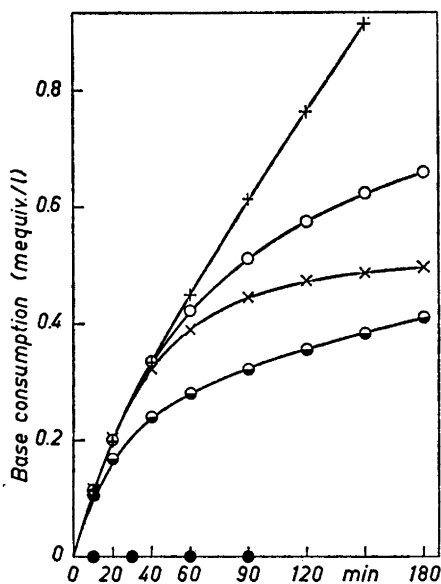
Fig. 2. Effect of cations on the activity of sulphatase A at 20°C and pH 5. The substrate concentration was 3 mM. +—+—+: 0.05 M CaCl₂, MgCl₂ or SrCl₂, O—O—O: 0.05 M Zn(NO₃)₂, ×—×—×: 0.05 M BaCl₂, ◐—◐—◐: 0.05 M MnCl₂, ●—●—●: 0.05 M HgCl₂ or saturated CuCl₂.

Mixtures of KCl and BaCl₂ were used in the experiments where the influence of different barium ion concentrations was investigated, so that the chloride concentration also in this experiment was kept at 0.10 M.

All the experiments were performed at pH 5.0 and 20.0°C, and the substrate concentration was 0.002 M. Enzyme and substrate were prepared as described previously[1].

RESULTS

The progress of the reaction in presence of different cations is demonstrated om Figs. 1 and 2. It is interesting to see that in the case of Ba^{++}, Zn^{++} and Mn^{++} the inhibition of the reaction first begins some time after the start of the reaction. When the enzyme is incubated for some hours together with these cations at pH 5 and the reaction is then started, then the same reaction progress is found. This indicates that the reaction between enzyme and metal ions is not simply a slow one, but that the substrate is involved in some way.

It is not surprising that Cu^{++} and Hg^{++} completely inhibit the reaction as it has been found that the enzyme contains sulfhydryl groups necessary for its activity [2].
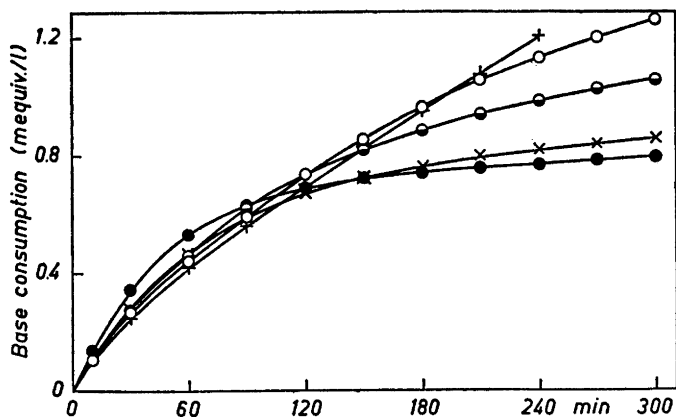
Fig. 3. Effect of BaCl$_2$ on the activity of sulphatase A at 20°C and pH 5. The substrate concentration was 3 mM. ●—●—●: 0.05 M BaCl$_2$, ×—×—×: 0.02 M BaCl$_2$, ◖—◖—◖: 0.01 M BaCl$_2$, O—O—O: 0.005 M BaCl$_2$, +—+—+: without addition of BaCl$_2$.

Co^{++} and Cd^{++} inhibit the reaction to some degree, and it is characteristic that there is a constant ratio between the activity when these ions are present and the activity of the noninhibited enzyme.

When K$^+$, Na$^+$, Mg^{++}, Ca^{++} or Sr^{++} are used no difference can be found between the progress of the reactions.

The influence of different concentrations of Ba^{++} on the reaction is shown in Fig. 3. The barium ions seem to have a double effect, first the enzyme is activated, later on inhibited. It was tried to reactivate the enzyme by removing the barium ions, but neither addition of K$_2$SO$_4$ nor dialyzing the reaction mixture against 0.1 M KCl for 24 h had any effect, so the inhibition is obviously irreversible. If the enzyme is incubated with BaCl$_2$ in the absence of substrate and the barium ions are removed by dialysis, then the enzyme shows the kinetics found in the absence of barium ions.

The reaction progress in the absence of heavy metal ions can be described by the expression:

$$\xi = At + B(1 - e^{-\lambda t})$$

where ξ is the amount of substrate split at the time t, and A, B and λ depend on the enzyme and substrate concentrations[1]. The same expression with a very small value of A can describe the reaction progress in the experiment with the highest concentration of barium ions but not in the experiments with smaller concentrations.

DISCUSSION

This formal similarity between the reaction progress when no barium ions are added and when they are present in excess can be explained if it is supposed that the reaction scheme is essentially the same in both cases, but that in the presence of barium ions the enzyme is somehow modified so that the velocity constants are altered.

The scheme previously proposed for the reaction when no barium ions are present was:

$$A + X_1 \rightleftharpoons X_2$$
$$X_2 \rightarrow X_1 + P$$
$$X_2 \rightleftharpoons X_3$$
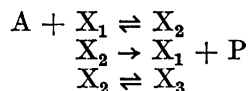
where X_1, X_2 and X_3 are different forms of the enzyme and A is the substrate and P is the reaction products. The transformation from X_2 to X_3 is assumed to be reversible and slow. If barium ions are present we should then have:

$$Ba^{++} + X_1 \rightleftharpoons X_1'$$
$$A + X_1' \rightleftharpoons X_2'$$
$$X_2' \rightarrow X_1' + P$$
$$X_2' \rightarrow X_3'$$
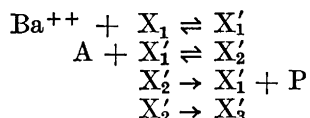
X_i' represent one of the forms of the enzyme modified by barium ions. The larger initial velocity when barium ions are present can be explained if this barium modification of the enzyme is more active towards the substrate than the unmodified enzyme.

If the concentration of barium ions is so high that practically all the enzyme is in the form of the barium modification, it should be expected that the reaction progress could be described by the expression $\xi = B(1-e^{-\lambda t})$. As mentioned this is not the case, but the existence of the term At could be explained if the reaction X_2' to X_3' is not irreversible as written in the scheme, but reversible with the equilibrium far to the right. The final velocity in the experiment with 0.05 M $BaCl_2$ is about twenty times smaller than the initial velocity. In equilibrium 95 % of the enzyme must then be present as X_3', and the reaction from X_2' to X_3' must be about twenty times as fast as the opposite reaction. It should therefore be expected that a measurable reactivation could be demonstrated after 24 h dialysis. This is as mentioned not the case.

When the concentration of barium ions used is not so high that all the enzyme is in the form of the barium modification, we should expect a reaction scheme composed of both schemes here mentioned. This will give a complex reaction which cannot be simply expressed, which is in accordance with the experiments.

Barium chloride has been used to remove excess of sulphate ions in some investigations of the kinetics of sulphatases [1,3]. The results here presented show that it must be used with caution, and that it is better to use strontium chloride as this can be used in high concentrations without having any effects on the enzyme. It is peculiar that there is such a difference between the effects of barium and strontium ions. One is reminded of the fact that barium salts are toxic to the organism whereas strontium salts are not.

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