BBA 63467

The product inhibition of sulphatase B

It was reported some years ago[1] that sulphatase B (aryl-sulphate sulphohydrolase, EC 3.1.6.1) from ox liver was noncompetitively inhibited by sulphate, one of the reaction products. Other workers[2] suggested that the inhibition was competitive, although they could not confirm this by kinetic measurements. Because of the importance of studies of product inhibition in elucidating the mechanism of enzyme reactions, this problem has been reinvestigated using a purified specimen of sulphatase B. The enzyme was a mixture of the isoenzymes Bα and Bβ which do not differ in their kinetic properties[3].

Sulphatase B (specific activity 70 units/mg) was prepared and assayed spectrophotometrically as previously described[3]. Kinetic constants were calculated by the method of WILKINSON[4].

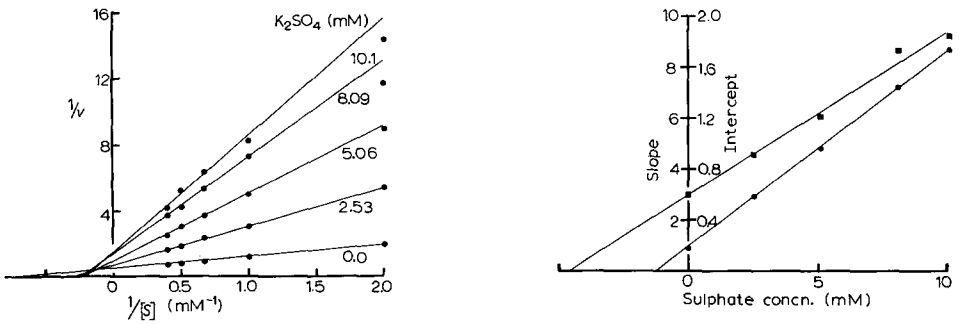

Fig. 1. Reciprocal plots showing the effect of sulphate on the activity of sulphatase B. Substrate, nitrocatechol sulphate; pH 5.4 in 0.5 M sodium acetate–acetic acid buffer; inhibitor, K_2SO_4 at the concentrations shown on the figure; incubation, 5 min at 37°.

Fig. 2. Replots of the slopes (●) and intercepts on the ordinate (■) of Fig. 1 against sulphate concentration.

The raw data are shown in Fig. 1 which clearly demonstrates the noncompetitive nature of the inhibition. The intersection of the lines above the abscissa shows that K_i calculated from the slopes of the lines must be less than that calculated from the intercepts on the ordinate[5]. Fig. 2 shows replots of the slopes and intercepts from Fig. 1 and gives values of 1.2 mM and 4.6 mM SO_4^{2-}, respectively, for $K_{i\ \text{slope}}$ and $K_{i\ \text{intercept}}$. The plots in Fig. 2 also show that the inhibition is linear noncompetitive[5].

The simplest reasonable mechanism for a hydrolytic enzyme is that of an ordered uni–bi reaction:

$$E + S \rightleftharpoons ES \rightleftharpoons EP_1P_2 \rightleftharpoons EP_2 + P_1 \rightleftharpoons E + P$$

The inhibition of such a reaction by P_1 is linear noncompetitive with $K_{i\ \text{slope}}$ and $K_{i\ \text{intercept}}$ being, in general, different, whereas the inhibition by P_2 is linear competitive. Assuming the reaction catalysed by sulphatase B to be of the above form, the present findings show that sulphate cannot be the last-released product although

it could be the first-released: this means that EP_2 must be a sulphatase B–phenol complex. The inhibition of sulphatase A by sulphate is, on the other hand, linear competitive (ref. 6 and personal communication from Dr. R. G. NICHOLLS) and therefore consistent with the last-released product being sulphate and EP_2 being a sulphosulphatase A. Confirmation of these postulated mechanisms could be obtained by determining the type of inhibition caused by the other product of the arylsulphatase reactions, a phenol (4-nitrocatechol in the present instance). Unfortunately this further information is unavailable because neither sulphatase A nor B is inhibited by 4-nitrocatechol at the highest concentration, 0.6 mM, practicable to use in a spectrophotometric assay.

These results nevertheless show that there is an important difference between the types of reaction catalysed by sulphatases A and B, and it seems that the difference between these enzymes may be more fundamental than has been suggested in the past[3,7].

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Received March 24th, 1970

Biochim. Biophys. Acta, 212 (1970) 365–366

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