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The substrate constant for yeast invertase

The constant for the dissociation of the enzyme–substrate complex (K_s) may be derived by an examination of systems in which k_0, the rate constant for the breakdown of the complex to enzyme and products, can be varied independently of K_s[1]. MAIN[2] determined K_s in this way by examination of LINEWEAVER–BURK plots[3] of data concerning butanol activation of human serum cholinesterase (arylesterase, EC 3.1.1.2) with *o*-nitrophenylbutyrate as substrate. Similar data have been presented by FOLK, WOLFF, SCHIRMER AND CORNFIELD[4] for the inhibition of carboxypeptidase B esterase activity by butanol with hippuryl-L-argininic acid as substrate. 0.05–0.5 M butanol was used by these workers. A precisely similar series of plots of the inhibition of invertase (β-D-fructofuranoside fructohydralase, EC 3.2.1.26) by aniline[5] in concentrations of 0.5–2 mM is reported in this communication.

A commercial solution of invertase (Nutritional Biochemical Corp., Cleveland, Ohio) was used as the source of soluble invertase. A preparation of yeast cell walls was used as a source of "bound" invertase. Compressed baker's yeast was suspended in water and shaken (12 cycles/sec) in a 65-ml stainless steel vertically reciprocating ball mill (Glen Creston, Stanmore, Great Britain) with glass beads about 0.4 mm in diameter. Samples were withdrawn at intervals and examined microscopically. When no intact cells could be seen in several fields the cell walls were separated by repeated differential centrifugation, suspended in water and stored at 5°. Before use the cell-wall suspension was centrifuged and the supernatant containing any soluble invertase released during storage discarded. A fresh suspension in water was used as the enzyme source in each experiment. The commercial invertase preparation was diluted 1:2000 before use and cell-wall suspensions were diluted as necessary to give approximately the same activity per ml.

Aniline of reagent quality was distilled before use. Buffer (0.25 M sodium maleate, pH 4.6), substrate and enzyme solutions were brought to 25° in a water bath. To 2 ml of substrate solution an equal volume of buffer was added (containing an amount of aniline giving the required final concentration) followed at a known time by 1 ml of enzyme. Immediate mixing was secured by blowing the enzyme solution rapidly into the buffer–substrate mixture from a wide tipped pipette. After exactly 3 min incubation a 1-ml sample was blown into 1 ml of SOMOGYI copper reagent[6] to stop the reaction. Total reducing sugar was then measured by the NELSON procedure[7]. Each determination at a given substrate and inhibitor concentration was made in triplicate with a blank determination on a tube containing water in place of enzyme solution. Enzyme blanks were also run. When the enzyme source was yeast cell walls the sample was 2 ml and was blown into 2 ml of reagent, the mixture was centrifuged and a 2-ml sample of supernatant taken for analysis. 1 unit of invertase activity was that amount of enzyme which hydrolysed 1 μmole of sucrose per min at 25° when acting on a solution of 0.12 M sucrose. Enzyme solutions were used at a dilution such that they showed an activity of 1.5 units/ml when assayed in 0.1 M sodium maleate buffer (pH 4.6).

Activity measurements were made in the presence of 0.5, 1.0, 1.5 and 2.0 mM aniline at sucrose concentrations of 20, 25, 33, 50 and 100 mM. The results were plotted according to the DIXON modification[8] of the LINEWEAVER–BURK plot[3].

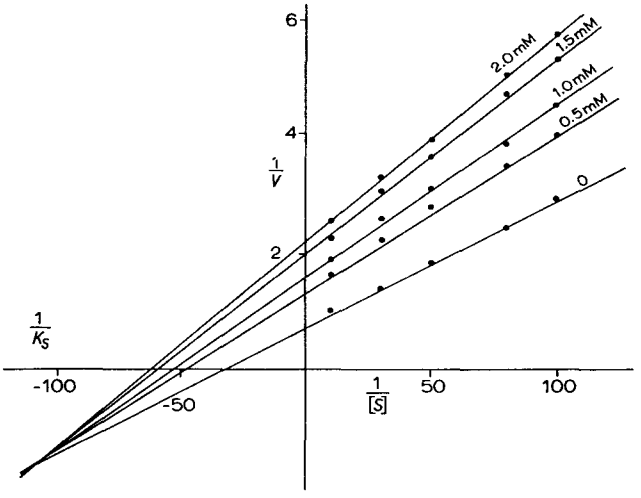

Fig. 1. LINEWEAVER–BURK plot of reciprocal activity against corresponding reciprocal sucrose concentration for 4 different aniline concentrations. (V in μmoles sucrose/min; [S] in mM sucrose).

Lines passing through the experimental points for a particular inhibitor concentration were produced and were found to intersect at a common point in the lower left quadrant of the diagram (Fig. 1). K_s evaluated from the point of intersection[2] was found to be 9.1 mM for the soluble invertase preparation and 9.1 and 9.25 mM for the cell-wall suspension carrying invertase in the bound form. A plot of V against K_m for the results of one experiment with soluble invertase and two with bound invertase as suggested by SLATER[1] is shown in Fig. 2.

Since both MAIN[2] and FOLK *et al.*[4] had shown that ethanol had a similar, though

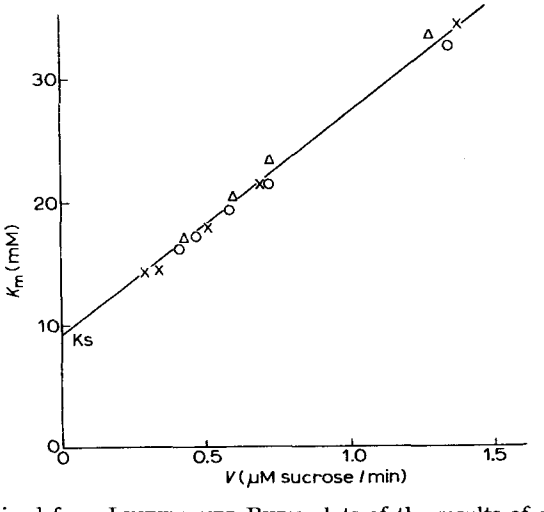

Fig. 2. Plot of V (derived from LINEWEAVER-BURK plots of the results of one experiment with soluble and two with cell-wall invertase similar to that shown in Fig. 1) against K_m derived from the same plots. Extrapolation to the K_m axis gives a value for K_s.

lesser, effect to that of butanol in the systems they studied the effect of ethanol on the invertase system was reexamined. In the concentration range of 2–10% ethanol, inhibition was of the purely non-competitive type; a K_m of 33 mM and 44 mM for cell wall and soluble invertase, respectively, being deduced from the common points of intersection on the horizontal axis in LINEWEAVER–BURK plots. The lower K_m for cell-wall preparations may reflect some hindrance imposed by the site of the bound enzyme. It was unlikely to be due to a local variation of effective pH at the site of the bound enzyme for it was shown that the K_m of the bound invertase did not increase until the pH of the medium had been lowered to between pH 3 and 4 and in this region there was also an increase in the K_m of the soluble enzyme.

As pointed out by FOLK *et al.*[4] these results may be interpreted either on the basis of modification of k_0 (rate constant for dissociation of enzyme–substrate complex to products and free enzyme) or of modification of the free enzyme. Such a large number of kinetic studies of invertase action are available that it appears likely that it may prove a useful system for further investigation of this effect in spite of possible complication by the demonstrable transferring activity of invertase.

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Urea biosynthesis in invertebrates: [^{14}C]Urea formation in the land snail and earthworm

The failure to demonstrate urea synthesis by Helix hepatopancreas tissue in early studies of invertebrate nitrogen metabolism[1,2] and the controversial findings with Tetrahymena[3,4] have led to the general conclusion that urea synthesis does not take place in invertebrate animals by the Krebs–Henseleit ornithine cycle[5,6]. Observations on the earthworm[7,8] and more recent studies of the land snail, *Otala lactea*[9], have, however, shown that the question of urea synthesis and metabolism in certain of these animals should remain an open one. Otala hepatopancreas was shown to possess ornithine transcarbamylase (EC 2.1.3.3), argininosuccinate lyase (EC 4.3.2.1), and possibly argininosuccinate synthetase (EC 6.3.4.5) activity in addition to high levels of arginase (EC 3.5.3.1)[9]. Ornithine transcarbamylase activity has also been shown