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Abstract

Control of aromatic biosynthesis

Day, C.H. Control of aromatic biosynthesis in Neurospora crassa.

3-Deoxy-D-arabino-heptulosonic acid 7-phosphate synthetase (DAHP synthetase) is the first enzyme of aromatic biosynthesis in micro-organisms and in E. coli has been shown to be a regulatory system of at least 3 isoenzymes (Doy and Brown 1965 Biochim. Biophys. Acta 104:377). Control is by feedback inhibition (phenylalanine and tyrosine) and repression (phenylalanine, tyrosine and tryptophan (Brown and Day 1966 Biochim. Biophys. Acta 118:157).

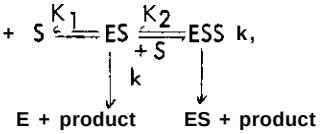
DAHP synthetase has now been examined in dialysed crude extracts of wild type N. crassa 74A, grown an Vogel's minimal medium at 25° for 48 hrs. Under the conditions stationary phase had not been reached. Extracts were made by grinding with glass and KH_2PO_4- NaOH buffer 0.1M pH 6.4 and dialysing against 0.025M of the same buffer. The supernatant was used after centrifuging the debris. DAHP synthetase was estimated essentially as described by Day and Brown.

The substrates are erythrose 4-phosphate and phosphoenolpyruvate and initial velocity measurements were determined by varying one substrate (10^{-5}M - 2 x 10^{-3}M) in the presence of excess of the other (2 x IO-3M). By plotting v against s, sigmoid curves were obtained which, within experimental error, had a positive initial slope. Reciprocal Plats of 1/v against 1/s show the characteristics more clearly. Parts of these data replotted as 1/v against $1/s^2$ yield a straight line as required if 1/v against I/s is a parabola. However, it appears likely that this is fortuitous and that the present data are more consistent with the characteristics of a non-rectangular hyperbola. it is important to make this distinction.

A parabolic 1/v against 1/s curve is consistent with a model: $E + S \underset{}{\overset{K_1}{\rightleftharpoons}} ES \overset{K_2}{\underset{+S}{\rightleftharpoons}} ESS \xrightarrow{k} E + product.$ -ES

That is, the formation of product requires the compulsory addition of two molecules of substrate. Only the second molecule of substrate gives product and the first can be regarded as an activator, or modifier, which reacts with enzyme to form the active rite. The initial velocity equation, $v = \frac{VS^2}{K_1K_2 + K_2S + S^2}$ gives a sigmoid curve when v is plotted against S and has an initial slope of zero.

A second model is:



For this model the first molecule of substrate is both an activator and is converted to product. The initial velocity equation requires a positive initial slope and in reciprocal plot yields a non-rectangular hyperbola. If k is very small, or if the enzyme is predominantly in the ESS form, the kinetic characteristics approximate to the first model. It is hoped to test these mechanisms further, although the work may be limited by the accuracy of the assay method. However, computer fitting of the data might provide a definitive answer.

This approach has been made in order to explore the possibility that in the cell the DAHP synthetase function is integrated for purposes of control. If later work shows the existence of isoenzymes, the reactions between substrate molecules and different isoenzymes must be identical for the present kinetic interpretations to be valid.

In the above models, modifier sites might also exist which recognize allosteric inhibitors and result in the inhibition of enzymic function. Inhibition by the aromatic end products has been tested. Both tyrosine and phenylalanine are inhibitors; chorismate and prephenate are not. With all extracts and throughout the concentration range 10^{-5} - 2 x 10^{-3}M, tyrosine was a better inhibitor than phenylalanine. With either inhibitor against either substrate, inhibition was non-competitive. It should be realized that although tangents to the reciprocal plots can be drawn, the kinetic constants so derived are not meaningful. In the present examples the data are considered consistent with non-competitive inhibition because the maximum velocity is changed, but the pseudo-apparent K_m values are not.

When an equimolar mixture of tyrosine and phenylalanine was used, the inhibition was greater than the sum of the inhibitions obtained with individual inhibitors and greater than the inhibition by the corresponding molar amount of either. This demonstrates that there is interaction between the modifiers so that inhibition is enhanced in the presence of both molecular species. Thus, if the Investigation later shows the existence of separate isoenzymes (or modifier sub-units) recognizing different inhibitor species, it can be concluded that these isoenzymes are capable of interaction. The data are also consistent with interaction between separate sub-units recognizing the inhibitors and units bearing active sites.

With E. coli it was difficult +a be certain whether or not tryptophan was an inhibitor. An isoenzymic fraction was obtained with sufficient activity to be certain that this was essentially not inhibited, but it was recognized that this might be a product of the procedure rather than a true reflection of the native state. With the present extracts of N. crassa the same difficulties exist, but by taking large amounts of enzyme in the presence of 10^{-3}M tyrosine and 10^{-3}M phenylalanine it has been shown that the addition of tryptophan results in further inhibition. Half-maximum inhibition was observed at about 10^{-5}M tryptophan and the maximum effect was given by about 4 x 10^{-5}M tryptophan. In terms of total DAHP synthetase activity, the tryptophan inhibited portion is about 11%. A small portion of activity (about 2%) remains in the presence of high concentrations (10^{-3}M) of all three aromatic amino acids. Tryptophan may also be an inhibitor in the absence of the other modifiers but results are still inconclusive. It is possible that inhibition by tryptophan is facilitated in the presence of phenylalanine and tyrosine.

In E. coli, feedback inhibition is complemented by repression of the individual isoenzymic components. No such relationship could be demonstrated far N. crassa. Extracts with lowered activity have been obtained by growing N. crassa in the presence of the aromatic amino acids singly and in various combinations. No consistent pattern has emerged beyond the conclusion that individual end products do not repress specific portions of activity: for example, tyrosine does not repress the portion inhibited by tyrosine. It appears likely that, when variation in activity has been observed, this is due to factors other than repression. The rationalization can be offered that repression is important to an organism like E. coli, where the natural exogenous environment is sometimes rich in aromatic amino acids, but this situation is unlikely to occur for N. crassa and consequently repression may have no selective advantage and therefore hove remained undeveloped. Operon-like situations found in N. crassa (for example, 5 aromatic pathway enzymes, N. Giles, personal communication) moy represent one means of controlling the organization of multiple enzymic function proteins. For example, anti-polarity mutations can be considered as indicators of polypeptide structure important to more than one enzymic function.

Resent work is oimed at relating the biology, biochemistry and genetics of DAHP synthetase in N. crassa. Part of the present account was presented to the Third Neurospora Information Congress (by D.G. Cotcheside) but not reputed in NN#9. - - - Deportment of Genetics, The John Curtin School of Medical Research, Australian National University, Canberra, A.C.T., Australia.