3-DEOXY-D-ARABINO-HEPTULOSONIC ACID 7-PHOSPHATE SYNTHETASE AS AN "ALLOSTERIC" FUNCTION AND LIGAND INTERACTIONS WITH THIS ENZYME FROM NEUROSPORA CRASSA

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This communication presents evidence for homo- and hetero-molecular cooperation between the ligands of 3-deoxy-D-arabino-heptulosonic acid 7-phosphate synthetase (DAHP synthetase) from Neurospora crassa.

In studies concerning the DAHP synthetase of E. coli the enzyme has frequently been refered to as an allosteric function (Doy and Brown, 1965; Brown and Doy, 1966). This was with regard to the original use of the term allosteric, meaning that the substrates and inhibitors were not steric analogues (Monod and Jacob, 1961). More recently the description allosteric has come to imply the existence of distinct conformers participating in an equilibrium in which one state, or another, is favoured when different ligands bind with the enzyme (Monod, Wyman and Changeux, 1965; Rubin and Changeux, 1966). DAHP synthetase was actually listed as an example of the V type of allosteric enzyme.

Allosteric functions are usually recognised by a departure from linearity in reciprocal plots of the Lineweaver and Burk type. In practice the published kinetic data on the isoenzymes

of E. coli shows no departure from linearity (Smith, Ravel, Lax and Shive, 1962). A recent note (Staub and Dénes, 1967) indicates that the phenylalanine sensitive DAHP synthetase isoenzyme of E. coli is an example of a "Ping pong" reaction (Figure 1) (Cleland, 1963). Again the data shows no departure from linearity. In contrast experiments with dialysed extract of Neurospora crassa wild type 74A yield reciprocal plots approximating to non-rectangular hyperbolas when any of the ligands, substrates (E4P and PEP), or inhibitors (phenylalanine and tyrosine) are varied in concentration. Although trypophan is an inhibitor (Doy, 1967) it has not been fully tested kinetically and will not be further discussed.

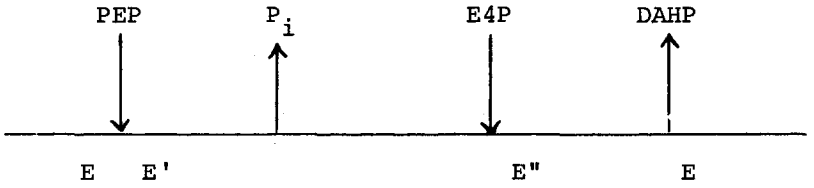

Figure 1. DAHP synthetase - the possible "Ping pong" mechanism of reaction.

Plots for each of the substrates are given in Figures 2 and 3. The parts of the curves approximating to asymptotes are extrapolated to cut the abscissa. I have termed the constants so obtained as pseudo-apparent K_m's. In Figure 4 the extent of inhibition is plotted in an analogous manner. Approximations to non-rectangular hyperbolas were again obtained with the exception of inhibition by a mixture of phenylalanine and tyrosine. This latter approximates to a straight line. In some experiments tyrosine has also given a straight line. Detailed interpretations of these curves (and the limitations of these approaches) will be discussed elsewhere (Doy, Manuscript in preparation). It is

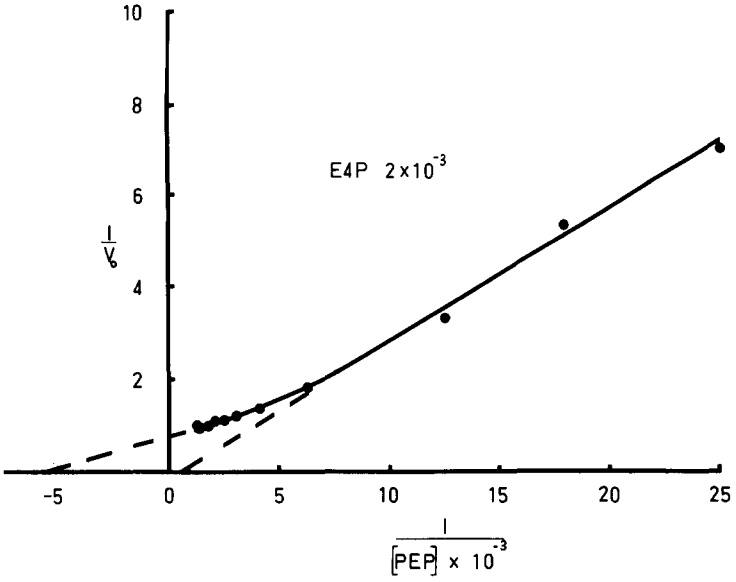

Figure 2. Double-reciprocal plot of initial velocity, v_0 ($A_{549m\mu}$) with varying PEP and saturating E4P. The broken lines are extrapolations of the two approximations to asymptotes. At high substrate concentration the "pseudo-apparent" $K_m = 1.8 \times 10^{-4}$. For these experiments and all others in this paper extracts of N. crassa were made by grinding with glass and 0.1M KH_2PO_4-NaOH buffer, pH6.4, centrifuging and dialysing the supernatant for 2 x 2 hr. against 0.025M of the same buffer. DAHP synthetase was assayed as described by Doy and Brown (1965). Approximately 0.1mg.protein was used per 0.25 ml reaction mixture.

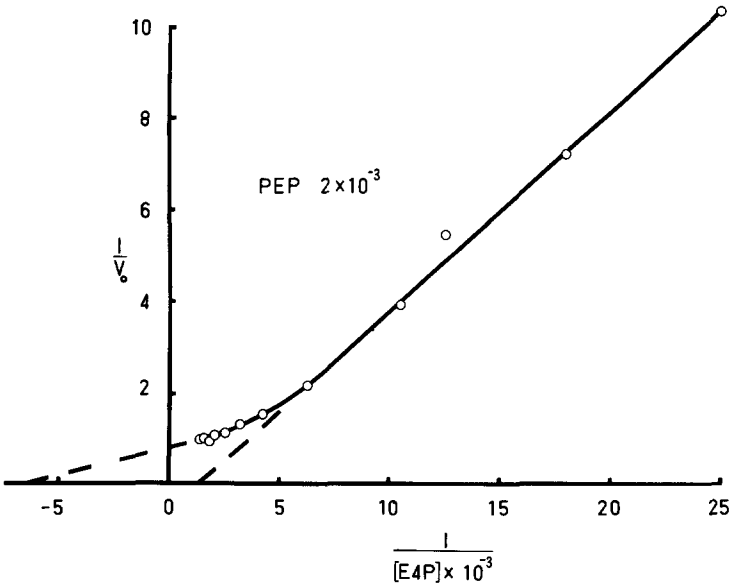

Figure 3. As for Figure 2 except that E4P varies. The "pseudo-apparent" $K_m = 1.75 \times 10^{-4}$.

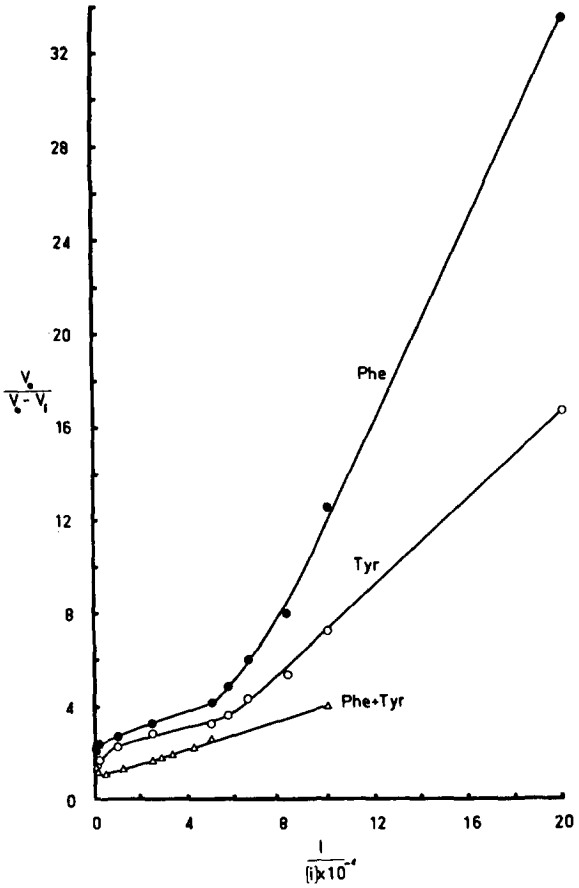

Figure 4. Double-reciprocal plots of the inhibition of DAHP synthetase. Both substrates saturating (2 x 10^{-3}M). In the experiments with Phe + Tyr an equi-molar nurture was used and plotted as total molarity.

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clear that homo- and hetero-molecular interaction occurs and that for inhibition by phenylalanine plus tyrosine this has a synergistic effect. The simplest general interpretation is that individual ligand-enzyme interactions approximate to:

$$E + L \rightleftharpoons EL, \qquad EL + L \rightleftharpoons ELL$$

when other appropriate ligands are saturating. In the example of the substrates, both EL and ELL can yield product and the ELL

form is fully activated. In the example of the inhibitors the ELL form is the most deactivated. Such an interpretation is independent of the allosteric conformational theory although it can be anticipated that conformational changes are involved.

In other experiments the appropriate titrations and plots (Cleland, 1963) indicate that at relatively high substrate levels the data approximated to that required for a "Ping pong" type of reaction. Superimposed on this are the molecular interactions already discussed. Inhibition data suggests that phenylalanine and tyrosine interfere with the binding of E4-P rather than the initial binding of PEP. These aspects will be reported in detail elsewhere (Doy, manuscript in preparation).

It seems that the DAHP-synthetase of N.crassa has more complicated properties than for this function in E. coli, or perhaps the analogous interactions have not yet been revealed for the latter. Control by repression also differs between these organisms. Little if any selective, or non-selective repression occurs in response to endproducts added to the exogenous environment of N.crassa. However derepression can be demonstrated with aromatic mutants. From the absence of mutants lacking DAHP synthetase it can be deduced that more than one structural gene probably codes for the DAHP synthetase function. Purification is hindered by extreme instability, but there is some indication that at least three isoenzymes, or molecular states exist.

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