Dihydropteroate Synthase: Affinity Chromatography and Mechanism of Action

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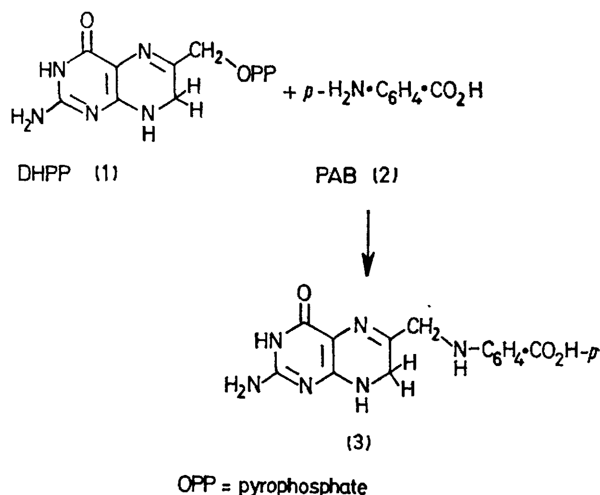
Summary The purification of dihydropteroate synthase by affinity chromatography on a sulphonamide linked to Sepharose provides insight into the mechanism of action of the enzyme.

DIHYDROPTEROATE synthase (E.C. 2.5.1.15) catalyses the substitution reaction between the pyrophosphate (**1**) (DHPP) and *p*-aminobenzoic acid (PAB) (**2**) to give 7,8-dihydropteroate (**3**), an intermediate in the bacterial synthesis of 7,8-dihydrofolate. The enzyme has chemotherapeutic significance because it is strongly inhibited by sulphonamides, which are analogues of PAB, and is the principal site of action of these antibacterial agents.[1]

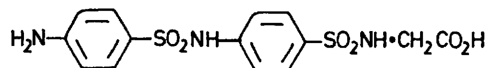
The enzyme has been partially purified by classical techniques[2] and we now report the specific purification by affinity chromatography.[3]

Sepharose-4B was activated with cyanogen bromide[4] and coupled to the spacer-arm di-(3-aminopropyl)amine.[5] The sulphonamide (**4**) was condensed with the Sepharose conjugate using 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulphonate in 50% aqueous dimethylformamide. This sulphonamide is a potent inhibitor of the enzyme (K_I 8 × 10^{-7}M).



When a saturated solution of crude enzyme from *E.coli* in either 0·2M Tris–HCl buffer, pH 8·5, or 0·1M potassium phosphate buffer, pH 8·0, was applied to a 60 × 8 mm

column of the sulphonamide–Sepharose, the protein and enzymic activity† were eluted together, although the enzyme was slightly retarded by the column. However, when the buffer contained in addition 18 μM DHPP and



(4)

5 mM dithiothreitol (DTT) essentially all the enzyme activity was retained on the column whilst the bulk of the protein was eluted. Immediately upon removal of DHPP and DTT from the buffer, enzymic activity emerged from the column. The eluted enzyme represents a purification of 180 fold in one step.

The obligatory presence of DHPP in the enzyme solution applied to the column and the negative elution effected by its removal are good evidence for the biospecificity of the process. Similar chromatographic behaviour has been observed with lactate dehydrogenase.[7] In this case kinetic experiments[8] indicate that NADH must be bound to lactate dehydrogenase before pyruvate binds and accordingly pig heart lactate dehydrogenase only binds to an inhibitor (oxamate)-containing column in the presence of NADH. It thus seems highly probable that dihydropteroate synthase requires that DHPP binds before PAB. This conclusion has an important bearing upon the mode of action of the anti-bacterial sulphonamides and upon the design of alternative chemotherapeutic inhibitors of dihydropteroate synthase.

The award of an S.R.C. studentship to J.R.S. is acknowledged.

(*Received*, 9*th January* 1975; *Com.* 025.)

† Enzyme activity was assayed by the method of Richey and Brown.[2] Protein was determined by the method of Lowry.[6]

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