MECHANISM OF 3-DEOXY-D-ARABINO-HEPTULOSONATE 7-PHOSPHATE (DAHP) SYNTHETASE*

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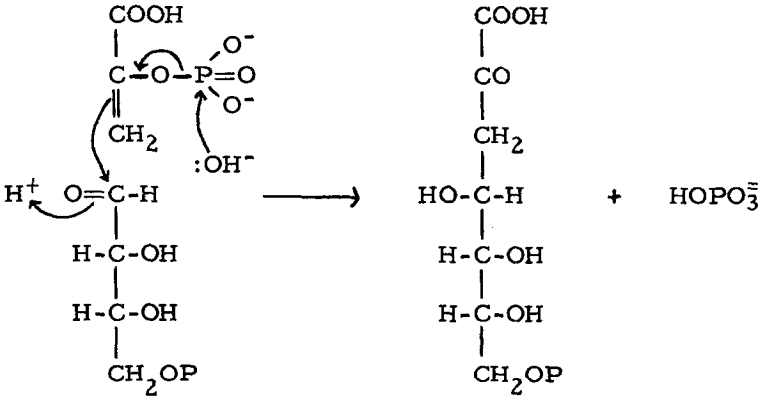
The condensation of enolpyruvate-P and erythrose-4-P to form DAHP (Scheme 1) was "tentatively regarded as a nucleophilic attack on



Scheme 1

enolpyruvate-P, by a reagent symbolized as OH^-, resulting in the release of phosphate and the formation of the open chain form of DAHP" (Srinivasan and Sprinson, 1959). Recent kinetic studies of the phenylalanine sensitive

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DAHP synthetase from Escherichia coli by Staub and Dénes (1967) have indicated a ping-pong mechanism (Cleland, 1967) for the reaction. It was also observed that the enzyme was stabilized by enolpyruvate-P against thermal inactivation, but not by erythrose-4-P. Hence it was suggested that the enzyme reacted first with enolpyruvate-P to release P_i, and then with erythrose-4-P to yield DAHP. The experiments described in the present report were designed to investigate the reaction with ^{18}O-enol-pyruvate-P and $H_2{}^{18}O$. It was found that P_i released was not labeled with ^{18}O from the water, but contained the ^{18}O of the C-O-P oxygen of enol-pyruvate-P. These results are in accord with the formation of a pyruvyl enzyme intermediate by addition of enyzme to enolpyruvate-P and elimination of P_i from the adduct.

Experimental

The enzyme preparation was a partially purified fraction of tyrosine sensitive DAHP synthetase from Salmonella typhimurium. It was obtained by chromatography on DEAE-cellulose, and had a specific activity of 50 μmoles of DAHP per mg of protein per hour. Like the phenylalanine isoenzyme from E. coli, this preparation was protected against thermal inactivation by enolpyruvate-P but not by erythrose-4-P. Erythrose-4-P was prepared by the method of Ballou and MacDonald (1963). ^{18}O-Enol-pyruvate-P was prepared (Knowles, Sprecher, and Sprinson, in press) from ^{18}O-bromopyruvic acid according to the procedure of Clark and Kirby (1963) for the unlabeled compound. It contained 13.2 atom % excess ^{18}O in the C-O-P oxygen. P_i was isolated as $MgNH_4PO_4$ which was converted to KH_2PO_4 (Harrison, Boyer, and Falcone, 1955). The latter was analyzed

for ^{18}O by the procedure of Ponticorvo and Rittenberg (1956).

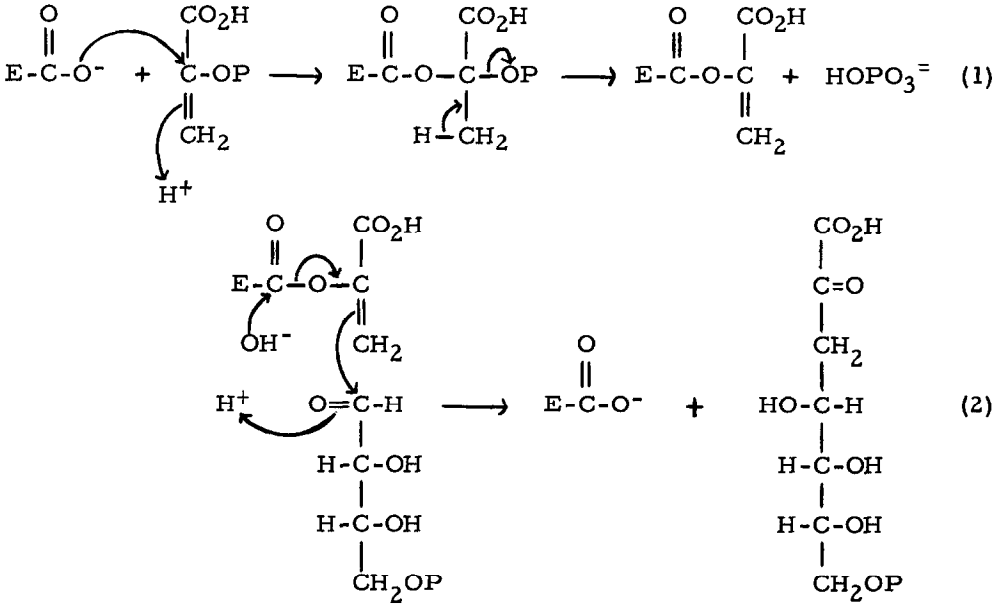
Solutions (110 ml) containing 110 μmoles of enolpyruvate-P, 110 μmoles of erythrose-4-P, 5.5 mmoles of Tris maleate buffer, pH 6.8, and enzyme (25 mg of protein), were incubated at 37° for 30 min. ^{18}O was present either in enolpyruvate-P or in water.

Results and Discussion

The P_i released in the $H_2{}^{18}O$ reaction mixture had no significant ^{18}O above the normal abundance, while the water evaporated from the solution had 1.38 atom % excess ^{18}O. In a similar incubation with ^{18}O-enolpyruvate-P the P_i had 2.40 and 2.55 atom % excess ^{18}O in two independent experiments. The calculated value for P_i resulting from C-O cleavage is $13.2/4 = 3.30$ atom % excess. The reasons for the low values obtained experimentally are unkown, and are under investigation. It is clear, however, that formation of DAHP is associated with C-O cleavage of the ester linkage of enolpyruvate-P, and that a nucleophilic attack on phosphorus (Scheme 1) does not occur.

The finding of ping-pong kinetics in DAHP synthetase, and of its stabilization by enolpyruvate-P have been interpreted as suggesting an initial reaction with enolpyruvate-P to release P_i, followed by reaction with erythrose-4-P to form DAHP (Staub and Dénes, 1967). Release of P_i by C-O cleavage is in accord with this interpretation. It may be tentatively suggested that electron donation by the ester oxygen of enolpyruvate-P results in protonation of the β-carbon, and is associated with attack on the α-carbon by a nucleophilic center of the enzyme. Elimination of P_i would give an enolpyruvyl enzyme

intermediate capable of reacting with erythrose-4-P. The above reactions, with a carboxyl group as the nucleophilic center of the enzyme, are shown in Scheme 2.



E = enzyme

H^+ = a proton from the medium, or from an electrophilic center of the enzyme.

Scheme 2

In support of this mechanism is the further observation that DAHP formed in a medium containing ^3H_2O was extensively labeled. The position of the tritium has not as yet been determined, but it is reasonable to assume that it was introduced at C-3 of DAHP. This is in accord with protonation of the β-carbon of enolpyruvate-P postulated in equation (1). An addition-elimination mechanism has been observed between shikimate-5-P and enolpyruvate-P in 3-enolpyruvylshikimate 5-phosphate

synthesis (Knowles, Sprecher, and Sprinson, in press). Further work is in progress to elucidate the questions raised by the present experiments.

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