STEREOSPECIFICITY OF THE 3-DEOXY-D-ARABINO-HEPTULOSONATE 7-PHOSPHATE SYNTHETASE REACTION.*

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Summary

As shown with PEP tritiated asymmetrically at C-3, the DAHP synthetase reaction is at least 60-70% stereospecific with respect to C-3 of PEP. This result excludes the intermediary formation of a methyl group at this carbon atom as an obligatory step in the reaction.

Kinetic studies (Staub and Dénes, 1967; Moldoványi and Dénes, 1969) of the condensation of phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E-4-P) to give 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) using the phenylalanine- and the tyrosine-sensitive enzymes from E. coli have indicated a "ping-pong" mechanism (Cleland, 1963) for this reaction. The evidence suggests that the enzyme first reacts with PEP. DeLeo and Sprinson (1968) found in ^{18}O experiments that the reaction involves C-O rather than P-O bond cleavage and, in accord with the kinetic data, proposed the mechanism shown in scheme 1. In this communication we report some results of experiments designed to determine the steric course of the reaction at C-3 of PEP using substrate labeled asymmetrically with tritium at this carbon atom.

Experimental

3-Phosphoglyceric acid (PGA)-3S, 3T and -3R, 3T were prepared from glucose-1T and mannose-1T, respectively, using hexokinase, glucose-6-phosphate isomerase or mannose-6-phosphate isomerase, phosphofructokinase, aldolase, triosephosphate isomerase, and 3-phosphoglyceraldehyde dehydrogenase in the presence of arsenate. On incubation with phosphoglyceromutase and enolase each of these two samples of PGA gives rise to an isomer of asymmetrically labeled PEP-3T. To improve the yield, this conversion was coupled to the subsequent reactions, the conversion of PEP-3T and non-labeled E-4-P into shikimate-6T by a modification of the procedures of Srinivasan et al. (1956, 1959) using a cell-free extract of E. coli mutant 83-24. Shikimate-7-^{14}C (prepared from

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PGA-1-^{14}C) was added as a reference label to the tritiated shikimic acids, which were then converted into chorismic acids by the method of Gibson and Gibson (1962) using an extract of A. aerogenes 62-1. An aliquot of each chorismate sample was analyzed for its T/^{14}C ratio and another was enzymatically converted into anthranilate (Zalkin and Kling, 1968) to confirm the position of the remaining tritium. The samples of shikimate and anthranilate were co-crystallized with carrier to assure constancy of the T/^{14}C ratios.

A sample of PEP-3T from PGA-3R, 3T obtained from Dr. L. A. Rose, was mixed with PEP-1-^{14}C, incubated with the E. coli extract and further treated as above.

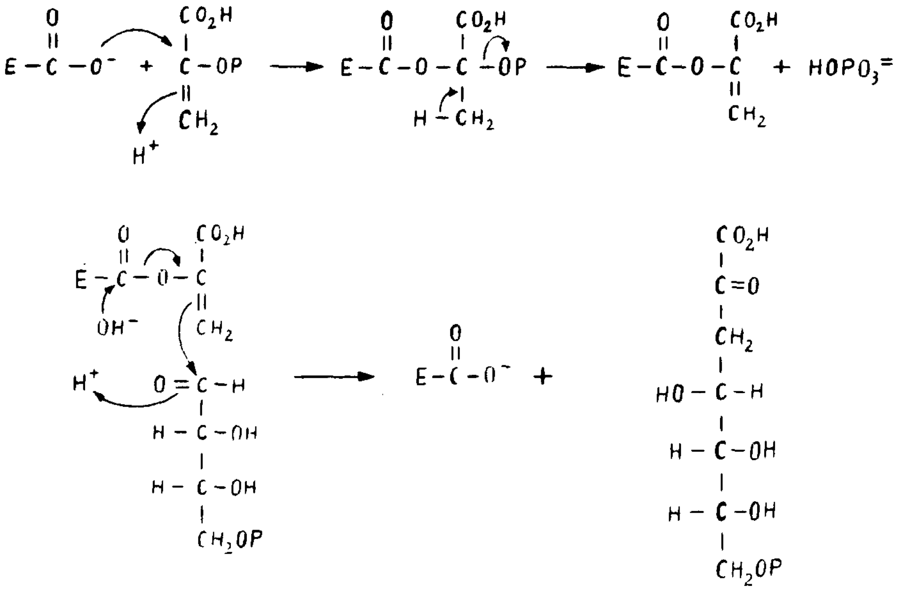
Radioactivity determinations were carried out in a Beckman LS 100 liquid scintillation counter using PPO and POPOP in toluene/methanol as the scintillation mixture and internal standards for each sample.

Results and Discussion

Table 1 shows the distribution of tritium at C-6 of shikimic acids obtained enzymatically from PGA-3R, 3T and from PGA-3S, 3T. Shikimate from PGA-3S, 3T was converted into chorismate with 18% retention of the tritium (expts. 1,3) whereas shikimate from PGA-3R, 3T retained 82 and 88% of the tritium (expts. 2,4). All these samples of chorismate upon reaction with anthranilate synthetase gave anthranilate which was devoid of tritium, showing that all the tritium was confined to the 6-position of the ring. These data indicate that the two hydrogens at C-3 of PEP predominantly retain their identity in the DAHP synthetase reaction. Using a sample of PEP-3T from PGA-3R, 3T which had been analyzed by conversion into malate and fumarate and found to be completely asymmetrically labeled (Rose, private communication), it was shown that the partial scrambling of the hydrogens observed was not already inherent in the starting PGA samples. This material mixed with

Table 1.

		Shikimate	Chorismate	
Expt.	Position of tritium in PGA	T/^{14}C	T/^{14}C	Retention of tritium
1	3S	14.0	2.5	18%
2	3R	14.3	11.7	82%
3	3S	12.6	2.3	18%
4	3R	5.9	5.2	88%



Proposed mechanism of the DAHP synthetase reaction (DeLeo and Sprinson, 1968)

PEP-1-^{14}C (T/^{14}C 2.78) gave shikimate (T/^{14}C 2.39) with 86% tritium retention, which in turn gave chorismate (T/^{14}C 2.11) with 88% tritium retention. The finding that a portion of the tritium equal to the degree of scrambling is lost also excludes the possibility that the chorismate synthetase reaction is partially non-stereospecific and suggests that the partial scrambling is due to either enolization of DAHP prior to its further conversion or to reversible protonation at C-3 as a side reaction of the enzyme-bound PEP moiety. The same phenomenon presumably also accounts for the incorporation of tritium from HTO into DAHP observed by DeLeo and Sprinson (1968). We conclude from these results that the DAHP synthetase reaction does not involve the transient formation of a methyl group at C-3 of the PEP moiety as an obligatory step.

Acknowledgment

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