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Chromatography of 3-Deoxy-D-Arabinoheptulosonic Acid-7-Phosphate Synthetase (trp) on Diethylaminoethyl Cellulose: a Correction

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In a previous communication (B. J. Wallace and J. Pittard, J. Bacteriol. **93**:237, 1967), we reported that chromatography on diethylaminoethyl (DEAE) cellulose of dialyzed extracts of *Escherichia coli* gave four peaks of 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP) synthetase activity. Two of these peaks were unambiguously identified as DAHP synthetase (phe) and DAHP synthetase (tyr), whereas it was concluded that the other two peaks occurring in fractions 9 to 10 and 34 to 35 represented DAHP synthetase (trp). Further work on the regulation of the DAHP synthetase isoenzymes has revealed that these two peaks do not represent DAHP synthetase (trp), but are artifacts. It is the purpose of this note to explain the nature of these artifacts and to present the elution profile of DAHP synthetase (trp).

To allow for maximal derepression of the DAHP synthetases an *aroB*$^-$ mutation had been introduced into many of the strains used in the previous work. The *aroB*$^-$ derivatives which are unable to carry out the second reaction in aromatic biosynthesis were then grown in a medium containing limiting amounts of shikimic acid and harvested for the preparation of cell-free extracts after growth had ceased. The peaks 9 to 10 and 34 to 35 found when such extracts are chromatographed do not represent enzyme activity, but represent the elution from the column of two compounds that give a positive reaction with the colorimetric test for DAHP. When the *aroB*$^-$ mutation is not present in the mutant strain, these compounds do not accumulate.

The compound eluting in fractions 34 to 35 appears to be DAHP, since synthetic DAHP (kindly provided by D. B. Sprinson) also elutes at this point. Both synthetic DAHP and this compound disappear when incubated with cell-free extracts of wild-type strains but do not disappear when the cell-free extracts are prepared from a strain lacking dehydroquinate synthetase.

The compound eluted in fractions 9 to 10 appears to be the dephosphorylated compound 3-deoxy-D-arabinoheptulosonic acid (DAH), as treatment of synthetic DAHP with alkaline phosphatase prior to chromatography produces a substance which still gives a positive color reaction but which elutes in fractions 9 to 10. As expected, the compound eluted in fractions 9 to 10 is not further metabolized by cell-free extracts prepared from a wild-type strain.

When a strain lacking DAHP synthetase (phe) and DAHP synthetase (tyr) but not containing the *aroB*$^-$ mutation is harvested from minimal medium in mid-exponential phase, the chromatography of its cell-free extract reveals a single peak of enzyme activity in fractions 37 to 40, as shown in Fig. 1. This peak is very unstable, losing 25% of its activity in 2 hr at 4 C. It is not significantly inhibited by phenylalanine, tyrosine, or tryptophan, either singly or together. Control tubes from which substrates were omitted gave values of absorbancy at 549 mμ of less than 0.025. When the same strain is grown in minimal medium containing phenylalanine, tyrosine, and tryptophan (it will not grow in the presence of tryptophan alone), the enzymatic activity found in fractions 37 to 40 is depressed sixfold. These results are also shown in Fig. 1. In a similar fashion, the specific activity of DAHP synthetase found in cell-free extracts prepared from cells harvested in mid-exponential phase from a medium containing phenylalanine, tyrosine, and tryptophan was only one-fifth that observed in cell-free extracts prepared from cells grown in minimal medium alone.

When cell-free extracts of a strain possessing no mutations in aromatic biosynthesis are

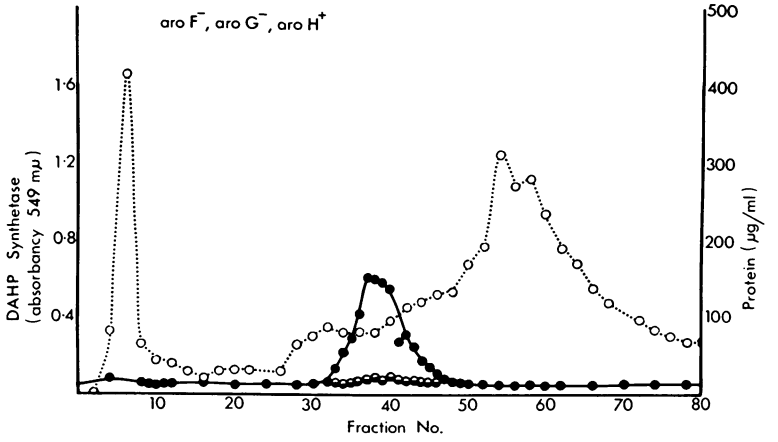

FIG. 1. *Chromatography of cell-free extracts of mutant strain AB2891 (aroF⁻, aroG⁻, aroH⁺). Symbols: ●, DAHP synthetase activity (cell-free extract prepared from cells grown in minimal medium); ◒, DAHP synthetase activity (cell-free extract prepared from cells grown in minimal medium supplemented with phenylalanine, tyrosine, and tryptophan); ○, protein. Conditions under which extracts were prepared, chromatographed, and assayed are described in a previous paper (B. J. Wallace and J. Pittard, J. Bacteriol. 93:237, 1967).*

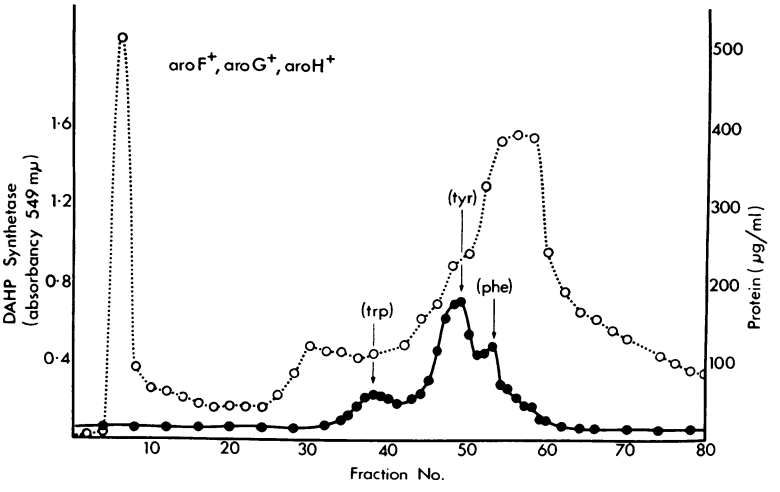

FIG. 2. *Chromatography of a cell-free extract of strain AB2825 (aroF⁺, aroG⁺, aroH⁺). Symbols: ●, DAHP synthetase; ○, protein; (phe), DAHP synthetase (phe); (tyr), DAHP synthetase (tyr); (trp), DAHP synthetase (trp).*

chromatographed, the DAHP synthetase (trp) peak can be seen, in addition to DAHP synthetase (phe) and DAHP synthetase (tyr). These results are shown in Fig. 2.

We are currently investigating our failure to observe this DAHP synthetase (trp) peak in some of our earlier experiments involving the *aroB*⁻ mutants.

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