BBA 63276

## Aromatic biosynthesis in yeast. II. Feedback inhibition and repression of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate synthase

In *Escherichia coli* 3-deoxy-*arabino*-heptulosonic 7-phosphate synthase (DAHP synthase, EC 4.1.2.15) the first enzymic function of the aromatic path is controlled by feedback inhibition (Phe, Tyr and Try?) and repression (Phe, Tyr, Try) of at least three isoenzymes[1-3]. In the course of a survey of the nature of DAHP synthase in other organisms (C. H. DOY, unpublished observations) the enzyme was examined from the wild-type *Saccharomyces cerevisiae* 24310 alpha-normal. As stated elsewhere[4], tyrosine and phenylalanine were inhibitors, but no combination of aromatic amino acids repressed DAHP synthase, or 5-dehydroquinate dehydratase (EC 4.2.1.10), the third enzymic function of the pathway. The present paper is to report the evidence on which these conclusions were based.

*S. cerevisiae* 24310 was grown and extracts prepared as described previously[4]. DAHP synthase was assayed essentially as described previously[1] using the cyclohexanone extraction modification. The specific activities of Table I are calculated on

TABLE I

DAHP SYNTHASE ACTIVITY OF *S. cerevisiae*. SPECIFIC ACTIVITY AND FEEDBACK INHIBITION RELATED TO SUPPLEMENTS OF AROMATIC AMINO ACIDS ADDED TO MINIMAL MEDIUM BEFORE GROWTH

Figures in parentheses: inhibition by Tyr corrected for possible cross-reaction on the Phe-sensitive portion. Figures in brackets: inhibition by Phe (observed) + Tyr (corrected). The possibility of cross-reaction is taken by analogy with the situation in *E. coli*[1].

| *Supplement ($10^{-3}$ M)* | | *Nil* | *Phe* | *Tyr* | *Try* | *Phe + Tyr + Try* | *Nutrient broth* |
|---|---|---|---|---|---|---|---|
| Specific activity (units/mg protein) | | 4.2, 3.6, 5.1 | 4.6, 3.6 | 3.7 | 6.8, 5.9, 4.6 | 4.1, 3.5 | 2.9, 2.6 |
| Proportion (%) of total specific activity inhibited using dialysed extracts and the end products ($10^{-3}$ M) | Phe | 35 | 37 | 45 | 33 | 36 | 49 |
| | Tyr | 68 (58) | 64 (56) | 65 (52) | 73 (61) | 71 (58) | 65 (45) |
| | Phe + Tyr | 93 [93] | 93 [93] | 92 [97] | 94 [94] | 94 [94] | 94 [93] |
| Proportion not inhibited by | Phe + Tyr | 7 | 7 | 8 | 6 | 6 | 6 |

the basis of a DAHP standard treated as an enzymic reaction mixture. In the survey from which this data is extracted, substrates (PEP and erythrose 4-phosphate) were each $2 \cdot 10^{-3}$ M and potential inhibitors at $10^{-3}$ M.

The data given in Table I illustrate the relationship between DAHP synthase activity and the environment during growth. It is clear that relative to minimal medium, little if any repression occurs when the medium is supplemented with aromatic amino acids. Repression in nutrient broth is slight, but probably significant. Sensitivity to inhibition by phenylalanine and tyrosine singly, or together, remains similar, although the increase in sensitivity to phenylalanine shown after growth in the presence of tyrosine may be significant. For *E. coli*, selective repression,

Abbreviation: DAHP, 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate.

especially of the tyrosine inhibited isoenzyme was readily demonstrated under conpitions analogous to the above[2]. Unlike *E. coli* repression below the "normal" wild-type level has not been detected. The failure to repress does not mean that repression is not a factor in control. The possibility exists that for *S. cerevisiae*, as with *Neurospora crassa*, a lower limit of repression occurs rather than a total absence of repression[12]. In the present work the level of 5-dehydroquinate dehydratase also was found to vary by less than 2-fold. It should be noted that in this paper repression and derepression refer to a change of specific activity measured using dialysed crude extracts.

Ammonium sulphate fractionation readily separated major DAHP synthase isoenzymes of *E. coli*[1] but was largely unsuitable for yeast in that much activity (>80%) was lost. However, the activity that remained showed a selective retention of sensitivity to phenylalanine with almost complete loss of sensitivity to inhibition by tyrosine (Table II). When discrete fractions were taken, inhibition by tyrosine

TABLE II

DAHP SYNTHASE OF *S. cerevisiae*. TENDENCY TO LOSE SENSITIVITY TO INHIBITION BY TYR AS THE RESULT OF $(NH_4)_2SO_4$ FRACTIONATION

| | *Inhibitor ($10^{-3}$ M) (% inhibition)* | | |
|---|---|---|---|
| | *Phe* | *Tyr* | *Phe+Tyr* |
| Crude extract (24 310 wild type) | 38 | 66 | 93 |
| $(NH_4)_2SO_4$ fractions (% saturation) | | | |
| 0–100 | 56 | 38 | 84 |
| >100 (*i.e.*, residue) | 71 | 13 | 80 |
| 50–60 | 31 | 0 | 36 |
| 60–70 | 74 | 13 | 73 |
| >70 (*i.e.*, residue) | 88 | 11 | 86 |

could be considered as almost entirely cross-reaction on the phenylalanine-sensitive site. It is clear that in some fractions the proportion of activity insensitive to inhibition by a mixture of phenylalanine and tyrosine is considerable. The recognition of this portion is not concomitant with the loss of sensitivity to tyrosine. It was not inhibited by tryptophan.

These findings suggest that separate sites recognise the modifying inhibitors phenylalanine and tyrosine, but it cannot be concluded unequivocally that isoenzymes exist. Since these conclusions were first reported[4], other workers[5] have independently confirmed inhibition by phenylalanine and tyrosine and lack of repression. However, those findings[5,6] and more recent work including genetic evidence[7,8], suggest more definitely that separate isoenzymes do exist.

In this laboratory detailed studies have been made of the DAHP synthase of *N. crassa*[9–12]. While resembling *E. coli* and *S. cerevisiae* in being inhibited by phenylalanine and tyrosine, it differs in that tryptophan can be demonstrated to be a potent inhibitor (as may be true for the native enzyme in other organisms[9]). While distinct sites recognising phenylalanine, tyrosine and tryptophan have been demonstrated and a mutant defective in the tyrosine sensitive portion has been obtained (D. M.

HALSALL and C. H. DOY, unpublished results) we are not yet prepared to state that separate, functionally independent isoenzymes exist. In *Bacillus subtilis* control differs in that the deactivating modifiers are chorismate and prephenate[13]. Work concerning the nature of DAHP synthase in micro-organisms is at present under review[3].

Mrs. G. LINDEGREN is thanked for the gift of *S. cerevisiae*, Dr. B. D. DAVIS for strains of *E. coli*, Dr. D. B. SPRINSON for DAHP. JULIE COOPER and SANDRA PAUL are thanked for technical assistance.

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Received July 17th, 1967
Revised manuscript received September 18th, 1967

*Biochim. Biophys. Acta*, 151 (1968) 293–295

BBA 63285

## Isocitrate lyase: Determination of $K_m$ values and inhibition by phosphoenolpyruvate

Published accounts of isocitrate lyase (*threo*-D$_s$-isocitrate glyoxylate-lyase, EC 4.1.3.1) give diverse values for the $K_m$ of the enzyme and for the extent of the inhibition by phosphoenolpyruvate (PEP). SMITH AND GUNSALUS[1] cited a $K_m$ value of 0.45 mM *threo*-D$_s$(+)-isocitric acid for the enzyme from *Pseudomonas aeruginosa* and similar values have been reported from other micro-organisms[2–4]. In contrast, $K_m$ values some 10 times smaller have been found chiefly by KORNBERG and his colleagues[5–7]. It is noticeable that nearly all the low values were obtained using a continuous spectrophotometric assay[8] while the higher values were obtained by methods that estimated the glyoxylate formed from isocitrate during a standard period of incuba-

Abbreviation: PEP, phosphoenolpyruvate.