Identification of the triose phosphate formed in the tryptophan synthetase reaction

In *Escherichia coli* tryptophan synthetase is a two-component enzyme catalyzing the following three reactions[1,2]:

Indole + L-serine ⟶ L-tryptophan (1)
Indole-3-glycerol phosphate + L-serine ⟶ L-tryptophan + triose phosphate (2)
Indole-3-glycerol phosphate ⇋ indole + triose phosphate (3)

Reactions (1) and (2) require pyridoxal phosphate as a cofactor. Reaction (2) is responsible for tryptophan synthesis in the normal cell[3]. When the enzyme is presented with indole-3-glycerol phosphate (InGP) or indole and a mixture of triose phosphates in the absence of L-serine, reaction (3) can be shown to occur. In the present experiments, in order to determine whether glyceraldehyde 3-phosphate or dihydroxyacetone phosphate is the triose phosphate concerned in reactions (2) and (3), purified *E. coli* tryptophan synthetase has been coupled with either glyceraldehyde 3-phosphate dehydrogenase* or α-glycerol phosphate dehydrogenase. Reactions were followed spectrophotometrically by noting the change in absorbancy of DPN or DPNH at 340 mμ.

The more acid-stable protein component of *E. coli* tryptophan synthetase, component A, was purified as previously described[1] from the supernatant of a $MnCl_2$-treated extract of a strain (B-8) producing large amounts of this component. Chromatography of a pH-4.0 supernatant on a DEAE-cellulose column resulted in a preparation of component A which was electrophoretically homogeneous[1], free of tryptophan synthetase component B, and nearly free of triose phosphate isomerase. Component B was prepared from a similar $MnCl_2$ supernatant of a strain (A-2) rich in this component. Ammonium sulfate fractionation followed by chromatography on DEAE-cellulose** resulted in a preparation which was free of component A and contained only small amounts of DPNH oxidase and triose phosphate isomerase activity.

Mixtures of components A and B, when added to cuvettes containing InGP, DL-serine and pyridoxal phosphate in addition to the glyceraldehyde 3-phosphate dehydrogenase system[4], rapidly reduced amounts of DPN stoichiometric to the amount of InGP added (Fig. 1). (No absorbancy change was observed when glyceraldehyde 3-phosphate dehydrogenase was omitted.) The same tryptophan synthetase components and substrates, when added to cuvettes containing an α-glycerol phosphate dehydrogenase system[4] caused only a slow oxidation of DPNH unless triose phosphate isomerase was also added (Fig. 2); the amount of DPNH oxidation observed in the absence of added triose phosphate isomerase was that expected from the small amounts of DPNH oxidase and triose phosphate isomerase contaminating the tryptophan synthetase preparations. It can be seen from Figs. 1 and 2 that no reaction was observed when one of the two *E. coli* components was omitted.

When reaction (3) was examined by omitting DL-serine and pyridoxal phosphate from the cuvettes, entirely analogous results were obtained. Again the amount of

Abbreviations: InGP, indole-3-glycerol phosphate; DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide; DEAE-, diethylaminoethyl.

* Glyceraldehyde 3-phosphate dehydrogenase, α-glycerolphosphate dehydrogenase, and a mixture of the latter enzyme and triose phosphate isomerase were all products of C. F. Boehringer and Sons, Mannheim.

** A similar purification of component B has been developed by O. SMITH AND C. YANOFSKY personal communication).

DPN reduced or oxidized was stoichiometric with the InGP added. The rate of reaction (3), however, was about one-tenth that of reaction (2). This finding is in agreement with previous results using different assay techniques and conditions[1]. Similar activity ratios have been reported by YANOFSKY AND RACHMELER[3] using the single component tryptophan synthetase of *Neurospora crassa*.

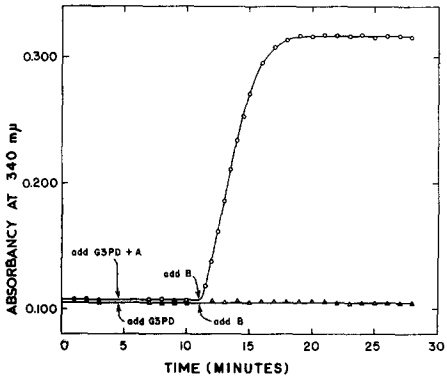

Fig. 1. Cuvettes contained 0.02 *M* NaP_2O_7, buffer, pH 8.5, 0.003 *M* Na_2HAsO_4, 1.5 μmoles DPN, 0.03 % DL-serine, 0.001 % pyridoxal phosphate, and 0.1 μmole InGP. 68 units* of glyceraldehyde 3-phosphate dehydrogenase (G_3PD), 11.6 units of component A and 5.6 units of component B were added at times indicated by the arrows.

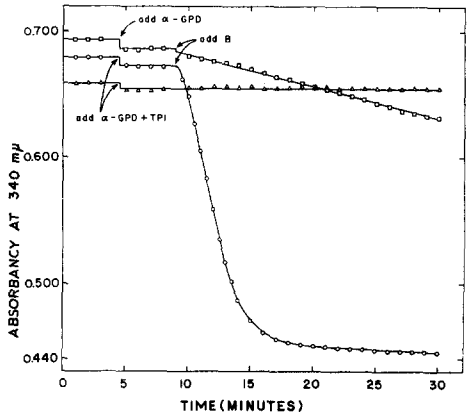

Fig. 2. Cuvettes contained 0.02 *M* NaP_2O_7 buffer, pH 8.5, 0.3 μmole DPNH, 0.3 % DL-serine, 0.001 % pyridoxal phosphate, 0.1 μmole InGP and 11.6 units of component A. 58 units of α-glycerol phosphate dehydrogenase (α-GPD) *or* 68 units of α-glycerol phosphate dehydrogenase plus excess triose phosphate isomerase (TPI), and 5.6 units of component B were added at times indicated by the arrows.

RACHMELER[5] found that when *N. crassa* tryptophan synthetase was presented with both indole and InGP as substrates for conversion to tryptophan, indole was utilized exclusively. In his experiments the fate of the triose phosphate moiety was unknown. When a similar experiment was performed with the *E. coli* enzyme by furnishing indole and InGP in equal concentrations in an experiment such as that of Fig. 1, about a 50 % decrease in the rate of DPN reduction was found. Even increasing the ratio of indole to InGP to 10:1 did not abolish glyceraldehyde 3-phosphate production. These results may indicate that *E. coli* tryptophan synthetase can utilize indole and InGP concurrently in the formation of tryptophan. Additional studies bearing on this problem are in progress.

The present experiments have demonstrated that glyceraldehyde 3-phosphate is the sole product formed from the glycerol phosphate side-chain of InGP during the tryptophan synthetase reaction. Obviously, the coupled reactions illustrated form a rapid and convenient way of assaying the purified enzyme in two of its reactions.

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* A unit of enzyme is that amount capable of causing the disappearance of 0.1 μmole of substrate or the appearance of 0.1 μmole of product in 20 min under the conditions of the experiment. Units of components A and B are determined in a 3-fold or greater excess of the other component.

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[2] C. YANOFSKY AND I. P. CRAWFORD, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1016.
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[4] E. RACKER, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 3, Academic Press, New York, 1957, p. 294–295.
[5] M. RACHMELER, *A Study of the Normal and Mutationally Altered Forms of Tryptophan Synthetase of Neurospora*, Doctoral Thesis, Western Reserve University, 1960.

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Structure chimique de la moitié N-terminale du lysozyme de blanc d'oeuf de poule

Au cours de l'étude de la structure chimique du lysozyme de blanc d'oeuf de poule, le pentapeptide N-terminal déjà connu[1]: H–Lys–Val–Phe–Gly–Arg a pu être intégré dans une séquence contenant soixante des quelques 130 acides aminés de la protéine. Huit peptides obtenus par hydrolyse trypsique ("unités trypsiques") du lysozyme réduit par l'acide thioglycolique puis traité par l'acide iodacétique[2] se sont en effet trouvés placés dans cet enchaînement N-terminal compte tenu des résultats acquis dans l'étude des peptides formés au cours de l'hydrolyse chymotrypsique ("unités chymotrypsiques") et contenant des résidus d'acides aminés basiques.

Sur les 8 unités trypsiques, 7 avaient déjà été décrites précédemment[1,3]:

T 1: Lys libre
T 2: Arg libre
T 3: Val–Phe-Gly–Arg
T 4: His–Gly–Leu–Asp–Asp(NH_2)–Tyr–Arg
T 5: Cys–Glu- Leu–Ala–Ala–Ala–Met–Lys
T 6: Phe–Glu–Ser–Asp(NH_2)–Phe–Asp(NH_2)–Glu(NH_2)–Ala–Thr–Thr–Asp(NH_2)–Arg
T 7: Asp–(Gly,Ser,Thr,Thr,Asp)–Asp–Tyr–Gly–Ileu–Leu–(Glu,Ileu,Asp,Ser)–Arg

La structure de la huitième unité:

T 8: Gly–Tyr–(Gly,Ser,Leu)–Asp(NH_2)–Try–Val–Cys–Ala–Ala–Lys

n'a été établie que récemment[4]. On sait de plus que T 2 s'enchaîne à T 5 car il a été possible d'isoler dans l'hydrolysat trypsique le peptide: Cys–Glu–Leu–Ala–Ala–Ala–Met–Lys–Arg.

Quelques unités chymotrypsiques du lysozyme avaient déjà été étudiées en partant de la protéine dénaturée par la chaleur[5]. Cependant parmi ces peptides aucun ne pouvait contenir de demi-résidu de cystine. Cette étude a été reprise en soumettant le lysozyme réduit par l'acide thioglycolique et traité par l'acide iodacétique[3] à l'action de la chymotrypsine (pH 7.5; 3 h; 37°; rapport enzyme/substrat: 1/50; concentration en protéine: 1 %). On sait qu'après réduction chaque résidu de cystine est transformé en deux résidus de S-carboxyméthylcystéine[2,3]; tous les autres acides aminés et même le tryptophane restent inchangés, à l'exception de la méthionine[6]: des peptides contenant à la fois des résidus de cystine et de tryptophane pouvaient ainsi être isolés; c'est en effet la présence de ces deux acides aminés qui rend si difficile l'étude du