The exclusion of free indole as an intermediate in the biosynthesis of tryptophan in *Neurospora crassa*

During the past fourteen years considerable evidence has been presented indicating that the following reaction constitutes the terminal step in the biosynthesis of tryptophan in *Neurospora crassa* and several other microorganisms[1,2]: indole + L-serine → L-tryptophan. Tryptophan synthetase (TSase), the enzyme catalyzing this reaction, requires pyridoxal phosphate (B_6alP) as cofactor[3].

Enzyme[4] and mutant[5] studies with *Escherichia coli* have shown that indole is formed as follows: anthranilic acid + phosphoribosylpyrophosphate → indoleglycerol phosphate (InGP); InGP → indole + triose phosphate. Since extracts of *E. coli* also contain TSase, tryptophan can be formed from anthranilic acid or InGP if the proper supplements are provided. *Neurospora* preparations also convert anthranilic acid and InGP to tryptophan, but, as can be seen in Table I, they do not form indole from InGP at a rate sufficient to account for the high rate of tryptophan formation from InGP. This observation, although disturbing, could be explained in several ways. One explanation, supported by the experimental results to be presented, is that free indole is not an intermediate in the conversion of InGP to tryptophan in *Neurospora*.

TABLE I

RATES OF VARIOUS REACTIONS INVOLVING INDOLE OR InGP

All reactions were carried out in phosphate buffer, pH 7.5. Added B_6alP was present in each tube. The enzyme preparation employed was 25-fold purified *Neurospora* TSase. Comparable relative rates are obtained with crude extracts of *Neurospora*.

Reaction	*μmoles taken up or formed/30 min/ml extract*
InGP → indole (—NH_2OH)	0.2
InGP → indole (+NH_2OH)*	0.3
Indole + hexose diphosphate + aldolase → InGP	0.2
InGP + L-serine → L-tryptophan	9.5
Indole + L-serine → L-tryptophan	20.0

* Hydroxylamine, at a concentration of 10^{-3} *M*, generally stimulates indole formation from InGP.

The rationale of the experimental approach employed in examining this possibility was as follows: If tryptophan formation in *Neurospora* involves free indole as an intermediate and proceeds by the sequence InGP → indole → tryptophan, then a *Neurospora* preparation capable of converting InGP to indole should form tryptophan from InGP equally well in the presence of TSase from *Neurospora* or *E. coli*. On the other hand, if the two reactions InGP → indole and indole → tryptophan are actually parts of a single reaction occurring on one enzyme, and if the indole formed is enzyme-bound and does not occur free during the course of the reaction, then a mixture of a *Neurospora* preparation capable of converting InGP to indole with an *E. coli* extract capable of forming tryptophan from indole would not be equivalent to a *Neurospora* preparation which could carry out the complete reaction, InGP → tryptophan.

A *Neurospora* preparation capable of forming indole from InGP but unable to convert indole to tryptophan was prepared by treating partially purified TSase with 10^{-3} *M* hydroxylamine for 5 min at 4°. The hydroxylamine presumably acts by combining with and effectively removing the free and enzyme-bound B_6alP. Excess hydroxylamine was removed by two precipitations of the treated TSase with ammonium sulfate. The preparation obtained by this procedure was unaffected in its ability to convert InGP to indole but could not form tryptophan from either indole or InGP. Upon addition of B_6alP both activities were regained at the full values expected. The *E. coli* preparation employed in this experiment was obtained from a tryptophan-requiring mutant, T-4, which is blocked in the conversion of InGP to indole[5]. Extracts of this strain exhibit TSase activity in the absence of added B_6alP but do not convert InGP to indole (Table II).

The results presented in Table II indicate that InGP is not converted to tryptophan at an appreciable rate in the absence of added B_6alP when T-4 TSase is added to the hydroxylamine-treated *Neurospora* preparation (Prep. A). Tryptophan is formed, however, upon addition of B_6alP. to the mixed preparations or to the *Neurospora* preparation alone.

The inability of *E. coli* TSase to substitute for *Neurospora* TSase activity in the conversion

TABLE II

INABILITY OF *E. coli* TSASE TO SUBSTITUTE FOR *Neurospora* TSASE ACTIVITY IN THE CONVERSION OF InGP TO TRYPTOPHAN

Each tube contained 0.2 μmole InGP or 0.4 μmole indole, 30 μmoles L-serine and 0.1 *M* phosphate buffer, pH 7.5, in a final volume of 1 ml. Following deproteinization with perchloric acid, the supernatant solutions were neutralized and assayed for tryptophan microbiologically[6]. Prep. A is hydroxylamine-treated *Neurospora* TSase. T-4 (*E. coli*) TSase cannot convert InGP to indole.

Additions	*Tryptophan (μmole) formed from*			
	Indole		*InGP*	
	$+B_6alP$	$-B_6alP$	$+B_6alP$	$-B_6alP$
0.02 ml Prep. A	0.24	0	0.1	0
0.02 ml Prep. A + 0.05 ml T-4 TSase	—	0.28	0.11	0.01
0.02 ml Prep. A + 0.1 ml T-4 TSase	—	—	—	0.005
0.05 ml T-4 TSase	0.46*	0.25	0	0

* Based on assays with smaller amounts of enzyme.

of InGP to tryptophan is not due to poorer affinity (larger K_m) of the *E. coli* enzyme for indole. This was confirmed most clearly in control experiments in which indole was generated gradually from tryptophan by tryptophanase. Under these conditions both *E. coli* and *Neurospora* TSases removed equivalent amounts of indole. Furthermore, the inability of the *E. coli–Neurospora* mixture to form tryptophan from InGP in the absence of added B_6alP is not due to excess hydroxylamine in the *Neurospora* preparation. As can be seen from the data in Table II, the *Neurospora* preparation did not inhibit the TSase activity of the T-4 extract. It would appear, therefore, that a B_6alP-requiring component of the *Neurospora* preparation must be activated to obtain rapid conversion of InGP to tryptophan.

In other studies with *Neurospora* preparations, in which toluene was employed as a trapping agent for free indole, it was found that considerable amounts of tryptophan were formed from InGP while only trace amounts of indole appeared in the toluene layer. Control experiments indicated that slowly generated indole would have been trapped if it were formed during the course of the reaction. Thus these studies too suggest that free indole is not an intermediate.

On the basis of the data obtained, the following mechanism is proposed for the conversion of InGP to tryptophan by *Neurospora*:

$$\text{InGP} + \text{Tsase} \longrightarrow \text{indole-TSase} + \text{triose phosphate}$$

$$\text{indole-TSase} + \text{L-serine} \longrightarrow \text{L-tryptophan} + \text{TSase}$$

Consistent with this proposal are the additional facts that (1) indoleglycerol or an indoleglycerol-like compound rather than indole appears to be the principal compound accumulated by *Neurospora* mutants which lack TSase and (2) no *Neurospora* mutant which responds to indole and accumulates indoleglycerol has been discovered.

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