carrier by the paraenzyme. The adsorption may serve as a means of orientating the molecules, and in itself may modify the tertiary structure of the protein moieties and hence influence their interaction.

Alternatively, for those allotopic complexes having a proteolytic action, one can imagine that little or no interaction between paraenzyme and active site carrier takes place, but that the substrate is more favourably bound to the complex than to the single enzyme. The observation by Esnouf that the esterolytic properties of factor X_a have not been enhanced by the formation of a prothrombinase complex hints in this direction[20].

Inhibition of tryptophan uptake in Aspergillus fumigatus by tryptamine

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Summary. The tryptophan uptake was inhibited considerably in tryptamine grown cells. This inhibition was due to feed-back inhibition and not to repression.

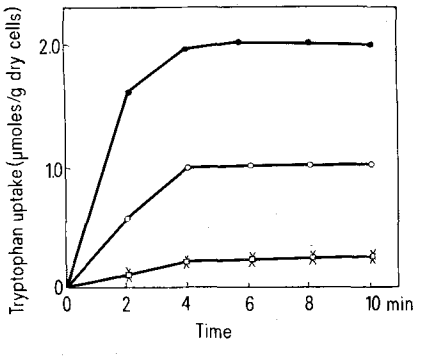
Tryptophan plays a central role in the biosynthesis of ergot alkaloids. In our previous work[3,4], we have shown that Aspergillus fumigatus mycelium can actively take up L-tryptophan from the medium and that a correleation exists between the ability of a strain to produce alkaloids and its ability to transport tryptophan. In continuation of this work on Aspergillus fumigatus, we have noticed that tryptamine inhibited the uptake of tryptophan. Wiley and Matchett[5,6] have reported the inhibition of tryptophan uptake by tryptophan and its analogues in Neurospora crassa. The purpose of this investigation was to study the mechanism of inhibition of tryptophan uptake in tryptamine grown cells.

Material and methods. A strain of A. fumigatus obtained from the Division of Mycology and Plant Pathology, IARI, New Delhi, was used in this investigation. The culture medium used was essentially that of Rao et al.[7]. Tryptamine was supplemented at the concentration of 200 mg/l. The cells were harvested after 48 h, washed with distilled water and pressed in between sheets of filter paper. Tryptophan uptake was assayed by the method of Brown and Romano[8]. The assay system contained 200 μmoles of phosphate buffer (pH 6.0), with 2.0 mg dry cells/ml in 10 ml volume; 10 μmoles of L-tryptophan were added after 30 min incubation to start the reaction. Uptake rate was calculated from initial values.

Results and discussion. We have found that tryptophan uptake was greatly reduced in tryptamine grown cells. The results presented in the table indicated that the tryptophan uptake was inhibited to the extent of 87.5% in tryptamine grown cells in comparison with ammonium citrate grown cells (considering 100% tryptophan uptake). 2 different experiments were designed to find out the mechanism of inhibition of tryptophan uptake. In one experiment, the tryptamine grown cells were washed, resuspended in ammonium citrate medium, and divided into 2 parts. Cycloheximide (50 μg/ml) was added to one culture to inhibit protein synthesis, and another culture was permitted to grow without restriction. If the cells were repressed in the presence of tryptamine and no tryptophan permease component was present, the growing cells would be expected to synthesize the transport component in the absence of tryptamine. Cycloheximide-treated cells would not be expected to synthesize this component and would remain repressed. During 48 h growth period, the cell mass of the growing culture increased significantly, indicating that protein synthesis had occurred. Tryptophan uptake studies with 48 h culture (figure) showed that there was a slight initial uptake

Inhibition of tryptophan uptake by tryptamine in Aspergillus fumigatus

Growth condition	Tryptophan uptake (μmoles/min/g dry cell)	%
Ammonium citrate	0.50	100.0
Tryptamine	0.04	12.5



Tryptophan uptake by Aspergillus fumigatus grown under various conditions. ○, Tryptamine grown cells resuspended in ammonium citrate medium. □, Tryptamine grown cells resuspended in ammonium citrate medium containing 50 μg of cycloheximide per ml. ●, Ammonium citrate grown cells (experiment 3). ×, Ammonium citrate grown cells with tryptamine added prior to experiment (experiments 1 and 2).

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(till 4 min) which then ceased. No tryptophan uptake was observed with cycloheximide-treated cells. These results led us to devise other experiments in a first attempt to distinguish between repression and feed-back inhibition. Ammonium citrate-grown cells were divided into 3 parts; 2 flasks (experiment 1 and 2) were supplemented with tryptamine and incubated for 0.5 h; tryptamine was removed from 1 culture by centrifugation and the cells were washed by subsequent centrifugation. These washed cells were transferred to ammonium citrate medium for additional 0.5 h (experiment 2). The third culture (experiment 3) served as control. 0.5-h-tryptamine supplemented culture (experiment 1) and the other 2 cultures were assayed for tryptophan uptake. The results in the figure indicated that no tryptophan was transported into either culture supplemented with tryptamine in contrast to unsupplemented control (experiment 3).

It can be concluded from the above results that the inability of tryptamine-grown cells for the uptake of tryptophan was caused by a severe feed-back inhibition of tryptophan uptake system, and not by repression of the formation of an uptake or transport component. Similar observations were reported in the case of glutamate transport inhibition by glutamine in Synchococcus cedrorum[9].

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Occurrence of sepiapterin deaminase in the normal type silkworm, Bombyx mori

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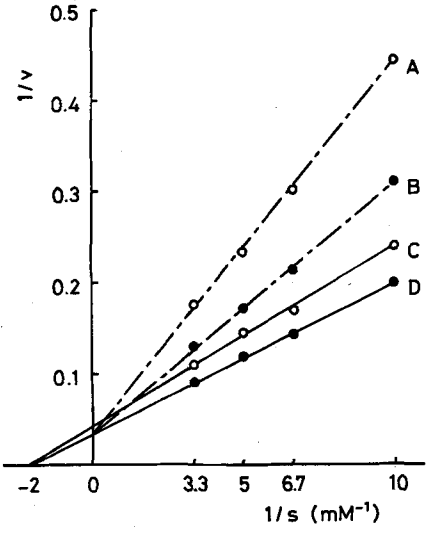
Summary. Sepiapterin deaminase was prepared from the normal strain of the silkworm, Bombyx mori. After inhibition experiments, this enzyme was found to have the same properties as that isolated from the lemon mutant strain. Several new inhibitors and their K_i values are described for the deaminase.

Sepiapterin deaminase was discovered in fat bodies of the lemon mutant strain of the silkworm, Bombyx mori[2]. Following this, the enzyme was purified from the integument of the same strain of silkworm and some of its properties were described[3]. Pterin and xanthopterin were shown at that time to be competitive inhibitors of the enzyme. The present paper reports the further study of the deaminase, in particular, the occurrence of reduced levels of the enzyme in the integument of the normal type silkworm, the mechanism of inhibition of additional inhibitors, and a comparison of the deaminase in the 2 types of silkworm, as well as with other pteridine deaminases.

Crystalline sepiapterin was obtained by the previously described method[4]. Other reagents were obtained from commercial sources. Sepiapterin deaminase was purified from both the normal type and the lemon mutant strains of Bombyx mori[3]. Data on the process of purification of the enzyme from the normal type silkworm is presented in table 1. The activity of the enzyme in crude extracts of normal type silkworm integument was from $^1/_{10}$ to $^1/_3$ less than that found with the lemon mutant strains.

Determination were made of the effect of several inhibitors on the deaminase prepared from both the normal type and lemon mutant strains of Bombyx. Reaction mixtures contained the following components in 1 ml of solution: potassium phosphate buffer, pH 8.0, 100 μmoles;



Double reciprocal plots of velocity versus sepiapterin concentration in the presence of inhibitors. Reaction mixtures are described in the text. Velocity is in terms of nmoles of sepiapterin decomposed per min. Plot A) 1×10^{-3} M 8-azaguanine present; B) 5×10^{-5} M amethopterin present; C) 5×10^{-4} M p-chloromercuribenzoate present; D) no inhibitor present.

Table 1. Summary of purification of sepiapterin deaminase from normal type silkworms

Step	Volume (ml)	Total activity (units)	Total protein (mg)	Specific activity	Yield
Crude extract	353	296.5	4006.6	1	100
Ammonium sulfate	42.5	416.5	850.0	6.6	140.4
DEAE-cellulose column	22.0	330.0	55.2	80.8	111.3
First hydroxylapatite column	30.0	67.5	9.07	100.5	22.8
Second hydroxylapatite column	6.7	28.5	0.58	658.1	9.6