experiments with unadenylylated enzyme (data not shown) indicated only 1 TNS/subunit in each type of site and much lower overall enhancement of TNS fluorescence. Thus, adenylylation appears to result in increased exposure of apolar binding area. This may correlate with decreased stability of the protein and enhanced sensitivity to feedback modifiers known to occur upon adenylation[2-5].

Fluorescence polarization studies. Witholt and Brand[14] have demonstrated that fluorescence polarization techniques allow one to distinguish among 3 distinct modes of fluorophor binding to ellipsodal macromolecules. Normalized Perrin plots for each are unique, and respond characteristically to changes in excitation wavelength: Class I, excited state dipoles of the fluorophor are fixed and symmetrically oriented relative to the axis of rotation of the ellipsoidal protein; Class II fluorophor dipoles are fixed but randomly oriented; Class III, covalently bound fluorophor rotates rapidly and freely.

Figure 2 shows normalized Perrin plots for TNS bound to glutamine synthetase ($E_{\overline{11}}$). As one increases the TNS from 10^{-5} to 10^{-4} M (and TNS bound/subunit from 1 to 3) the curve shape changes slightly but remains qualitatively the same. Comparison to Witholt and Brand's[14] model schemes seem to suggest Class I-type binding with higher levels of TNS causing some changes in the angle between fluorophor dipole and the ellipsoidal macromolecule rotational axis. The pattern response to excitation wavelength (λ) is also typical of Class I binding. The rate of displacement of TNS from the enzyme surface by substrates or modifiers is relatively slow ($t_{1/2} \simeq 1$ min), and this argues against any Class III-type binding. When polarization was observed at $t = 0$ with subsaturating (10^{-5} M) TNS, the pattern is more like that expected for Class II binding. Thus, the association of TNS molecules with enzyme may involve a time-dependent transition from a somewhat disordered state to a more orderly one. At saturating levels of TNS (10^{-4} M) however, at $t = 0$ the dye appears to bind immediately in an ordered manner. Finally, in figure 3, the normalized Perrin plots for a fluorophor with a much longer excited lifetime, pyrene butyrate ($\tau > 100$ ns[17,18]) are also typical of patterns for symmetrical fluorophor binding (Class I).

Conclusion. The present evidence indicates that orientation of the excited state dipole of the bound dyes, relative to the axis of rotation of the oblate ellipsoid of E. coli glutamine synthetase, is regular and symmetrical. This in turn, may be of significance in the mechanisms of regulation of this highly complex key enzyme[2-5,8,19], i.e., spatial ordering of modifier sites.

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Hepatic induction of threonine dehydratase and tryptophan pyrrolase in tyrotoxic rats

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Summary. The levels of hepatic threonine dehydratase and tryptophan pyrrolase are elevated in 5% tyrosine-fed rats, and these increases are dependent on the dietary tyrosine level. Experiments with RNA and protein synthesis inhibitors indicate that the appearance of these new enzyme activities are dependent on concomitant new protein synthesis and the inducer operates at a transcriptional level.

Threonine supplementation has an alleviating effect on tyrosine toxicity in relation to growth depression and onset of toxic symptoms[1-3]. Hepatic tyrosine transaminase is increased in tyrotoxic rats developed by excess tyrosine feeding[4-6]. While studying the effect of threonine supplementation on tyrosine toxicity, it has been observed that besides tyrosine transaminase, the activity of threonine dehydratase is also increased in tyrosine-fed rats[7].

In this paper, there is evidence that the levels of threonine dehydratase and tryptophan pyrolase are elevated in excess tyrosine-fed rats, and the activities are inhibited by administration of inhibitors of RNA and protein synthesis e.g. actinomycin D, puromycin and cycloheximide.

Materials and methods. Weanling albino (Holtzman strain) rats, weighing 25–40 g were used in these experiments. A basal diet containing 9% casein was prepared according to Benton et al.[8]. In order to develop tyrosine toxicity, L-tyrosine (3% or 5%) was added to the basal diet at the expense of equivalent amount of starch. Animals were kept in temperature and light controlled room. For the induction experiments, rats kept on tyrosine diet were killed at different times by cervical dislocation and the activities of hepatic threonine dehydratase and tryptophan pyrrolase were determined by the method of Bottomley et al.[9] and of Knox et al.[10]. Protein was measured by the method of Lowry et al.[11]. At 0 h, actinomycin D (50 µg/100 g b.wt) or cycloheximide (0.5 mg/100 g b.wt) or puromycin was (3.5 mg/100 g b.wt) was administered intraperitoneally to the rats fed on either tyrosine

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Effect of actinomycin D, puromycin, cycloheximide on the induction of threonine dehydratase and tryptophan pyrrolase in 5% tyrosine fed rats

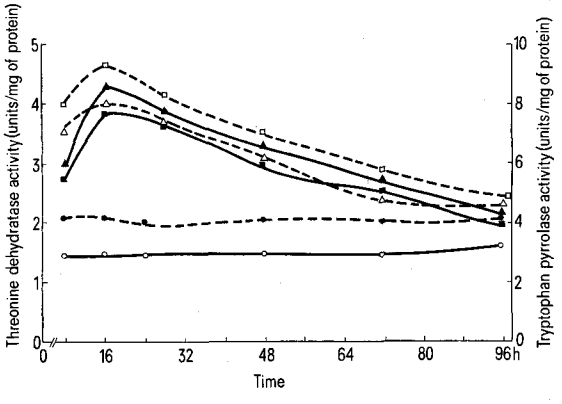
Dietary supplementation	Treatment	Specific activity of threonine dehydratase	Inhibition (%)	Specific activity tryptophan pyrrolase	Inhibition (%)
–	–	1.50 ± 0.2	–	4.4 ± 1.4	–
5% tyrosine	–	4.33 ± 0.2*	–	9.3 ± 1.2*	–
5% tyrosine	Actinomycin D (50 µg/100 g b. wt)	1.63 ± 0.2	95.5	4.71 ± 1.2	94.4
5% tyrosine	Puromycin (3.5 mg/100 g b. wt)	1.78 ± 0.2	90	5.15 ± 1.3	85
5% tyrosine	Cycloheximide (0.5 mg/100 g b. wt)	1.71 ± 0.2	92.6	4.81 ± 1.1	91

At zero time each inhibitor was administered to 1 group of tyrosine-fed rats and another group of tyrosine-fed rats received no injection. The same treatment was done in control group also. At 16 h, after the administration of antibiotic, rats were killed and the enzyme activities in the cell extract were determined. Enzyme activity is expressed as mentioned in the figure. There is no change in both the enzyme activities in control rats after the treatment with antibiotic (not presented in the table). * The results are expressed as ± SEM of et least 8 different experiments of each group containing 4 rats and it significantly differs from control value. $p < 0.001$.

containing diet or basal diet. Saline solution was injected to the control rats. After 16 h, rats were killed and the level of liver threonine dehydratase and tryptaphan pyrrolase were determined.

Results and discussion. The activities of threonine dehydratase and tryptophan pyrrolase in livers of excess tyrosine-fed rats are shown in the figure. It is evident that the activities of these 2 enzymes are significantly increased with increasing the level of dietary tyrosine. After having the experimental diet, the level of these enzymes reaches maximum at 16 h and then it starts to decline. To find out the mechanism of stimulation in the activities of threonine dehydratase and tryptophan pyrrolase, several inhibitors of RNA and protein synthesis were tested (table). The induction of tryptophan pyrrolase and threonine dehydrase in 5% tyrosine-fed rats requires continuous protein synthesis and is prevented if cycloheximide (0.5 mg/100 g b. wt) or puromycin (3.5 mg/100 g b. wt) is injected to the rats at 0 h of the experiment (at a dose which is effective to stop protein synthesis). Further, administration of cycloheximide at any time after the beginning of induction prevents any further increase in enzyme activity and causes it to level off (K. Datta, unpublished work). This result suggests that the induction involves new protein synthesis and that the induction does not function by simply converting a precursor of the enzyme into an active form.

Actinomycin D is a potent inhibitor of RNA biosynthesis and has been used to analyse the mechanism of regulation of protein biosynthesis in many systems. Administration of actinomycin D (at a dose of 50 µg/100 g b. wt) also prevents the increase of threonine dehydratase and tryptophan pyrrolase. Tomkins et al.[12] reported a paradoxical increase in enzyme concentration in the presence of actinomycin D for inducible tyrosine aminotransferase in cultured hepatomacells. A phenomenon sometimes termed 'superinduction' is rather frequently observed in other eukaryotic cells[13]. In the present study, no such 'superinduction' was observed for threonine dehydratase and tryptophan pyrrolase in the presence of actinomycin D. All these findings suggest that the induction of threonine dehydratase and tryptophan pyrrolase involve induced synthesis of specific mRNAs and possibly selective promotion of gene transcription by the inducer.



Activities of hepatic threonine dehydratase and tryptophan pyrrolase in 3% or 5% tyrosine fed rats. Specific activity of threonine dehydratase (○—○, 9% casein, ■—■, 3% tyrosine, ▲—▲, 5% tyrosine) is expressed as µmoles of α-ketobutyric acid formed/30 min/mg of protein at 37 °C under the assay condition. Specific activity of tryptophan pyrrolase (●---●, 9% casein, △---△, 3% tyrosine, □---□, 5% tyrosine) is expressed as µmoles of kynurenine formed /h/mg of protein at 37 °C under the assay condition.

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