radius of the particle, k the Boltzman constant and T the absolute temperature, if τ decreases the radius r must decrease. This would mean that histidine is causing a conformational change in the enzyme that renders it tighter (smaller apparent size). This is in agreement with the conclusions of Bell and Koshland[4], who showed that histidine produced a higher exposure to the surrounding media of the essential SH group. The drop in the signal height (figure 2) can be explained as an increment of the dipolar interactions (there is a real possibility of several labelling sites per enzyme molecule): due to the conformational change the labels draw closer together.

At higher histidine concentrations (over 15–25 μM), τ increases. However, the signal height goes on decreasing throughout the range of histidine concentrations used, which means that the increase of dipolar interactions continues as an effect of histidine action. The only possible explanation, for these 2 simultaneous facts, is to assume that the enzyme size increases as a consequence of histidine action and that this increase (association) causes more dipolar interaction between the nitroxides bound to the enzyme molecules. It is known that histidine associates the E. coli enzyme from dimer to hexamer[6,7]; in those studies, histidine, in the range 0.05 to 1 mM, was able to aggregate the enzyme as shown in ultracentrifugation experiments[7] and 0.4 mM histidine readily associated it as followed by gel filtration[6]. In the present report, using a finer technique, we have been able to detect association of the enzyme with as little as 10 μM histidine. By calorimetric measurements of the binding of histidine to the enzyme, we have detected association at even lower concentrations (manuscript in preparation).

The nitroxide chain length does not affect the hyperfine splitting constant but produces different line-widths and correlation times, yielding broader lines and slower correlation rates as the nitroxide chain length is reduced. Labelling of the protein with one nitroxide or another affects the position of the minimum. This position depends on the relative contribution of the 2 superimposed processes, conformational change of the protomer and its association, to the overall effect produced by the feedback inhibitor histidine.

Interaction of extrinsic fluorescent probes with E. coli glutamine synthetase[1]

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Summary. Binding of 2-p-toluidinylnaphthalene-6-sulfonate (TNS) to adenylylated ($E_{\overline{11}}$) glutamine synthetase is cooperative and time-dependent, with 3 dye sites per subunit. In fluorescence polarization experiments TNS and pyrene butyrate give normalized Perrin plots that indicate a symmetrical arrangement of dye excited state dipoles, relative to the rotational axis of the oblate ellipsoid of the dodecameric native enzyme.

Glutamine synthetase plays a key role in nitrogen metabolism for a variety of organisms[2–4]. The enzyme from E. coli (12 subunits of 50,000 daltons each) has been studied extensively with regard to its regulation and physical characteristics[5]. Covalent attachment of an AMP group to any subunit markedly alters its pH optimum, metal ion specificity, substrate binding constants, and sensitivity to bound feedback modifiers[6]. The present study is a continuation of attempts to probe critical features of this complex system by optical spectroscopy, the first of which involved introduction of chromophoric rare earth ions in the active site region[7].

Experimental. Adenylylated glutamine synthetase ($E_{\overline{11}}$) was prepared according to Shapiro and Stadtman[8]. 2-p-Toluidinyl-naphthalene-6-sulfonate (TNS) was prepared and recrystallized by the procedures of McClure and Edelman[9]. Sucrose (ultrapure) was a product of Schwarz/Mann. 1-Pyrene butyrate was a product of Eastman, and was recrystallized three times from ethanol-water. Fluorescence data were obtained with a Turner 430 spectrofluorometer (bandwidth 7.5 nm), equipped with thermostatted ($\pm$ 0.1 °C) cell holder and polarization filters, connected to a Sargent SLRG recorder.

Enzyme (0.5 mg/ml) was buffered at pH 6.5 with 10 mM 3,3-dimethylglutarate and 1 mM $MnCl_2$. % sucrose was varied by mixing 2 buffer solutions (A = 0%, B = 50% sucrose) in different ratios to the same volume (2.0 ml). Concentrations of TNS were varied similarly. Viscosities of sucrose solutions were determined at 37 °C with an Ostwald viscometer and compared to published[10] values. % sucrose (η, cp) were- 5% (0.815), 10% (0.955), 20% (1.305), 35% (2.65), 50% (9.55), respectively.

Binding data were analyzed by Scatchard[11,12] plots, by the equation

$$Y/[D] = K(n - Y)$$

where Y is the average moles of dye (D) bound, and n is the number of binding sites for D, per mole protein.

Polarization of fluorescence of enzyme-bound fluorescent dyes was calculated by the equations[13,14]:

$$P = \frac{I_{VV} - GI_{VH}}{I_{VV} + GI_{VH}}$$

where I_{VV}, I_{VH}, etc, refer to the intensity of the emission with polarizing filters on the excitation and emission sides oriented both vertically or vertically-horizontally, respectively. G is the grating correction factor, equal to (I_{HV}/I_{HH}). The normalized form of this equation:

$$\frac{U}{Uo} = \frac{1/P - 1/3}{1/Po - 1/3} = 1 + \frac{3\tau}{\varrho h}$$

where Po is the polarization observed where T/η is zero, or at infinite viscosity, was used for plots of the data as U/Uo vs T/η.

Results and discussion. Binding of TNS. As reported earlier by Miller et al.[15], binding of TNS to glutamine synthetase occurs with such enhancement of fluorescence that unbound dye is essentially non-fluorescent. The binding process is a biphasic process and time-dependent: rapid initial complexation of TNS with enzyme gives a maximal fluorescence emission within the mixing time, then this signal decays to about 70% of the original value in a first-order process having $t_{1/2} \simeq 1$ min. The initial kinetically-favored product apparently provides

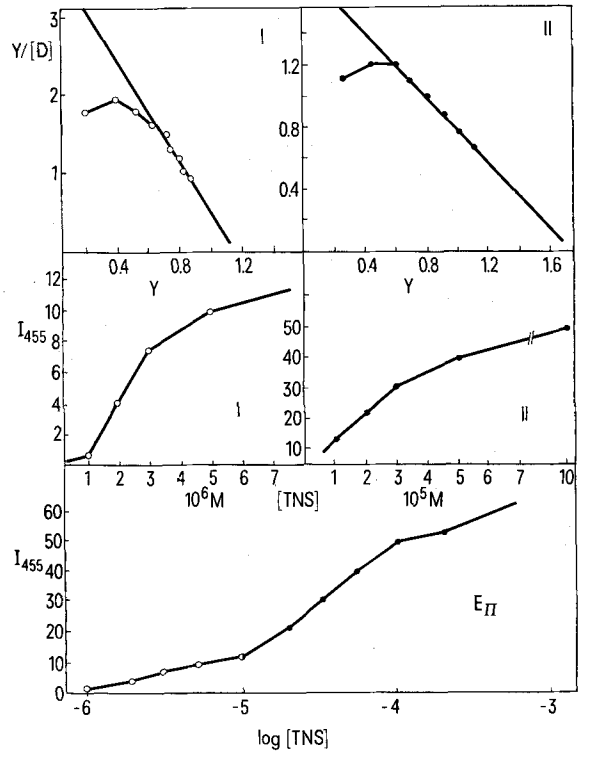

Fig. 1. Fluorescence titration of adenylated $E_{\overline{II}}$ E. coli glutamine synthetase with TNS, pH 6.5, 37 °C. Bottom: I_{455} (emission) vs log [TNS]; middle: linear plot of I_{455} vs [TNS] in each region of binding; top: Scatchard plots for each separate class of binding sites, indicating stoichiometry of association. (See experimental section.)

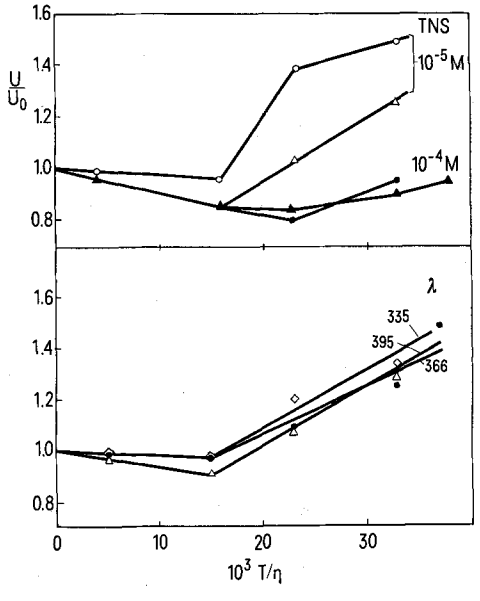

Fig. 2. Normalized Perrin plot of fluorescence polarization for the TNS-$E_{\overline{II}}$ complex of glutamine synthetase at 37 °C, pH 6.5. Top: TNS at 10^{-5} M (open symbols) or 10^{-4} M (closed symbols) and at t = 0 (circles) or at t = 10 min (triangles). Bottom: effect of excitation wavelength on the plots: 366 nm (triangles); 355 nm (filled circles); 395 nm (diamonds); with TNS = 10^{-4} M, λ_{EX} = 366 nm.

maximal apolar interactions and thus more intense emission of fluorescence, as discussed by Beyer et al.[16], but perhaps unfavorable juxtaposing of cationic protein groups relative to the anionic sulfonate moiety. In the thermodynamically-favored product, however, the binding is apparently tighter, perhaps due to decreased unfavorable ionic interactions, but so that the overall environment is less apolar, yielding somewhat lower emission.

Titration of the adenylylated enzyme with TNS (figure 1) produces a biphasic binding curve. Some cooperativity is seen in the linear plots of I_{455} vs [TNS] for the first set of sites (I). The Scatchard plots at the top of figure 1 indicate 1 TNS bound/subunit for the tightest (I) sites, but 2 TNS bound/subunit in the looser (II) sites. Similar

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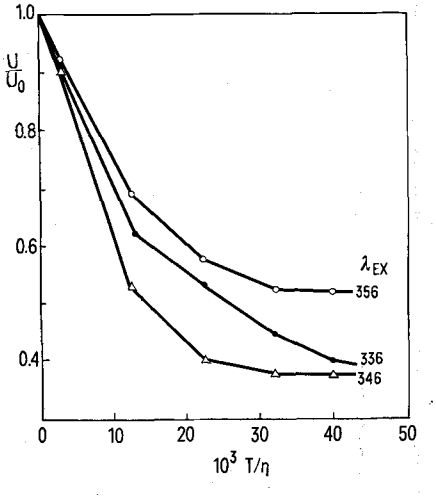

Fig. 3. Normalized Perrin plot of fluorescence polarization for the pyrene butyrate-$E_{\overline{II}}$ complex of glutamine synthetase, 37 °C, pH 6.5, at a series of excitation wavelengths. Data obtained at λ_{EM} = 372 and 393 nm were essentially identical.

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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