nucleotides located in the loop region (with the exception of nucleotides 7825 and 7826) could be modified without affecting the splicing pattern both in myoblasts and in myotubes (*11*).

We compared the sequences of the chicken β-tropomyosin gene with the corresponding *Xenopus laevis* and rat genes as far as stem I is concerned. In this region, the three genes have similar structures and the mutually exclusive exons 6A and 6B have identical splicing expression patterns (*1, 11, 12*). A single nonconservative change is present in stem I both for the rat and the *X. laevis* genes at position 7820, which causes an internal G-A mismatch instead of a A·U base pair (*13*), while most of the changes are located in the large loop or lead to G·T versus G·C base pair substitution. A similar conservation is not present in the cognate chicken α-tropomyosin gene (three nonconservative base changes can be observed in the stem I), in spite of the high conservation of the protein sequence in this region. This was expected because of the different expression pattern of exon 6B in the α tropomyosin gene (*14*).

The mechanism of this interference of splicing by secondary structures is still unclear. The integrity of stem I is necessary but not sufficient for the establishment of the negative control around exon 6B. Moreover, further data are needed to unambiguously define the overall structure of the region. We have described other nonoverlapping mutations (located in the upstream intron) capable of independently exerting the same activating influence as the stem mutations described above (*3, 4*). We have also obtained evidence that additional negative cis elements are present upstream of the branchpoint of exon 6B but not in the intron between exons 6B and 7 (*15*).

We favor the hypothesis that intron and exon cis elements are part of a complex regulatory system in which secondary structures and possibly trans acting factors act together competing with the binding of splicing factors to the branchpoint upstream of exon 6B in myoblasts. Finally, data in myotubes, when both exons 6A and 6B can be recognized by the splicing apparatus, a competition between the two exons is responsible for the preferential choice of the skeletal muscle specific exon (*15*).

REFERENCES AND NOTES

1. D. Libri, M. Lemonnier, T. Meinnel, M. Y. Fiszman, *J. Biol. Chem.* **264**, 2935 (1989).
2. D. Libri, J. Marie, E. Brody, M. Y. Fiszman, *Nucleic Acids Res.* **17**, 6449 (1989).
3. D. Libri, M. Goux-Pelletan, E. Brody, M. Fiszman, *Mol. Cell. Biol.* **10**, 5036 (1990).
4. M. Goux-Pelletan *et al.*, *EMBO J.* **9**, 241 (1990).
5. L. P. Eperon, J. P. Estibeiro, I. C. Eperon, *Nature* **324**, 280 (1986); X. Y. Fu and J. L. Manley, *Mol. Cell. Biol.* **7**, 738 (1987); D. Solnick, *Cell* **43**, 667 (1985); _____ and S. I. Lee, *Mol. Cell. Biol.* **7**, 3194 (1987).
6. L. P. Eperon, I. R. Graham, A. D. Griffiths, I. C. Eperon, *Cell* **54**, 393 (1988).
7. K. Chebli, R. Gattoni, P. Schmitt, G. Hildwein, J. Stevenin, *Mol. Cell. Biol.* **9**, 4852 (1989).
8. A. Watakabe, K. Inoue, H. Sakamoto, Y. Shimura, *Nucleic Acids Res.* **17**, 8159 (1989).
9. B. Clouet-d'Orval, Y. d'Aubenton-Carafa, P. Sirand-Pugnet, E. Brody, J. Marie, *Science* **252**, 1823 (1991).
10. The theoretical foldings of the same region in the two mutants are quite different in spite of the little sequence difference (D. Libri, unpublished data). To further prove that the mutation of the descending arm of stem I (region of MutA16) has indeed an activating effect on exon 6B splicing, we have constructed ten more mutants of different sequence in the same region and shown that most of them activates the exon. Similar results were obtained for the region of Mut16 (D. Libri *et al.*, in preparation).
11. D. Libri, unpublished data.
12. D. M. Helfman, S. Cheley, E. Kuismanen, L. A. Finn, Y. Yamawaki-Kataota, *Mol. Cell. Biol.* **6**, 3582 (1986); D. M. Helfman, R. F. Roscigno, G. J. Mulligan, L. A. Finn, K. S. Weber, *Genes Dev.* **4**, 98 (1990); M. Y. Fiszman, unpublished results.
13. It is possible that this change does not affect considerably the stability of the stem, as previously proposed for some G-A mismatches in double-stranded RNA [H. F. Noller, *Annu. Rev. Biochem.* **53**, 119 (1984)] and DNA [W. D. Wilson, M.-H. Dotrong, E. T. Zuo, G. Zon, *Nucleic Acids Res.* **16**, 5137 (1988)].
14. M. Lemonnier, D. Libri, M. Y. Fiszman, *Nucleic Acids Res.* **17**, 5400 (1989).
15. D. Libri *et al.*, in preparation.
16. M. Zuker and P. Stiegler, *Nucleic Acids Res.* **9**, 133 (1980).
17. S. M. Freier *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9373 (1986).
18. S. H. Erster, L. A. Finn, D. A. Frendewey, D. M. Helfman, *Nucleic Acids Res.* **16**, 5999 (1988).
19. T. A. Kunkel, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 488 (1985).
20. F. L. Graham and A. J. Van der Eb, *Virology* **52**, 456 (1973).

21. We thank E. Brody, J. Marie, B. Clouet-d'Orval, Y. Daubenton-Carafa, M. Gallego, V. Mouly, and M. Lemonnier for helpful discussions and constant interest; and B. Dujon and F. Michel for critical reading of the manuscript. Supported in part by fellowships from the Association des Myopathes de France (D.L.), the Erasmus program (A.P.); and grants from the CNRS, the CEA, the Ministère de l'Industrie et de la Recherche, the Fondation pour la Recherche Médicale Française, the ARC, the Ligue Française contre le Cancer, and the Association des Myopathes de France.

4 December 1990; accepted 17 May 1991

Subunit Communication in the Anthranilate Synthase Complex from *Salmonella typhimurium*

MAUREEN G. CALIGIURI AND RONALD BAUERLE*

The anthranilate synthase–phosphoribosyl transferase complex of the tryptophan biosynthetic pathway in *Salmonella typhimurium* is an allosteric, heterotetrameric ($TrpE_2$-$TrpD_2$) enzyme whose multiple activities are negatively feedback-regulated by L-tryptophan. A hybrid complex containing one catalytically active, feedback-insensitive and one catalytically inactive, feedback-sensitive mutant TrpE subunit was assembled in vitro and used to investigate communication between regulatory and catalytic sites located on different subunits. The properties of the hybrid complex demonstrate that the binding of a single inhibitor molecule to one TrpE subunit is sufficient for the propagation of a conformational change that affects the active site of the companion subunit.

REGULATION OF CARBON FLOW IN metabolic pathways is often achieved by the ligand-dependent modulation of the activity of strategically located allosteric enzymes. Allosteric enzymes are generally oligomeric in structure and have multiple, topologically distinct binding sites for substrates and regulatory effectors. Ligand binding is accompanied by conformational changes in the enzyme that alter its affinity for the binding of additional ligand molecules and are usually manifested by cooperativity in the reaction kinetics (*1, 2*). A pivotal question in the elucidation of the allosteric mechanism is whether the binding of a single effector molecule to one of the regulatory sites of an enzyme is sufficient to elicit the conformational effects that modulate the functioning of all of its catalytic sites. We report results of experiments that test the possibility of such a concerted transition in the feedback-regulated anthranilate synthase–phosphoribosyl transferase (AS-PRT) complex of the tryptophan (Trp) biosynthetic pathway from *S. typhimurium*.

The AS-PRT complex is a multifunctional, heterotetrameric enzyme composed of the two TrpE and two TrpD polypeptides, each with a molecular weight of ~57 kD (*3, 4*). The complex catalyzes the first two steps of Trp pathway: (i) the conversion of chorismate (Chr), the branchpoint intermediate in aromatic amino acid biosynthe-

Department of Biology and Molecular Biology Institute, University of Virginia, Charlottesville, VA 22901.

*To whom correspondence should be addressed at the Department of Biology, University of Virginia, Charlottesville, VA 22901.

sis, to anthranilate (AS activity) and (ii) the transfer of the phosphoribosyl group of 5-phosphorylribose-1-pyrophosphate to anthra-nilate, forming *N*-phosphoribosylanthranilate (PRT activity). In the AS reaction, the ortho amino group of anthranilate, which becomes the nitrogen atom of the indole ring of Trp, is derived from the amide group of Gln. The reaction (AS-Gln activity) is catalyzed at a compound active site where ammonia (NH_3) is released from Gln by the Gln amidotransferase (GAT) activity of the NH_2-terminal domain of the TrpD subunit (*5*), and anthranilate is then synthesized from Chr and the nascent NH_3 at a catalytic site on the TrpE subunit. Whereas the ability to use Gln is a property unique to the complex, the complex as well as the free TrpE subunit can use NH_3 directly for the amination of Chr (AS-NH_3 activity) (*6*). The PRT activity of the complex is catalyzed in turn at an autonomous active site in the COOH-terminal domain of the TrpD subunit (*5*).

The AS-Gln, AS-NH_3, GAT, and PRT activities of the complex are all subject to negative feedback regulation by Trp (*3*). Feedback inhibition results from the binding of one molecule of Trp to each of the TrpE subunits (*7, 8*) and is competitive with respect to Chr and noncompetitive with respect to the other substrates. Mutational analysis and proteolytic probing of the TrpE subunit has indicated the existence of distinct catalytic and regulatory sites that reside in separate domains within TrpE (*4, 8*). Significant conformational effects accompany inhibitor binding, as indicated by positive cooperativity in Trp binding (*8, 9*), Trp-induced cooperative kinetics for Chr in both the AS-Gln (*8, 10*) and AS-NH_3 (*11*) reactions (*12*), and Trp-induced alterations in the affinity of the enzyme for dye-conjugated chromatographic media (*4, 8*).

We analyzed communication between the TrpE subunits of the AS-PRT complex by the isolation and kinetic characterization of a hybrid enzyme complex containing one catalytically inactive, feedback-sensitive ($TrpE^{T425I}$) (*13*) and one catalytically active, feedback-insensitive ($TrpE^{S40F}$) TrpE subunit. The hybrid complex was assembled in vitro from a mixture of the two mutant TrpE subunits and the wild-type (wt) $TrpD^+$ subunit with methods developed for the spontaneous assembly of the wt holoenzyme (*14*).

The $TrpE^{T425I}$ and $TrpE^{S40F}$ mutant subunits both assemble normally with the $TrpD^+$ subunit to form stable tetrameric mutant complexes that are totally defective in either AS activity [$(TrpE^{T425I})_2(TrpD^+)_2$] or feedback regulation [$(TrpE^{S40F})_2(TrpD^+)_2$], but are otherwise fully functional (*15*). However, each mutant complex displays a different aberrant behavior upon dye-ligand chromatography. The wt complex binds avidly to Orange A agarose (Matrex Gel Orange A, Amicon Corporation, Lexington, Massachusetts) and is specifically eluted by buffers containing low concentrations of Trp, apparently as a result of an induced conformational change (*4, 8*). The feedback-resistant $(TrpE^{S40F})_2(TrpD^+)_2$ complex binds in a normal fashion to the Orange A gel but is refractory to elution by Trp because of a complete loss of Trp binding (*8*). In contrast, the catalytically inactive $(TrpE^{T425I})_2$-$(TrpD^+)_2$ complex has reduced affinity for the gel and binds only poorly under the conditions effective for the wt and feedback-resistant enzymes (*16*).

If random association of subunits occurred, three enzyme complexes would result from the spontaneous assembly of a tripartite mixture of the two mutant TrpE subunits and the $TrpD^+$ subunit, namely the homologous $(TrpE^{T425I})_2(TrpD^+)_2$ and $(TrpE^{S40F})_2(TrpD^+)_2$ complexes and the hybrid $(TrpE^{T425I})(TrpE^{S40F})(TrpD^+)_2$ complex. Only the latter two assemblages, con-

Table 1. Orange A agarose fractionation of mutant AS complexes assembled in vitro. Each partially purified TrpE subunit (1.25 mg) (*21*) was mixed either individually or in combination with a twofold molar excess of monomeric $TrpD^+$ subunit (*22*) in a final volume of 2.5 ml. We incubated the mixtures on ice for 30 min to allow spontaneous complex formation and then applied the mixtures to 5-ml columns of Orange A agarose that had been equilibrated with standard buffer [100 mM potassium phosphate (pH 7.0), 1 mM dithiothreitol (DTT), 0.1 mM EDTA, and 10% glycerol]. After the protein mixtures entered the gel, the columns were closed off for 30 min, then washed with 30 ml of buffer, and developed with 20 ml of buffer containing 50 μM L-Trp, followed by 20 ml of buffer containing 1.5 M KCl. All operations were carried out at 4°C. The column fractions were assayed for AS-Gln activity (*4*). Essentially identical results were obtained in numerous repeats of the experiment.

Fraction	AS-Gln activity [total units (U)]		
	$TrpE^{S40F}$	$TrpE^{T425I}$	$TrpE^{S40F}$ + $TrpE^{T425I}$
Column load	936	0	951
Column wash	4	0	28
Trp elution	6	0	305
KCl elution	780	0	500

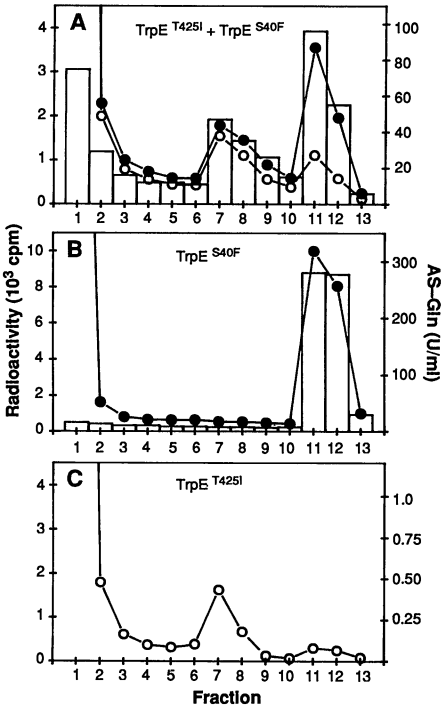

Fig. 1. Orange A agarose fractionation of AS-PRT complexes assembled in vitro from [^{3}H]$TrpE^{S40F}$ and [^{14}C]$TrpE^{T425I}$ mutant subunits. The cells from 1-liter cultures of *E. coli* CB96/pSTG39 ($TrpE^{S40F}$) and CB96/pSTH16 ($TrpE^{T425I}$), grown in the presence of [^{3}H]L-Leu and [^{14}C]L-Leu, respectively (*21*), were collected by centrifugation, washed in 0.9% NaCl, and resuspended in 10 ml of standard buffer (100 mM potassium phosphate, pH 7.0, 1 mM DTT, 0.1 mM EDTA, and 10% glycerol). One-half of each suspension was combined and mixed with a suspension of the cells from a 1.3-liter culture of *E. coli* strain CB25/pSTG119 ($TrpD^+$); the other half was mixed individually with a similar suspension of CB25/pSTG119 cells. We treated the cell mixtures with sonication, centrifuged the sonification products at 49,000*g* for 30 min to remove cellular debris, and fractionated the crude extracts by ammonium sulfate precipitation (40% saturation) (*4, 23*). The concentrated, partially purified preparations were desalted over Sephadex G25 (Pharmacia) and fractionated by dye-ligand chromatography on Orange A agarose as described in Table 1. The elution program was as follows: fractions 1 to 6, standard buffer; 7 to 10, standard buffer and 50 μM L-Trp; 11 to 13, standard buffer and 1.5 M KCl. Fraction volumes were as follows: fractions 1 to 3, 10 ml; 4 and 5, 5 ml; 6, 2.5 ml; and 7 to 13, 5 ml. The peaks of ^{3}H (●) and ^{14}C (○) counts coincident with the Trp-eluted peak of AS-Gln activity (open bars) of the $TrpE^{S40F}$ and $TrpE^{T425I}$ assembly mixture (**A**) demonstrated the presence of both mutant subunits (*24*). However, the inconstant ^{3}H/^{14}C ratio across the AS-Gln peak in (A), the wash-off of radioactivity and AS-Gln activity in the $TrpE^{S40F}$ control (**B**), and the minor Trp-eluted peak of ^{14}C counts in the $TrpE^{T425I}$ control (**C**) all indicated that under the conditions of this experiment the isolated hybrid enzyme was not homogeneous. Thus, fractions 8 and 9 of (A) were combined, concentrated by ultrafiltration, and refractionated on Orange A agarose as before. The ^{3}H/^{14}C ratio of the Trp-eluted hybrid complex was constant across the peak, indicating that homogeneity was achieved. This was verified by SDS–polyacrylamide gel electrophoresis. The experiment was performed twice with essentially identical results.

taining two and one functional active sites, respectively, should contribute to the total AS-Gln activity in the subunit mixture. Of these two, only the $(TrpE^{T425I})(TrpE^{S40F})$-$(TrpD^{+})_2$ complex, containing one functional feedback site, would have the potential for wt chromatographic behavior. In agreement with these assumptions, an enzyme species accounting for one-third of the applied AS-Gln activity was eluted by Trp in the Orange A agarose fractionation of an assembly mixture containing both mutant TrpE subunits (Table 1). In contrast, essentially none of the activity of the $TrpE^{S40F}$ control mixture was recovered by Trp elution, and, as expected, no AS-Gln activity was discovered in the $TrpE^{T425I}$ control mixture, nor was any detected in the fractionation. These results indicate that in the tripartite mixture the two mutant TrpE subunits had indeed assembled together with $TrpD^{+}$ to generate a catalytically active, Trp-responsive $(TrpE^{T425I})(TrpE^{S40F})(TrpD^{+})_2$ hybrid complex. We verified that the Trp-eluted enzyme did in fact contain both mutant TrpE subunits by performing a similar assembly experiment with ^{3}H-labeled $TrpE^{S40F}$ and $[^{14}C]TrpE^{T425I}$ subunits (Fig. 1).

The AS-Gln activity of the purified hybrid complex was then characterized by steady-state kinetic analysis. Its rate constant, k_{cat}, was one-half that of the homologous $TrpE^{S40F}$ mutant and wt complexes, whereas the apparent Michaelis constants, K_m^{Chr}, of the enzymes were virtually identical (Table 2). In addition, the hybrid complex displayed sensitivity to Trp inhibition similar to the wt (Fig. 2, A and B). The hybrid's inhibition constant K_i^{Trp} was only slightly elevated (Table 2), and inhibition was competitive with respect to Chr. However, the complex lacked the Trp-induced cooperativity for Chr use displayed by the wt enzyme. These kinetic characteristics are evidence that the hybrid complex contained a single functional active site, contributed by the $TrpE^{S40F}$ subunit, and a single functional regulatory site, contributed by the $TrpE^{T425I}$ subunit. Thus, we conclude that binding of Trp to the regulatory site of the $TrpE^{T425I}$ subunit of the hybrid was effective both in inducing conformational changes in the complex that caused its release from Orange A agarose and in cross-regulating the active site of its $TrpE^{S40F}$ subunit.

Also of significance is the hyperbolic nature of the secondary plot of the kinetic data of the hybrid enzyme (Fig. 2A, inset). This feature indicates that feedback inhibition in the hybrid was partially competitive in nature, in contrast to the pure competitive inhibition seen with the wt enzyme (Fig. 2B, inset). Unlike pure competitive inhibition, in which occupancy of the enzyme by inhibitor or substrate strictly excludes binding of the other, partial competitive inhibition results when substrate and inhibitor can occupy the enzyme simultaneously and when the enzyme-subtrate-inhibitor complex produces product at the same rate as the enzyme-substrate complex (*17*). A corollary of this is that at high concentrations of substrate, the enzyme is not inhibited, regardless of inhibitor concentration. Consistent with this we found that at 250 μM of Chr ($100 \times K_m^{Chr}$), the AS-Gln activity of the hybrid enzyme was completely insensitive to feedback inhibition, even at Trp concentrations as high as 500 μM. Under these same conditions, the wt complex was completely inhibited.

Table 2. Kinetic constants of the hybrid AS complex in the AS-Gln reaction. The $(TrpE^{T425I})(TrpE^{S40F})(TrpD^{+})_2$ hybrid complex was prepared and purified as described in Fig. 1. Details of the kinetic methods were as described in (*8*). The Chr concentration was varied between 0.5 and 12.5 μM, with the Gln concentration held constant at 20 mM. The concentration of Trp was varied between 0 and 20 μM. The derived kinetic constants for the hybrid complex are the mean values from two independent determinations. Deviation from the mean in the two experiments was ±2% for k_{cat}, ±20% for K_m^{Chr}, and ±5% for K_i^{Trp}. Kinetic constants for the $(TrpE^{+})_2(TrpD^{+})_2$ wt complex and the $(TrpE^{S40F})_2(TrpD^{+})_2$ mutant complex were taken from (*8*).

$(TrpE)_2(TrpD^{+})_2$ complex	k_{cat} (s^{-1})	K_m^{Chr} (μM)	K_i^{Trp} (μM)
$(TrpE^{T425I})(TrpE^{S40F})$	5.8	2.5	4.3
$(TrpE^{S40F})_2$	12.0	2.4	>300
$(TrpE^{+})_2$	12.0	2.3	1.3

It might be argued that the catalytic and regulatory properties of the hybrid complex derive from a conformational correction (*18*) of one or both of the individual defects of the mutant TrpE subunits on complex formation. However, the kinetic properties of the hybrid enzyme do not support this interpretation. If the active site of the $TrpE^{T425I}$ subunit had been reactivated through its association with the $TrpE^{S40F}$ subunit, the k_{cat} of the hybrid complex should have been greater than the rate predicted for a complex with a single $TrpE^{S40F}$ subunit (*19*). Also, the pattern of feedback inhibition would be expected to be mixed, rather than partially competitive (*17*). On the other hand, if the feedback site of the $TrpE^{S40F}$ subunit had been reactivated by complex formation, the hybrid enzyme should have exhibited pure competitive inhibition. The speculation that either the AS active site or the feedback site (or possibly both sites) of the complex is made up of residues contributed by both TrpE subunits also seems unlikely in view of the fact that the monomeric TrpE subunit has a robust AS-NH_3 activity that is feedback-inhibited by Trp (*6*).

Our results demonstrate that the binding of Trp to one TrpE subunit of the AS-PRT complex elicits conformational and kinetic effects that are propagated to the unliganded TrpE subunit. This is somewhat analogous to the situation in the *Escherichia coli* aspartate transcarbamoylase, where it has been shown by different methods that the holoenzyme undergoes a concerted transition from the tense (T) state to the relaxed (R) state in response to the binding of a single ligand molecule to one of its six active sites (*20*). However, the properties of the AS-PRT complex are not readily reconcilable

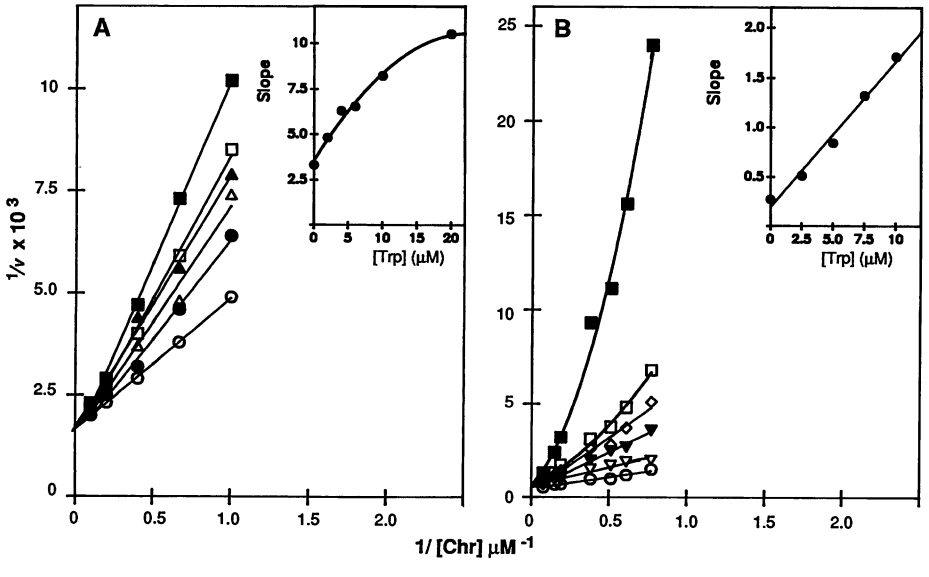

Fig. 2. Kinetics of Trp inhibition of the hybrid mutant (**A**) and wt (**B**) AS-PRT complexes. Conditions were as described in Table 2. Concentrations of Trp were as follows: none (○), 2.0 μM (●), 2.5 μM (▽), 4.0 μM (△), 5.0 μM (▼), 6.0 μM (▲), 7.5 μM (◇), 10 μM (□), and 20 μM (■); *v*, velocity.

with the classical two-state, concerted model for allosteric enzymes (*1*). The cooperative binding of both inhibitor and substrate is inconsistent with the model. Furthermore, because Trp inhibition of the hybrid complex is partially competitive with respect to Chr, whereas inhibition of the wt complex and the monomeric TrpE subunit is strictly competitive, it can be inferred that the conformational and kinetic consequences of Trp binding to a single TrpE subunit of the complex are not symmetrical. Instead, the effects appear to be more stringent for the active site of the liganded TrpE subunit than for the active site of the unliganded subunit. Finally, the results of dye-ligand chromatography of the wt and various mutant enzymes have indicated at least three conformational states for the complex, with the unliganded enzyme being conformationally distinct from that of the Trp-liganded and Chr-liganded enzymes (*16*). Thus the properties of the AS-PRT complex appear to be better described by a variation of the sequential model for allosteric enzymes (*2*) where multiple conformational states arise as a result of the tertiary and quaternary effects of the successive binding of ligands by the subunits of the enzyme.

REFERENCES AND NOTES

1. J. Monod, J. Wyman, J. Changeux, *J. Mol. Biol.* **12**, 88 (1965).
2. D. Koshland, G. Nemethy, D. Filmer, *Biochemistry* **5**, 365 (1966).
3. H. Zalkin, in *Multifunctional Proteins*, H. Bisswanger and E. Schmincke-Ott, Eds. (Wiley, New York, 1980), pp. 123–149.
4. R. Bauerle, J. Hess, S. French, *Methods Enzymol.* **142**, 366 (1987).
5. M. Grieshaber and R. Bauerle, *Nature (London) New Biol.* **236**, 232 (1972); M. Grieshaber, *Z. Naturforsch.* **33**, 235 (1978).
6. H. Zalkin and D. Kling, *Biochemistry* **7**, 3566 (1968).
7. H. Nagano and H. Zalkin, *J. Biol. Chem.* **245**, 3097 (1970).
8. M. Caligiuri and R. Bauerle, *ibid.* **266**, 8328 (1991).
9. E. Henderson and H. Zalkin, *ibid.* **246**, 6891 (1971).
10. E. Henderson, H. Nagano, H. Zalkin, *ibid.* **245**, 1416 (1970).
11. M. Caligiuri and R. Bauerle, unpublished results.
12. Similar inhibitor-induced substrate cooperativity has been noted in other regulated enzymes [B. Valdez, E. Younathan, S. Chang, *J. Biol. Chem.* **264**, 131 (1989); J. Foreman and W. Niehous, *ibid.* **260**, 10019 (1985); L. Kuo, W. Lipscomb, E. Kantrowitz, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2250 (1982)].
13. Mutant TrpE subunits are designated by the nature and location of the amino acid change. The single amino acid code (F, Phe; I, Ile; S, Ser; and T, Thr) is used. Thus, $TrpE^{S40F}$ and $TrpE^{T425I}$ represent mutant subunits with amino acid changes of Ser^{40} → Phe and Thr^{425} → Ile, respectively.
14. D. Smith and R. Bauerle, *Biochemistry* **8**, 1451 (1969).
15. The $(TrpE^{T425I})_2(TrpD^+)_2$ complex lacks AS-Gln activity but has wt PRT activity with normal sensitivity to feedback inhibition (*16*). The $(TrpE^{S40F})_2(TrpD^+)_2$ complex has wt AS-Gln and PRT activities, both of which are completely resistant to inhibition by Trp (*8*).
16. J. Sennewald and R. Bauerle, unpublished results.
17. I. Segel, *Enzyme Kinetics* (Wiley, New York, 1975).
18. F. Crick and L. Orgel, *J. Mol. Biol.* **8**, 161 (1964).
19. I. Zabin and M. Villarejo, *Annu. Rev. Biochem.* **47**, 295 (1975).
20. J. Foote and H. K. Schachman, *J. Mol. Biol.* **186**, 175 (1985).
21. Mutant *S. typhimurium* TrpE subunits were prepared from *E. coli* strains CB96/pSTG39 ($TrpE^{S40F}$) and CB96/pSTH16 ($TrpE^{T425I}$). Wild-type *S. typhimurium* TrpD subunit was prepared from *E. coli* strain CB25/pSTG119 ($TrpD^+$). Plasmids pSTG39 and pSTG119, derivatives of pBR327, and plasmid pSTH16, a derivative of pBR322, carry their respective *trp* genes under the control of the native *trp* promoter. Host strains CB96 (C600 *ΔtrpED24 leuB6 hsdR thi-1*) and CB25 (*W3110 ΔtrpE-A2 tna2 bglR*) were provided by C. Yanofsky. Overexpression of the mutant TrpE and wt $TrpD^+$ polypeptides was achieved by derepression of the genes in cultures grown under conditions of Trp limitation (*8*) in supplemented minimal medium [K_2HPO_4 (10.5 g/liter), KH_2PO_4 (4.5 g/liter), $(NH_4)_2SO_4$ (1.0 g/liter), $MgSO_4$ (0.1 g/liter), glucose (2.5 g/liter), thiamine (50 μM), L-Trp (3 mg/liter), Leu (25 mg/liter), each of the other 18 protein amino acids (50 mg/liter), and ampicillin (25 mg/liter)]. Crude extracts were prepared and partially purified by ammonium sulfate precipitation (38% saturation) as described (*4*). For the preparation of radiolabeled $TrpE^{S40F}$ and $TrpE^{T425I}$ subunits, cultures of CB96/pSTG39 and CB96/pSTH16 were grown as above except that either 100 μCi [U-^{14}C]L-leucine (184 mCi/mmol) or 600 μCi [3,4,5-^{3}H]L-leucine (108 mCi/mmol) were added at the time of inoculation, as indicated.
22. The $TrpD^+$ subunit dimerizes when present in vitro at high concentrations [M. Grieshaber and R. Bauerle, *Biochemistry* **13**, 373 (1974)]. Although the dimeric form has undiminished PRT activity, it is not competent for assembly with the TrpE subunit. We estimated the amount of the assembly-competent monomer in the $TrpD^+$ preparation immediately before its use in the assembly experiment by assaying both its PRT activity and its ability to complement TrpE in the AS-Gln reaction (*4*). Any increase in the PRT/AS-Gln activity ratio over that of the wt complex (1.4) was used to estimate the proportion of monomer present and to calculate the amount of the preparation needed for the experiment.
23. The sonication of mixtures of whole cells, instead of the mixing of partially purified subunits as used in the experiment of Table 1, minimized the problem of spontaneous dimerization of the $TrpD^+$ subunit, but prevented the use of a defined stoichiometry of subunits because of the different levels of expression of the three subunits.
24. The ^{3}H and ^{14}C radioactivities were determined in 0.50-ml samples of each fraction in the LKB model 1215 liquid scintillation counter (Pharmacia LKB) with Ready-Solv counting fluid (Beckman). In Fig. 1A, counts were corrected for spillover, which was <1% for ^{3}H and ~8% for ^{14}C. Offscale counts in fraction 1 of each experiment were as follows: ^{3}H, 45,800 cpm; ^{14}C, 36,200 cpm (Fig. 1A); ^{3}H, 42,500 cpm (Fig. 1B); and ^{14}C, 36,000 cpm (Fig. 1C). AS-Gln activity was assayed as described (*4*).

25. Supported by NIH research grant GM35889 and training grant GM07082. We thank C. Yanofsky for providing bacterial strains and C. Stephens for helpful discussions.

1 March 1991; accepted 26 April 1991

A Corticosteroid Receptor in Neuronal Membranes

MILES ORCHINIK,* THOMAS F. MURRAY, FRANK L. MOORE

Steroids may rapidly alter neuronal function and behavior through poorly characterized, direct actions on neuronal membranes. The membrane-bound receptors mediating these behavioral responses have not been identified. [^{3}H]Corticosterone labels a population of specific, high-affinity recognition sites (dissociation constant = 0.51 nanomolar) in synaptic membranes from an amphibian brain. These binding sites were localized by receptor autoradiography in the neuropil, outside the regions of perikarya. The affinities of corticoids for this [^{3}H]corticosterone binding site were linearly related to their potencies in rapidly suppressing male reproductive behavior. Thus, it appears that brain membranes contain a corticosteroid receptor that could participate in the regulation of behavior.

IN THE CLASSIC MODEL OF STEROID hormone action, steroids bind to intracellular receptors, which act as ligand-dependent transcription factors that regulate gene expression (*1*). In addition to these well-known actions of steroid receptors, steroids may alter brain function through nongenomic mechanisms (*2*). For example, in rats, short-term exposure to the gonadal steroid progesterone is associated with rapid changes in behavior, and this effect occurs in the absence of new protein synthesis (*3*). Gonadal and adrenal steroid hormones can alter neuronal firing activity within milliseconds to minutes of administration (*4*), and these responses can occur in brain regions lacking classic steroid receptors (*5*) or if steroid access to intracellular receptors is blocked (*6*). These events appear to be mediated by direct steroid action on neuronal membranes, but there is little information concerning steroid binding to membrane-bound recognition sites in the brain (*7, 8*).

To investigate the possibility that glucocorticoid receptors occur on neuronal membranes, we performed radioligand binding studies on synaptic (P2) membranes from brains of the amphibian *Taricha granulosa*, which is known to have rapid behavioral responses to corticosterone (CORT) (*9*). Equilibrium saturation binding experiments (*10, 11*) indicated that ^{3}H-labeled CORT binding to brain membranes was specific, saturable, and of high affinity [dissociation

M. Orchinik and F. L. Moore, Department of Zoology, Oregon State University, Corvallis, OR 97331.
T. F. Murray, Department of Pharmacy, Oregon State University, Corvallis, OR 97331.

*To whom correspondence should be addressed.