strategy for Parkinson's disease. Therefore, it should be regarded as preliminary and in need of confirmation. Fortunately, a large multi-institutional study, DATATOP (*23*), which was designed to determine whether or not deprenyl alone or in combination with vitamin E can slow the course of Parkinson's disease, is now nearing completion. As the DATATOP study has enrolled 800 subjects, it should provide definitive evidence for (or against) the conclusions reached here. These issues are not minor, as setting a precedent for one age-related human neurodegenerative disease is likely to have consequences for others, such as Alzheimer's disease and amyotrophic lateral sclerosis.

There are several possible mechanisms by which deprenyl might achieve the effects reported here (*9*), including prevention of the formation of a pyridinium species after exposure to an exogenous or endogeous tetrahydropyridinium (*24*). Deprenyl could also be moderating oxidative stress by preventing oxidation of dopamine by MAO B (*25*). It will be important to explore this question, because determining the mechanism of this effect could provide a clue to the cause of Parkinson's disease. Knoll and colleagues (*26*) have dramatically increased the life-span of rats with chronic deprenyl administration, raising the possibility that more than dopaminergic neurons are being protected.

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Triggering of Allostery in an Enzyme by a Point Mutation: Ornithine Transcarbamoylase

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The origin of allostery is an unanswered question in the evolution of complex regulatory proteins. Anabolic ornithine transcarbamoylase, a trimer of identical subunits, is not an allosteric enzyme per se. However, when the active-site residue arginine-106 of the *Escherichia coli* enzyme is replaced with a glycine through site-directed mutagenesis, the resultant mutant enzyme manifests substrate cooperativity that is absent in the wild-type enzyme. Both homotropic and heterotropic interactions occur in the mutant enzyme. The initial velocity saturation curves of the substrates, carbamoyl phosphate and L-ornithine, conform to the Hill equation. The observed cooperativity depends on substrate but not enzyme concentration. The finding underscores the possibility that a single mutation of the enzyme in the cell could turn transcarbamoylation into a regulatory junction in the biosynthesis of L-arginine and urea.

COOPERATIVITY OF PROTEINS IN ligand binding has been known for more than 80 years since Bohr's observation of heme-heme interactions in hemoglobin (*1*). All allosteric proteins are either oligomeric or contain multiple interacting domains within one polypeptide chain, and models (*2, 3*) have been advanced to explain the binding properties of allosteric ligands. However, most oligomeric enzymes are not cooperative, and reasons such as promotion of stability have been offered for their existence. Recently, several oligo-

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meric enzymes that are not known to be allosteric have been discovered to exhibit cooperative properties in the presence of an inhibitor (*4–6*) or when chemically modified (*7*).

Anabolic ornithine transcarbamoylase (E.C. 2.1.3.3) is a trimeric enzyme composed of identical subunits. This enzyme catalyzes the first reaction in the urea cycle, the synthesis of L-citrulline by transfer of the carbamoyl group from carbamoyl phosphate to L-ornithine:

$$H_2N(CO)PO_4^{2-} + H_2N\text{-}(CH_2)_3CH(NH_3^+)CO_2^- \rightarrow H_2N(CO)\text{-}NH\text{-}(CH_2)_3CH(NH_3^+)CO_2^- + HPO_4^{2-}$$

Although this multimeric enzyme catalyzes a reaction similar to that catalyzed by aspartate transcarbamoylase, which is a classic allosteric enzyme, both substrates of ornithine transcarbamoylase display noncooperative saturation kinetics.

We report a site-directed point mutant of *Escherichia coli* ornithine transcarbamoylase that displays allostery toward both of its substrates. Together with the earlier reports (*4–7*), this finding raises the intriguing question of whether allostery is a natural and perhaps common property of oligomeric enzymes. Is allostery an inherent phenomenon in oligomeric proteins that may be selected for when metabolic need arises?

Analysis of the three-dimensional structure of the closely related enzyme aspartate transcarbamoylase leads us to believe that Arg^{106} is one of two arginine residues that may form ionic bonds with the oxygen atoms of the phosphate group of carbamoyl phosphate [see Kuo *et al.* (*8*) for a comparison of *E. coli* ornithine and aspartate transcarbamoylases]. The other residue, Arg^{57}, has been shown to be indispensable for isomerization and catalysis (*8*). Site-directed mutagenesis at the Arg^{106} site of *E. coli* K12 ornithine transcarbamoylase was conducted with the procedure of Zoller and Smith (*9*) as modified by Norris *et al.* (*10*). A 21-base oligonucleotide was synthesized (Beckman System-1-Plus DNA Synthesizer) to introduce the specific C→G base change necessary for the substitution of a glycine for the wild-type arginine at residue 106 of the enzyme. The criteria applied to ensure that only the designated site was modified has been described together with details of the mutagenic experiments (*8*). Enzyme purification, steady-state kinetic assays, and data analysis were performed as reported previously (*8, 11*).

We use the two-state model of Monod, Wyman, and Changeux (*2*) to interpret our kinetic data, although the sequential model of Koshland *et al.* (*3*) is equally applicable. An allosteric enzyme in the MWC model exists in two conformational forms, R (relaxed) and T (tense), in spontaneous equilibrium. A noncooperative ligand does not disrupt this equilibrium because its affinity for both states is identical, and its saturation curve in the form of an Eadie-Scatchard plot is linear with a slope of −1. A cooperative ligand binds the R form preferentially and thereby shifts the R-T equilibrium toward R, so that its saturation curve in an Eadie-Scatchard plot is a concave-up parabola. If two types of allosteric ligands, *A* and *B*, bind to different sites, both ligands would displace the R-T equilibrium of the enzyme, and their interactions may be coupled to yield a heterotropic effect. Inherent in the MWC model is that heterotropic ligands affect homotropic interactions exclusively by shifting the R-T equilibrium. A systematic analysis on the heterotropic effect of different combinations of ligands has been conducted by Rubin and Changeux (*12*).

The most commonly used index to quantify the degree of ligand cooperativity is the Hill coefficient (*13*), n_H, which may be obtained by fitting equilibrium binding or kinetic velocity data to the equation:

$$\overline{Y} = \frac{[S]^{n_H}}{[S_{0.5}]^{n_H} + [S]^{n_H}}$$

where $\overline{Y}$ is the fractional saturation of the enzyme by the substrate S or the fraction of maximal velocity, and $[S_{0.5}]$ is the concentration of the substrate at half-saturation. For an allosteric system, n_H varies from unity (no cooperativity) up to a maximum value equaling the number of interacting sites (infinite cooperativity). Its value increases with the number of interacting binding sites and with the strength of the interactions. The physical meaning of n_H has been discussed by Wyman (*14*).

The saturation curves of L-ornithine and carbamoyl phosphate obtained under steady-state conditions are shown in Fig. 1. Both curves are parabolic (concave-up) in shape signifying positive cooperativity. These and similar initial velocity data are analyzed with the Hill approximation. The observed kinetic parameters are compared in Tables 1 and 2.

The Hill coefficient for ornithine (n_H^{orn}) decreases from 1.85 to 1.27 as the concentration of carbamoyl phosphate increases from 0.05 m*M* [apparent Michaelis constant (K_m^{app})] to 2.5 m*M* (~50 × K_m^{app}) (Table 1). The influence of carbamoyl phosphate on n_H^{orn} signifies a heterotropic effect. Since a noncooperative ligand cannot influence the R-T equilibrium, the results in Table 1 indicate that both carbamoyl phosphate and L-ornithine must bind cooperatively (*15*). Corroborating this statement is the reciprocal effect seen on the Hill coefficient of carbamoyl phosphate (n_H^{cp}). The value of n_H^{cp} decreases as the ornithine concentration increases (Table 2). However, the apparent Hill coefficient of carbamoyl phosphate saturation is low (n_H^{cp} = 1.3) even at subsaturating ornithine levels. When the ornithine concentration approaches saturation, the carbamoyl phosphate cooperativity becomes recondite, and the residual n_H^{cp} value is ~1 (*16*). The combined data in Tables 1 and 2 reveal that ornithine has a stronger cooperativity than carbamoyl phosphate; its binding to the enzyme tips the scale of the conformational balance to the R form.

In addition, the $S_{0.5}$ value for saturation of the Gly^{106} enzyme by either substrate increases as the apparent n_H decreases and approaches the K_m^{app} values of the wild-type enzyme at saturating substrate concentrations (Table 1 and 2). A decreasing n_H coupled with an increasing $S_{0.5}$ occurs only if the substrates bind to both the R and T forms of the enzyme (*12*). This is a more realistic situation than one in which the substrates bind exclusively to one of the two conformational states.

The Gly^{106} enzyme has a turnover rate

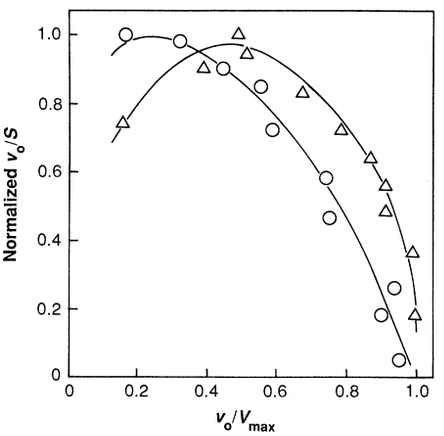

Fig. 1. Eadie-Scatchard plots of L-ornithine (△) and carbamoyl phosphate (○) saturation of Arg^{106}→Gly ornithine transcarbamoylase. The vertical scale has been adjusted so that the maximum point of each curve corresponds to 1.0. Initial velocity experiments were conducted at 25°C in 50 m*M* tris acetate buffer, *p*H 8.5, as described in earlier reports (*8, 11*); steady-state and initial velocity conditions were strictly maintained. For ornithine saturation, the amino acid was varied from 0.08 to 6 m*M* and the concentration of carbamoyl phosphate was kept at 0.6 m*M*. For carbamoyl phosphate saturation, the substrate was varied from 0.01 to 2.5 m*M* with ornithine fixed at 0.10 m*M*. The respective values for k_{cat} are given in Tables 1 and 2. (When both substrates are saturating, the limiting k_{cat} is 2.45 × 10^4 min^{-1}.) For the data shown, the enzyme concentration used in the assays was 0.4 n*M*; the same Hill coefficients were observed for both saturation curves at 4 n*M* enzyme.

Table 1. Steady-state kinetic parameters of L-ornithine saturation of the $Arg^{106} \rightarrow Gly$ ornithine transcarbamoylase. Kinetic assay conditions are as stated in the legend to Fig. 1. The standard error of the values shown is ±7%. The wild-type K_m^{app} of carbamoyl phosphate saturation as determined in steady-state initial velocity assays is 0.05 ± 0.001 m*M* (*8, 11*).

[Carbamoyl phosphate]		n_H^{orn}	$[S_{0.5}]$ (m*M*)	$k_{cat} \times 10^4$ min^{-1}
(m*M*)	($\times K_m^{app}$)			
0.05	1	1.85	0.11	0.75
0.1	2	1.69	0.20	1.39
2.5	50	1.27*	0.29	2.45

*A residual $n_H^{orn} > 1$ at saturating concentration of both substrates provides indicative evidence that the observed sigmoidicity in Fig. 1 cannot be the manifestation of a random mechanism.

Table 2. Steady-state kinetic parameters of carbamoyl phosphate saturation of the $Arg^{106} \rightarrow Gly$ ornithine transcarbamoylase. Experimental conditions are as stated in legend of Fig. 1. The standard error of the values shown is ±7%. The wild-type K_m^{app} of L-ornithine saturation from initial velocity kinetics is 0.32 ± 0.01 m*M* (*8, 11*).

[L-Ornithine]		n_H^{cp}	$[S_{0.5}]$ (μ*M*)	$k_{cat} \times 10^4$ min^{-1}
(m*M*)	($\times K_m^{app}$)			
0.3	1	1.36	29	0.93
0.6	2	1.32	33	1.47
7	20	1*	49	2.22

*See (*16*).

one-fifth that (*8*) of the wild-type ornithine transcarbamoylase. This reduced catalytic efficiency as a result of the site-directed mutation could be due to three factors: (i) the mutant enzyme is impaired since Arg^{106} is probably involved in carbamoyl phosphate binding [see (*8*)]; (ii) the *p*H optimum of the mutant enzyme may be slightly altered (*17*); and (iii) the mutant enzyme, even when completely saturated by substrates, fails to adopt the R conformation to the same extent as the wild type. In the case of the homologous enzyme aspartate transcarbamoylase, the activities of the holoenzyme, which is allosteric, and the catalytic trimer, which is not allosteric, differ by a factor of 1.45 (*18*). The efficiency of an allosteric protein rarely, if ever, exceeds that of its nonallosteric counterpart that is always in the R conformation. The nonallosteric form may also have a less complicated or less constrained structure (for example, myoglobin versus hemoglobin).

To confirm our interpretation, we have investigated the possibility of a substrate-induced protomer-oligomer equilibrium phenomenon. The saturation data of both substrates at two protein concentrations yield identical kinetic parameters (see legend of Fig. 1). Hence, the situation of a polymerizing mutant enzyme with different affinities or different catalytic velocities for the oligomer and protomer is unlikely. The possibility of kinetic cooperativity through a random mechanism as responsible for the observed cooperativity can also be ruled out. For an oligomeric enzyme having independent sites and two substrates that follow a random and steady-state addition, second-order rate equations may result, which give rise to apparent cooperativity (*19, 20*). In such an event, the positive cooperativity observed in the velocity saturation curve of one substrate would depend on the concentration of the other and would disappear when the second substrate is saturating. This phenomenon is not seen for the $Arg^{106} \rightarrow Gly$ mutant (Table 1). A random mechanism would also lower the catalytic rate (k_{cat}) much more drastically because of the kinetically significant ordered addition of substrates in ornithine transcarbamoylase (*21*).

We have shown that the Zn^{2+} ion binds ornithine transcarbamoylase cooperatively (*4, 6*) to the L-ornithine site (*22*). In doing so, the metal induces ornithine cooperativity by being a competitive inhibitor of this substrate (*23*). Carbamoyl phosphate binding is unaffected. In the absence of both substrates, zinc further promotes a slow protein isomerization leading to irreversible inactivation; the metal ion is entrapped in the isomerized enzyme (*6*). One of the protein ligands to the metal has been identified as the thiol of Cys^{273} (*22*). Although the gross mechanism of action of zinc on ornithine transcarbamoylase is known, its regulatory role is unclear. The effective concentration of zinc for modulation of transcarbamoylation is in the micromolar range, so its action may be physiological. The control of enzyme catalysis by small allosteric cofactors is not well understood but such action implies that the enzyme in concern must be capable of transmitting binding information among its subunits. Therefore, it is not entirely surprising to find that such an enzyme can be transformed into an "intrinsically" allosteric protein without the aid of extraneous cofactors. However, conversion of a noncooperative enzyme into a cooperative one by mutation of a single amino acid residue is a new phenomenon (*24*). This finding thus makes available to us a system to probe (*25*) how allosteric constraints may be incorporated in the enzyme polypeptide scaffold to transform a "relaxed" oligomer into a "tensed" one. The finding further underscores the possibility that such a mutation of the enzyme in the cell could turn transcarbamoylation into a regulatory junction in the biosynthesis of L-arginine and urea. This reaction is hitherto not known to be regulatory of the cycle. Our discovery shows that perhaps some oligomeric proteins may have the potential to be allosterically controlled. This potential may be a "built-in" evolutionary adaptation in which a protein can switch from being noncooperative to being cooperative if physiologically necessary.

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