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## The homotropic cooperative effect in the case of *N*-acetylglutamate 5-phosphotransferase

ATP:*N*-acetylglutamate 5-phosphotransferase catalyzes the feedback-sensitive step in the arginine biosynthesis of *Chlamydomonas reinhardti*. As reported, the inhibition caused by arginine is apparently competitive with respect to acetylglutamate and noncompetitive with the other substrate, ATP. The enzyme has a separate binding site for arginine[1]. The conformation stabilized by arginine has a negligible, if any, affinity towards acetylglutamate, since 10 mM arginine causes more than a 95% inhibition in the presence of 75 mM acetylglutamate. (The $K_m$ for acetylglutamate is about 4 mM.)

This enzyme fits into the group of the "K" type allosteric enzymes, as classified by MONOD *et al.*[2]. A homotropic cooperative effect is indicated in the inhibition, depending on the second or higher power of arginine concentration; however the substrates obey the Michaelis–Menten kinetics both in the absence and presence of the inhibitor[1]. According to the symmetry model[2] in those systems in which an allosteric effector modifies the apparent affinity of the substrate, the substrate also should exhibit cooperative homotropic interactions. In the case of *N*-acetylglutamate 5-phosphotransferase, the substrate does not obey this prediction.

The homotropic cooperative effect exhibited by arginine has been investigated in detail. Using the Hill plot seemed to be the most convenient way to analyze quantitatively this effect under different circumstances. Straight lines are obtained by this method, with a Hill coefficient of 2 or about 2, in every case when the measurements are carried out between pH 6 and 7.5. Fig. 1 shows the Hill plot of inhibition in the presence of different acetylglutamate concentrations at pH 7.5. The change in the concentration of the apparent competitive substrate results in parallel straight

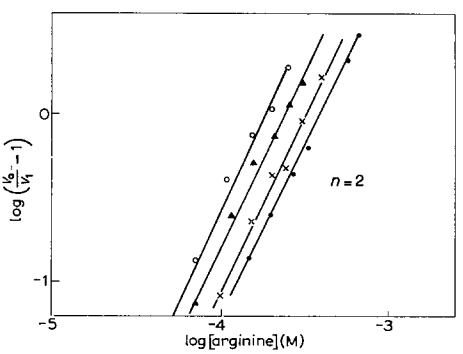


Fig. 1. The Hill plot of inhibition caused by arginine in the presence of 75 mM (●—●), 25 mM (×—×), 5 mM (▲—▲) and 2.5 mM (○—○) acetylglutamate. The experiment was carried out at 37° in Tris–HCl buffer (pH 7.5). The reaction mixture contained 250 μmoles of Tris base, 200 μmoles of hydroxylamine hydrochloride, 20 μmoles of ATP and 20 μmoles of $MgCl_2$ in a total volume of 2.0 ml. The reaction was arrested by adding the hydroxamic acid reagent, and the hydroxamic acid formed was determined photometrically as described previously[1].

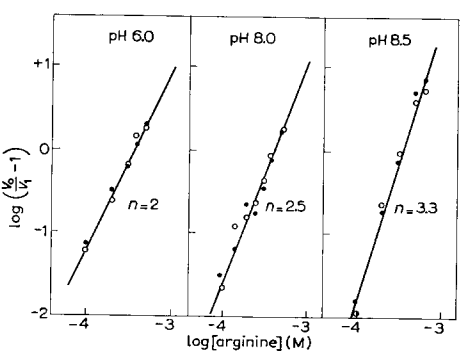


Fig. 2. The Hill plot of inhibition at different pH's in the presence of 75 mM acetylglutamate and 10 mM (●—●), or 5 mM (○—○) ATP.

lines. The decrease in the substrate concentration is accompanied by a decrease in the arginine concentration causing a 50% inhibition, ($I_{1/2}$), but the slope of the lines remains 2. There cannot be found any tendency indicating that the cooperative effect of arginine is weakening with a decreasing substrate concentration, although this would be expected on the basis of the model of Monod. The concentration of the noncompetitive substrate, ATP, does not influence the position or the slope of the straight line in the Hill plot.

The value of the Hill coefficient of inhibition does not change either when the affinity of the enzyme towards the inhibitor increases or when it decreases with several orders of magnitude. As described the inhibition of *N*-acetylglutamate 5-phosphotransferase is an exothermic process[3], and from 37 to 15° there is a 20-fold increase in the apparent affinity of the enzyme towards arginine ($I_{1/2}$ 0.4 mM and 20 $\mu$M, respectively) but without any alteration in the cooperativity.

TABLE I

THE HILL COEFFICIENT AND THE ARGININE CONCENTRATION CAUSING A 50% INHIBITION ($I_{1/2}$) UNDER DIFFERENT CIRCUMSTANCES

| *pH* | *Temperature* | *Acetyl-glutamate (mM)* | *n* | $I_{1/2}$ *(μM)* |
|---|---|---|---|---|
| 6 | 37° | 75 | 2 | 400 |
| 7 | 37° | 75 | 2 | 400 |
| 7 | 25° | 75 | 2 | 100 |
| 7 | 15° | 75 | 2 | 25 |
| 7.5 | 37° | 75 | 2 | 400 |
| 7.5 | 37° | 25 | 2 | 300 |
| 7.5 | 37° | 5 | 2 | 250 |
| 7.5 | 37° | 2.5 | 2 | 180 |
| 8 | 37° | 75 | 2.5 | 430 |
| 8.5 | 37° | 75 | 3.3 | 300 |
| 9 | 37° | 75 | 2.3 | 500 |

The mode of action of arginine analogues, L-canavanine and L-citrulline, on the activity of the enzyme is the same as that of arginine; however, the $I_{1/2}$ of these materials is in the range of $10^{-2}$ M. While at and below pH 7.5 the Hill coefficient remains 2, under circumstances in which the arginine concentration causing a 50% inhibition is changing, a shift in the pH from 7.5 to the alkaline direction causes different "*n*" values without great difference in $I_{1/2}$ (Table I). The Hill plot also gives straight lines at an alkaline pH (Fig. 2). The Hill coefficient increases, reaches its maximum at pH 8.3 and then decreases again. The maximal "*n*" value is about 3.5. In the range (from pH 6 to 9) measured, no value lower than 2 has been found.

The homotropic cooperative effect, which is one of the most frequently discussed problems connected with allosteric enzymes[2,4,5], means an interaction between identical ligands of a protein. The sigmoidal saturation curve of a ligand may be the manifestation of this effect. The kinetic data, however, give only indirect information about the real saturation conditions. The binding of an allosteric modifier is accompanied by a change in the enzyme conformation; and when measuring the

inhibition, we only can observe the final result of several distinct steps. Experiments carried out with partially purified enzyme preparations suggest that the homotropic cooperative effect which is a permanent and characteristic property in the inhibition of *N*-acetylglutamate 5-phosphotransferase cannot be found in the arginine saturation curve obtained by equilibrium dialysis.

Further investigations concerning this question are in progress.

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