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1 E. Y. LASFARGUES, *Exptl. Cell. Res.*, 13 (1957) 553.
2 H. SOBEL, *J. Embryol. Exptl. Morphol.*, 6 (1958) 518.
3 R. W. HINZ AND J. T. SYVERTON, *Proc. Soc. Exptl. Biol. Med.*, 101 (1959) 19.
4 J. W. GROVER, *Exptl. Cell Res.*, 26 (1962) 344.
5 D. J. CAVANAUGH, W. O. BERNDT AND T. E. SMITH, *Nature*, 200 (1963) 261.
6 M. RODBELL, *J. Biol. Chem.*, 239 (1964) 375.
7 J. N. FAIN, N. REED AND R. SAPERSTEIN, *J. Biol. Chem.*, 242 (1967) 1887.
8 S. L. HOWELL AND K. W. TAYLOR, *Biochem. J.*, 108 (1968) 17.
9 I. MANDL, *Advan. Enzymol.*, 23 (1961) 163.
10 T. KONO, *Biochemistry*, 7 (1968) 1106.
11 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
12 D. S. MIYADA AND A. L. TAPPEL, *Anal. Chem.*, 28 (1956) 909.
13 H. E. MORGAN, M. J. HENDERSON, D. M. REGEN AND C. R. PARK, *J. Biol. Chem.*, 236 (1961) 253.
14 Y. TAKEDA, A. ICHIHARA, H. TANIOKA AND H. INOUE, *J. Biol. Chem.*, 239 (1964) 3590.

Received September 16th, 1968
Revised manuscript received December 24th, 1968

*Biochim. Biophys. Acta*, 178 (1969) 397–400

BBA 63385

## Differences between the active and the inhibited conformations of the allosteric *N*-acetylglutamate 5-phosphotransferase

The catalytic activity of the first enzyme in the arginine pathway of *Chlamydomonas reinhardti*, ATP:*N*-acetylglutamate 5-phosphotransferase is inhibited by the end product arginine. As reported, the kinetic relationship between acetylglutamate and arginine is competitive, while between ATP and the allosteric modifier it is non-competitive. Urea in low concentrations (less than 1.5 M) suppresses the inhibition caused by arginine without impairing the catalytic activity of the enzyme[1].

These data suggest that phosphotransferase has separate binding sites for the substrates and the inhibitor, and has at least two different conformations. One of these conformations is catalytically active and binds the substrate acetylglutamate, while the other one is inactive, binding the inhibitor arginine. The two functional and structural forms of the enzyme are mutually exclusive.

A sigmoidal kinetic pattern has been found in the inhibition of phosphotransferase, indicating that subunit structure plays some role in the allosteric transition[2]. For this reason the molecular weight of the enzyme has been estimated under different circumstances. The estimation is carried out by Sephadex G-200 gel filtration, according to the method of ANDREWS[3]. For calibration of the column (50 cm × 2.5 cm diameter) $\beta$-galactosidase (EC 3.2.1.23) and crystalline D-glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) are used. Their elution volumes are 90 ± 2 ml and

130 ± 2 ml, respectively, in good agreement with the results of ANDREWS[3]. Phosphotransferase used in these experiments has a specific activity of 30 units/mg protein (purified 100-fold, as described[1]).

Phosphotransferase is found in the eluate immediately after β-galactosidase, with an elution volume of 96 ± 2 ml (Fig. 1). Its position in the elution pattern does

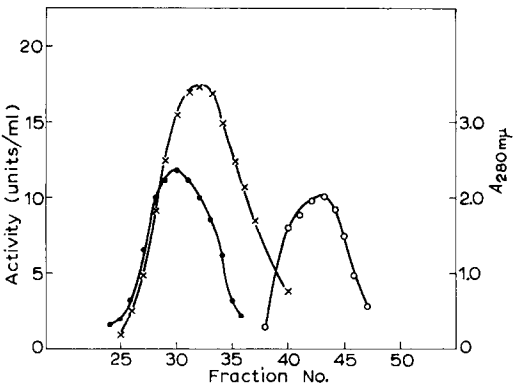


Fig. 1. Elution pattern of phosphotransferase on Sephadex G-200. The column (50 cm × 2.5 cm diameter) was equilibrated by 0.1 M Tris–HCl buffer (pH 7.5) and the filtration procedure was carried out at 5°. Fractions of 3 ml were collected. The activity of phosphotransferase (×—×) was measured in the standard assay mixture as described previously[1], the activity of β-galactosidase (●—●) was determined using the chromogenic substrate *o*-nitrophenyl-β-D-galactoside[5], and the amount of crystalline D-glyceraldehyde-3-phosphate dehydrogenase (○—○) was obtained by its absorbance at 280 mμ.

not change in the presence of $10^{-4}$ M arginine (saturating concentration at 5°), or in the presence of $2.3 \cdot 10^{-2}$ M acetylglutamate. Since β-galactosidase has a molecular weight of about 500 000, the molecular weight of phosphotransferase is approx. 400 000. This value allows the supposition that the enzyme consists of subunits, but under the conditions of our experiments it does not dissociate. The change from one functional conformation to the other must be less radical than a complete association–dissociation process.

A great difference has been found between the stability of the active and the inhibited forms of phosphotransferase. This enzyme is very sensitive to agents influencing the ternary structure of proteins. Materials reducing the weak interactions essential for the native conformations, such as dioxane, dimethylformamide, guanidine · HCl, in relatively low concentrations produce a rapid loss in the catalytic activity of the free phosphotransferase. Arginine in all cases protects the enzyme, while the rate of inactivation caused by dioxane and dimethylformamide is increased in the presence of acetylglutamate (Figs. 2A and 2B). The effect of arginine and acetylglutamate on the inactivation caused by dioxane and dimethylformamide is similar to that obtained in the presence of 4 M urea as denaturating agent[1], but only urea, and no other material tested, can produce the reversible suppression of allosteric inhibition.

Phosphotransferase is stable over a relatively wide pH range (pH 5.5–8.5). At acidic pH (pH 4) there is no difference in the inactivation rate of the enzyme in the

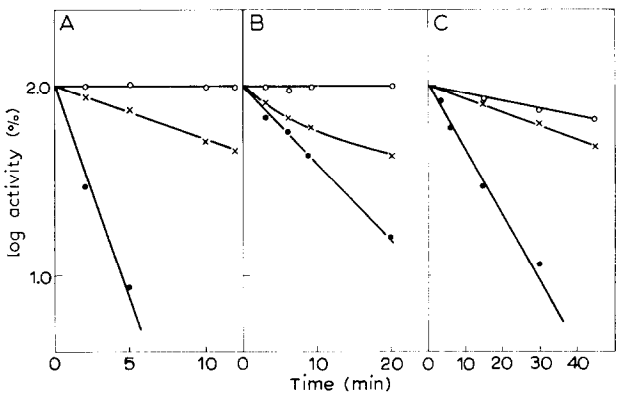


Fig. 2. The inactivation of phosphotransferase caused by 5% dioxane (A), 20% dimethylformamide (B), and by alkaline pH (pH 10.0, C). The curves represent the inactivation in the absence (×—×) and in the presence of $10^{-3}$ M arginine (○—○), or $7.5 \cdot 10^{-2}$ M acetylglutamate (●—●). The effect of the organic solvents was measured in 0.1 M Tris–HCl buffer (pH 7.5), while the alkaline pH was produced by 0.1 M $Na_2CO_3$ buffer (pH 10.0). The incubation was carried out at 37° in a final volume of 3 ml, containing 45 units of phosphotransferase.

presence of acetylglutamate or arginine, while at pH 10.0 arginine decreases and acetylglutamate increases the inactivation rate (Fig. 2C). The protecting effect of arginine and the opposite effect of acetylglutamate is also found in the case when phosphotransferase is inactivated by mushroom tyrosine oxidase[4].

From these data it seems probable that the inhibited form of the enzyme is stabilized by more or stronger interactions than the active conformation. Arginine presumably produces a compact enzyme structure, while acetylglutamate brings about a more open form. This difference in the ternary structure of the active and inhibited enzyme can be responsible for the allosteric inhibition. It is possible that the compact structure of the enzyme, formed by the effect of arginine, is accompanied by partial masking of the active center, and the substrate binding site becomes inaccessible for acetylglutamate. On the contrary, the binding of acetylglutamate induces an open structure in which the interactions necessary for the binding of arginine cannot be formed.

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1 A. FARAGÓ AND G. DÉNES, *Biochim. Biophys. Acta*, 136 (1967) 6.
2 A. FARAGÓ AND G. DÉNES, in preparation.
3 P. ANDREWS, *Biochem. J.*, 96 (1965) 117.
4 A. FARAGÓ AND G. DÉNES, *Acta Biochim. Biophys. Acad. Sci. Hung.*, in the press.
5 S. HESTRIN, D. S. FEINGOLD AND M. SCHRAMM, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. I, Academic Press, New York, 1955, p. 241.

Received December 6th, 1968

*Biochim. Biophys. Acta*, 178 (1969) 400–402