DEPENDENCE OF HUMAN ERYTHROCYTE PRPP SYNTHETASE SPECIFIC ACTIVITY ON CONCENTRATION

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1. Introduction

The enzyme 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase (EC 2.7.6.1) catalyzes the synthesis of PRPP from ribose-5-phosphate (R-5-P) and ATP in the presence of magnesium and inorganic phosphate [1–3]. In the course of our studies on normal and mutant human erythrocyte PRPP synthetase [4], we have observed that the specific activity of this enzyme exhibits in dilute solution a marked dependence on enzyme concentration. In the present communication we document these findings.

2. Materials and methods

2.1. *Enzyme preparation*

PRPP synthetase was partially purified (50-fold) from stroma-free charcoal-adsorbed normal hemolysate by treatment with DEAE-cellulose as described by Hershko et al. [5]. Incubation mixtures contained 50 mM Tris buffer (pH 7.4), 5 mM $MgCl_2$, 0.5 mM R-5-P, 0.5 mM ATP, 0.3 mM [8-^{14}C] hypoxanthine (10–20 mCi/mmole), 2.5 mM mercaptoethanol, K_2HPO_4 and the partially purified enzyme at the specified concentration, in a final volume of 200 μl. The samples were incubated at 37° and the reaction was terminated by the addition of 40 μl of 2.5 M perchloric acid. The incorporation of [8-^{14}C] hypoxanthine into inosinic acid was measured following thin-layer chromatography on microcrystalline cellulose [6]. In several experiments, in order to verify the validity of the results obtained by the standard procedure, the uncoupled method described by Fox and Kelley [7] was used in addition.

3. Results and discussion

In dilute PRPP synthetase solution the formation of PRPP exhibited a "lag phase" in relation to time. This property was undetectable at higher enzyme concentration (fig. 1). Following the "lag phase" the dilute enzyme achieved maximal specific activity similar to that of concentrated enzyme solution, the specific activities for the varying enzyme concentrations (specified in the legend to fig. 1) ranging from 5.14–5.68 nmoles PRPP formed/mg protein/min.

The "lag phase" was manifest over a wide range of inorganic phosphate concentration (0.5–10 mM; maximal PRPP synthetase activity obtained at 8–10 mM) and was not altered by varying the pH within the range of significant enzyme activity (pH 6–9) nor by preincubation of the enzyme with its substrates and activators, whether singly or in incomplete combination.

The dependence of the enzyme activity on the enzyme concentration at short reaction times (5–10 min) is most clearly illustrated when plotting the latter against enzyme specific activity (fig. 2). Identical results were obtained with the coupled and uncoupled assays proving that the "lag phase" was not an artifact of the kinetically coupled assay.

The mechanism by which dilution of PRPP synthetase renders the enzyme temporarily less active has not yet been established. The dependence of the enzyme activity on enzyme concentration, as manifest in the "lag phase", might be ascribed to a change in

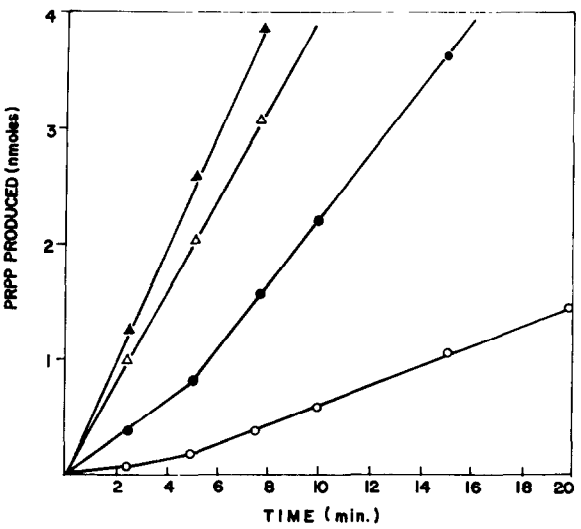

Fig. 1. Activity of PRPP synthetase at varying concentration in relation to time. Inorganic phosphate concentration 1 mM, enzyme concentrations (mg/ml): (o—o—o) 0.082; (•—•—•) 0.25; (△—△—△) 0.375; (▲—▲—▲) 0.5.

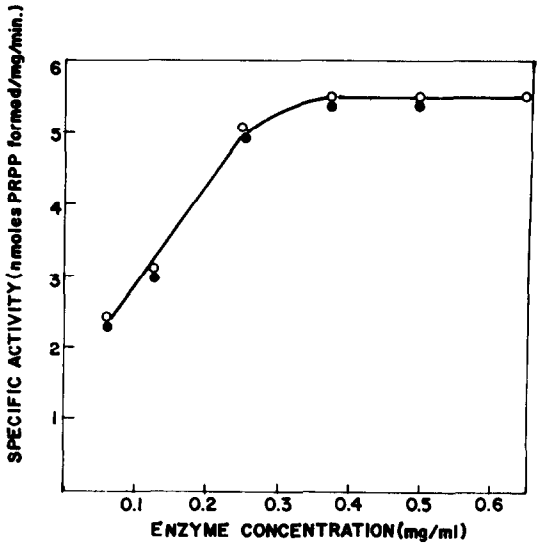

Fig. 2. Specific activity of PRPP synthetase as a function of enzyme concentration. (o—o—o) Coupled assay; (•—•—•) uncoupled assay; inorganic phosphate concentration 10 mM, incubation time 10 min.

the state of aggregation of the enzyme, it being dissociated by dilution into a less active form. This assumption is compatible with the observations of Fox and Kelley [7] who found that human erythrocyte PRPP synthetase can reversibly associate and dissociate in presence of substrates or dissociating substances, the associated form of the enzyme being the active form.

References

[1] A.W. Murray and P.C.L. Wong, Biochem. Biophys. Res. Commun. 29 (1967) 582.
[2] R.L. Switzer, J. Biol. Chem. 244 (1969) 2854.
[3] P.C.L. Wong and A.W. Murray, Biochemistry 8 (1969) 1608.
[4] O. Sperling, P. Boer, S. Persky-Brosh, E. Kanarek and A. de Vries, Europ. J. Clin. Biol. Res., in press.
[5] A. Hershko, A. Razin and J. Mager, Biochim. Biophys. Acta 184 (1969) 64.
[6] O. Sperling, M. Frank, R. Ophir, U.A. Liberman, A. Adam and A. de Vries, Europ. J. Clin. Biol. Res. 15 (1970) 942.
[7] I.H. Fox and W.N. Kelley, J. Biol. Chem. 246 (1971) 5739.