OXIDATION *IN VIVO* OF THE –SH GROUPS OF *STREPTOMYCES VIOLACEORUBER* FRUCTOKINASE

B. SABATER*

Instituto de Enzimologia del C.S.I.C., Departemento de Bioquimica, Facultad de Medicina, Universidad Autónoma, Madrid (34), Spain

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1. Introduction

An inducible fructokinase (ATP:fructose-6-phosphotransferase) has been recently found in *Streptomyces* [1]. This enzyme has –SH groups and it readily loses its activity when exposed to air which is recovered after incubation with dithioerythritol (DTE). Substantial increases of fructokinase activity on incubation with DTE are observed in extracts of *Streptomyces* made near the stationary phase of growth, which suggest the presence of an –SH oxidation-mediated fructokinase inactivation *in vivo*.

2. Materials and methods

Growth conditions of *Streptomyces violaceoruber* (Spanish Type Culture Collection no. 3086), preparation of extracts and fructokinase activity measurements, have been described elsewhere [2]. Extracts were usually made with 8 volumes the weight of fresh mycelia of an extraction medium composed of: 50 mM phosphate buffer, pH 7; 1 mM EDTA-Na_2, and 1 mM DTE. Extracts with about 1 mg of protein/ml were obtained. Proteins were estimated by Lowry's method [3]. A unit of enzyme activity is defined as the amount of enzyme that transforms 1 μmole of substrate/min.

* Present address: Cátedra de Fisiologia Vegetal, Facultad de Ciencias, Universidad Complutense, Madrid (3), Spain.

3. Results

Fig. 1 shows the fructokinase activity of an extract of *S. violaceoruber* made at the end of growth at different times after addition of 5 mM DTE and incubation at 0–4°. A 120% increase of activity is observed after 3 days incubation. The control, without 5 mM DTE, does not show an appreciable change of activity. The final activity is indistinguishable from the initial one, by the following criteria: thermosensitivity, K_m for fructose and MgATP (0.5 and 0.2 mM, respectively), activation by Mg^{2+} ions and sigmoid satura-

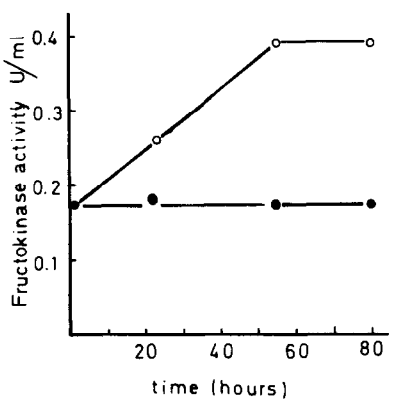

Fig. 1. Recovery of fructokinase activity after DTE addition. *S. violaceoruber* was grown with shaking in minimal medium [2] plus 0.5% fructose. At the end of growth (7 days) an extract was made with 2 volumes of extractive medium and it was incubated at 0–4° with 5 mM DTE. At the times indicated fructokinase activity (○—○—○) was measured with 5 mM fructose, 5 mM ATP and 10 mM $MgCl_2$. (●—●—●) Control without DTE addition.

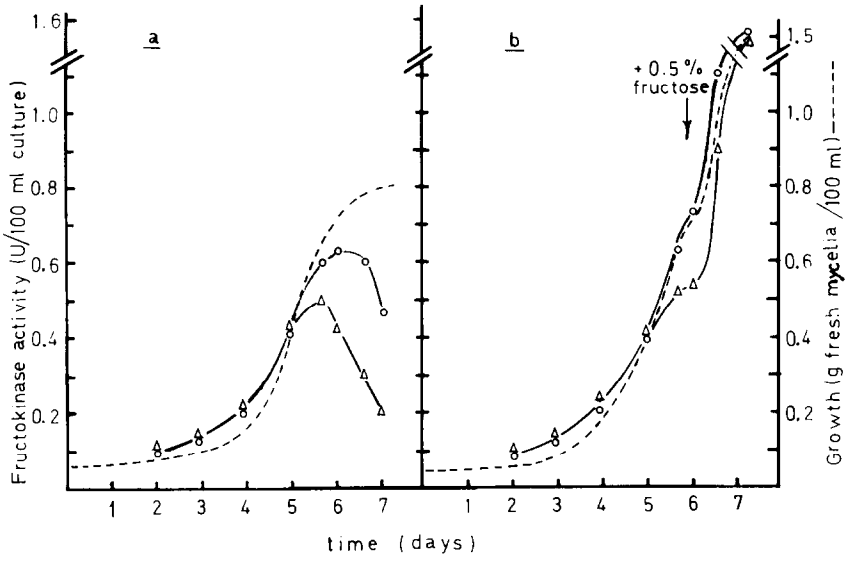

Fig. 2. Fresh and whole fructokinase activity during growth of *S. violaceoruber*. Cells were grown as in fig. 1, aliquots of 25 or 50 ml were extracted at different times with 8 volumes of extractive medium. Fructokinase activity was measured as in fig. 1, in fresh extracts (△—△—△), and in extracts after 3 days of incubation with DTE (○—○—○). In experiment 2b, 0.5% fructokinase was added to the culture at the time indicated. Dashed lines represent the growth curves.

tion curve for MgATP at a low Mg^{2+} concentration in excess of the ATP concentration [1]. Purified fructokinase preparations, without DTE, lost almost all their activity after 7 days exposure to air at 0–4° but activity was almost quantitatively recovered by a new addition of DTE and 3 days incubation [1]. Apparently the recovery of activity both in crude extracts and in purified preparations is mediated by chemical reduction by DTE of oxidized –SH groups present in the inactivated enzyme. No apparent recovery of activity was found after 2 hr incubation with DTE at 30°.

Fig. 2a shows the fructokinase activity detected in fresh extracts of *S. violaceoruber* during growth, and the activity of the same extracts after 3 days of incubation with DTE. When enzyme synthesis is falling off and presumably the need for it is decreasing, fructokinase is markedly inactivated by oxidation of its –SH groups, only later does fructokinase seem to be further inactivated by other mechanisms, plausibly by proteolysis; when this happens activity is not recovered by addition of DTE. The –SH-mediated inactivated enzyme, as measured in these experiments, corresponds to the fraction reactivatable by DTE.

Fig. 2b shows the effect of addition of fructose to the culture when fructokinase began to be oxidized. After 30 hr from this addition no activation by DTE is observed.

When *S. violaceoruber* is grown on glucose, the level of fructokinase is about 10% of that obtained when grown on fructose, but the patterns of recovery of activity with DTE are similar: no activation with extracts of mycelia during exponential growth but activation with mycelia at the end of growth.

If mycelia during exponential growth on fructose (all fructokinase protein apparently in the active form) are transferred to a medium with 0.25% lactate plus 0.25% glycerol, the activity 40 hr later was decreased 50% in fresh extracts, incubation with DTE raises the activity in these extracts by 25%, whereas the control without DTE lost 30% of activity after 3 days at 0–4°.

4. Discussion

Experiments reported here clearly suggest the existence of an –SH oxidation mechanism *in vivo*

which inactivates fructokinase, a key enzyme for the energy-yielding metabolism of fructose by *Streptomyces*. At present, it is not possible to establish if the *in vivo* –SH-mediated conversion of fructokinase is enzymatic and if it is reversible.

Plausibly, the *in vivo* enzyme oxidation reported here may be an example of a widespread mechanism which could act, according to the redox state of the cell, by sensitizing enzymes with –SH groups to proteolytic attack when nutrients are depleted. The observation reported here was possible because of the easy chemical reduction of the oxidized inactivated fructokinase and, additionally, because of the resistence of the oxidized enzyme to proteolytic attack.

Opposite redox control of enzyme activity has been reported for nitrate reductase of photosynthetic *Chlamydomonas* [4] and *Chlorella* [5]. In these ammonia represses the activity and the inactivation presumably due to a raising of the cellular level of reducing power, which in turn causes the reduction of the enzyme.

Many enzymes have disulfide bridges in their active forms, on the other hand transhydrogenase activities have been described which oxidize the corresponding reduced polypeptide chains [6, 7] yielding active enzymes. Many other enzymes are active only with their –SH groups in the reduced state. Frequently antagonic metabolic routes have enzymes with opposite redox state of their cysteine–cystine residues. This is clearly the case with the systems of proteins and nucleic acid synthesis and degradation; proteases and nucleases have disulfide bridges [6, 8], and thiol-oxidizing agents inhibit protein and nucleic acid synthesis [9, 10]. The possibility arises of a redox control of antagonic routes by the oxidation–reduction of enzyme –SH groups together with other regulatory mechanisms. This might be involved in heterotrophic cells in the control of biosynthetic and energy-yielding routes as opposed to non energy-yielding degrading reactions.

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