Regular paper

Hysteretic properties of NADP-malic enzyme from sugarcane leaves

Alberto A. Iglesias* & Carlos S. Andreo
Centro de Estudios Fotosintéticos y Bioquímicos (Consejo Nacional de Investigaciones Científicas y Técnicas, Fundación Miguel Lillo y Universidad Nacional de Rosario), Suipacha 531, Rosario (2000), Argentina; *Address for correspondence: Dr. Alberto A. Iglesias, Department of Biochemistry, Biochemistry Building, Michigan State University, East Lansing, MI, 48824, USA

Received 9 September 1991; accepted in revised form 4 October 1991

Key words: malic enzyme, hysteresis, C₄ metabolism, sugarcane

Abbreviations: Diamide – azodicarboxylic acid bis(dimethylamide); DHAP – dihydroxyacetone-phosphate; DTT – dithiothreitol; Ga₃P – glyceraldehyde-3-phosphate; NADP-ME – NADP-dependent malic enzyme; PEP – phosphoenolpyruvate; 3PGA – 3-phosphoglycerate

Abstract

NADP-malic enzyme highly purified from sugarcane leaves exhibited hysteretic properties. This behavior resulted in a lag phase during activity measurement of the enzyme preincubated in the absence of substrates. The lag was inversely proportional to the protein concentration during preincubation, which suggests that changes in the aggregational state of the enzyme are responsible for hysteresis. The pH conditions as well as the presence of different compounds in the preincubation medium modified the hysteretic properties of the enzyme. Mg²⁺ eliminated the lag period and increased the enzyme activity by nearly 2-fold. NADP⁺, 3-phosphoglycerate, ATP and dithiothreitol shortened the lag phase. The substrate L-malate inhibited the enzyme by decreasing the steady state velocity and increasing the lag time in a concentration-dependent manner. NADPH, triose-phosphates and high ionic strength increased the lag phase. Results are consistent with the view that the level of different metabolites and the pH conditions at the chloroplast regulate the activity of NADP-malic enzyme in a coordinate and effective manner.

Introduction

The C₄ pathway of photosynthesis is an additional metabolic route for carbon fixation occurring in certain higher plants. This route involves a number of specific enzymes which are located in two different photosynthetic cells (Edwards and Huber 1981, Ashton et al. 1990). In C₄ plants, atmospheric CO₂ is first fixed in mesophyll cells, rendering dicarboxylic acids. These are transported to bundle-sheath cells where they are decarboxylated, being the CO₂ thus produced finally fixed through the Calvin cycle (Edwards and Huber 1981, Ashton et al. 1990). Although the carboxylation phase at the mesophyll cells through the PEP¹ carboxylase is common to all C₄ plants, there are three different C₄ subgroups based on differences in the decarboxylating step. NADP-ME-type C₄ plants comprises, among others, the most notable C₄ crops: maize, sugarcane and sorghum (Edwards and Huber 1981). NADP-ME [L-malate:NADP⁺ oxidoreductase (oxaloacetate: decarboxylating), EC 1.1.1.40] has been highly purified from the C₄ species Zea mays (Asami et al. 1979b, Häusler et al. 1987) and sugarcane (Iglesias and Andreo 1989). The primary sequence of the maize leaves enzyme has been determined (Rothermel and Nelson...
Several studies have shown that the C₄-plant enzyme is an aggregate of a 62 kDa single subunit (Asami et al. 1979b, Häusler et al. 1987, Thorniley and Dalziel 1988, Iglesias and Andreo 1989, 1990a, Rothermel and Nelson 1989). Different oligomeric states of NADP-ME have been detected depending on the conditions (Thorniley and Dalziel 1988; Iglesias and Andreo 1990a,b). The enzyme from C₄ plants exhibits a high affinity for L-malate and NADP⁺ (Asami et al. 1979b, Häusler et al. 1987, Iglesias and Andreo 1989, 1990a), it produces CO₂ as the inorganic carbon product (Häusler et al. 1987), and requires a divalent metal ion as an essential cofactor (Asami et al. 1979b, Iglesias and Andreo 1989, Drincovich et al. 1990, 1991).

Taking into account the operation of C₄ metabolism and the key role played by NADP-ME in the C₄ pathway, it is conceivable that the activity of the enzyme is controlled in vivo. To be effective, such control should operate coordinately with the Calvin cycle. An earlier report from Asami et al. (1979a), pointed out that NADP-ME from maize leaf appears to be regulated by an interaction between pH, Mg²⁺ and L-malate as well as by the NADPH to NADP⁺ ratio. Although this and other works have shown regulation of NADP-ME from C₄ plants by different metabolites (Davies et al. 1974, Asami et al. 1979a, Iglesias and Andreo 1989, Ashton et al. 1990), the characterization of the regulatory properties of the protein is far from complete.

In this work we report that NADP-ME highly purified from sugarcane leaves is a hysteretic enzyme. That is, it is modified by different effectors at rates slower than the rate of catalysis (Frieden 1971, Neet and Ainslie 1980). Results are discussed in terms of the importance that this hysteretic behavior could have for the regulation of C₄ metabolism.

Materials and methods

Enzyme purification and assay. NADP-ME was purified to electrophoretic homogeneity from sugarcane leaves by a procedure previously described (Iglesias and Andreo 1989). Specific activities of 53 and 75 U mg⁻¹ (see definition of units below) were determined for the purified enzyme at pH 7.0 and 8.0, respectively.

Enzyme activity was assayed at 30 °C by following the change in absorbance at 340 nm with a HITACHI 150-20 spectrophotometer. The standard assay medium contained (unless otherwise specified) Tricine-Mops (25 mM each) adjusted at pH 7.0 or 8.0 with NaOH; 0.5 mM NADP⁺; 4 mM L-malate; 5 mM MgCl₂ and NADP-malic enzyme (about 50 ng of protein) in a final volume of 1 ml. The reaction was started by addition of the enzyme previously incubated as stated below. One unit (U) of enzyme is defined as the amount catalyzing the formation of 1 μmol of NADPH per min under the specified conditions.

Preincubation of NADP-ME. The purified enzyme was incubated at 30 °C in a medium containing Tricine-Mops (25 mM each) adjusted at pH 7.0 or 8.0 with NaOH with the specified additions. After 15 min of incubation, an aliquot was withdrawn and injected into the mixture that was used to assay NADP-ME activity. Unless otherwise specified, protein concentration in the preincubation medium was about 5 μg ml⁻¹. In experiments in which an effector was added into the preincubation medium, controls were carried out in parallel to check that the amount of hysteretic effector introduced from the preincubation mixture into the assay medium had no effect on catalysis. In that way, it was assured that the observed differences in enzyme activity reflected changes that occurred only during the preincubation phase.

Data analysis. The lag time (τ, the inverse of the apparent rate constant for the transition between the initial and steady state velocity) was determined according to Neet and Ainslie (1980). Thus, the natural log of the difference between the steady state rate and the observed rate at time t was plotted against time using Eq. (1):

\[ \ln(V_{ss} - V) = \ln(V_{ss} - V_i) - t/\tau \]

where \( V, V_i, \) and \( V_{ss} \) are the instantaneous, the initial, and the steady state velocities, respectively. Velocity values were obtained from the first derivative trace of the spectrophotometric assay.