# ALLOSTERIC PROPERTIES OF YEAST PYRUVATE DECARBOXYLASE

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## 1. Introduction

Pyruvate decarboxylase (EC 4.1.1.1) of yeast was discovered by Neuberg and Karczag [1] and subsequently isolated and partially purified by several groups of investigators [2,3]. The authors reported, that the overall kinetics of the enzyme follow the Michaelis-Menten equation. However, as pointed out earlier [4] in studies on the control mechanism of glycolysis we found that the enzyme has allosteric properties. We here report the results of kinetic measurements carried out with cell-free extract and highly purified yeast enzyme.

## 2. Materials and methods

Yeast extract was prepared from brewer's yeast (*Saccharomyces carlsbergensis*, strain ATCC 4228) as described elsewhere [5]. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH, EC 1.1.1.1) were purchased from C.F. Boehringer GmbH, Mannheim. Before use the enzymes were subjected to dialysis or gelfiltration in order to remove ammonium sulphate. Dithioerythritol (DTE) and bovine serum albumin (BSA) were purchased from SERVA, Heidelberg. All other chemicals were analytical grade and obtained from E. Merck, Darmstadt.

The kinetic measurements were performed at 25° in cuvettes of 1 cm light path. Absorbancy changes at 366 nm were recorded with the Eppendorf photometer equipped with a T/A converter and recorder of Netheler and Hinz, Hamburg. The basic reaction mixture contained the following final concentrations in a total volume of 3 ml:

| | | |
|---|---|---|
| 100 mM | potassium citrate or potassium phosphate buffer pH 6.0 | |
| 0.3 mM | DTE | |
| 0.3 mM | TPP | (thiamine diphosphate) |
| 0.3 mM | $Mg^{2+}$ | |
| 0.33 mM | NADH | |
| 1% | BSA | |
| 18 I.U. | ADH | (sufficient to maintain a linear response with a threefold increase in maximal PDC activity |
| 0.05 I.U. | PDC | (purified enzyme or yeast extract) |
| 0.05 to 50 mM | | sodium pyruvate |

In order to ensure saturation with the coenzyme [6] yeast extract as well as dialyzed purified enzyme were preincubated for 30 min at 25° in 30 mM potassium citrate or potassium phosphate, pH 6.8, containing 30 mM TPP, 30 mM $Mg^{2+}$, 30 mM DTE and 1% BSA. The kinetic data were evaluated with the aid of a computer programme for the determination of kinetic parameters from sigmoidal steady-state kinetics [7]. Enzyme activities are given in international units per liter.

## 3. Results and discussion

Kinetics of pyruvate decarboxylase in yeast extract clearly exhibit cooperativity as shown in fig. 1a in an activity vs. pyruvate concentration plot. Furthermore, the degree of cooperativity depends on the concentration of phosphate in the medium. In the absence of phosphate the enzyme has only a moderate degree of cooperativity ($n_H = 1.37$). In this case, we find a Michaelis constant between 1.1-1.3 mM pyruvate for the purified enzyme as well as with the yeast extract. This is in good agreement with the data published by

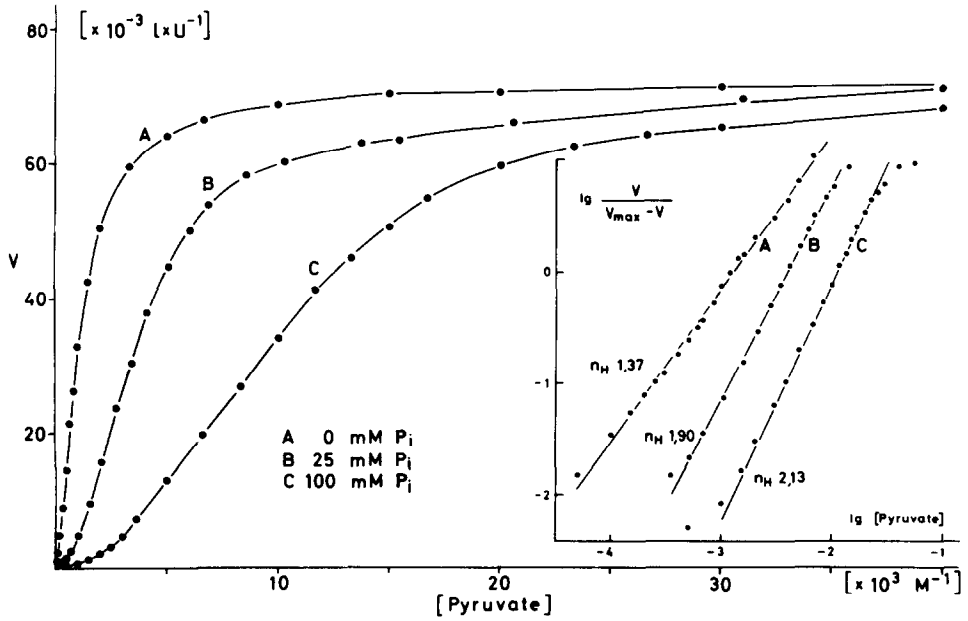


Fig. 1. a) Michaelis-Menten plot of yeast PDC velocity vs. pyruvate concentration. The measurements were performed with yeast extract, the purified enzyme gives the same results. Conditions as given in methods. Curves A, B and C were obtained with the indicated phosphate concentrations. b) *Insert*: Hill plot calculated from the same set of data demonstrating different degrees of cooperativity.

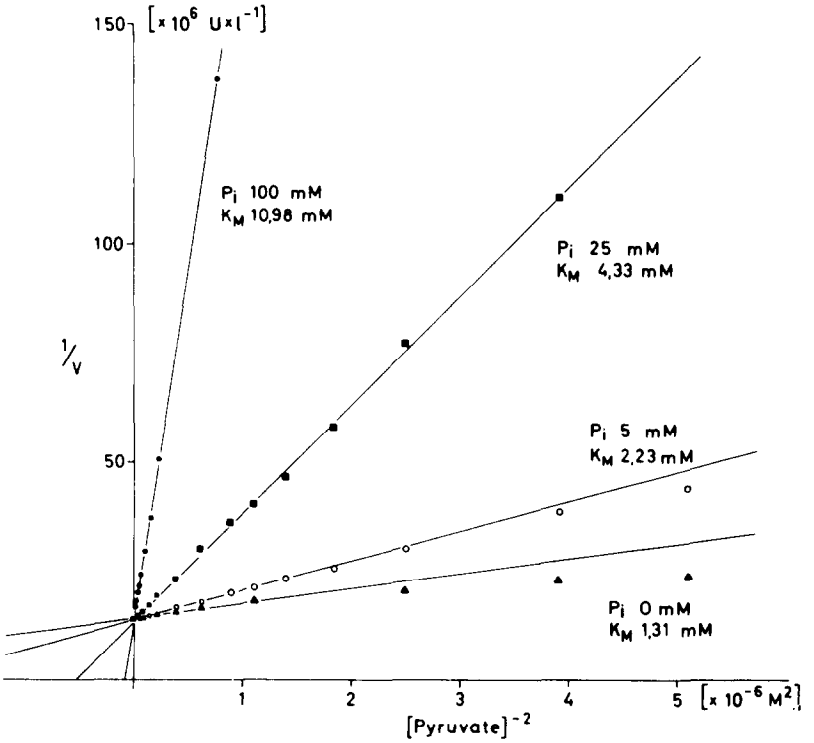


Fig. 2. Modified Lineweaver-Burk plot of yeast PDC with pyruvate as substrate. Reciprocal velocity vs. squared reciprocal pyruvate concentration. Conditions as given in methods. The different curves and $K_m$ values were obtained with the indicated phosphate concentrations.

Holzer et al. [3]. Obviously, with increasing phosphate concentration, the allostericity of the enzyme is enhanced. In fig. 1b, the Hill-plot demonstrates increasing cooperativity rising from $n_H = 1.37$ to $n_H = 2.13$ with increasing phosphate concentration.

The increasing cooperativity is also reflected in a modified Lineweaver-Burk plot of fig. 2 where the reciprocal activity is plotted against the squared reciprocal pyruvate concentration. With increasing phosphate concentration, the experimental data can clearly be fitted to a straight line on the basis of a quadratic function. These experiments classify pyruvate decarboxylase as an enzyme belonging to the K-system, as defined by Monod et al. [8]. The figures indicate at least two binding sites of the enzyme for the substrate pyruvate. This is in agreement with recent ultracentrifuge studies, which revealed that yeast pyruvate decarboxylase consists of two subunits [9].

Fig. 2 also demonstrates that the enzyme is modified by phosphate not only with respect to substrate cooperativity but also with respect to the affinity for its substrate. Phosphate increases the cooperativity and at the same time decreases the apparent affinity for pyruvate. Under the specified conditions of fig. 2 the Michaelis constant ($K_m$) is shifted about one order of magnitude from 1.3 mM to 11 mM pyruvate. This inhibition is restricted to an effect upon $K_m$ while the maximum activity ($V_{max}$) is not affected. Therefore, the inhibitory action of phosphate on pyruvate decarboxylase can be classified as being competitive.

As is well known, the classical competitive inhibitor binds reversibly in place of the substrate on the active site of the enzyme yielding a straight line in a Dixon plot [10]. In fig. 3, the kinetics of phosphate inhibition of pyruvate decarboxylase are plotted in this manner. Instead of straight lines, a curvature is found indicating cooperative effects for phosphate with respect to its inhibitory action. Analogous to the Hill equation system, we use a cooperativity factor

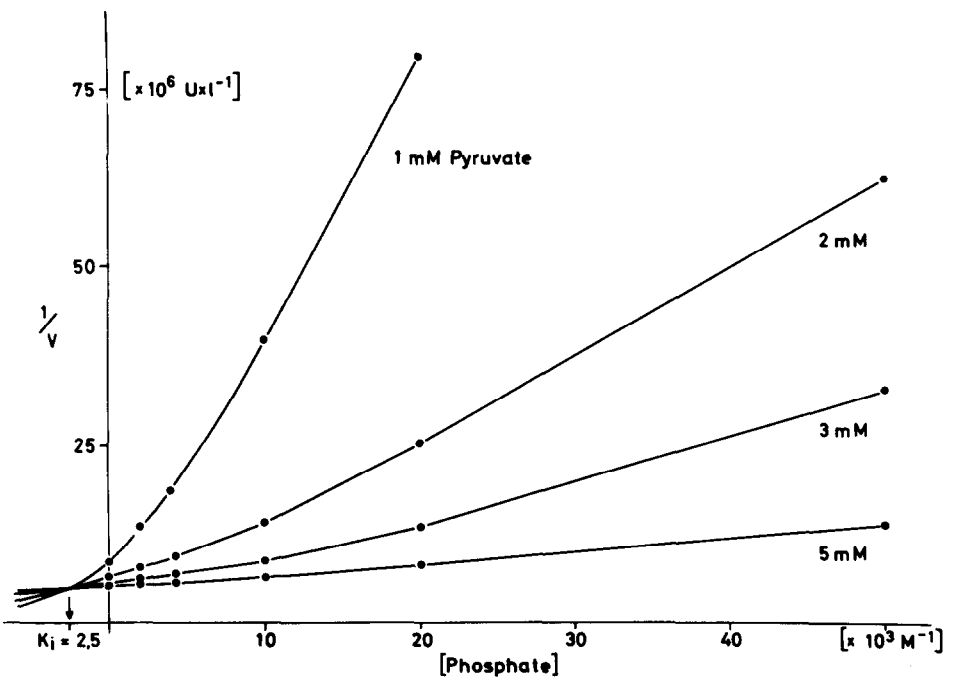


Fig. 3. Dixon plot of yeast PDC with pyruvate as substrate. Reciprocal velocity vs. phosphate concentration. Conditions as given in methods. The different curves were obtained with the indicated pyruvate concentrations.

$n_H$ to describe the degree of cooperativity. Using a computer program to fit the experimental results [11] a cooperativity of $\tilde{n}_H = 1.3$ was obtained. Obviously, the classical picture of competitive inhibition has to be modified here to account for the observed allosteric effect. The mechanism is under current investigation.

It is interesting to note that the inhibitor constant for phosphate ($K_i = 2.5$ mM) is of the same order of magnitude as the Michaelis constant for pyruvate ($K_m = 1.3$ mM). This makes the enzyme about equally sensitive to variation of phosphate and pyruvate concentrations. Inside the yeast cell pyruvate decarboxylase is exposed to varying concentrations of pyruvate and phosphate, and therefore to the simultaneous action of allosteric activation and inhibition. Holzer et al. [3] observed decreasing $K_m$ values (5–1 mM) with prolonged periods of standing after plasmolysis, when testing pyruvate decarboxylase activity in liquid air treated yeast cells. This unexplained phenomenon is probably caused by the allosteric properties of the enzyme.

The observed positive homotropic cooperative effect of pyruvate and the negative heterotropic cooperative effect of phosphate on the enzyme could be very effective in the control of glycolytic flux in the yeast cell, and it will be necessary to study to what extent this enzyme may be involved into the active regulation of metabolism.

While preparing this manuscript, our observations were partially confirmed. The positive cooperative effect of pyruvate has been observed independently by J. Ullrich (personal communication) in a purified preparation of pyruvate decarboxylase from *S. carlsbergensis*.

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