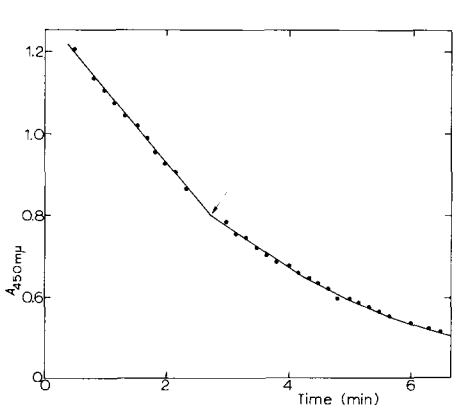
BBA 61123

The inhibition of succinate dehydrogenase by oxaloacetate

It is generally believed that the inhibition of succinate dehydrogenase (EC 1.3.99.1) by oxaloacetate[1] is competitive in nature[2]. In this laboratory DERVARTANIAN AND VEEGER[3] found that this is the case with the purified enzyme[4] and reported an inhibition constant of 1.5 μM. WOJTCZAK and co-workers[5,6], however, have reported that a non-competitive inhibition is obtained if the initial reaction rate is measured, and a typically competitive inhibition only if the steady-state rate of oxidation is taken. Furthermore, they state that neither the inhibition by oxaloacetate nor its partial reversal by succinate is instantaneous. A K_i of 0.2 μM was reported for the constant rate of oxidation.

We have re-examined the problem with the purified enzyme isolated by a modification[3] of the method of WANG, TSOU AND WANG[4]. Fig. 1 shows that, in disagreement with WOJTCZAK *et al.*[5,6], the inhibition is instantaneous. However, in agreement with WOJTCZAK *et al.*, after a steady rate of oxidation for about 1.5 min, the degree of inhibition increased, to reach a new level after about 30 sec to (as in the experiment illustrated in Fig. 1) several minutes. The increased inhibition is not due to the formation of fumarate. The addition of 1 mM fumarate had no appreciable effect on the activity of the enzyme in the absence of oxaloacetate, or on the degree of inhibition by oxaloacetate, either that directly measured or after the secondary inhibitory state is reached.

In disagreement with WOJTCZAK *et al.*[5,6], the inhibition found immediately on the addition of oxaloacetate was competitive. Fig. 2 shows a typical experiment from which values of K_i of 4.5 and 4.3 μM may be calculated. Similar results were obtained



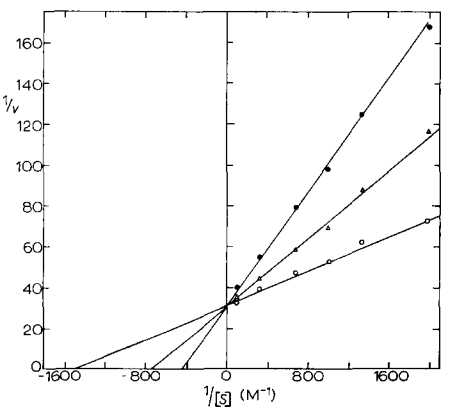


Fig. 1. Inhibition of succinate dehydrogenase by oxaloacetate. Phosphate buffer (pH 7.8), 100 mM; $K_3Fe(CN)_6$, 5 mM; succinate, 10 mM; bovine serum albumin, 1 mg/ml; enzyme, 0.06 mg/ml. At the arrow, oxaloacetate was added to a concentration of 6.3 μM.

Fig. 2. Double-reciprocal plot of primary inhibition of succinate dehydrogenase by oxaloacetate. Phosphate buffer (pH 7.8), 100 mM; $K_3Fe(CN)_6$, 6 mM; bovine serum albumin, 1 mg/ml; EDTA, 1 mM; enzyme (240 units[4]/mg protein), 0.08 mg protein per ml. The velocity is in arbitrary units. ○—○, no oxaloacetate; △—△, 5.4 μM oxaloacetate; ●—●, 10.8 μM oxaloacetate. The oxaloacetate concentrations were determined with malate dehydrogenase and NADH. The oxaloacetate used contained 10% pyruvate (as determined with lactate dehydrogenase and NADH).

over a large range of oxaloacetate concentrations (0.6–135 μM) and succinate concentrations (0.2–100 mM). The K_i varied between 1.7 and 6.3 μM in 14 measurements in this range. In the experiment shown in Fig. 2, the reaction was started by adding enzyme to an otherwise complete reaction mixture. Inhibition was also instantaneous and competitive (K_i = 2.6–3.1 μM) when the order of addition was the same as in Fig. 1.

The secondary inhibition was also found to be purely competitive, with a K_i of 0.6–0.7 μM.

When the enzyme was pre-incubated for 10 min with a high concentration of oxaloacetate and then diluted to the concentrations usual in the assay of enzyme activity before adding the succinate or $K_3Fe(CN)_6$, the greater inhibition was found immediately. In this case, however, the inhibition was partly non-competitive and partly competitive. The K_i of the competitive component was 0.7 μM, and that of the non-competitive component 0.6 μM.

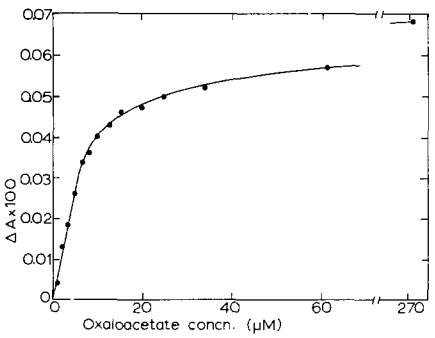


Fig. 3. Titration of the succinate dehydrogenase with oxaloacetate, followed spectrophotometrically at 570 mμ. To 1.8 ml of enzyme (240 units[4]/mg protein; 3 mg protein per ml) in a 1-cm cell was added from a Hamilton microsyringe various amounts of 1 mM oxaloacetate. Finally an excess oxaloacetate was added in the form of a 10 mM solution. The total volume added was 0.3 ml. The absorbances were corrected for dilution. The initial absorbance was 0.200.

The reaction between the enzyme and oxaloacetate, as measured by the increase in absorbance at 570 mμ on adding the inhibitor to the enzyme[3,7], has also been further studied. Experiments with the stopped-flow apparatus showed that the reaction was always complete in 4 sec. In close agreement with the value previously reported[3,7], a dissociation constant of 3 μM may be calculated from the data given in Fig. 3. (In this titration, the concentrations of enzyme and oxaloacetate are of the same order of magnitude, and in calculating the K_D a correction for the amount of inhibitor bound to the enzyme was made.)

The rapid reaction between enzyme and oxaloacetate, as followed spectrophotometrically, and the agreement between the K_i obtained for the primary inhibition and the K_D from the titration supports the conclusion[3,7] that the spectroscopically identified compound is the EI complex of the primarily inhibited enzyme.

The nature of the secondary inhibition is under study.

We wish to thank Professor C. VEEGER and Dr. D. V. DERVARTANIAN for useful suggestions and Mr. S. VAN DALSEM for technical assistance.

Laboratory of Biochemistry,
B. C. P. Jansen Institute,*
University of Amsterdam,
(The Netherlands)

W. P. ZEYLEMAKER
E. C. SLATER

1 N. B. DAS, *Biochem. J.*, 31 (1937) 1116.
2 A. B. PARDEE AND V. R. POTTER, *J. Biol. Chem.*, 176 (1948) 1085.
3 D. V. DERVARTANIAN AND C. VEEGER, *Biochim. Biophys. Acta*, 92 (1964) 233.
4 T. Y. WANG, C. L. TSOU AND Y. L. WANG, *Sci. Sinica Peking*, 5 (1956) 73.
5 A. B. WOJTCZAK, in J. M. TAGER, S. PAPA, E. QUAGLIARIELLO AND E. C. SLATER, *Regulation of Metabolic Processes in Mitochondria*, BBA Library, Vol. 7, Elsevier, Amsterdam, 1966, p. 539.
6 L. WOJTCZAK, A. B. WOJTCZAK AND L. ERNSTER, *Abstracts Third Meeting of the Federation of European Biochemical Societies, Warsaw, 1966*, Academic Press, London, and PWN, Warsaw, 1966, p. 124.
7 D. V. DERVARTANIAN, W. P. ZEYLEMAKER AND C. VEEGER, in E. C. SLATER, *Flavins and Flavoproteins*, BBA Library, Vol. 8, Elsevier, Amsterdam, 1966, p. 183.

Received October 13th, 1966

* Postal address: Plantage Muidergracht 12, Amsterdam-C.

BBA 61121

β-D-Xylosidase in pig kidney

It has recently been reported[1] that rat liver possesses β-xylosidase activity. Little is known of the distribution of this enzyme in other mammalian tissues, although highly active preparations have been demonstrated in bacteria, plants and fungi[2,3]. It is possible that the enzyme occurs in a number of organs where the cleavage of the link between xylose and serine in chondroitin sulphate–protein complexes may be an important step in the catabolism of the mucoid residues, but lack of a suitably sensitive method of assay has so far hampered investigation of this enzyme.

By using the fluorigenic substrate, 4-methylumbelliferyl β-D-xyloside[4], we have been able to detect and examine a β-xylosidase in pig kidney. The substrate (1 ml of 1 mM solution) in 0.2 M phosphate–citrate buffer is incubated with 1 ml of a 0.04% (w/v) homogenate of the tissue in water for 30 min before adding 3 ml of 0.5 M glycine–NaOH buffer (pH 10.4) to stop the reaction and develop the fluorescence of the liberated aglycone. Fluorescence was measured on a Locarte LF.5 fluorimeter at 440 mμ, (activation at 340–380 mμ).

Under these conditions the enzyme had a broad pH optimum of 5–6 (Fig. 1), the rapid loss of activity in more acid conditions being caused by irreversible denaturation. The working substrate concentration used does not allow complete saturation of the enzyme (Fig. 2), but higher concentrations are precluded by its low solubility.

It has been shown that the rat liver enzyme is sedimented in isotonic sucrose homogenates along with the acid phosphatase-rich particles, and the β-xylosidase activity is latent until released by detergent or hypotonic conditions[1]. If thus appears