SUMMARY

A differential equation is presented describing the Michaelis-Menten scheme under the assumption that [E] $\gg$ [ES], an approximation which is valid in the initial and in the last stages of an enzymic reaction. The solution of this equation relates the product concentration to a two-term exponential function in time. Under most conditions only one term is significant during the terminal phase of the reaction. When $K_m \gg$ [E], this dominant term is given by the Briggs-Haldane "steady state" approximation.

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THE REACTION OF β-MERCAPTOPYRUVATE WITH LACTIC DEHYDROGENASE OF HEART MUSCLE*

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INTRODUCTION

Recent studies dealing with the mechanism of desulfuration of cysteine in certain microorganisms (KUN *et al.*[1,2]) suggested that β-mercaptopyruvic acid is a precursor of H_2S. An enzyme system catalyzes the release of some form of sulfur from β-mercaptopyruvate which then oxidizes 2 moles of cysteine to cystine and H_2S. A similar series of reactions has been suggested previously by MEISTER *et al.*[3] and shown to occur in mammalian liver. These workers demonstrated that β-mercaptopyruvate may arise by transamination from cysteine. CHATAGNER AND SAURET-IGNAZI[4], postulating an analogous mechanism, also attribute a central rôle to β-mercaptopyruvate in the H_2S producing multienzyme system of mammalian liver. Furthermore the participation of β-mercaptopyruvate as a sulfur donor in enzymic transsulfuration reactions was pointed out by WOOD *et al.*[5,6] who used cyanide as a sulfur acceptor. Since the crystalline rhodanase of SÖRBO[7] does not react with β-mercaptopyruvate it is likely that liver cells contain several transsulfurases.

This apparently manifold rôle of β-mercaptopyruvate in intermediary metabolism led to a search for further enzymic reactions which this substance may undergo. As a part of this study this paper describes the reaction of reduced diphosphopyridine nucleotide (DPNH) and crystalline lactic dehydrogenase (LDH) with β-mercaptopyruvate.

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EXPERIMENTAL

Synthesis and properties of β-mercaptopyruvate

Preparative procedures were previously reported by SCHNEIDER AND REINEFELD[8] and PARROD[9]. These methods did not yield uniform products in our laboratory. Therefore, certain modifications were introduced. Bromopyruvic acid was prepared according to SPRINSON AND CHARGAFF[10], with strict precautions to exclude any moisture during bromination. The bromopyruvic acid was recrystallized after charcoal treatment from hot chloroform. 50 ml of concentrated (15.1 *N*) NH_4OH were chilled in an ice bath to 0° C and dry H_2S gas was passed through a capillary into the solution for a period of 2 to 3 hours. 11.5 g dry, finely pulverized bromopyruvic acid were then added in small portions (in solid form) at a rate which permitted the temperature of the solution ot remain below 6 to 8° C. H_2S gas was continuously bubbled through the reaction mixture to secuer adequate mixing. The addition of the total amount of bromopyruvic acid is completed in 45 minutes, whereupon 400 ml of 95% ethanol are admixed. The quickly precipitated light yellow product is filtered off (6.5 g), then dissolved in 90 ml of distilled H_2O, treated with charcoal, and filtered. To the water clear filtrate 800 ml of 95% ethanol are added and crystallization initiated by scratching the wall of the container with a glass rod. The fine colorless crystals are collected by filtration after 24 hours of standing at 4° C. The product (3.4 g of the ammonium salt) gives the nitroprusside test and its 2,4-dinitrophenylhydrazone melts at 164–165° C with decomposition (reported melting points of this 2,4-dinitrophenylhydrazone are: 161–162° C[3] and 163–165° C[6]). The 2,4-dinitrophenylhydrazone derivative of β-mercaptopyruvic acid was analyzed also by paper chromatography[11] and found to be completely homogeneous. The infrared spectrum of ammonium β-mercaptopyruvate clearly indicates that this compound is in the enol form. Further molecular properties of β-mercaptopyruvate will be discussed elsewhere.

Aqueous solutions of β-mercaptopyruvate show a pH dependent characteristic enol spectrum with a maximum at 290 mμ (Fig. 1). The salt is sufficiently stable in neutral or moderately alkaline aqueous solutions for *in vitro* work (at pH 10.1 for at least 20–30 minutes at 20–25° C). This was ascertained by chromatographic analyses[11] of aqueous solutions at different H^+ concentrations.

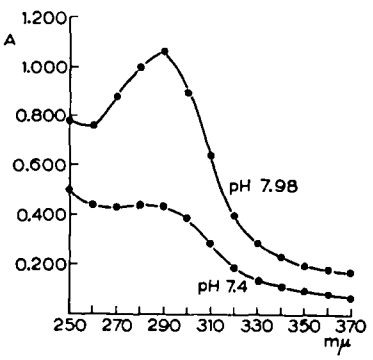

Fig. 1. Absorption spectrum of β-SH-pyruvate (3 μmoles/3 ml). Absorption spectra were determined in the Beckman spectrophotometer in quartz cuvettes of 1 cm light path, charged with 3 ml of 0.01 *M* potassium phosphate buffer, containing 1 μmole/ml ammonium β-mercaptopyruvate.

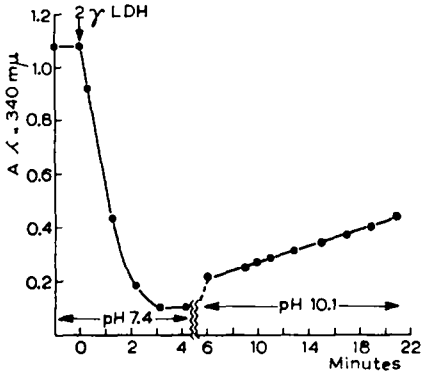

Fig. 2. Reversibility of the reduction of β-SH-pyruvate by LDH. The test system contained 3 μmoles of ammonium β-mercaptopyruvate and 400 γ DPNH, dissolved in 0.01 *M* potassium phosphate buffer of pH 7.4. 0.05 ml (2 γ) heart muscle lactic dehydrogenase (*cf.* J. B. NEILANDS, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. I, Academic Press. Inc., New York, 1955, p. 449) was added at 0 time and DPNH oxidation followed in the Beckman spectrophotometer. At 5 minutes 0.06 ml of *N* NaOH was added which raises the pH to 10.1.

Reaction with DPNH and LDH

A solution of ammonium β-mercaptopyruvate rapidly oxidizes DPNH in the presence of LDH. The Michaelis constant for pyruvate was found to be $5.4 \cdot 10^{-5}$ at pH 7.4 (0.01 *M* potassium phosphate buffer) and $8.2 \cdot 10^{-4}$ for β-mercaptopyruvate. MEISTER[12]

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reported a Michaelis constant for pyruvate of $5.2 \cdot 10^{-5}$. According to these measurements β-mercaptopyruvate has about 1/15th the affinity of pyruvate for LDH which corresponds to those of α, γ-diketocaproic and α-ketobutyric acids[12]. While the oxidation of DPNH by β-mercaptopyruvate proceeds rapidly at pH 7.4, the oxidation of β-mercaptolactate by DPN takes place at more alkaline pH. This reversible oxidation–reduction is shown in Fig. 2. The absorbance of the enolate ion at 340 mμ has been subtracted in these measurements in order to obtain true values of DPNH formation at pH 10.1. Chromatographic analyses of the test samples revealed no decomposition of β-mercaptopyruvate under given experimental conditions.

DISCUSSION

The broad substrate specificity of LDH has been extensively studied by MEISTER[12]. The addition of β-mercaptopyruvate to the list of mono- and diketo acids which react with LDH is of particular interest. Preliminary observations in this laboratory indicate that β-mercaptopyruvate, but not β-mercaptolactate is a substrate for enzymic transsulfuration, thus in the presence of LDH the availability of DPNH governs the rate of transsulfuration. This might be an example of chemical regulation of multienzyme systems in which β-mercaptopyruvate is one of the intermediary substrates.

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SUMMARY

A modified procedure for the preparation of pure ammonium β-mercaptopyruvate has been described. Evidence was obtained which indicates that the salt exists in solid state as the enol. LDH reversibly reduces β-mercaptopyruvate by DPNH. It is suggested that this reaction may be of physiological significance.

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