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Mechanism of Decarboxylation of Pyruvic Acid in the Presence of Hydrogen Peroxide

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ABSTRACT

The purpose of this work was to probe the rate and mechanism of rapid decarboxylation of pyruvic acid in the presence of hydrogen peroxide (H_2O_2) to acetic acid and carbon dioxide over the pH range 2–9 at 25°C, utilizing UV spectrophotometry, high performance liquid chromatography (HPLC), and proton and carbon nuclear magnetic resonance spectrometry (^{1}H, ^{13}C-NMR). Changes in UV absorbance at 220 nm were used to determine the kinetics as the reaction was too fast to follow by HPLC or NMR in much of the pH range. The rate constants for the reaction were determined in the presence of molar excess of H_2O_2 resulting in pseudo first-order kinetics. No buffer catalysis was observed. The calculated second-order rate constants for the reaction followed a sigmoidal shape with pH-independent regions below pH 3 and above pH 7 but increased between pH 4 and 6. Between pH 4 and 9, the results were in agreement with a change from rate-determining nucleophilic attack of the deprotonated peroxide species, HOO^-, on the α-carbonyl group followed by rapid decarboxylation at pH values below 6 to rate-determining decarboxylation above pH 7. The addition of H_2O_2 to ethyl pyruvate was also characterized.

Introduction

The purpose of this work was to probe the pH dependence of the reaction rates, as well as the mechanism of rapid decarboxylation of pyruvic acid in the presence of hydrogen peroxide (H_2O_2). The physicochemical properties (hydration equilibrium and dissociation) of pyruvic acid as well as a series of other α-ketocarboxylic acids was examined in an earlier study.[1] Here, the fast kinetics and mechanism of decarboxylation of pyruvic acid in the presence of H_2O_2 were studied at 25°C using a UV spectrophotometry technique and HPLC and nuclear magnetic resonance (NMR) product analysis. A mechanism of reaction, involving the tetrahedral intermediates (2-hydroperoxy-2-hydroxypropanoate[2]) (Scheme 1), was proposed that could explain the pH-rate profile.

The decarboxylation of pyruvic acid and a few other α-ketocarboxylic acids in the presence of peroxides has been studied earlier by others.[2-4] Most of these studies were incomplete or probed only in a narrow pH range. The novel use of carbon isotope effect on the decarboxylation by Melzer and Schmidt[4] suggested the possibility of a change in rate-determining step in the reaction but how this affected the pH-rate profile was not specifically examined. Hence, important mechanistic details of the reaction between pyruvic acid and H_2O_2 remain obscure.

Hydrogen peroxide and other peroxide species, such as organoperoxides (ROOR′) or hydroperoxides (ROOH), are reactive impurities present in many pharmaceutical excipients.[5] These peroxides may be introduced into excipients during the manufacturing process[6] or their concentrations could increase under storage conditions when exposed to oxygen.[7] Stability of active pharmaceutical ingredients is often compromised by these reactive peroxide species, examples of which appear throughout the literature.[7-13] Polymeric excipients, in particular, such as povidone,[7] crospovidone,[14] hydroxypropylcellulose,[6] polysorbate 80,[8-10] and polyethylene oxide,[15] are major sources of peroxides.

Peroxides can impact drug product stability by several mechanisms.[16] Peroxides can contribute to three types of oxidative

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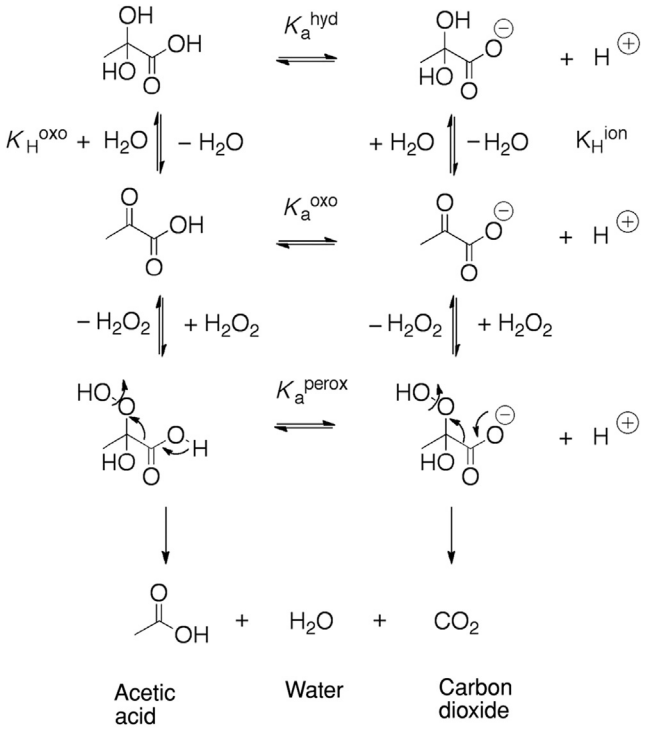

Scheme 1. Proposed overall reaction and ionization scheme involving the reaction of various pyruvic acid specie with H_2O_2.

chemical reactions with drugs: nucleophilic addition, electrophilic displacement, and radical reactions.[17] Therefore, it is important to remove peroxides during the manufacturing and before the packaging processes as well as preserve the stability of formulations during the storage using scavenger antioxidants.[6,7,17] Pyruvic acid is an excellent H_2O_2 scavenger.

It is also known that several endogenous or exogenous molecules in our body, such as cysteine, glutathione, methionine, ascorbic acid, and α-keto acids, including pyruvic acid, alter oxidation of proteins and important cellular components caused by H_2O_2 and reactive oxygen species.[18]

Materials and Methods

Materials

Sodium pyruvate ReagentPlus (≥99%), acetic acid ACS reagent (≥99.7%), sodium acetate 99%, H_2O_2 50% solution in water, sodium chloride BioXtra ≥99.5%, HCl 37% A.C.S. reagent, and dimethyl sulfoxide d_6 (DMSO d_6) 99.96% were commercially available from Sigma-Aldrich (Milwaukee, WI) and used without purification. NaOH certified A.C.S. pellets, NaH_2PO_4 H_2O, and Na_2HPO_4 anhydrous certified A.C.S. were purchased from Fisher Scientific (Fair Lawn, NJ). Tris-HCl +99% was commercially available from Acros Organic (NJ). Deionized water was used to prepare all the samples for the UV, NMR, and HPLC analysis.

Methods

UV Spectophotometric Analysis

Separate sodium pyruvate (1.5 mM) and H_2O_2 solutions with concentrations between 5 and 30 mM were prepared using buffers tris (pH 9), phosphate (pH 6-8.0), and acetate (pH 4.0-5.5) at 20 and 40 mM and HCl 0.01 and 0.003M, with ionic strength adjusted to 0.15 with NaCl. Equal volume of the pyruvate solution was mixed with each H_2O_2 solution in a quartz cuvette (Starna Cells, Inc., Atascadero, CA) equilibrated at 25°C in a water bath. Even though H_2O_2 and pyruvic acid absorbed at the similar wavelengths, it was possible to monitor the overall drop in absorbance at 220 nm as pyruvate was consumed by H_2O_2 (Scheme 1) to form acetic acid and CO_2. A UV spectrophotometer Spectramax® PLUS^{384} (Sunnyvale, CA) was used to monitor the fast kinetics.

HPLC Analysis

A Shimadzu SIL 10-A system including a SIL-10A autoinjector, a SPD-10A UV–Vis detector, a SCL-10A system controller, LC-10AT pumps, and Class-VP version 4.10 software obtained from Shimadzu Scientific Instruments (Columbia, MD) was used. A Phenomenex Synergi 4 μm Hydro-RP 80 Å 250 × 4.6 mm^2 column thermostated at 30°C was used. An isocratic separation was performed using 25 mM NaH_2PO_4 with pH 3 as mobile phase at a flow rate of 0.7 mL/min. The UV detection of pyruvic acid and acetic acid (the degradation product) was carried out at 220 nm. An injection volume of 5 μL was used in all experiments.

^{1}H, ^{13}C-NMR, Heteronuclear Single Quantum Correlation, and Heteronuclear Multiple Bond Correlation

The starting concentration of sodium pyruvate and ethyl pyruvate were 150 mM, and the concentration of H_2O_2 was 450 mM. Equal volumes of 0.25 mL of sodium pyruvate and H_2O_2 solutions both at pH 2 and ionic strength 0.15 with sodium chloride were mixed in a 5-mm NMR tube and the spectra were acquired. Ethyl pyruvate and H_2O_2 solutions were also titrated to the desired pH 5 using concentrated hydrochloric acid such that the final ionic strength was 0.15 with sodium chloride. Equal volumes of 0.25 mL of each solution were mixed in a 5-mm NMR tube and the spectra were acquired. The pH of each sample was measured directly in the NMR tube using a 5 mm pH electrode purchased from Wilmad Labglass (Vineland, NJ). The samples of ethyl pyruvate in presence of H_2O_2 and sodium pyruvate alone were stable during the analysis and no variation in spectra and pH values was observed when the runs were repeated. 1D and 2D ^{1}H and ^{13}C NMR spectra were acquired on a 500-MHz Bruker AVIII spectrometer (Rheinstetten, Germany) equipped with a carbon-enabled cryoprobe. Sample temperature was set to 25°C. For kinetics experiments, the Bruker automation program "multi_zgvd" was employed to acquire 1D ^{13}C spectra (64 scans) every 3 min until the reaction was judged complete. The following parameters were used for 2D ^{1}H-^{13}C heteronuclear single quantum correlation (HSQC) experiment: number of scans, 1; number of complex data points (experiments) in F1, 148; number of complex data points in F2, 512; sweep width in F1 and F2, 165 and 16 ppm, respectively; spectrometer offset for ^{1}H and ^{13}C, 4.7 and 70 ppm, respectively; and interscan delay, 2 s. The following parameters were used for 2D ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC) experiment: number of scans, 2; number of complex data points (experiments) in F1, 128; number of complex data points in F2, 2048; sweep width in F1 and F2, 222 and 13 ppm, respectively; spectrometer offset for ^{1}H and ^{13}C, 6 and 100 ppm, respectively; interscan delay, 1.5 s. Data were processed with the software MestreNova (MestreLab Research S. L., Santiago de Compostela, Spain). For 2D, spectra were zero filled to 512 data points in the indirect dimension and multiplied by cosine-square apodization function in both dimensions prior to Fourier transform and phase correction.