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ENZYMATIC CATALYSIS IN A SUPERCRITICAL FLUID

T.W Randolph*, H.W. Blanch, J.M. Prausnitz, C.R. Wilke
Department of Chemical Engineering
College of Chemistry
University of California
Berkeley, California 94720, USA

ABSTRACT

The enzyme alkaline phosphatase, EC 3.1.3.1, was found to be active in a supercritical carbon dioxide solvent system. A batch reaction of disodium p-nitrophenyl phosphate with a 0.1 vol. % water solution in supercritical CO_2 at 100 atm and 35°C produced p-nitrophenol when catalyzed by alkaline phosphatase.

INTRODUCTION

A developing research area in enzymatic catalysis is the use of solvents other than pure water. Many enzymes show higher activity in mixtures of organic solvents with water than in pure water (Butler, 1979), and enzymes have even been shown to be active and stable in an environment composed of only alcohols (Zaks,1984).

Non-aqueous solvent systems for enzymic reactions are attractive for several reasons. In their native cellular microenvironment enzymes do not function in pure water, but in an environment composed of lipids, proteins, and various ionic species in addition to water. An enzyme in a non-aqueous solvent may experience solvent/enzyme interactions similar to those in its native environment, and thus show higher activity than in pure water. Also, substrates may be more soluble in a non-aqueous solvent, yielding a higher rate of reaction in the non-aqueous solvent. Other potential advantages include facilitated separations steps and enhanced enzyme specificity.

An interesting class of alternate solvents is supercritical fluids. Supercritical fluids are currently enjoying a surge of industrial interest, particularly in extraction processes. Commercial processes using supercritical carbon dioxide are now in operation for the extraction of caffeine from coffee (Kurzals, 1982) and for hops extraction (Gardner, 1982).

Supercritical fluids offer a number of advantages over conventional liquid solvents. Among these advantages are high diffusivities (one to two order of magnitude higher than liquid solvents), and low viscosities (about one order of magnitude lower than liquid solvents) (de Fillipi, 1982). Another advantage is the ability to manipulate solubilities by changing either temperature and/or

pressure. Of particular interest is the high sensitivity of solubility to changes in temperature and pressure. In the area of the critical point, small changes in these variables can sometimes result in 100-fold changes in solubility, greatly simplifying separations.

In particular, supercritical carbon dioxide is of special interest to the pharmaceutical and biochemical industries for several reasons. CO_2 has a critical temperature of 31.1°C, allowing heat labile organics to be processed without thermal denaturation or decomposition. Carbon dioxide is a very inert solvent, especially towards proteins, which are not solubilized. Its non-toxicity, non-flammability, availability, and low cost have made CO_2 the most popular supercritical fluid for investigation to date.

While the emphasis in research on supercritical fluids has centered on extraction, the possibility of carrying out chemical reactions in a supercritical solvent is intriguing. The combination of high diffusivities along with the ability to control solubilities by varying temperature and pressure could lead to faster rates of reaction and permit a simplified separations step. Little work has been done in this area, although one study reported the hydrolysis of biomass in a supercritical water-SO_2 mixture (Vick Roy, 1984).

EXPERIMENTAL

The experiment was conducted in the batch reactor shown in Fig. 2. In the stainless steel reaction vessel (200 ml volume) were placed 0.2 ml de-ionized water and 40 mg disodium p-nitrophenyl phosphate. 2.0 mg alkaline phosphatase (EC 3.1.3.1) were sealed inside a glass tube, and then placed inside the reaction vessel. Air was flushed out of the reaction vessel with several volumes of carbon dioxide. At a constant temperature of 35°C, carbon dioxide was pumped into the vessel until the pressure reached 100 atmospheres. At this time, the pump was shut off and the vessel sealed closed. To initiate the reaction, the vessel was shaken to shatter the glass vial containing the enzyme.

After reacting for a measured length of time, the reaction vessel was frozen in liquid nitrogen, stopping all reactions and solidifying the carbon dioxide. The vessel was then opened, and 40 ml 4N NaOH added. The reactor was next allowed to warm to room temperature, subliming the carbon dioxide. The remaining liquid was collected and analyzed for p-nitrophenol by light absorbance at 410 nm.

In control experiments, the above procedure was followed, except that no enzyme was added.

RESULTS AND CONCLUSIONS

In the control experiments, no reaction of the water and the disodium p-nitrophenyl phosphate in supercritical CO_2 could be detected after 24 hours at 100 atm. and 35°C.

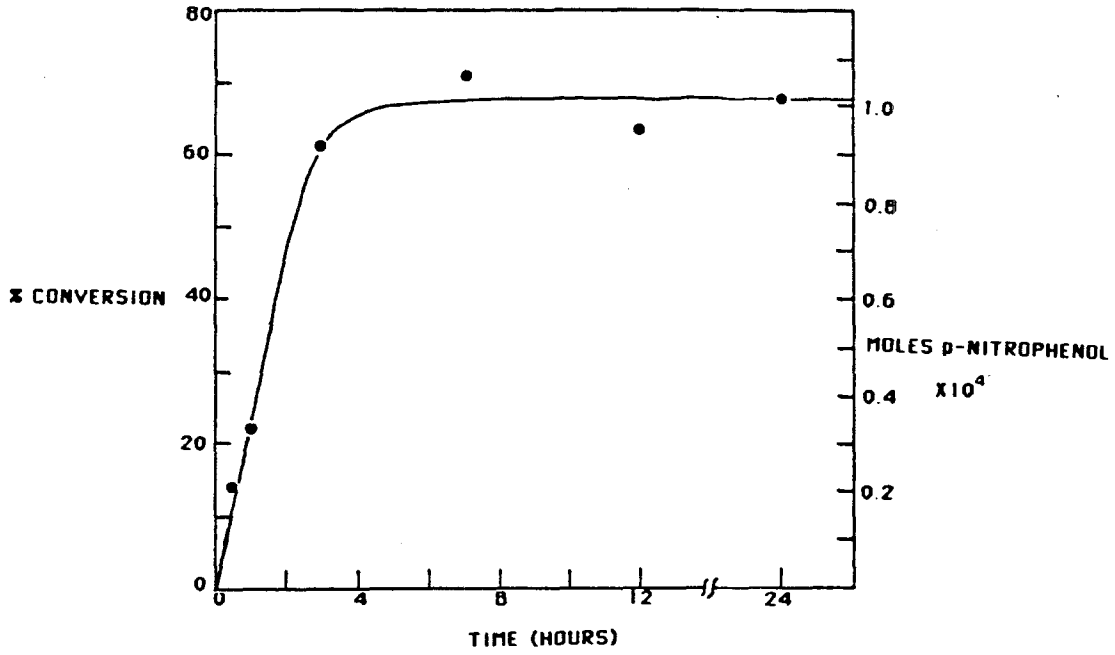

FIGURE 1. BATCH RESULTS FOR CONVERSION OF DISODIUM p-NITROPHENYL PHOSPHATE TO p-NITROPHENOL IN SUPERCRITICAL CARBON DIOXIDE @ 100 ATM., 35 °C

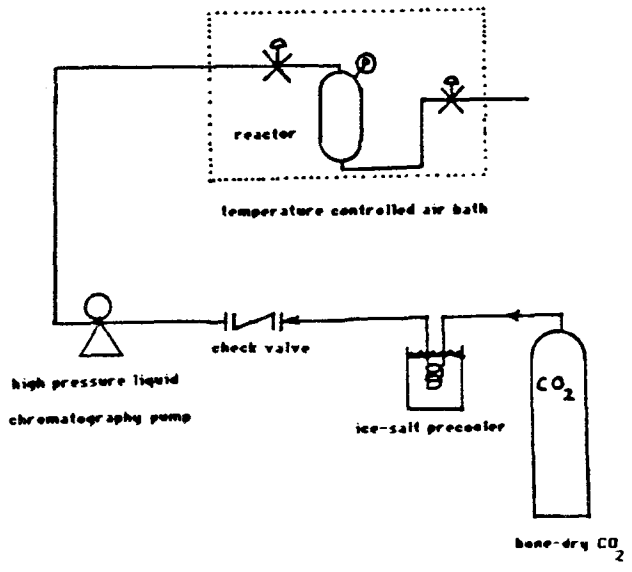

FIGURE 2. SCHEMATIC OF EXPERIMENTAL APPARATUS

When alkaline phosphatase was present in the reactor, formation of p-nitrophenol was detected in yields of up to 71%. Figure 1 shows the results of batch runs quenched at various times. Other experiments showed that, for batch reactions quenched after the same length of time, the amount of disodium p-nitrophenyl phosphate converted was insensitive to halving or doubling the amount of enzyme added. It is surmised that the reaction is limited not by the rate of reaction at the enzyme surface, but by the rate of dissolution of the disodium p-nitrophenyl phosphate into the CO_2 solution.

Additional experiments were conducted in which the enzymes, after exposure to supercritical CO_2 for various periods of time, were removed from the reactor without quenching them by adding NaOH. The enzymes were then tested for activity in aqueous solutions. Enzymes tested in this fashion showed activity in aqueous solutions after up to 24 hours of exposure to supercritical CO_2 at 100 atmospheres and 35°C.

REFERENCES

Butler, L,G., Enzyme Microb. Technol., vol. 1, pp.253-259, (1979).

de Fillipi, R., Chemistry and Industry, p. 390, June 19, 1982.

Gardner, D.S.J., "Industrial Scale Hops Extraction," presented at the Soc. Chem. Ind. Food Engineering Panel Symposium, CO_2 in Solvent Extraction, London, 1982.

Kurzals, H., "Caffeine Extraction," presented at the Soc. Chem. Ind. Food Engineering Panel Symposium, CO_2 in Solvent Extraction, London, 1982.

Vick Roy, J.R., and A.D. Converse, "Biomass Hydrolysis with Sulfur Dioxide and Water in the Region of the Critical Point," presented at the 1984 AIChE Annual Meeting, San Francisco, November, 1984.

Zaks, A., and A.M. Klibanov, Science, vol. 224, pp.1249-1251, June 15, 1984.

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