ENZYME ASSISTED FORMATION OF FATTY ACID HYDROPEROXIDE

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Introduction

Lipoxygenase (EC 1.13.11.12) catalyzes the dioxygenation of polyunsaturated fatty acids to form their corresponding hydroperoxides. Reduced derivatives of these have the potential to replace ricinoleic acid in industrial products. Currently ricinoleic acid is obtained from castor oil, a commodity that is imported into the U.S. at the level of thirty thousand metric tons per year (1). A method for immobilizing lipoxygenase was developed, and the use of this immobilized preparation to produce the hydroperoxide of linoleic acid (HPOD) in aqueous buffer-organic solvent mixtures was investigated.

Methods

Soybean lipoxygenase (Sigma, lipoxidase, type 1-B) was covalently immobilized on a commercially available carbonyl di-imidazole activated matrix (Reacti-Gel 6X, Pierce) (2). In a typical assay, 40 mg of linoleic acid was dissolved in 15.0 ml of water-saturated octane in a 125 ml glass-stoppered Erlenmeyer flask. After washing the immobilized lipoxygenase (IMM-LOX) with 0.2 M borate buffer (pH 9.0), 0.3 g of IMM-LOX containing 1.5 mg protein and 6 ml of 0.2 M borate buffer, pH 9.0, were added to the solution of linoleic acid. The reaction was allowed to proceed at 15°C while the mixture was agitated at 250 rev./min. After 1 h, the gel was filtered from the reaction mixture. The pH of the water layer was lowered to 3.0, and HPOD was extracted with diethylether. The amount of HPOD generated was determined with Xylenol Orange reagent (3). Reaction products were also examined with thin layer chromatography. Silica gel preadsorbent HPTLC-HL TLC plates (10 X 10 cm, coating thickness: 150 microns) were dipped in 5% boric acid in methanol and allowed to air dry prior to spotting. The TLC plates were developed sequentially in the following solvent systems: diethyl ether, benzene, ethanol, acetic acid (40:50:2:0.2; v/v/v/v); air drying; isooctance, diethyl ether, acetic acid (50:50:2; v/v/v) (4).

Results and Discussion

Reaction medium parameters were methodically varied to find those in which the greatest amount of HPOD was generated. It was found that a milieu containing water-saturated octane and 35% (v/v) 0.2 M borate buffer, pH 9.0, supported the maximal rate of HPOD generation. The rate of oxidation of LA catalyzed by IMM-LOX was determined when

different organic solvents were added to aqueous buffer (Figure 1). Water saturated solvents were used to ensure that the level of free water was constant

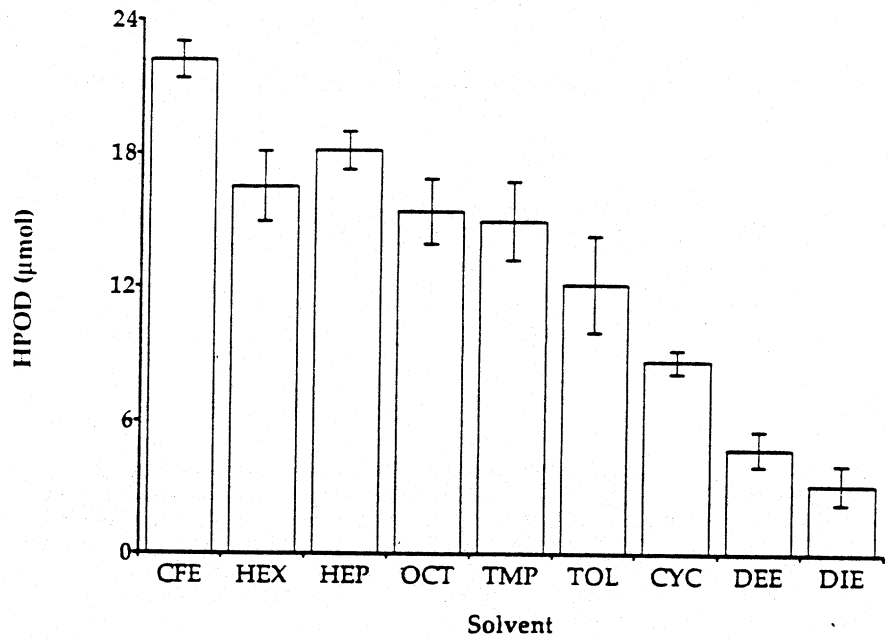

Figure 1. The influence of organic solvent upon HPOD formation catalyzed by IMM-LOX.

as the solvents were varied. The highest amounts of HPOD were obtained with 1,1,2-trichlorotrifluoroethane (CFE). Approximately equal amounts of HPOD were formed when the solvents hexane (HEX), heptane (HEP), octane (OCT) and 2,2,4-trimethylpentane (TMP) were added, but the amount of HPOD was slightly reduced from that formed in 1,1,2-trichlorotrifluoroethane. Use of toluene (TOL) and cyclohexane (CYC) resulted in a further decrease in the amount of HPOD formed. The lowest detectable levels of HPOD were found in reactions that contained diethyl ether (DEE) and diisopropyl ether (DIE). HPOD formation was not detected in reactions conducted with butanone or octanone. That the ethers and ketones inhibit the action of IMM-LOX more than the less polar solvents tested is consistent with other work that shows that generally polar organic solvents are detrimental to enzymatic activity.

The time course of HPOD formation was examined with nonlinear regression analysis over a 6 h period at 15°C using IMM-LOX that contained 3.0 mg protein. Curve fit estimates were obtained using Equation 1,

$$P_{obs} = \frac{P_{max} k_1^n t^n}{1 + k_1^n t^n} \tag{1}$$

where P_{obs} is the amount of product observed at time t. P_{max} is the maximum amount of product formed. k_1 is the rate of reaction at one-half P_{max}, and n is the cooperativity parameter that is constrained to integer values. The reciprocal of k_1 yields the time needed to generate one-half P_{max} and is therefore the reaction half time (5). That a cooperative model (n = 2) provided the best fit of the data was demonstrated by several observations. First, TLC analysis showed that a portion of LA was converted to byproducts as indicated by materials that had lower R_f values than HPOD. Thus a potential HPOD yield of about 100%, as was given when n was set equal to one, is not realistic, while when n was equal to two, the yield was 84%. Second, when the value of n was set equal to two, the calculated reaction half time, $t_{1/2}$, is 42 min which closely corresponds to that indicated by visual observation of TLC plates. Finally, when n was increased from one to two, the errors of the coefficients decreased.

Conclusion

HPOD yields of greater than 80% can be obtained in mixtures of aqueous buffer and organic solvent using IMM-LOX as the catalyst. This yield is as high as that obtained previously using pure oxygen, rather than air (6), although somewhat lower than that obtained in the presence of both organic solvent and surfactant (7). However, the present procedure avoids the difficult problem of separating the surfactant and HPOD.

References

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