IMMOBILIZATION OF LIPOXYGENASE IN AN ALGINATE-SILICATE SOLGEL MATRIX: FORMATION OF FATTY ACID HYDROPEROXIDES

1. Introduction

Lipoxygenase (EC1.13.11.12) (LOX) catalyzes the positionally specific dioxygenation of polyunsaturated fatty acids to their corresponding hydroperoxy derivatives. Reduction of the latter compounds gives the corresponding hydroxy acids which are potential substitutes for ricinoleic acid, an industrially important fatty acid. LOX has been conventionally immobilized by adsorption to glutenin, glass wool, talc, polymer beads and ion exchange supports or by covalent linkage to matrices such as CNBr-activated Sepharose, agarose, or oxirane acrylic beads (1,2) or a carbonyl diimidazole activated polymer (3,4). In this study, a novel method for immobilizing LOX entrapped in an alginate-silicate sol-gel matrix was developed. The stability and reusability of LOX immobilized by this improved method was investigated.

2. Materials and Methods

Sodium alginate (4%, w/v) in 0.2 M sodium borate buffer (pH 9.0) was mixed well with an equal volume of soybean LOX (5 mg/ml in 0.2 M sodium borate buffer, pH 9.0). Droplets of this mixture were dispensed through a pasteur pipette into a cold solution of 0.2 M $CaCl_2$ (100 ml) to form single beads. The beads were collected within 10 min. and transferred to a beaker containing enough hexane to cover the beads. Tetramethoxy-ortho-silicate (TMOS, 1-1.5 vol/vol of the beads) was added and the mixture was left overnight at room temperature to complete the polymerization process. The beads were left to dry overnight under vacuum at room temperature. The beads then were either soaked in 0.2 M borate buffer (sodium pH 9.0), 75 % glycerol in water, or borate buffer saturated with isooctane for 6 h. Finally, the beads were removed from the solution, air dried and assayed for lipoxygenase activity by measuring the hydroperoxide formation from linoleic acid described previously (5). The level of hydroperoxide was determined spectrophotometrically by xylenol orange method (6).

3. Results and Discussions

The formation of the product of the linoleic acid oxidation, 13-hydroperoxyoctadecadienoic acid (HPOD), was maximal at pH 9.0. The enzymatic activity of immobilized LOX in

calcium alginate beads was affected by drying treatment. Vacuum drying was most efficient in removing water from samples while retaining the highest LOX activity. The average entrapped activity relative to that of free LOX varied from 15 to 30%.

The enzymatic activity of entrapped LOX after immobilization could be enhanced by soaking the beads in 75% glycerol or in borate buffer or borate buffer saturated with isooctane. For long term storage, samples were soaked in 75% glycerol because samples that were not stored in glycerol lost more than 50% of their activity after one week at room temperature. The entrapped LOX was stable at room temperature with no loss in activity after 25 days of storage (Fig. 1). In contrast, free LOX in solution lost most of its activity after 24 hours at room temperature. Other studies on immobilized β-glucosidase in alginate silicate gel (7) also showed that the enzymatic activity was stable at ambient temperature for at least several months. In contrast, LOX covalent bound to a carbonyl-imidazole activated support (3) had a half-life of only 75 hours at 15°C. Other conventionally immobilized LOX preparations had half lives on the order of only several hours (1,2) at room temperature.

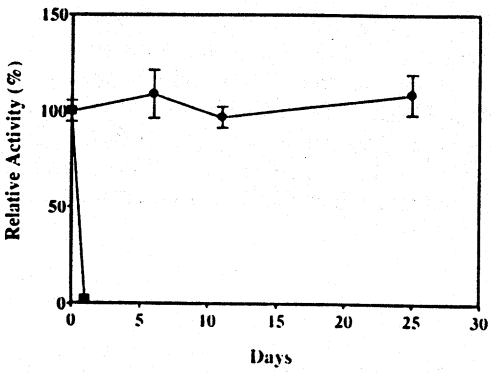

Fig. 1. Comparison of the immobilized (●—●) and free (■—■) lipoxygenase at room temperature. At the times indicated, the samples were assayed to measure the lipoxygenase. Zero day of free enzyme or immobilized lipoxygenase is expressed as 100%

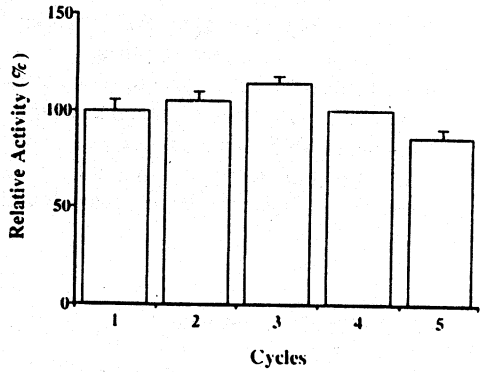

Fig. 2. Reusability of the immobilized lipoxygenase. The immobilized beads were incubated with linoleic acid for five cycles. The activity of lipoxygenare during the first cycle is expressed as 100%.

In our study, the reusability of LOX immobilized in calcium-alginate beads was determined by repeatedly using the same beads for five successive reactions with linoleic acid (LA) (Fig. 2). The LOX activity was measured after each cycle, and the beads were recovered and washed with sodium borate buffer before reuse. To initiate the next cycle of oxidation, LA was added to the incubation mixture containing the recovered beads. The data (Fig. 2) demonstrates that LOX immobilized in beads can be reused at least five times without substantial loss in enzyme activity. In contrast, free lipoxygenase is typically inactivated by hydroperoxide accumulation and the partial anaerobic condition that develops in the reaction mixture (8).

5. Conclusion

In this study, lipoxygenase was successfully entrapped by a novel immobilization method within a calcium-alginate silicate gel matrix. The entrapped lipoxygenase had stability better than LOX immobilized by other methods. The immobilized preparation also was reusable. This procedure should enhance the potential of LOX as a biocatalysts in the preparation of oxygenated fatty acid derivatives that have unique chemical and physical properties.

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