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Protection of enzymes from photodegradation by entrapment within alumina

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ABSTRACT

Most enzymes are highly sensitive to UV-light in all of its ranges and their activity can irreversibly drop even after a short time of exposure. Here we report a solution of this problem by using sol-gel matrices as effective protectors against this route of enzyme inactivation and denaturation. The concept presented here utilizes several modes of action: First, the entrapment within the rigid ceramic sol-gel matrix, inhibits denaturation motions, and the hydration shell around the entrapped protein provides extra protection. Second, the matrix itself – alumina in this report – absorbs UV light. And third, sol-gel materials have been shown to be quite universal in their ability to entrap small molecules, and so co-entrapment with well documented sun-screening molecules (2-hydroxybenzophenone, 2,2′-dihydroxybenzophenone, and 2,2′-dihydroxy-4-methoxybenzophenone) is an additional key protective tool. Three different enzymes as models were chosen for the experiments: carbonic anhydrase, acid phosphatase and horseradish peroxidase. All showed greatly enhanced UV (regions UV-A, UV-B, and UV-C) stabilization after entrapment within the doped sol-gel alumina matrices.

1. Introduction

Stabilization of enzymes by sol-gel entrapment methods is a well-developed field. Stabilization against temperature increase, denaturing pH values, and exposure to harsh chemicals is well documented [1–5]. To the best of our knowledge, the use of sol-gel matrices for protection against photodegradation has not been reported, despite the fact that most enzymes are highly sensitive to UV-light in all of its ranges. Here we report how to utilize sol-gel matrices as effective shields against this route of enzyme inactivation as well.

The main photodegradation of route of enzymes has been associated with the protein chromophores of tryptophan, tyrosine, histidine, phenylalanine, and cysteine, in the 200–400 nm region [6]. It is believed that this photodegradation is due to the formation of radicals, to photoionization, and to the cleavage of disulfide bonds. Some of these photoactivated species may also form destructive singlet oxygen [7]. Photodegradation is manifested in changes in the primary, secondary and tertiary protein structures, and in fragmentation and aggregation of proteins, all of which lead to loss of enzymatic activity [8]. Enzyme protection from UV light is a relatively unexplored filed, and some representatives studies are the use of sunscreens and antioxidants such as green tea flavonoids, melatonin, Trolox, glutathione, threo-1,4-dimercapto-2,3-butanediol, and ascorbic acid [9–15].

The photo-protection concept presented here utilizes several modes of action: First, the entrapment within the rigid ceramic sol-gel matrix, inhibits denaturation motions, and the hydration shell around the entrapped protein provides extra protection [16]. Second, the matrix itself – mainly alumina in this report – absorbs UV light. And third, as sol-gel materials have been shown to be quite universal in their ability to entrap small molecules, co-entrapment with well documented sun-screening molecules is an additional key protective tool. We recall that sunscreens operate by an efficient mechanism of UV energy dissipation, the excited states of which are not involved in photochemical reactions. Previously [17–19], some sol-gel composites with sunscreen molecules have been successfully prepared and even commercial product – UV-Pearls® [20] are already available on the market. The sunscreens selected for this study are of the benzophenone family, 2-hydroxybenzophenone, 2,2′-dihydroxybenzophenone, and 2,2′-dihydroxy-4-methoxybenzophenone, and were used for protection of three enzymatic models – carbonic anhydrase, acid phosphatase,

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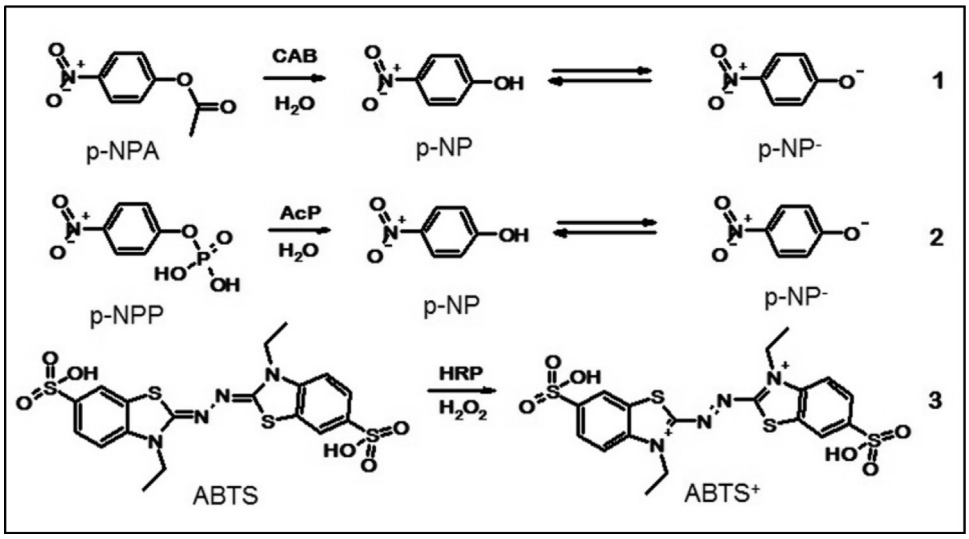

Fig. 1. The enzyme-catalyzed reactions used in this study.

and horseradish peroxidase, all at three UV radiation ranges: UV-A (320–400 nm), UV-B (280–320 nm) and UV-C (200–280 nm). The entrapment of these enzymes within alumina and ferria (magnetite) sol-gel matrices has been the subject of earlier studies [16,23,24], where it was found that the entrapment induces major thermal stability, while reducing the reaction rates due to the porosity of the matrix.

2. Experimental details

2.1. Chemicals

Aluminum isopropoxide ≥99%, iron(II) chloride tetrahydrate ≥98.5%, iron(III) chloride hexahydrate ≥99%, carbonic anhydrase from bovine erythrocytes (CAB), acid phosphatase from potato (AcP), horseradish peroxidase (HRP), aqueous solution of ammonia ≥25%, *p*-nitrophenylphosphate (pNPP), *p*-nitrophenyl acetate (pNPA), acetone ≥99.6%, 2-hydroxybenzophenone (2-HBP), 2,2′-dihydroxybenzophenone (2,2′-DHBP), 2,2′-dihydroxy-4-methoxybenzophenone (2,2′-DH-4-MBP), methanol 100%, hydrogen peroxide 30%, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), glycine ≥99.5%, tris-buffer were all obtained from Sigma-Aldrich.

2.2. Sol-gel synthesis of alumina and ferria (magnetite) sols

2.2.1. Alumina

3.3 g (16.1 mmol) of aluminum isopropoxide was hydrolyzed in 50 mL deionized water pre-heated to 85 °C under constant stirring (400 rpm) for 15 min. Then, the resulting suspension was subjected to ultrasonic treatment (37 kHz, 110 W) under constant stirring (300 rpm) for 120 min [21].

2.2.2. Ferria

For preparation of magnetite ferria (Fe_3O_4) sol, 2.5 g $FeCl_2{\cdot}4H_2O$ (12.6 mmol) and 5 g $FeCl_3{\cdot}6H_2O$ (18.5 mmol) were dissolved in 100 mL of deionized water under constant stirring (500 rpm). Then, 12 mL of 7.1 M aqueous ammonia solution was added drop-wise to this solution under constant stirring (500 rpm) at room temperature for 5 min. The washed black precipitate was mixed with 100 mL of deionized water and subjected to ultrasonic treatment (37 kHz, 110 W) under constant stirring (300 rpm) for 75 min [22,23].

2.3. Entrapping the enzymes within sol-gel alumina

Entrapment of the enzymes was carried out as follows [24]: For the entrapment of CAB, 200 μL of 0.3 M freshly prepared alumina

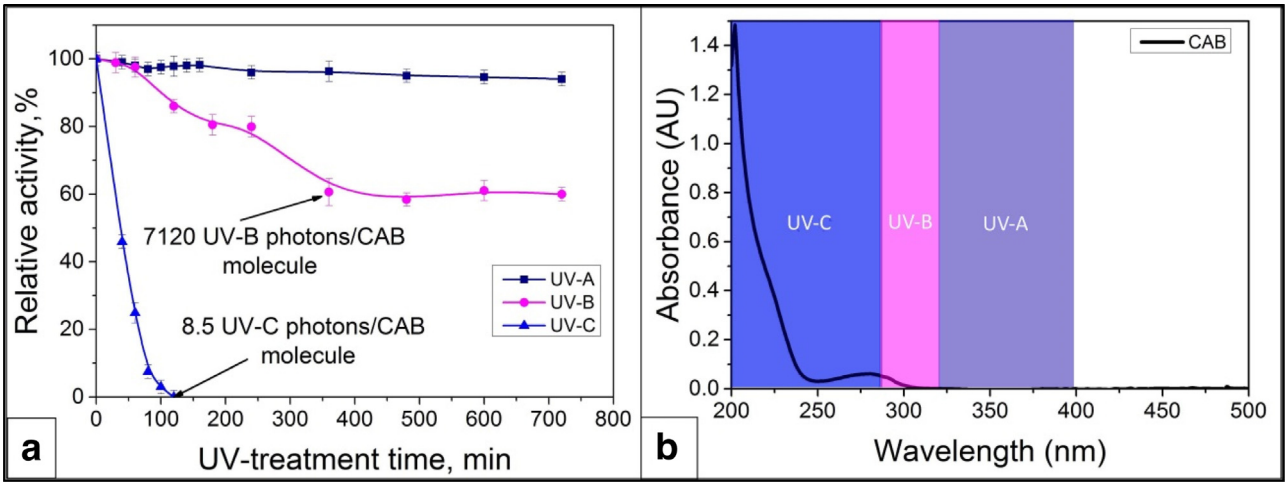

Fig. 2. (a) Relative enzymatic activity of CAB as a function of radiation time for all UV ranges. (b) The absorption spectrum of CAB in relation to the three UV-ranges.