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Spontaneous deposition of horseradish peroxidase into polyelectrolyte multilayer capsules to improve its activity and stability

Changyou Gao,*[a] Xingyu Liu,[a] Jiacong Shen[a] and Helmuth Möhwald[b]

[a] *Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China. E-mail: cygao@mail.hz.zj.cn; Fax: +86-571-87951948; Tel: +86-571-87951108*

[b] *Max-Planck-Institute of Colloids and Interfaces, D-14424 Golm/Potsdam, Germany*

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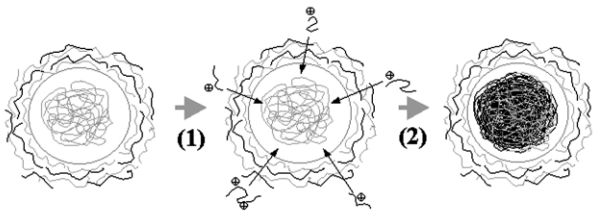
Horseradish peroxidase (HRP) was encapsulated in pre-formed polyelectrolyte multilayer (PEM) microcapsules by spontaneous deposition with remarkably improved stability and catalytic activity.

In recent years, polyelectrolyte multilayer nano- and micro-capsules prepared by step-wise adsorption of oppositely charged polyelectrolytes onto various colloidal templates have received considerable attention because of their high scientific interest and technological importance in the fields of drug delivery, microreactors as well as bioseparation.[1] However, many of these technological applications are closely associated with the problem of how to encapsulate desired substances such as biomacromolecules into capsules without losing their biological activity during or after the encapsulation process. Materials such as enzymes or drugs can be made into a form of core template or be attached to core template particles prior to core decomposition so as to trap them inside hollow capsules.[2–5] Attempts were made to load macromolecules into pre-formed capsules, using *in situ* polymerization of monomers inside the capsules *via* a 'ship in bottle' synthesis[6] or by changing the permeability of the capsule wall by altering the bulk pH.[7] The recent novel finding that various water soluble substances can spontaneously deposit into the interior of pre-formed capsules templated on melamine formaldehyde (MF) colloidal particles inspired a more convenient and effective way to incorporate bioactive macromolecules.[8] This communication reports the results of HRP deposition into poly(styrene sulfonate, sodium salt)(PSS)/poly(diallyldimethylammonium) chloride (PDADMAC)$_5$ capsules and its stability and catalytic activity by comparing with free HRP in solution.

A brief procedure for the preparation of HRP-deposited microcapsules is shown in Scheme 1. According to this spontaneous deposition mechanism, the existence of the negatively charged PSS/MF complex in the capsule interior is thought to be the driving force inducing the spontaneous deposition of HRP (for details see Ref. 8). Equal amounts of a (PSS/PDADMAC)$_5$ capsule suspension (~1million capsules mL^{-1}, intact capsule ratio >90%, for fabrication see Ref. 9) and HRP solution (1mg mL^{-1}, pH = 4.5) were mixed and incubated for 1.5 h at room temperature. The suspension was then rinsed with Millipore water 5 times by using a membrane filtration technique until no enzyme was detected in the filtered solution.[10] It is worth noting that the pH value of the suspension should be adjusted lower than the isoelectric point of HRP (pI = 9), for only in that condition can HRP be effectively deposited.[8]

Confocal laser scanning microscopy (CLSM, Bio-Rad Radiance 2100) has directly verified the occurrence of spontaneous deposition of HRP (labelled with rhodamine B isothiocyanate) as shown in Fig. 1a. The fluorescence intensity, which is proportional to the HRP concentration, in the capsule interior is much stronger than in the bulk. Scanning force microscopy (SFM, tapping mode, Seiko SPI3800N) in the dry state further confirmed the observation of CLSM in the wet state. Through comparison of the top view (Fig. 1b, 1c) and the 3-D view (Fig.1, insets) of the control and the HRP deposited capsules, one can calculate that the average height of the collapsed microcapsules increased from ~40 nm to ~200 nm. It has to be pointed out that the contribution of the simply adsorbed HRP on the capsule wall should be less than 10 nm for an enzyme double layer. So there is over a 100 nm increase of average height caused by the HRP deposition in capsules. In both the SFM and the CLSM observations, there exist some capsules that lack this spontaneous deposition phenomenon (Fig.1a arrow). This is because of the release of the PSS/MF



Scheme 1 The procedure for the preparation of enzyme-deposited PEM microcapsules. (1) PSS/PDADMAC microcapsule after MF particle decomposition. The inner PSS layer releases from the multilayers forming a negatively charged complex with MF during the core removal process. The PSS/MF complex is confined inside the intact capsules due to its larger size. (2) Enzyme with positive charge in low pH deposits into the PSS/MF complex.

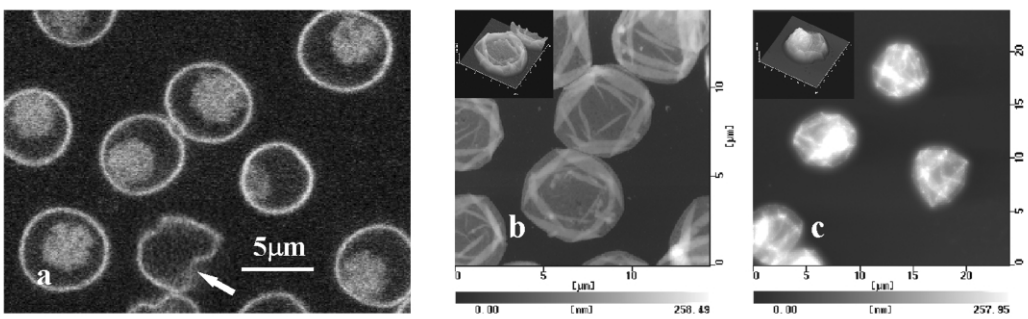


Fig. 1 (a) CLSM image of polyelectrolyte multilayer microcapsules after spontaneous deposition of HRP labelled with rhodamine B isothiocyanate. SFM images of polyelectrolyte multilayer microcapsules (b) before and (c) after HRP deposition. A typical 3-D view is shown in the insets. The arrow shows a broken capsule without spontaneous deposition.

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complex which is crucial for the spontaneous deposition from the broken capsule interior during the core decomposition process.[8] Suppose that each microcapsule adsorbed an HRP monolayer with a thickness of about 5 nm, then the total amount HRP adsorbed on the surface of the capsules (M_{ads}) can be evaluated ($\sim$1 million capsules 1mL^{-1}, capsule diameter 5.6 μm). Since the total amount of active HRP loaded within the capsules (M_{tot}) can be determined from an HRP standard curve (for activity test see Ref. 11, using 2,2′-azino-bis-ethylbenzthiazoline-6-sulfonic acid (ABTS) as substrate, data not shown), the radio of HRP encapsulated in the capsules ($M_{encap} = M_{tot} - M_{ads}$) to that absorbed on the capsule surface (M_{ads}) can be estimated as about 20 : 1, which indicates that more than 90% was deposited into the interior of the PEM microcapsules and only a small amount of HRP was absorbed on the capsule surface. It is noteworthy that the amount of HRP deposition strongly depends on the age of the capsule. Freshly prepared capsules (< 1month) are not favourable for this spontaneous phenomenon.

The practical application of these kinds of enzyme-containing microcapsules relies on their stability and activity under various conditions. In the experiments on enzyme stability, both the free (a) and the deposited HRP (b) decreased their relative activity with temperature increase as shown in Fig. 2. However, the activity-losing rate of the deposited HRP was obviously slower than that of the control. A temperature independent regime was observed between the 30–60 °C region for deposited HRP while a sharp reduction for free HRP in this region occurred. At 60 °C when deposited HRP still retains 71% of its initial catalytic activity, more than 70% of its initial activity was lost for free HRP. This improvement of the catalytic stability of the deposited HRP was also observed in a similar experiment on measuring the activity at 47 °C. Free HRP in solution lost half of its activity after 3 h while deposited HRP still retained above 90% of its activity (Fig. 3). It is believed that the organic solvents strip water from the enzymes,

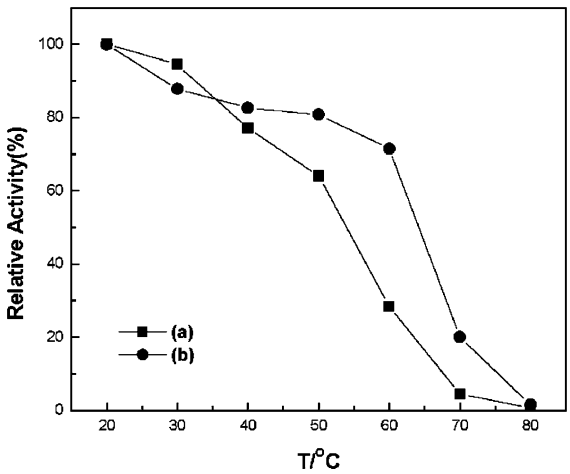

Fig. 2 The relative activity of (a) free (b) deposited HRP as a function of temperature. Relative activity is defined as the ratio of retained activity to starting activity of HRP. Incubation time for each sample is 15 min.

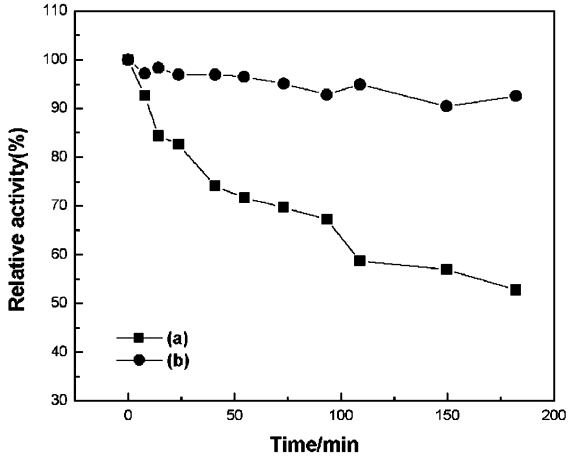

Fig. 3 Stability of (a) free and (b) deposited HRP in water at 47 °C.

leading to the unfolding of the enzyme with exposure of the inner hydrophobic residues and that this denaturation occurs at a much faster rate than in a pure aqueous system.[12] In a test of HRP activity in 20% dioxane aqueous solution, deposited HRP also exhibited an enhanced resistance to denaturation (data not shown).

In conclusion, HRP has been deposited in pre-formed polyelectrolyte microcapsules with significant improvement of stability and catalytic activity. Encapsulation of other biomacromolecules into PEM microcapsules by similar procedures can be expected to have widespread applications. The possibility of spontaneous deposition while retaining (bio)activity opens a new gate to make use of PEM microcapsules in various fields.

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