JOURNAL OF THE CHEMICAL SOCIETY

Chemical Communications

Number 9
1986

Initiation–Cessation Control of an Enzyme Reaction using pH-Sensitive Poly(styrene) Microcapsules with a Surface-coating of Poly(iminoethylene)

Etsuo Kokufuta,* Touru Sodeyama, and Takeshi Katano

Institute of Applied Biochemistry, University of Tsukuba, Sakura-mura, Niihari-gun, Ibaraki 305, Japan

Hydrolysis of maltotriose in an aqueous suspension of poly(iminoethylene)-coated poly(styrene) microcapsules containing β-amylase is initiated at pH 5.5 and stopped at pH 6.5; the initiation and cessation can be repeated because of a pH-induced reversible alteration in the permeability of the capsules by the substrate.

Since enzyme-loaded microcapsules were prepared by Chang,[1] many attempts have been undertaken to encapsulate aqueous solutions of enzyme within polymer membranes. Recently, Okahata *et al.*[2] have demonstrated several methods for regulating permeation through capsule membranes of water-soluble substances by outside stimuli such as temperature, pH, light, and so forth. This prompted us to study a new type of microencapsulated enzyme in which capsules serve as a 'cell' having the ability to control enzymatic processes by external stimulation.

In order to use microcapsules for regulating enzyme reactions, it is necessary to control their permeability by substrates using an appropriate externally applied signal which has no influence on the activity of the encapsulated enzyme. Here we report that an aqueous suspension of β-amylase-loaded poly(styrene) (PSt) microcapsules, surface-coated with poly(iminoethylene) (PIE), can be used to control reversibly the initiation–cessation of enzymatic hydrolysis of maltotriose by changing the ambient pH. This is because the permeability of the capsules is dramatically altered in response to small differences in pH (±0.5 units).

Stable PSt microcapsules (mean diameter 9.74 μm), as semipermeable membranes, were prepared by depositing the polymer around emulsified aqueous droplets *via* the following three procedures; (i) primary emulsification of an aqueous solution (20 ml) of 1% sodium dodecylbenzenesulphonate (SDS; as emulsifier) in dichloromethane (40 ml) containing 4 g PSt (weight-average molecular weight 1.76×10^5 units) with a homoblender for 5 min, (ii) secondary emulsification of the obtained water/organic-type emulsion in an aqueous 1% SDS solution (600 ml) under vigorous agitation for 10 min, and (iii) complete removal of dichloromethane from the resulting (water/organic)/water-type complex emulsion according to the literature.[3] To encapsulate the enzyme, the primary emulsification was carried out on 20 ml of 0.1 M phosphate buffer containing both 20 mg sweet potato β-amylase (Sigma) and 200 mg Triton X-100 (as emulsifier and also as stabilizer[4] for the enzyme). The prepared microcapsules were centrifuged, thoroughly washed with distilled water, and then subjected to the polymer coating procedure, in which the capsules were stirred in 0.1 M phosphate buffer (pH 8; 100 ml) containing 1 g of fractionated PIE[5] (branching type having primary, secondary, and tertiary nitrogens in the ratio 1 : 2 : 1; weight-average molecular weight 1.10×10^5 units) at room temperature for 10 h. The polymer-coated capsules were recovered by centrifugation and purified by repeated washing with 0.1 M phosphate buffer (pH 8) and 0.1 M acetate buffer (pH 4) until no PIE was detected in the washing extracts as determined by colloid titration.[5] The amount of polymer coating was 23.3 μg/cm^2, as estimated from the amount of PIE remaining in the supernatant liquid.

The pH-dependence of the permeation constant (P) for n-propyl alcohol (PA) was first investigated to determine the pH range which caused a change in the permeability of the PIE-coated capsule membrane. The P value was calculated from studies of the time-dependent decrease in PA after quick mixing of 8% v/v aqueous PA (20 ml) and 90% v/v capsule suspension (20 ml), which were previously kept at the same temperature (25 °C) and adjusted to the same pH (3—8) with 0.1 M acetate or phosphate buffer. The concentration of PA was determined by h.p.l.c. with 20 μl of each sample (0.1 ml) separated through a 0.1 μm filter at suitable time intervals. Figure 1(a) shows the curves of P *vs.* pH for PIE-coated and uncoated PSt microcapsules. In the case of the uncoated, original capsule membrane, P is independent of pH and remains constant ($3.14 \pm 0.10 \times 10^{-5}$ cm s^{-1}). In the polymer-coated capsules, however, a remarkable difference between the P values at pH $\leqq 5.5$ ($1.47 \pm 0.3 \times 10^{-5}$ cm s^{-1}) and at pH $\geqq 6.5$ ($5.0 \pm 2.0 \times 10^{-7}$ cm s^{-1}) is observed. The pH range that causes a pronounced change in the permeability is very narrow compared to that (pH 2 to 8 or *vice versa*) of pH-sensitive capsules already reported.[2] This is very desirable for the regulation of β-amylase-catalysed hydrolysis, since its starch-hydrolysing activity[6] is kept approximately constant in the pH range where the permeability is controllable.

Included in Figure 1 (b and c) for comparison are the previously obtained results[7] of viscometric and electrophoretic measurements for PIE. A rapid increase in the viscosity with decreasing pH could indicate a conformational transition of PIE from a tightly coiled chain (pH $\geqq 6.5$) to an

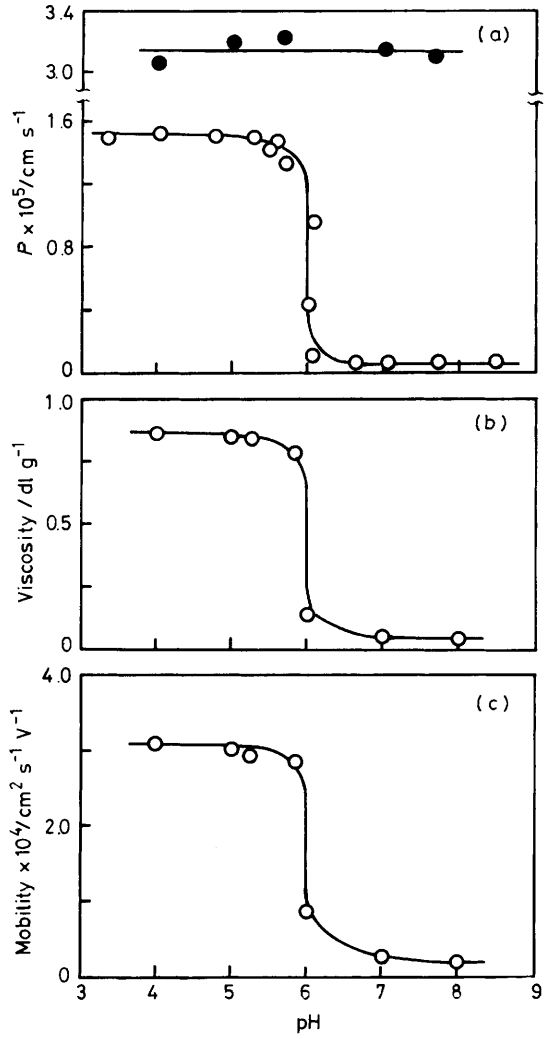
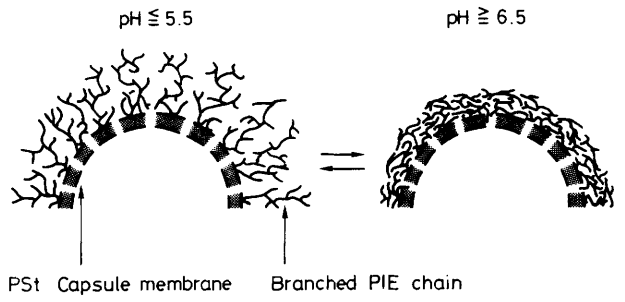


Figure 1. Dependence of P on pH (a) for PSt microcapsules coated with PIE (○) and uncoated (●), and the comparisons of the pH curves with those[7] of intrinsic viscosity (b) and limiting mobility (c) for PIE. The viscosity and mobility were measured at 25 °C using phosphate or acetate buffer adjusted to ionic strength 0.1.

extended one (pH ≦ 5.5), which takes place *via* a quick change of the polyion charges as characterized by the mobility curve. Since pH-induced alteration of the configuration of PIE on the membrane surface seems to be analogous to that in the conformation in aqueous medium, the polymer coating would seem to be present in a flat layer at pH ≧ 6.5 and in a looped or extended layer at pH ≦ 5.5 (see Scheme 1). This could function as a kind of permeation valve to reduce and to enhance the permeability towards PA as required. This can be supported by the fact that the pattern of the P *vs.* pH curve for the PIE-coated capsules shows a striking resemblance to those of the viscosity and mobility curves.

Figure 2 shows typical time courses of maltose formation *via* the enzyme-catalysed hydrolytic degradation† of maltotriose at pHs 5.5 and 6.5, using the PIE-coated or uncoated capsules into which β-amylase was loaded. The maltose concentration was measured by h.p.l.c. analysis, after mixing a substrate solution (100 ml) containing 8 mmol maltotriose with an aqueous 20% v/v suspension (100 ml) of the capsules including 17.8 mg of the enzyme. Utilizing the uncoated capsules, the encapsulated enzyme catalyses the reaction not only at pH 5.5

† Glucose, in amounts equimolar to maltose, was also obtained.



Scheme 1

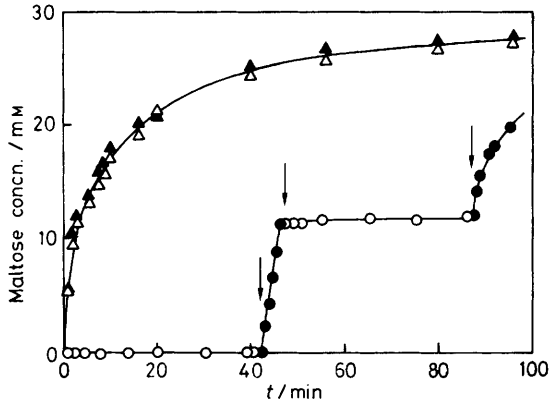


Figure 2. Formations of maltose from maltotriose in an aqueous suspension of PIE-coated (○, ●) or uncoated (△, ▲) PSt microcapsules containing β-amylase at pHs 6.5 (○, △) and 5.5 (●, ▲). The arrows show the pH control, which was made by quick addition of a small amount of 2 M HCl or NaOH into the capsule suspension adjusted to ionic strength 0.1 with phosphate salts and thermostated at 37 °C.

but also at pH 6.5, to form maltose in the same yield. In contrast, when the PIE-coated capsules were employed at pH 6.5, the catalytic action of the loaded enzyme is almost or entirely depressed (maltose concentration < 0.005 mM), but the maltose formation is initiated by adjusting the pH of the outer medium to 5.5. Such initiation–cessation control could be repeated reversibly throughout a single run of the measurement, and also reproduced over at least 10 runs which were carried out with a freshly prepared substrate solution. The relative activity of the enzyme loaded into the PIE-coated capsules was *ca.* 95% of that in the uncoated capsules, as estimated from comparing the initial rates of maltose formation with both encapsulated enzymes.

In addition, PIE-coated PSt microcapsules were found to be useful for control of trypsin-catalysed hydrolyses of oligopeptides.

Received, 12th November 1985; Com. 1599

References

1 T. M. S. Chang, *Science*, 1964, **146**, 524.
2 Y. Okahata, K. Ozaki, and T. Seki, *J. Chem. Soc., Chem. Commun.*, 1984, 519, and references therein.
3 M. Kitajima and A. Kondo, *Bull. Chem. Soc. Jpn.*, 1971, **44**, 3201.
4 Y. Takeda and S. Hizukuri, *Biochim. Biophys. Acta*, 1972, **268**, 175.
5 E. Kokufuta, *Macromolecules*, 1979, **12**, 350.
6 J. A. Thoma, D. E. Koshland, Jr., R. Shinke, and J. Ruscica, *Biochemistry*, 1965, **4**, 714.
7 E. Kokufuta, M. Hirata, and S. Iwai, *Kobunshi Ronbunshu (Jpn. Ed.)*, 1974, **31**, 234; *ibid. (Engl. Ed)*, 1974, **3**, 1383.