Poly(vinyl methyl ether) Gel for the Construction of a Thermosensitive Immobilised Enzyme System Exhibiting Controllable Reaction Initiation and Termination

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In an aqueous solution with a poly(vinyl methyl ether) gel containing immobilised *exo*-1,4-α-D-glucosidase, the hydrolysis of maltose proceeds at temperatures below 37 °C and stops above this temperature; the initiation and termination of this enzymatic process can be repeated by a rapid temperature-switching, *e.g.*, from 32 to 42 °C and back to 32 °C, because of a thermally induced reversible swelling–collapsing change of the gel support.

Evidence has accrued which indicates that the volume of gels results from a balance between the repulsion and attraction of the crosslinked polymer chains in their networks, which arise from a combination of four intermolecular forces: ionic, hydrophobic, van der Waals and hydrogen bonding.[1] Hence, external factors affecting these intermolecular forces, such as temperature, pH and solvent composition, will cause changes in gel volume. In particular, temperature-induced volume changes have attracted much attention in connection with the development of thermosensitive immobilised biocatalysts and drug delivery devices. For example, Hoffman *et al.*[2] immobilised enzymes or microorganisms within a thermosensitive copolymer hydrogel consisting of *N*-isopropylacrylamide (NIPAAm) and acrylamide, and showed that thermally induced swelling and shrinking changes in the gel volume have a marked influence on the activity of immobilised biocatalysts due to an alteration in mass transfer rates within the gel support. Bae *et al.*[3] also reported that the release of indomethacin from NIPAAm gel can be regulated with considerable precision by changing the ambient temperature. In such biotechnological or biomedical fields, however, the thermosensitive gels previously employed were limited to the polymers of acrylamide or its alkyl derivatives, substances the toxicity of which has been pointed out in several studies.[4]

Poly(vinyl methyl ether), PVME, is known to be highly soluble in water and to be transformed into a polymer gel with γ-ray irradiation in aqueous solution.[5] The resultant gel exhibits a thermosensitive characteristic: it shrinks above 38 °C and swells below this phase transition temperature (T_c). These facts prompted us to study a novel and promising

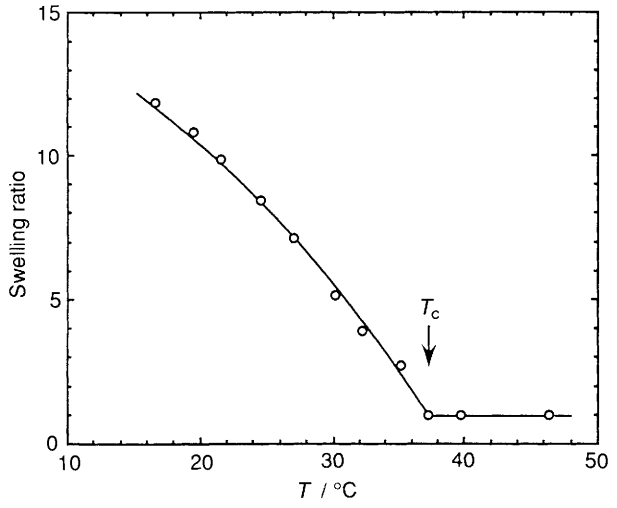

Fig. 1 Swelling ratio of PVME gel as a function of temperature. Data were normalised by dividing the gel volume at an arbitrary temperature by the volume of the completely collapsed gel at temperatures above 37 °C.

application of PVME gel in the preparation of a functional immobilised enzyme whose catalytic action starts and stops in response to alterations in temperature.

A sheet gel was prepared by pouring a well degassed 30% (w/v) aqueous solution of PVME (Tokyo Kasei Kogyo Co. Ltd.) into the space (0.8 mm) between two glass plates and then irradiating with γ-rays (11 kGy h^{-1}) from ^{60}Co for 10 h at 23 °C. The gel obtained was washed thoroughly with distilled water, and its swelling ratio and permeability were measured. The swelling ratio was determined by measuring the length of one side of a rectangular section (2 mm × 2 mm × 0.8 mm) cut from the sheet. The measurements were made in distilled water at different temperatures using a microscope. The permeation experiments were carried out at 32 and 42 °C by monitoring the diffusion of glucose through the sheet gel (effectve area = 4.7cm^2) from one side to the other of a permeation cell. The glucose concentration was measured by the mutarotase–glucose oxidase method.[6]

The enzyme immobilisation was carried out in the same manner as used in the sheet-gel preparation, except for the use of a PVME solution containing 0.2% (w/v) of *Aspergillus niger exo*-1,4-α-D-glucosidase (EC 3.2.1.3; Boehringer-Mannheim Ltd). The immobilised preparation was cut with a slicer into cubic segments of fixed size (each side *ca.* 0.8 mm) and purified by repeating the swelling and shrinking procedures several times in a citrate buffer (50 mmol dm^{-3}; pH 5.0). The activities of the native and gel-entrapped enzymes were assayed with stirring in the same buffer solution which included 1% (w/v) of maltose as the substrate. The concentration of glucose formed was measured as described above.

Fig. 1 shows the temperature dependence of the swelling ratio. The gel swells and expands below 37 °C, while above this temperature it shrinks. This behaviour is reversible. The T_c of PVME gel obtained here was thus estimated to be 37 °C. The permeability of the gel for glucose dramatically changed below and above the T_c: 2.0 × 10^{-6} cm^2 s^{-1} at 32 °C and 7.3 × 10^{-11} cm^2 s^{-1} at 42 °C. These features of PVME gel indicate that it might be beneficially applied to the construction of an immobilised enzyme system with the function of controlling enzymatic reactions.

The temperature–activity profile was studied since enzyme activity is generally known to vary with temperature. As shown in Fig. 2(*a*), the rate of glucose formation from maltose by the native enzyme increases monotonically with the rise in temperature. In contrast, the catalytic action of the gel-entrapped enzyme is almost or entirely depressed at temperatures above T_c, but a decrease in the temperature from 37 to

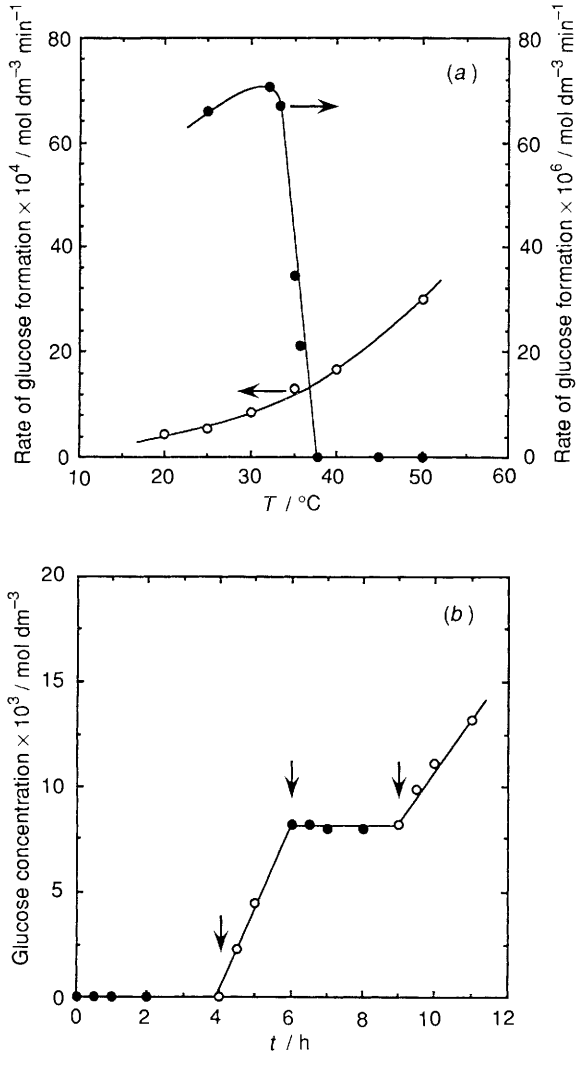

Fig. 2 (*a*) Effect of temperature on the activities of the native and immobilised enzymes and (*b*) the temperature-responsible initiation–termination control of the glucose formation by the immobilised enzyme. The activities of the native (○) and immobilised enzymes (●) in (*a*) are expressed in terms of the formation rate of glucose form maltose. As shown in (*b*), the enzymatic reaction was depressed at 42 °C (●) but commenced as the temperature changed to 32 °C within 2 min (○). The immobilised enzyme activity can be switched on and off repeatedly by rapidly decreasing the temeprature from 42 to 32 °C and rapidly increasing it from 32 to 42 °C at the time indicated by the arrows. The average rate of glucose formation in the two 'on' states during a single run at 32 °C changed from 58 ± 8 mol dm^{-3} min^{-1} (initial run) to 45 ± 8 mol dm^{-3} min^{-1} (final run) when the experiment was repeated in a series of 20 runs [(*b*) shows results of the initial run].

30 °C brings about a rapid restoration of activity. This difference in the immobilised enzyme activities below and above T_c is due largely to the change in substrate diffusion within the gel phase caused by the temperature-dependent swelling characteristic of PVME gel (see above).

The activity of the gel-entrapped enzyme is found to be about 20% of the native enzyme activity when the initial hydrolysing rates of both enzymes at 25 °C are compared in Fig. 2(*a*). This seems to be related to (*i*) the denaturation of the enzyme molecules during the immobilisation and (*ii*) the increasing mass transfer resistance within the gel matrix even when it is in a swollen state. However, the immobilised preparation obtained here displayed an excellent capacity for the initiation–termination control of the enzymatic hydrolysis of maltose [see Fig. 2(*b*)]. When the immobilised enzyme was utilized, glucose formation from maltose halted at 42 °C but

immediately recommenced when the temperature was lowered to 32 °C. Such initiation–termination control could be repeated reversibly throughout a single run of the measurements, and also reproduced without a serious loss in activity for at least 20 runs carried out with a freshly prepared substrate solution.

Enzyme immobilisation with thermosensitive PVME gel does not require a complicated procedure, and the application of this immobilisation method to trypsin also makes possible the initiation–termination control of enzymatic hydrolyses of oligopeptides. PVME was believed to be a harmless chemical up to this time,[7] although more detailed and strict examinations for toxicity to the human body are required. Therefore, it is suggested that PVME gel-entrapped enzymes have the potential for use in the biotechnological or biomedical fields.

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