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IMMOBILIZATION OF ENZYMES WITH USE OF PHOTOSENSITIVE POLYMERS BEARING STILBAZOLIUM GROUPS

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Reaction of poly[(2-chloroethyl vinyl ether)-co-(N-vinylpyrrolidone)] with γ-stilbazole afforded water-soluble photosensitive polymer, in which enzymes were entrapped on irradiation. The activities of entrapped invertase, glucoamylase and catalase were 30, 60, and 35%, respectively.

Among a number of methods to immobilize enzymes,[1)] entrapping methods are considered superior, since enzyme molecules suffer no chemical reactions which are unfavorable for sustaining enzymic activities. In this respect, photo-initiated entrapping methods have acquired increasing interest because of their mild reaction conditions. The most commonly used material is photochemically crosslinked polyacrylamide, while recent papers describe the use of polyethylene glycol dimethacrylate[2)] and bis-azide type crosslinking agent.[3)] The crosslinking of these types of materials, though photo-initiated, involves such highly reactive and non-specific species as free radicals and nitrenes. The denaturation of bioactive materials can not be completely eliminated because the entrapping matrices bind the biocatalyst covalently. Photocrosslinking of the polymer having γ-stilbazolium (SbQ) groups[4)] takes place highly specifically because the reaction is cyclodimerization.[5)] The application of this polymer to the entrapment of enzymes is reported here.

Polymers having SbQ groups are known to be readily insolubilized by irradiating with light of wavelength shorter than ca. 600 nm.[4)] Since the polymers prepared so far are often scarcely soluble in water, initial efforts have been put on the synthesis of water-soluble polymer. The reaction of cationically polymerized 2-chloroethyl vinyl ether (CV) ($\eta_{sp}/C=0.40$ in $CHCl_3$) with a slight excess of trans-γ-stilbazole (Sb) in 2-ethoxyethyl alcohol (EEA) gave quarternized polymers with good photosensitivity but with poor water-solubility (Table 1). To improve the water-solubility, CV was copolymerized radically with N-vinylpyrrolidone (VP). The copolymer (poly(CV-VP)) was then treated with Sb in a similar manner. Precipitation of the resulting polymers into ethyl acetate gave hygroscopic resins which were readily soluble in cold water (Table 2).

The fluorescence of the polymers was considerably red shifted when compared with the value for N-benzyl-(γ-stilbazolium) chloride (λ_{max}=430 nm) and was understood ascribable to the excimer of SbQ.5) The intensity of green emission at 490 nm increased with the increase in the content of SbQ in the polymer (Fig. 1). The observation that the stilbazolium chloride exhibited the red shifted emission (λ_{max}=ca. 495 nm in water) only in highly concentrated solution ($\sim 5 \times 10^{-2}$ M) indicates that SbQ attached to the polymer was locally and effectively concentrated in the polymeric domain. By irradiating a thin film of CV-3 with the light of wavelength $>$350 nm, the absorbance of the long-wavelength band at 345 nm due to trans-SbQ decreased with a concomitant increase in the intensity of a new band at 264 nm which was absent in cis-SbQ. Absorbances of the spectra at two different wavelengths changed in the same ratio when the film was irradiated at certain intervals (linear extinction differences diagram).7) These reveal the preference of the dimerization to the trans-cis photoisomerization of SbQ in the solid state. All these photochemical behaviors reflect the high photosensitivity of this type of polymers. In connection with the enzyme entrapment, it is noteworthy to mention that neither photodimerization nor trans-cis photoisomerization was affected by hydrophilic amino acid residues like those of serine (3.2 M) and cystein hydrochloride (2.8 M) and by acetamide (4 M). This fact suggests that the crosslinking proceeds without the influence of proteins.

Table 1. Reaction of poly-CV with Sb at 130°C in EEA

Sample no.	React. time (hr)	Mol% of SbQ introduced	Solubility*a)			Sensitivity*b)
			acetone	ethanol	water	
CV-1	1	7	+	+	-	1
CV-2	7	32	+	+	-	16
CV-3	24	51	-	+	±	39
CV-4	48	55	-	+	±	58

*a) +:soluble, -:insoluble, ±:soluble only in hot water. *b) This was obtained by the so-called gray scale method and is expressed by relative values against the standard of 1.0 for poly(vinyl cinnamate) sensitized by 10w/w% 5-nitroacenaphthene.6)

Table 2. Reaction of 3 g of poly(CV-VP) with Sb at 130°C for 15 hr in EEA

Sample no.	CV:VP in copolym.	Weight of Sb (g)	Mol% of SbQ introduced	Sensitivity*a)	η_{sp}/C in MeOH
CVVP-1	16:84	2.39	3.4	0	1.19
CVVP-2	22:78	2.40	6.1	1.4	0.98
CVVP-3	28:72	2.82	14.1	2.0	1.06

*a) See footnote *b In Table 1.

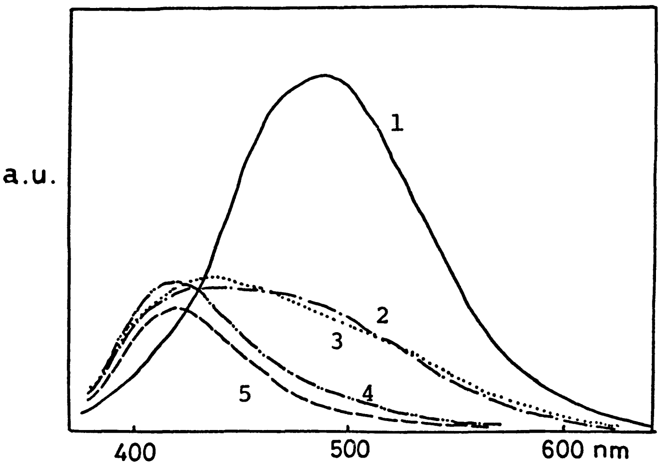
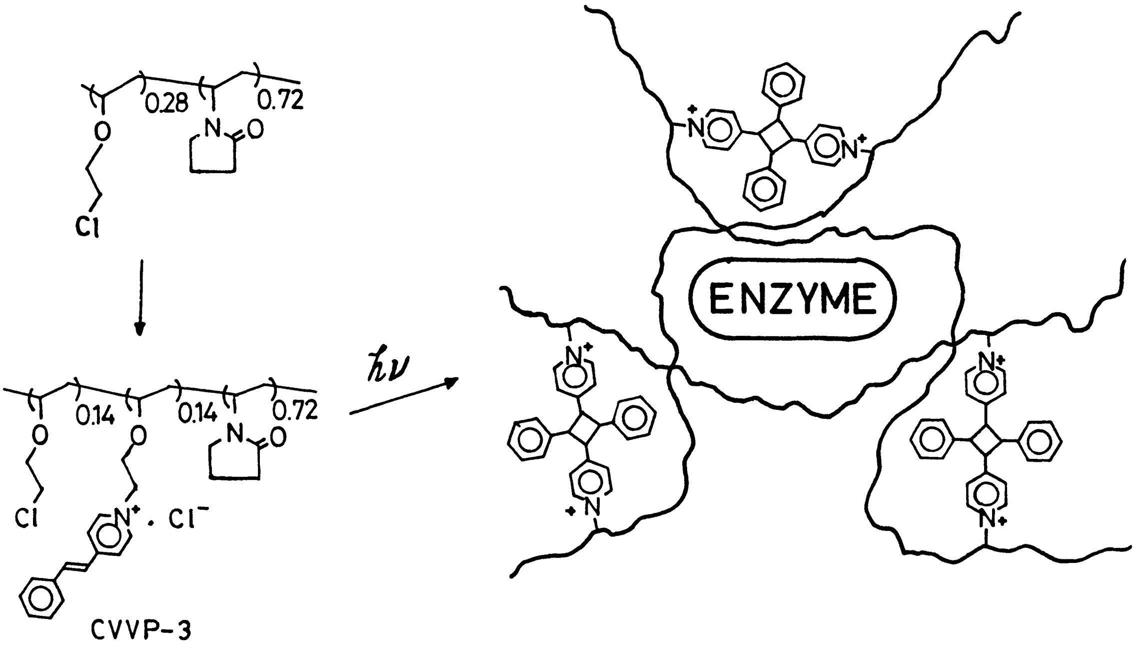


Fig. 1. The fluorescence spectra of photosensitive resins in EEA (1; CV-3, 2; CV-2, 3; CVVP-3, 4; CVVP-1, and 5; 1-benzyl-(4-stilbazolium) chloride).

For the purpose of entrapping enzymes, CVVP-3 was employed because of the good water-solubility as well as the high photosensitivity. A concentrated solution of CVVP-3 containing enzyme (0.1-0.5w/w%) was spread on a glass plate, and a coated thin film was irradiated with light of wavelength longer than 350 nm. By treating with water, the irradiated film cracked into small pieces which were collected by filtration, washed with water thoroughly and dried in vacuo. The amount of the washed out protein was measured by the method of Lowry et al.[8)] Fig. 2 shows the yield of the gel and the amount of invertase entrapped at various irradiation period with light intensity of 3.5 mW/cm^2. The results indicate that the gel yield parallels the amount of the entrapped enzyme, and therefore, the weight ratio of the enzyme to the polymer in the gel is approximately equal to the value before irradiation. Glucoamylase and catalase were entrapped in a similar way.

The activity of invertase and glucoamylase was measured with the aid of the Glucostat reagent, using sucrose and maltose as substrates, respectively.[9)] The catalase activity was obtained by measuring volumetrically the rate of oxygen evolution. As shown in Fig. 3, glucoamylase entrapped photochemically has ca.



60% activity of the native at optimal pH. Invertase and catalase in the gels were found to maintain ca. 35 and 30% activity, respectively. It was confirmed that no enzymes escaped from the gels during the course of the enzymic reactions.

Although optimization of the present method awaits further investigation, this type of photoreactive polymer is better suited for entrapping intact biomaterials by the reason that the photocrosslinking proceeds specifically at the photofunctional groups and indifferent to co-existing of proteins.

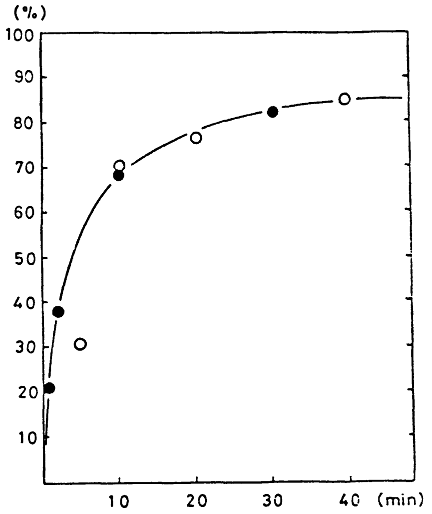


Fig. 2. The yield of photocrosslinked gel (—○—) and the amount of entrapped invertase (-●-) at various irradiation period.

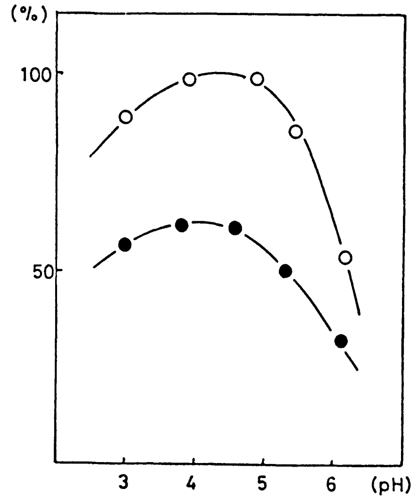


Fig. 3. The activity of native (-○-) and entrapped (-●-) glucoamylase, using 5% maltose as a substrate.

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