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PHOTOCONTROL OF UREASE ACTIVITY IN SPIROPYRAN COLLAGEN MEMBRANE

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Summary

1. Collagen fibrils were modified with β-1-[3,3-dimethyl-6′-nitrospiro-(indoline-2,2′-2*H*-benzopyran)] propionic anhydride.

2. Urease (urea amidohydrolase, EC 3.5.1.5) was immobilized in spiropyran collagen membrane. The activity of the urease-spiropyran collagen membrane was found to increase in the dark and then decrease with visible light irradiation.

3. The optimum pH of the urease-spiropyran collagen membrane under visible light was lowered in the dark.

4. The apparent Michaelis constant (K'_m) of the urease-spiropyran collagen membrane in the dark was almost the same as that under visible light. The apparent maximum velocity was increased in the dark.

5. The diffusion coefficient of urea through the spiropyran collagen membrane in the dark was 1.4 times that under visible light. However, the increase of the diffusion rate was not responsible for the activity increase of the urease-spiropyran collagen membrane.

Introduction

Substances which undergo reversible color formation under light irradiation are called photochromic compounds [1]. Photosensitive enzymes were previously prepared by modifying α-amylase, α-chymotrypsin and urease with a photochromic spiropyran compound [2]. Substrate affinity of the enzymes was found to be changed by the modification, possibly because of the change in polarity of the spiropyran compound [2].

The activity control of immobilized enzymes also has a potential application in a switch system or controller of a bioreacter. The activity of immobilized enzymes can be controlled by two methods. One method is to control di-

rectly, with external energy, the activity of modified enzymes bound on or entrapped in insoluble carrier. Based on this consideration, photocontrol of the spiropyran-urease activity in collagen membrane has been investigated [3]. The other method is to change the environmental characteristics around the enzymes with an external source of energy. The reversible change of properties of the carrier with light irradiation may cause a change in the activity of the immobilized enzymes.

The photosensitive membrane can be prepared from collagen fibrils modified with a photochromic spiropyran compound.

In this paper, the collagen fibrils were modified with β-1-[3,3-dimethyl-6′-nitrospiro-(indoline-2,2′-2*H*-benzopyran] propionic anhydride. Then, urease (urea amidohydrolase, EC 3.5.1.5) was entrapped in the membrane prepared from spiropyran collagen fibrils. The photocontrol of the urease activity in spiropyran collagen membrane is described.

Materials and Methods

Materials. Purified collagen fibril was obtained from bull calf skin as described previously [4]. Urease (from jackbeans, 2900 units/g) was purchased from Sigma Chemical Co. A photochromic compound, β-1-[3,3-dimethyl-6′-nitro-(indoline-2,2′-2*H*-benzopyran] propionic anhydride was prepared by the method reported previously [5].

Modification of collagen. Modification of collagen fibril was carried out by the following procedure. 1.0 g of collagen fibrils was added to 100 ml of acetone containing 300 mg of spiropyran anhydride in solution, and the reaction mixture was allowed to stand at room temperature for 20 h with stirring. After reaction, the spiropyran collagen fibrils were collected by filtration and washed with acetone to remove unreacted spiropyran compound. The amount of bound spiropyran compound, determined colorimetrically, was 67 mg per 1 g of collagen. The degree of modification can be made to vary by varying the ratio of spiropyran compound to collagen.

Preparation of urease-spiropyran collagen membrane. 30 mg of urease was added to 30 g of 1.0% spiropyran collagen fibril suspension at pH 4.5. (The ratio of enzyme to collagen was 1 : 10). The urease-spiropyran collagen membrane was prepared by pouring the mixed suspension on a Teflon plate and drying it at room temperature for 15 h. Urease-spiropyran collagen membranes were treated with 0.1% glutaraldehyde solution for 1 min and dried at 4°C. The thickness of the membrane was about 90 μm. No leakage of urease was observed from the spiropyran collagen membrane.

Enzyme assay. Unless otherwise noted, standard assays of native urease and the urease-collagen membrane were carried out as described previously [3]. Ammonia concentration was determined by the method of Sumner [6]. The formation of 1 μmol ammonia per min at 25°C was defined as 1 activity unit.

Activities were measured both in the dark and under visible light. A tungsten lamp (750 W projector lamp) was used as the visible light source.

Diffusion coefficient measurement. Diffusion coefficients of urea and ammonium ion in the modified membrane were also determined in the dark and un-

der visible light irradiation. The apparatus and procedure for measuring fluxes were similar to those used previously [7].

Results

Spiropyran compound bound to collagen fibrils was photoisomerized reversibly by light irradiation in a similar fashion to free spiropyran. The spiropyran collagen membrane showed reverse photochromism. It was colored red in the dark and bleached with visible light irradiation (Fig. 1). This photochromic behavior of the membrane was similar to that observed in the case of free spiropyran in dioxane/water solution [5].

The activity of the urease-spiropyran collagen membrane in the dark was about 30% of that of native urease.

The relationship between the activity of urease and spiropyran content of the collagen membrane are shown in Fig. 2. The activity of the urease-spiropyran collagen membrane in the dark was higher than that under visible light. The ratio of the activity in the dark to that under visible light gradually increased with increase in the spiropyran content of the collagen membrane.

On the other hand, the activity of native urease was the same under visible light and in the dark.

Fig. 3 shows the pH vs. activity profiles of the urease-spiropyran collagen membrane under visible light and in the dark. At pH 6.0, the activity of the urease-spiropyran collagen membrane in the dark was 2.0. times that under visible light. The shape of pH vs. activity curve in the dark was different from that under visible light. The optimum pH of the urease-spiropyran collagen membrane under visible light was 6.8 which was the same as that of native urease and shifted in the acidic direction in the dark.

The hydration of the spiropyran collagen membrane was determined as described previously [4]. The maximum hydration of the spiropyran collagen membrane was attained after 20 min incubation in water at 25°C. 1 g of the spiropyran collagen membrane absorbed 6.8 g water under visible light and 7.8 g in the dark.

Table I shows the apparent diffusion coefficients of urea and ammonium ion through spiropyran collagen membrane. The same experiment was repeated three times. The diffusion coefficient of urea in the dark was found to be 1.4 times that under visible light. This result seemed to be consistent with the activity increase in the dark. On the other hand, the diffusion coefficient of ammonium ion under visible light was larger than that in the dark.

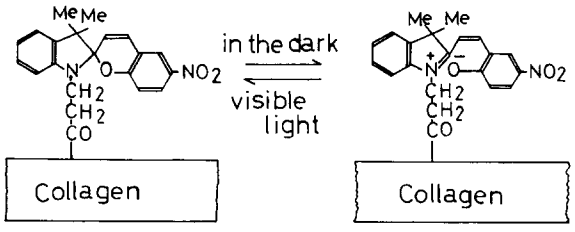
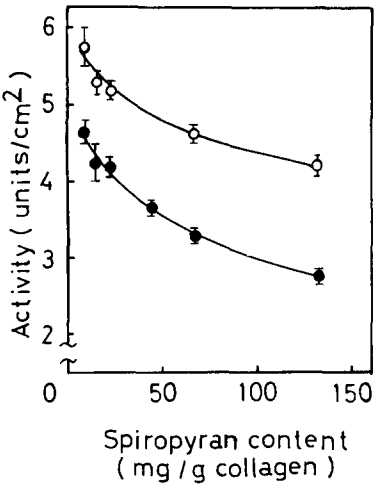


Fig. 1. Photoreversible isomerization of spiropyran compound.



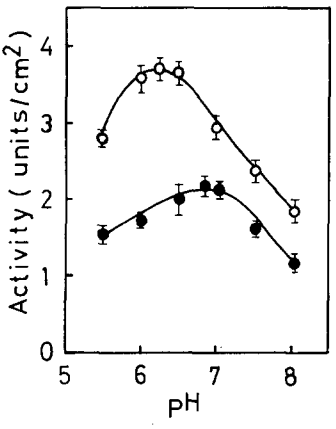


Fig. 2. Relationship between the activity of urease and the spiropyran content of the collagen membrane. The enzyme assay was carried out under standard conditions. Urease-spiropyran collagen membrane (○) in the dark, (●) under visible light.

Fig. 3. pH vs. activity profiles of urease-spiropyran collagen membrane. 0.2 M phosphate buffer solution was employed. Urease-spiropyran collagen membrane (○) in the dark, (●) under visible light.

Table II shows the kinetic parameters of the urease-spiropyran collagen membrane under visible light and in the dark. The same experiment was repeated four times. The urease reaction in the spiropyran collagen membrane followed Michaelis-Menten kinetics. The apparent Michaelis constant, K'_m, of urease-spiropyran collagen membrane was larger than that of native urease (0.01 mol/l). K'_m of the spiropyran collagen membrane in the dark was almost the same as that under visible light. However, the apparent maximum velocity of reaction, V, under visible light was smaller than that in the dark.

The activity reversibility of the urease-spiropyran collagen membrane was examined. The activity was determined under visible light and after the activity measurement, the urease-spiropyran collagen membrane was stored for 12 h in the dark. Then the activity was measured in the dark. The activity of the urease-spiropyran collagen membrane increased in the dark. Then the activity of

TABLE I

APPARENT DIFFUSION COEFFICIENTS OF UREA AND AMMONIUM ION THROUGH SPIROPYRAN-COLLAGEN MEMBRANE

0.1 M urea or NH_4Cl solution (in 0.2 M phosphate buffer, pH 6.5) was employed. Apparent diffusion coefficient, D, was calculated from the equation: $J = (A/l) \cdot D \cdot \Delta C \cdot t$ where J is solute flux, A is membrane surface area, l is membrane thickness, ΔC is the concentration difference between the two compartments, and t is diffusion time.

	$D_{(NH_2)_2CO}$ ($\times 10^{-6}$ cm^2/s)	$D_{NH_4^+}$ ($\times 10^{-6}$ cm^2/s)
In the dark	2.0 ± 0.1	1.9 ± 0.1
Under visible light	1.4 ± 0.2	2.5 ± 0.1

TABLE II

KINETIC PARAMETERS OF THE UREASE-SPIROPYRAN COLLAGEN MEMBRANE

	K'_m (M)	V (μmol NH_3/min per mg membrane)
In the dark	0.030 ± 0.002	7.6 ± 0.3
Under visible light	0.030 ± 0.003	4.9 ± 0.2

the urease-spiropyran collagen membrane was measured under visible light. It was not restored to its initial activity. Furthermore, no activity increase of the spiropyran collagen membrane was observed after incubation in the dark.

Discussion

No difference of stability was observed between urease in the collagen membrane and urease in the spiropyran collagen membrane within the experimental period.

The activity of the urease-spiropyran collagen membrane in the dark increased to twice that under visible light. The mechanism of the activity change is different from that of the spiropyran-urease in collagen membrane [3].

It is known that spiropyran compound changes in polarity with isomerization [8].

The spiropyran collagen membrane showed reverse photochromism. That is, colorless spiropyrans are converted into colored form (red) when they are placed in the dark. The spiropyran compound bound to the collagen fibrils bore a charge in the dark. On the other hand, it was uncharged under visible light. Therefore, the spiropyran collagen membrane in the dark was more hydrophilic than that under visible light. This phenomenon was also demonstrated by hydration experiments conducted on the spiropyran collagen membrane. Adsorption of water [9] to the spiropyran collagen membrane was increased in the dark. As previously reported [7], the enzymatic reaction of the urease-collagen membrane was limited by the diffusion of substrate in the collagen membrane. Therefore, it was assumed that the diffusivity increase of the substrate in the dark was responsible for the activity increase of the urease-spiropyran collagen membrane. In practice, the diffusion coefficient of urea through the spiropyran collagen membrane in the dark was larger than that under visible light. However, the apparent Michaelis constant (K'_m) of the urease-spiropyran collagen membrane in the dark was identical to that under visible light. This result did not bear out the above speculation.

However, the maximum velocity (V) of the urease-spiropyran collagen membrane was larger in the dark than under visible light. The rate of the decomposition of ES complex to product [3] in the dark was greater than that under visible light. Therefore, it was concluded that the hydrophilic microenvironment around the immobilized urease in the spiropyran collagen membrane may increase the velocity of urea hydrolysis.

A shift of the optimum pH of the urease-spiropyran collagen membrane was observed in the dark. Urease hydrolyzes urea to ammonium ion and bicarbon-

ate ion in phosphate buffer [10]. As described above, the diffusion coefficient of ammonium ion through the spiropyran collagen membrane in the dark was smaller than that under visible light. Therefore, ammonium ion interacted with charges of spiropyran compound bound to the collagen membrane. Ammonium ion may increase the local pH near the immobilized enzyme in the membrane. The increase of local pH would cause the displacement of the pH vs. activity curve of the membrane toward lower pH values.

The reversibility of the activity change of the urease-spiropyran collagen membrane was poor. It is known that spiropyran compounds undergo irreversible isomerization with light irradiation [1]. This irreversible isomerization of spiropyran compound bound to the collagen membrane decreases the reversibility of the activity changes of urease-spiropyran collagen membrane.

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