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PREPARATION OF CELLULOSE-HYDROUS TITANIUM OXIDE COMPOSITE FIBRE ENTRAPPED WITH GLUCOSE OXIDASE

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SUMMARY
The fibre of a cellulose-TiO_2 composite was prepared by the reaction of cellulose acetate with titanium isopropoxide. Glucose oxidase was entrapped in the fibre. This immobilization can be easily and simply formed under mild conditions. The fibre is stable in common solvents, high ionic solutions and over the wide pH range of 3-10.

INTRODUCTION
There are many investigations concerning about the reactions using immobilized enzymes as bio-catalysts. Polymer matrices are used exclusively, they have poor structure stability, however. Previously, hydrous TiO_2 and ZrO_2 have been shown to be suitable as matrices on which enzymes can be immobilized with retention of enzyme activity (Kennedy and Kay, 1976). However, if they are very fine (3-10 mμ), then a certain amount of enzyme leaches from the matrix by washing or during the reaction. In addition, it is difficult to shape them into beads, fibre and film. Natural cellulose polymer is excellent for vital suitability and shaping into various forms. Here, we developed a new type fibre of a cellulose-hydrous titanium oxide composite containing enzyme.

EXPERIMENTAL
All chemicals were of the highest grade commercially available and used without further purification. D-glucose oxidase (EC,1.1.3.4, a specific activity 15units/mg) was obtained from Tokyo Kasei Co. Stock solution of glucose were prepared with 1M D-glucose. A 10wt% cellulose acetate acetone solution was made by dissolving 38.8% acetyl cellulose acetate in acetone. Oxidase moistened with water was dispersed into these solutions in a 1:10 weight ratio proportion. A 10wt% titanium alkoxide acetone solution was made by dissolving titanium iso-propoxide (Nacalai Tesque Inc.) into acetone. The former oxidase-dispersed solution was pressed out slowly into the latter solution using a syringe. It was allowed to stand for 30 minutes. The resultant fibre (diameter:0.2-0.5mm) is soft, elastic and transparent. The fibre was removed from the solution and washed with ethanol. The transparent gel fibre was then hydrolyzed by 0.1M phosphate buffer. One gram of wet fibre was added to 200ml of glucose solution. The pH was adjusted by ace-

tate, phosphate or tris buffer. The reaction was followed by the amount of oxygen removed using a dissolved oxygen sensor. The experiments were carried out in a thermostated bath at about 25°C and under an atmosphere of air. CP/MAS ^{13}C-NMR spectra were recorded using a JEOL GX-270 spectrometer (resonance frequency 67.8MHz). All samples were cut into small pieces in order to fill the rotor. Spectra were accumulated 100000 runs.

RESULTS AND DISCUSSION

We have investigated the formation of the polymer-inorganic composite and its properties. In such an investigation, we found a gel formation when cellulose acetate solution was in contact with alkoxide solution. On the other hand, it is reported that an oil-like substance is formed when polysaccharide solution is in contact with titanium triethanolamine (Kramer and Prud'homme, 1986). However, the reaction mechanism is not well established. The gel formation between alkoxide and cellulose acetate may be due to ester or alcohol exchange reactions between the hydroxyl or acetyl groups on the pyranose rings and the titanium alkoxide. However, the contact of cellulose triacetate with alkoxide did not produce gel formation. In addition to the above exchange reactions, alkoxide can undergo a multiple hydrolysis reaction followed by contact with water. In the first step of the exchange reaction, enzymes are loosely entrapped in the gel network, cross-linking the cellulose and alkoxide through the exchange reaction previously described.

The NMR spectra of fibre, cellulose gel film and cellulose powder are given in Fig.1. A distinction between C_1 and C_6 signals is clearly observed, but C_2,C_3,C_4 and C_5 are superimposed. It is necessary to use a higher resolution spectrometer to elucidate these points. No substantial displacement of the signals was seen between spectra.

Photo. shows the fibres immersed in a petri dish. The fibre contains 10-20wt% TiO_2 which depends on its preparation conditions. It shows no X-ray diffraction pattern (Cu-Kα, 40kV, 15mA), indicating very fine particles (<2nm). The freezed dried fibre has a specific surface area of 1-5m^2/g as determined by N_2 adsorption. One gram fibre was immersed in buffer solution (pH 4,7 and 9) for 30 days. Liberation of Ti from the fibre during this storage was not detected by ICP atomic emission spectrometry.

It seems that the initially hydrolyzed-species has a linear polymeric chain of $TiO(OH)_2$. Two hydroxyl groups are known to be pendent to each Ti atom of the chain. Interaction between enzyme and matrix may arise through displacement of the hydroxyl group of the hydrous TiO_2 by ligands of the enzyme. In addition to the hydroxyl groups, the enzyme has amine and carboxyl groups. These must be considered to act as ligands to titanium. In view of these considerations, hydrous TiO_2 is expected to exhibit a general affinity for the binding of the other proteins. Moreover, it is considered that the precipitation of hydrous TiO_2 in the presence of enzyme may yield a physical trapping of an enzyme in the matrix owing to the higher surface area of the growing particles.

Glucose oxidase catalyses the oxidation of β-D-glucose by O_2 to the corresponding lactone. Activity adopted here is the initial rate of dissolved oxygen disappearance. The initial amount of dissolved O_2 in test solution is easily changed by an atmosphere and temperature. Therefore, the linear sections of the response-time curve were used for

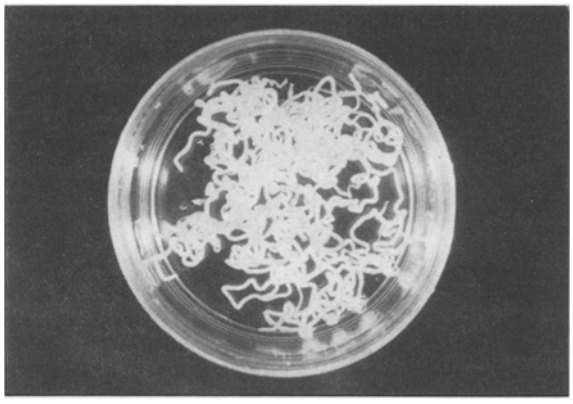

Photo. Cellulose-TiO_2 composite gel fibre.

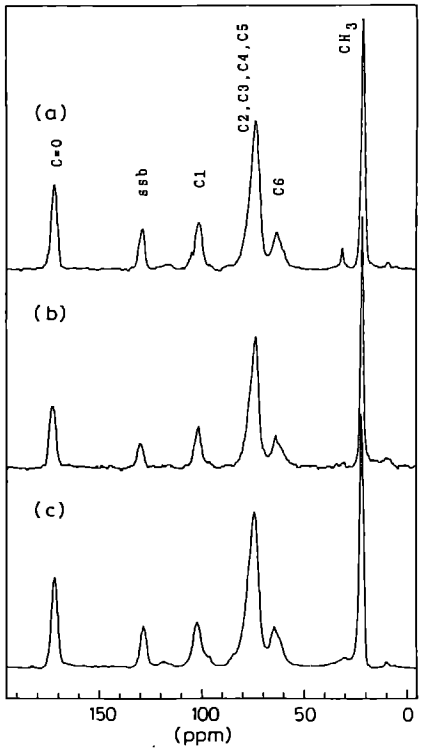

Fig.1. ^{13}C NMR spectra of gels. (a)CA gel film (b)CA-TiO_2 gel fibre washed with acetone (c)CA-TiO_2 gel fibre washed with ethanol.

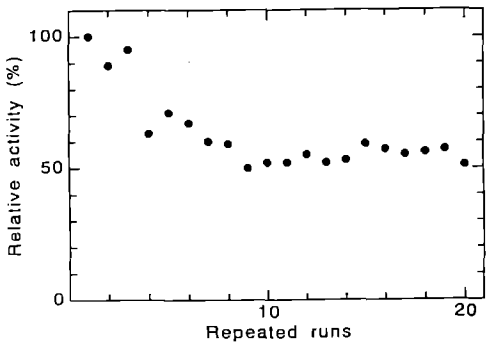

Fig.2. Effects of repeated runs on relative activity of immobilized enzyme.(25°C,pH7.0)

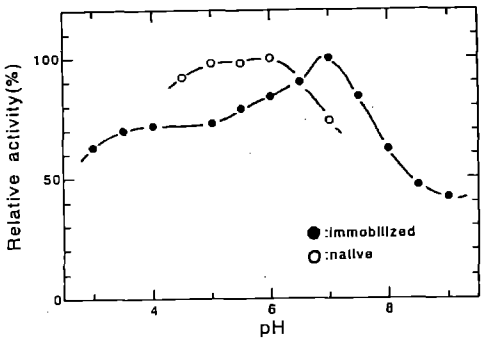

Fig.3. Effect of pH on activity of immobilized enzyme.

determining the initial rate. Fig.2 shows the stability of immobilized enzyme for repeated runs. Relative activity decreases rapidly during an initial stage and afterwards maintains a constant value. In entrap immobilization, it is difficult to prevent the enzyme from being leached into solution during use. The decrease may be attributable to the fact that loosely adsorbed enzyme is removed and that only tightly bound enzyme remained.

The effect of pH on activity is given in Fig.3. The activity at optimum pH has been set equal to 100%. It shows a narrow range of pH 4-9 with a maximum at pH 7.0, compared to native activity. There is a shift toward a more alkaline pH value of 1.5 compared to native enzyme. The behaviors are similar to these in the glucose oxidase on nylon membrane (Da Silva et al.,1991). They may be mainly influenced by ionic matrix. Titanium oxide gives a negatively charged surface above its isoelectric point (5.5) and a positively charged surface below that point. The shift may be due to the negatively charged surface of the matrix, thereby creating a microenvironment for the bound enzyme that has a higher hydrogen ion concentration (lower pH) than the concentration in the surrounding solution where the pH is actually measured.

This new immobilization method has advantages over other methods in that it can be performed easily and simply under mild conditions. This composite matrix is insoluble in all the common solvents and in highly ionic aqueous solutions. This method can also be conveniently extended to the immobilization of other proteins, peptides, antibodies and amino acids. Further work on this immobilization is now in progress.

REFERENCES

Kennedy,J.F.,Kay,I.M.(1976).J.Chem.Soc.,Perkins Trans.I,329-335.

Kramer,J., Prud'homme,R.K.(1986). J.Colloid Inter.Sci.,118, 294-296.

Da Silva,M.A. et al.(1991). J.Polym.Sci.Polym.Chem.29,275-279.