ml of glucose. Specific activity of the enzyme used was 255 unit/mg.

RESULTS

1. *Effect of CMC on the activity of glucose isomerase*

The enzyme activity was supposed to be affected with CMC by the modification of free carboxyl groups or other structures in the protein. Therefore effect of CMC on the activity of free (mobile) glucose isomerase was investigated. As shown in Fig. 1, the activity was gradually reduced to 70% of initial value after incubation with 1% CMC for 6 hr. But in the presence of excess D-glucosamine, a monomer sugar of chitosan, glucose isomerase showed little loss in activity. From these result it was expected that the inhibition of enzyme activity by CMC treatment would also be small in the presence of chitosan.

Disc gel electrophoretic pattern of CMC treated glucose isomerase is shown in Fig. 2. Delay of migration and broadening of the band were observed corresponding to incubation time. These effects were probably due to the neutralization of negative charge of enzyme by the modification of carboxyl radicals or others with CMC or aggregation of enzyme molecules caused by CMC treatment.

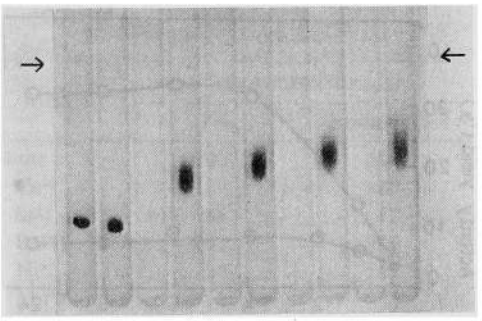

FIG. 2. Disc Electrophoresis of CMC Treated Glucose Isomerase on Polyacrylamide Gels (7.5%, pH 9.4).

Enzyme was incubated with 1% of CMC at room temperature. At time intervals 10 μl of incubation mixture was loaded on top of the gel. The current was 3 mA per gel. The gels were stained with 1% Amide black 10 B.

Migration was downward toward the anode. Control; native enzyme, →; top of the gels.

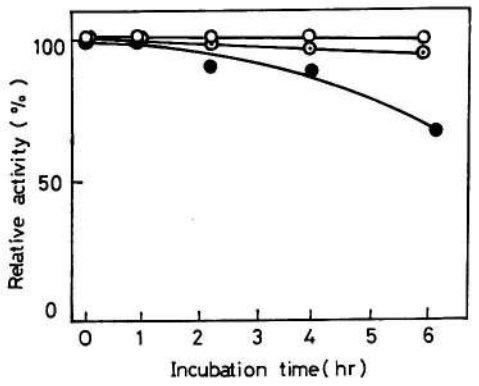

FIG. 1. Effect of CMC on the Activity of Glucose Isomerase.

Enzyme was incubated with 1% CMC at room temperature and then, remaining activity was estimated. Composition of incubation mixture was as follows: ○—○ control, 1 ml of glucose isomerase (0.97 mg) and 5 ml of 0.02 M phosphate buffer (pH 6.0), ●—● 60 mg of CMC was added to the control, ⊙—⊙ 60 mg of CMC and 60 mg of D-glucosamine·HCl were added to the control.

2. *Activity yield of immobilized glucose isomerase*

The effects of CMC concentration and reaction time on the activity yield of immobilized glucose isomerase are shown in Figs. 3 and 4.

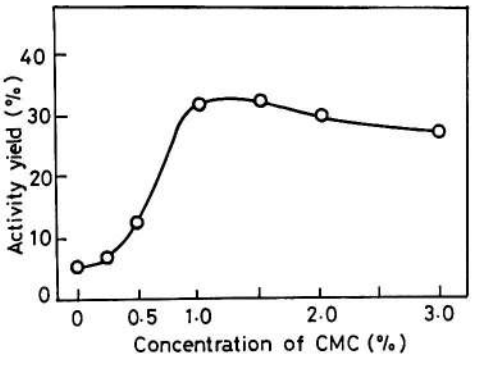

FIG. 3. Effect of CMC Concentration on Binding of Glucose Isomerase to Chitosan.

Binding reaction was continued for 5 hr. The activity yield was expressed as a percent of immobilized activity to added activity.