Studies of Polystyrene-Based Ion-Exchange Fiber. IV. A Novel Fiber-Form Material for Adsorption and Immobilization of Biologically-Active Proteins†

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A new polystyrene-based ion-exchange fiber (IONEX) has a large surface area per unit weight and has been studied for its ability to adsorb and immobilize biologically-active proteins. A strong cation IONEX was found to be able to effectively adsorb hemoglobin and albumin, invertase, and glucose isomerase were readily adsorbed to a strong anion IONEX. The adsorption of these proteins to IONEX was found to take place through an electrostatic force. The adsorption capacity for proteins became exceedingly large (ca. 300 mg g^{-1}) with an increase in the water-holding capacity of the fiber. The activities of the invertase and glucose isomerase immobilized to the fiber exhibited 40—50 and 75—80%, respectively, of those of native enzymes. A continuous inversion of sucrose was also carried out using immobilized invertase. From these results, this fibrous ion exchanger with a high water-holding capacity is considered to be an excellent material for the adsorption and immobilization of proteins.

In other papers we have reported the preparation methods, fundamental characteristics, and acid-base catalytic properties of a polystyrene-based ion-exchange fiber (IONEX).[1-3] The ion-exchange fiber has a large surface area and, accordingly, is expected to have a large capacity for adsorbing macromolecular ionic substances.

Recently, the immobilization of enzymes has been intensively studied and some immobilized emzymes have been used in industry. There have been many investigations regarding methods for immobilizing enzymes, such as bonding to a supporter, entrapping in a crosslinked gel, and crosslinking by a bi- or multifunctional reagent.[4] An immobilization of enzymes to an ion-exchange fiber based on poly(vinyl alcohol) has also been examined and several reports have been published.[5-7] We have undertaken an investigation regarding the immobilization of emzymes using IONEX while considering the following advantages:[8]

(1) The capacity for adsorbing macromolecular proteins is expected to be large since IONEX has a large surface area per unit weight.

(2) The yield of the activity is expected to be high because immobilization probably occurs on the surface through ionic bonding. Thus, steric hindrances for the active site of proteins and for the diffusion of the substrates would be smaller.

(3) IONEX exhibits a high mechanical strength and, thus, it can be utilized in various forms, such as a cut fiber, a knitted fabric, a woven fabric, a braid, a felt, a nonwoven fabric, and a paper.

In the present report, the adsorption and immobilization of some proteins and enzymes to IONEX are described. The importance of the water-holding capacity of the fiber for protein adsorption and enzyme immobilization is also described.

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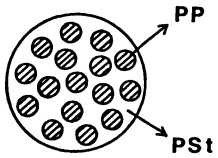

Fig. 1. Schematic cross section of composite fiber. Pst: polystyrene as *sea* ingredient; PP: polypropylene as *island* ingredient.

Experimental

Polystyrene to be converted into an ion exchanger (as the predominant *sea* ingredient) and polypropylene for reinforcement (as the *island* ingredient) were spun into composite filaments having an *islands-in-a-sea* type sectional structure (Fig. 1) by the conventional method. A cation IONEX with sulfo groups and an anion IONEX with trimethylammonio groups were prepared by the introduction of crosslinking groups and corresponding ion-exchange groups into the polystyrene part according to the procedures described in a previous paper.[1] The capacity of strong acidic- and basic-type IONEX was ca. 2.5 mequiv g^{-1}. A cation IONEX in the Na form or an anion IONEX in the Cl form was immersed in deionized water at room temperature for a sufficient period in order to reach an equilibrium water sorption. The fiber was centrifugally dehydrated at 3000 r.p.m. for five minutes, and then, the wet weight (W) was determined immediately. The water content of the fiber was determined using the following equation:

$$\text{Water content} = (W - W_0)/W_0,$$

where W_0 is the dry weight of the fiber. The water content means the water-holding capacity of a fiber. The content was controlled by choosing the conditions of the crosslinking reaction.[1-3] The water content becomes higher as the degree of crosslinking is lowered.

Hemoglobin and albumin from bovine blood and invertase from *Candia utilis* were obtained from Sigma Co. and Seikagaku Kogyo Co., Ltd., respectively. Glucose isomerase was extracted by the lysozyme methods from *Streptomyces*

phaeochromogenus supplied by Nagase Sangyo Co., Ltd. The adsorption capacity of the fiber for proteins was evaluated by measuring the concentrations of the proteins in solutions before and after an adsorption treatment by means of the copper-Folin[9] or optical-density (at 270 nm) methods. The amount of the enzyme adsorbed to the fiber (A) was calculated by subtracting the activity of the enzyme which remained unadsorbed from the activity of the added enzyme. The yield of the activity (B/A) was determined by measuring the activity of the enzyme immobilized to the fiber (B). One unit (U) of invertase activity was defined as the amount of enzyme which produced 1 μ mole of glucose per minute at 30°C in a 10 wt% sucrose solution containing a 0.01 M (1 M=1 mol dm^{-3}) phosphate buffer of pH 5.0. The activity of the native invertase was 170 U mg^{-1}. On the other hand, one unit (U) of glucose isomerase activity was defined as the amount of enzyme which produced 1 mg of fructose per hour at 60°C in a 0.5 M glucose solution (pH 8.2) containing 0.05 M $NaHCO_3$ and 0.01 M $MgSO_4$. The amount of glucose or fructose produced was determined by the polarimetric methods.[10]

Results and Discussion

Adsorption of Hemoglobin and Albumin. The adsorption of hemoglobin and albumin was carried out by the following procedures. A strong cation exchanger (20—100 mg in the Na form) was added to 10 ml of a 0.1% hemoglobin (isoelectric point; pH 6.9, molecular weight; ca. 70000) solution containing a 0.05 M phosphate buffer of pH 6.6. The mixture was then shaken at 30°C for 3 h. Similarly, a strong anion exchanger (20—100 mg in the Cl form) was added to 10 ml of a 0.1% albumin (isoelectric point; pH 4.7, molecular weight; ca. 70000) solution containing a 0.05 M phosphate buffer of pH 7.0; then, the mixture was shaken at 30°C for 3 h. The results of hemoglobin adsorption to cation exchangers and albumin adsorption to anion exchangers are summarized in Tables 1 and 2, respectively.

The adsorption capacity of a cation IONEX for hemoglobin was apparently dependent on its water content. IONEX with a water content of 1.5 exhibited an adsorption capacity of less than 20 mg g^{-1}. In contrast, IONEX with the highest water content among those tested exhibited a capacity of 300 mg g^{-1}. The granular cation-exchange resins, Amberlite IR-120B and IR-200C, and the crushed resin, Powdex PCH, were also examined for their capacity to adsorb hemoglobin. These resins had a water content in a range from 0.9 to 1.6 and showed adsorption capacities similar to that of IONEX with the lowest water content. In the case of albumin, protein was readily adsorbed to an anion IONEX having a trimethylammonio group as the ion-exchange site. The adsorption capacity was again dependent on the water content. Macroreticular-type granular anion-exchange resins, such as Amberlite IRA-900, IRA-904, and IRA-938, showed low adsorption capacities of 12—40 mg g^{-1}. Gel-type granular anion-exchange resins, Amberlite IRA-400 and IRA-401, and the crushed resin, Powdex PAO, exhibited no appreciable adsorption capacities for albumin.

Table 1. Results of Hemoglobin Adsorption to Cation Exchangers in Na Form

Cation exchanger	Water content	Adsorption capacity[a] mg g^{-1}
IONEX	1.5	<20
	3.0	50
	5.0	300
Amberlite IR-120B	0.9	<20
Amberlite IR-200C	0.9	<20
Powdex PCH	1.6	<20

a) 0.1% Hemoglobin solution containing 0.05 M phosphate buffer (pH 6.6), 30°C, 3 h.

Table 2. Results of Albumin Adsorption to Anion Exchangers in Cl Form

Anion exchanger		Water content	Adsorption capacity[a] mg g^{-1}
IONEX		1.5	30
		2.5	200
		3.5	300
Amberlite	IRA-400	0.8	<10
	IRA-401	1.6	<10
	IRA-900	1.6	13
	IRA-904	1.4	40
	IRA-938	2.7	12
Powdex PAO		1.4	<10

a) 0.1% Albumin solution containing 0.05 M phosphate buffer (pH 7.0), 30°C, 3 h.

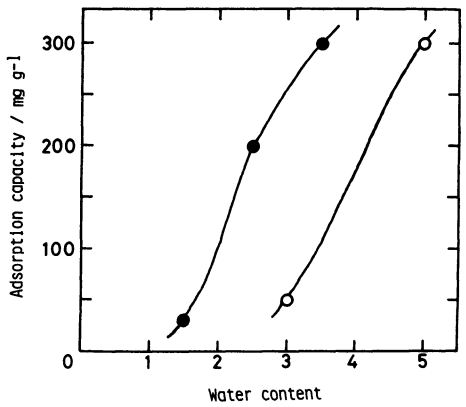

Fig. 2. Water content dependence of protein adsorption capacity of IONEX. Adsorption was carried out at 30°C for 3 h in 0.1% protein solution containing 0.05 M phosphate buffer.
○: Hemoglobin adsorbed to cation IONEX at pH 6.6, ●: albumin adsorbed to anion IONEX at pH 7.0.

The dependences on the water content of the adsorption capacities of IONEX for the proteins are shown in Fig. 2. Apparently, water in the polystyrene network of IONEX plays an important role in macromolecular protein's ability to have access to the ion-exchange site. In fact, IONEX comprises a polystyrene network crosslinked between the phenyl

Table 3. Results of Invertase Immobilization to Anion IONEX in Cl Form by Batch Method

Water content	Amount of IONEX	Adsorbed[a] enzyme (*A*)	Observed activity (*B*)	Yield of activity (*B/A*)
	mg	U	U	%
1.5	250	610	230	38
	500	1170	460	39
	1000	2125	860	40
2.5	50	625	350	56
	150	1940	1025	53
	500	2125	1140	54

a) Enzyme added: 2125 U, 12.5 mg (50 ml of 0.01 M phosphate buffer, pH 5.0), 20°C, 2 h.

groups by methylene linkages. The lattice of the network becomes wider as the degree of crosslinking is decreased; that is, the water content of the fiber increases, and thus, a macromolecule can diffuse into the network and more easily interact with the ion-exchange sites.

The higher adsorption capacity of IONEX (diameter; ca. 30 μm), compared with gel-type granular resins (diameter; ca. 500 μm) or with crushed resins (particle size; 37—250 μm) with similar water contents, would come from the larger surface area of IONEX. The surface areas of IONEX with a water content of 1.5—5.0 were 2.0—2.5 $m^2 g^{-1}$, as determined by the BET methods using nitrogen gas. On the other hand, the surface areas of the crushed resins and the gel-type granular resins were 0.5—0.8 $m^2 g^{-1}$ and less than 0.1 $m^2 g^{-1}$, respectively. In addition, the lattice sizes of the polystyrene network of IONEX are presumably larger than those of the usual styrene-divinylbenzene-based resins. This also contributes, at least in part, to the larger adsorption capacity of IONEX.

When a fiber which had adsorbed albumin was washed with distilled water and then treated with 10 wt% NaCl, more than 90% of the adsorbed protein was released. These results indicate that a protein is adsorbed to the fiber through an electrostatic force.

Immobilization of Some Enzymes. The results described above suggest the possibility of IONEX as an excellent material for the immobilization of enzymes. In order to prove this possibility, the immobilization of two enzymes, invertase and glucose isomerase, to IONEX was investigated with special reference to their activities.

Invertase (isoelectric point; pH 3.8, molecular weight; 270000) was first immobilized to an anion IONEX with two different water contents (1.5 and 2.5) by the following methods. A fixed amount of the anion IONEX in the Cl form was added to 50 ml of a 0.01 M phosphate buffer solution (pH 5.0) containing 2125 U of invertase. The mixture was shaken at 20°C for 2 h. Then, the fiber to which the enzyme was adsorbed was washed five times with distilled water and stored in the same buffer solution.

As expected, invertase was readily adsorbed to the anion IONEX and its adsorption capacity for this

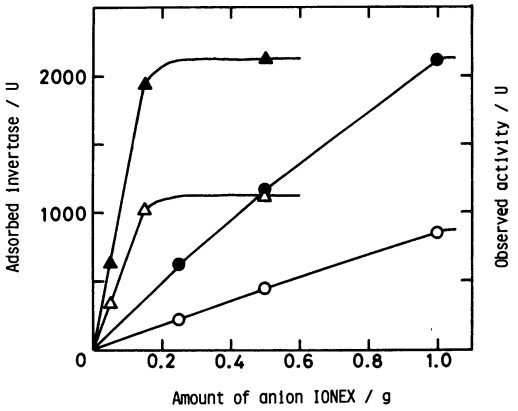

Fig. 3. Adsorbed invertase and observed activity vs. amount of anion IONEX in Cl form. Immobilization was carried out in 50 ml of 0.01 M phosphate buffer solution (pH 5.0) containing invertase of 2125U (20°C 2 h). ●: Adsorbed invertase, ○: observed activity (water content of IONEX=1.5), ▲: adsorbed invertase, △: observed activity (water content of IONEX=2.5).

enzyme was greater when the water content of the ion exchanger was higher (Table 3). When the water content was 1.5, the increase in the amount of the adsorbed enzyme was almost linear to the amount of IONEX (see Fig. 3) up to 1.0 g under the conditions used. In contrast, when the water content was 2.5, saturation occured in the vicinity of 0.2 g. These results indicate that the capacity for immobilization increases by five times when the water content of the fiber increases from 1.5 to 2.5. This fact is attributable to the difference in the crosslinked structure described above. The reasons that the yield of the activity is lower than 100% are considered to be (1) that the enzyme is inactivated during the immobilization process, (2) that the active site of the immobilized enzyme is sterically hindered, and (3) that the diffusion of the substrate to the active site of the immobilized enzyme is hampered. The yield of the activity was enhanced from ca. 40% to ca. 50% when the water content of the fiber was increased from 1.5 to 2.5 (Table 3). These results may indicate that two effects, (2) and (3), are eliminated by a loosening of the crosslinked structure.

Invertase was also immobilized to anion exchangers

Table 4. Results of Invertase Immobilization to Anion Exchangers in Cl Form by Column Method

Anion exchanger	Water content	Packed[a] amount	Adsorbed[b] enzyme (*A*)	Observed activity (*B*)	Yield of activity (*B*/*A*)
		mg	U	U	%
IONEX	1.5	500	600	265	44
	2.5	150	1700	865	51
Amberlite IRA-900	1.4	1500	600	260	43

a) Column diameter: 7.5 mm. b) Enzyme added: 2125 U, 12.5 mg (50 ml of 0.01 M phosphate buffer, pH 5.0), 20 °C, 1 h.

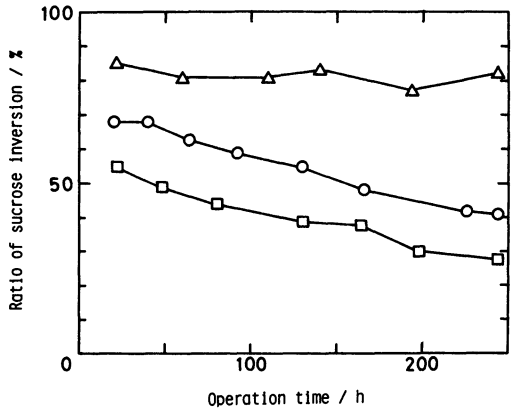

Fig. 4. Stability of immobilized invertase to continuous inversion of sucrose by a column method. 10 wt% Sucrose solution containing 0.01 M phosphate buffer of pH 5.0 was passed through the column (7.5 mmϕ) filled with the immobilized invertase at a flow rate of 50 ml h^{-1} at 25 °C.
○: 500 mg of anion IONEX with water content of 1.5 (265U). △: 150 mg of anion IONEX with water content of 2.5 (865U), □: 1500 mg of Amberlite IRA-900 (260U).

by a column method as follows. Anion exchangers were packed into a jacketed glass column with an inside diameter of 7.5 mm. The enzyme solution described above was circulated through the column at 20 °C for 1 h. The results are summarized in Table 4.

The column method gave results similar to the batch method, except that the amounts of adsorbed enzyme for IONEX with water contents (1.5 and 2.5) decreased to 55 and 85%, respectively. The decrease in the amounts of adsorbed enzyme may imply that some parts of IONEX are not utilized because of an irregular flow. The macroreticular-type resin, Amberlite IRA-900, has only one third as large an effective capacity for immobilizing invertase per unit weight as IONEX with a water content of 1.5.

Figure 4 shows the dependence on operation time of the ratio of sucrose inversion when the substrate solution was continuously passed through each column at 25 °C. The activity of an IONEX column with a water content of 2.5 decreased more slowly than that of an IONEX column with a water content of 1.5 and an IRA-900 column. The inversion ratio was not improved, although an enzyme solution with twice the decreased activity was circularly passed through each column. This fact indicates that the decrease in activity can be attributed to the inactivation of the enzyme, but not to the release of the enzyme.

Table 5. Results of Glucose Isomerase Immobilization to Anion IONEX of Water Content 4.0 in Cl Form

Amount of IONEX	Adsorbed[a] enzyme (*A*)	Observed activity (*B*)	Yield of activity (*B*/*A*)
mg	U	U	%
10	160	125	78
20	294	235	80
40	337	253	75

a) Enzyme added: 344 U (2 ml, pH 8.0), 20 °C, 6 h.

The immobilization of glucose isomerase was then examined by the following methods. A fixed amount of the anion IONEX with a water content of 4.0 in the Cl form was added to 2 ml of an extract solution (pH 8.0) of glucose isomerase. The mixture was shaken at 20 °C for 6 h. Then, the fiber to which the enzyme was adsorbed was washed five times with distilled water. The results of glucose isomerase immobilization are listed in Table 5.

Glucose isomerase was also readily adsorbed to the anion IONEX and the amount of the adsorbed enzyme was directly proportional to the amount of IONEX (up to 20 mg) under the conditions used. The yield of the activity was very high, that is, in the range from 75 to 80%. This shows that the enzyme is very stable, that the active site of the immobilized enzyme is not sterically hindered, and that the diffusion of glucose to the immobilized enzyme is not hampered.

Conclusion

1. The water content of the ion-exchange fiber as well as its surface area is very important for protein adsorption and enzyme immobilization. The adsorption capacity becomes much larger as the water content increases.

2. The immobilization of the enzymes by adsorption to the fiber gives high yields of the activity, that is, 40—50% for invertase and 75—80% for glucose isomerase.

3. The yield of the activity is improved as the water content of the fiber is increased.

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