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Sorption Characteristics of Anion-Exchange Resins Possessing a Spacer Arm for Bile Acids

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Polystyrene-based strong basic anion-exchange resins possessing a spacer arm, ω-oxoalkyl (–CO(CH_2)$_n$–) chain, were prepared and their sorption behavior for bile acids was studied. The spacer arm on the resins functioned effectively for the sorption of the bile acids through hydrophobic interaction. In the presence of diverse salts, the resin having a long spacer arm showed a high sorption capacity for bile acids as compared with Dowex 1-X2.

Keywords anion-exchange resin; spacer arm; bile acid; sorption isotherm; hydrophobic interaction; Dowex 1-X2

Previously, we reported the synthesis and properties of polystyrene-based anion-exchange resins that have a spacer arm between a quaternary ammonium group and the polymer matrix, and showed that the spacer arm plays an effective role in the sorption of some phenols bearing bulky alkyl groups.[1)] In this report, the role of the spacer arm on the ion-exchange resins in the sorption of bile acids is further explored.

Bile acids consist of a bulky hydrophobic steroid nucleus and an ionic group. The sorption of bile acids with an anion-exchange resin is significant pharmacologically, *i.e.*, cholestyramine resin, a strong basic anion-exchange resin which is chemically equivalent to Dowex 1-X2, has been used as an antihyperlipoproteic drug which acts to increase the fecal excretion of endogenous bile-salts.

We examined the sorption of bile-salts in various salt solutions with proposed anion-exchange resins having an ω-oxoalkyl spacer of various lengths by comparing their equilibration isotherms with those of Dowex 1-X2.

Experimental

Apparatus and Reagents A Hitachi U-3200 spectrophotometer was employed for the measurement of bile acids. A Hitachi 655A-12 liquid chromatograph equipped with a 2710-SA-IC column, L-3700 conductivity detector and D-2000 chromatointegrator was used for the determination of cyclohexanecarboxylic acid (CCA). The sodium salts of cholic acid (CA), deoxycholic acid (DCA), glycocholic acid (GCA) and taurocholic acid (TCA) were purchased from Sigma Chemical Co., Ltd. CCA was obtained from Tokyo Kasei Industrial Co., Ltd. Dowex 1-X2 used as a reference material was obtained from Muromachi Industrial Co., Ltd., and was washed with ethanol and then dried. Other chemicals were of reagent grade and were used without further purification.

Preparation of Resins I—III Polystyrene beads crosslinked with 2% divinylbenzene (gel-type, 35—60 mesh fraction) were prepared in our laboratory in the usual way and used as the starting material. Resins I—III were prepared by chemical modification of the beads, according to the procedure reported previously.[2)]

Characterization of Resins The total capacity, the water regain and the swelling ratio were determined by the same procedures as described previously.[1,2)]

Sorption Studies The bile acids were determined spectrophotometrically with 65% sulfuric acid.[3)] CCA was determined by nonsuppressed ion chromatography using 0.75 mM potassium biphthalate solution (pH 7.0) as an eluent at 40 °C. Unless otherwise stated, sorption experiments were carried out as follows: 20 mg of resin (Cl form) was placed in a 100 ml flask together with 50 ml of 1 mM sample solution (Na salt) without or with diverse salts, and mechanically shaken at 25 °C for 24 h. For the study of sorption isotherms, series of solutions of CCA, CA and DCA were prepared over the concentration range of 0.1—4.0 mM. An aliquot of each solution was pipetted off. After proper dilution, the concentration of each organic anion was determined by the above methods. The amount of organic anions sorbed on the resins was calculated from the difference between the initial concentration and the concentration in the solution after shaking.

Results and Discussion

Characterization of Resins Table I summarizes the analytical data and the physical properties of the resins shown in Fig. 1. Resin III and Dowex 1-X2 have the same chemical structure but different polymer matrices. The total capacity of each resin approximately corresponds to the nitrogen content determined by elemental analysis, and decreases with an increase in the spacer length. The volume-swelling ratios of the resins except for Dowex 1-X2 increase with a decrease in the total capacity and with a lengthening of the spacer arm. The values of water regain are not proportional to the amount of the functional group in the resins. Although the nitrogen content of resin II is considerably smaller than those of resin III and Dowex 1-X2 and furthermore the hydrophobic moiety of resin II apparently increases owing to the introduction of the spacer arm, resin II shows a relatively high water regain, comparable to that of Dowex 1-X2. The conflicting results of the water regain and the swelling ratio suggest that resin II is spatially capable of holding a larger amount of water of hydration around the functional group.

Sorption of Bile Acids The sorption behavior was ex-

Table I. Properties of Resins

Resin	N content (mmol/g)	Total capacity (meq/g)	Water regain (g/g)	Volume swelling ratio (ml/ml)
Resin I	2.46	2.46	1.373	1.577
Resin II	2.50	2.39	2.197	2.060
Resin III	2.95	2.94	1.518	1.267
Dowex 1-X2	3.60	3.31	2.237	1.878

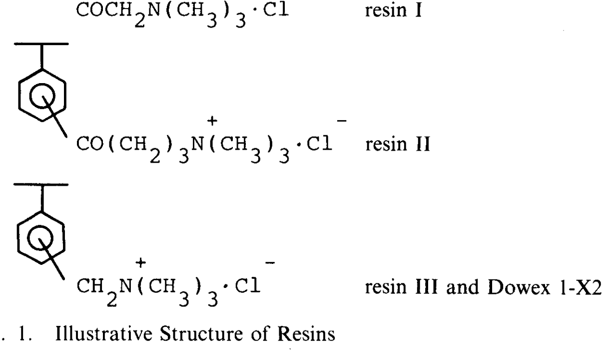

Fig. 1. Illustrative Structure of Resins

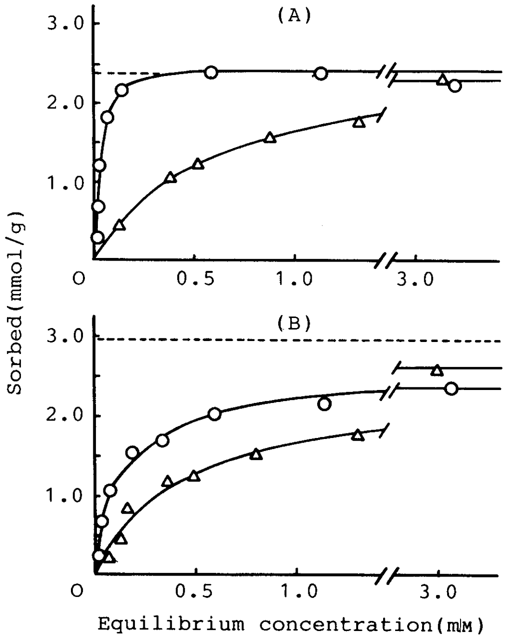

Fig. 2. Sorption Isotherms of Cholic Acid (CA) and Cyclohexanecarboxylic Acid (CCA) to Anion-Exchange Resins at 25 °C

(A), resin II; (B), resin III, ○, CA; △, CCA. The broken lines express the total capacity of each resin.

Table II. Langmuir Constants for Sorption of Organic Anions on Anion-Exchange Resins at 25 °C

Resin	CCA k_1 (l/mol $\times 10^4$)	CCA k_2 (mmol/g)	CA k_1 (l/mol $\times 10^4$)	CA k_2 (mmol/g)	DCA k_1 (l/mol $\times 10^4$)	DCA k_2 (mmol/g)
Resin I	0.143	2.60	1.290	1.82	3.610	2.10
Resin II	0.148	2.55	1.630	2.49	4.412	2.41
Resin III	0.150	3.00	1.086	2.29	3.353	2.41
Dowex 1-X2	0.136	3.68	0.905	3.01	3.490	3.12

CCA, cyclohexanecarboxylic acid; CA, cholic acid; DCA; deoxycholic acid.

Table III. Sorption of Bile Salts in the Presence of Diverse Salts

Sorbate	Resin	Sorbed (mmol/g) 0.05 M NaCl	0.05 M $NaHCO_3$	0.05 M CH_3COONa
CA	ResinII	1.12	1.23	1.40
	Dowex 1-X2	0.85	0.96	1.11
GCA	Resin II	1.08	1.32	1.42
	Dowex 1-X2	0.81	1.05	1.18
TCA	Resin II	1.33	1.44	1.57
	Dowex 1-X2	1.21	1.31	1.36
DCA	Resin II	1.55	1.74	1.99
	Dowex 1-X2	1.45	1.50	1.99

CA, cholic acid; GCA, glycocholic acid; TCA, taurocholic acid; DCA, deoxycholic acid.

plored in terms of the sorption isotherm. Figure 2 shows typical sorption isotherms of CA and CCA. All of the curves show a tendency to approach a plateau, and no sorption over the total capacity is observed. Under the experimental conditions, the starting material used for the preparation of resins I—III did not sorb these anions. Therefore, the mode of sorption of these organic ions is presumably a monomolecular exchange to a functional group of the resins.

The sorption isotherms were further examined by using the Langmuir-type equation,[4)]

$$\frac{C}{x/m}=\frac{C}{k_2}+\frac{1}{k_1k_2}$$

where C is the concentration of organic anion remaining in solution, x is the amount of organic anion sorbed on the resin, and m is the amount of resin employed. The constant k_1 is the association or affinity constant, which is related to the magnitude of the binding force. The capacity constant k_2 indicates the apparent maximum amount of organic anions that can be sorbed per gram of resin. All the sorption isotherms tested showed a good linear relationship between $C/(x/m)$ and C.

Table II shows the obtained Langmuir constants. With all resins, the affinity constants for CA and DCA possessing a steroid nucleus are larger than those for CCA, and the k_1 values for DCA having two hydroxyl groups in the steroid nucleus are larger than those for CA having three hydroxyl groups. Although the primary mode of interaction of CA and DCA with the resins is an electrostatic interaction between the anionic group of the bile-salt and a cationic functional group of the resins, these data clearly demonstrate the presence of an additional hydrophobic interaction of the steroid nucleus with the hydrophobic portion of the resins, including the spacer arm. William and Theodore[5)] investigated the mechanism of sorption of various bile-salt anions to Dowex 1-X2, and they have pointed out that the steroid nucleus plays an additional role in the sorption. Rudman and Kendall[6)] studied the binding of bile-salt derivatives to human serum albumin, and proposed a similar sorption mechanism. Moreover, based on the finding that the affinity constants for CA and DCA increase with an increase in the spacer length, it is suggested that the additional interaction is reinforced by the spacer arm. Although the capacity constants of resin II for the anions are similar, the constants of the other resins for CA and DCA are somewhat smaller than those observed for CCA. These phenomena may be dependent on the bulkiness of the steroid nucleus rather than the number of substituted hydroxyl groups.

It is well known that diverse salts strongly affect ion-exchange reactions. The sorption behavior of the proposed resin for bile-salt anions in the presence of diverse salts was studied, and the results are summarized in Table III. The values decrease consistently in the following order: $CH_3COONa > NaHCO_3 > NaCl$. This can be attributed to the inherent differences in the selectivity of each resin for the diverse salt anions. Although the total capacity of resin II is considerably smaller than that of Dowex 1-X2, resin II having a long spacer arm has a higher capacity for bile-salt anions than Dowex 1-X2. This characteristic behavior may be explained by assuming that the additional hydrophobic interaction between the steroid nucleus of the bile-salt anion and the hydrophobic portion, including the spacer arm, of the resins is reinforced by the added diverse salts, although the extent of reinforcement will depend on the nature of the added salts. Further experiments on pharmaceutical applications are planned.

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