Sorption Characteristics of Anion-Exchange Resins Possessing ω -Oxoalkyl or ω -Hydroxyalkyl Spacer for Bile Acids

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In order to evaluate anion-exchange resins possessing a spacer arm (ω -oxoalkyl($-\text{CO}(\text{CH}_2)_n-$) or ω -hydroxyalkyl ($-\text{CH}(\text{OH})-(\text{CH}_2)_n-$) between the quaternary ammonium group and the polymer backbone, sorption behavior for bile acids was investigated for three series of resins prepared from polystyrene beads having different crosslinkages. In the highly crosslinked resins, the resins having the spacer arms gave higher exchange capacities for bile acids than conventional-type resins did. The sorption was also affected by the length and chemical structure of the spacer arm. Thus, a spacer arm allows for more favorable sorption of sterically hindered anions.

Keywords Anion-exchange resin, spacer arm, sorption, bile acid

Recently, we reported the preparation of new anion-exchange resins that have spacer arms between the quaternary ammonium group and the polymer backbone¹, and which were applied to the sorption of sterically hindered phenols.² The resins prepared from low-crosslinked polystyrene bead showed a characteristic sorption behavior for bile acids.³

In order to clarify the effect of the spacer arm, considerations should be given to not only the spacer length but also the influence of the physical properties, such as crosslinkage, porosity and surface area, of the matrix resin, since ion-exchange sorption is also affected by physical properties.⁴⁻⁶ In the present paper, with three series of resins derived from polystyrene beads of different crosslinkages, the ion-exchange sorption of bile acids was further investigated. The effect of the chemical structure of the spacer arm was also studied.

Experimental

Apparatus and reagents

The surface properties of resins were characterized with a Monosorb (Iwaki Co., Ltd.) and a mercury porosimeter (type 220, Carlo Erba). Sodium dodecyl sulfate (SDS) was purchased from Nacalai Tesque Inc. Amberlyst A-26, used as a reference resin, was obtained from Organo Co., Ltd. All other apparatus and reagents were the same as those used in the previous paper.³

Resins I - XII

Resins I - IX were similar to those used in earlier

publications.^{2,3} The starting material for resins X - XII, polystyrene beads containing 15% divinylbenzene (DVB), was prepared by the conventional suspension polymerization method in the presence of isooctane as a porogen. Resins X - XII were also derived by the chemical modification of the beads, as previously described.¹ Unless otherwise noted, the particle size of the resins employed was 35 - 60 mesh fraction.

Sorption studies

The study of sorption isotherms and the determination of organic anions were performed by the same procedures as reported previously.³ SDS was determined by spectrophotometry with Methylene Blue.⁷ The sorption rate study was carried out using a 300 ml three-necked round bottom flask equipped with a stirring apparatus at 25°C. Resins IV and V clogged with a 35 mesh sieve (420 μm) were collected for this study. Two hundred and fifty milligrams of the strictly fractionated resins and a given volume of deionized water were placed into the flask, and allowed to stand for 1 h. Measured amounts of cholic acid stock solution were added to the reaction flask to achieve desired concentrations. One milliliter aliquots were withdrawn from the flask at appropriate time intervals. The samples were diluted, if necessary, and spectrophotometrically assayed.

Results and Discussion

Properties of the resins

The chemical structures of the resins used are represented diagrammatically in Fig. 1, and their analy-

Table 1 Properties of anion-exchange resins

Resin	N content ^a /mmol g ⁻¹	Total capacity ^b /meq g ⁻¹	S.S.A./m ² g ⁻¹	P.V./ml g ⁻¹	P.P.R./nm
Resin I	2.46	2.46	0	0	0
Resin II	2.50	2.39	0	0	0
Resin III	2.95	2.94	0	0	0
Dowex 1-X2	3.60	3.31	0	0	0
Resin IV	2.78	2.76	23.6	0.568	38.2
Resin V	1.75	1.68	28.8	0.579	31.3
Resin VI	1.68	1.41	24.2	0.486	30.6
Resin VII	1.60	1.34	26.7	0.406	20.7
Resin VIII	1.45	1.17	17.1	0.325	19.9
Resin IX	2.38	2.31	14.6	0.970	10.4
Amberlyst A-26	3.64	3.34	17.4	0.150	13.8
Resin X	2.88	2.14	38.1	0.757	35.9
Resin XI	1.58	1.56	34.4	0.723	30.9
Resin XII	2.28	2.14	48.3	0.743	30.9

a. Determined by elemental analysis. b. See ref. 2. S.S.A., specific surface area; P.V., pore volume; P.P.R., peak pore radius.

Resin I	P ₂ -COCH ₂ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin II	P ₂ -CO(CH ₂) ₃ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin III	P ₂ -CH ₂ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin IV	P _{7.5} -COCH ₂ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin V	P _{7.5} -CO(CH ₂) ₃ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin VI	P _{7.5} -CO(CH ₂) ₄ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin VII	P _{7.5} -CH(OH)(CH ₂) ₃ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin VIII	P _{7.5} -CH(OH)(CH ₂) ₄ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin IX	P _{7.5} -CH ₂ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin X	P ₁₅ -COCH ₂ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin XI	P ₁₅ -CO(CH ₂) ₄ -N ⁺ (CH ₃) ₃ · Cl ⁻
Resin XII	P ₁₅ -CH ₂ -N ⁺ (CH ₃) ₃ · Cl ⁻

Fig. 1 Schematic diagram of anion-exchange resins. P_x: polystyrene beads crosslinked with *x*(2, 7.5, 15)% divinylbenzene.

tical data and surface properties are summarized in Table 1. In the resin series (*i.e.*, 2, 7.5 and 15% DVB resins), the nitrogen content and the total exchange capacity decrease consistently with an increase in the length of the spacer arm. Resins I – III and Dowex 1-X2 are gel types, while the other resins are macroreticular types which possess characteristic porous structures. In 7.5% DVB resins, no important differences in specific surface area, pore volume and pore radius are observed for resins IV – VI that have ω -oxoalkyl spacers. Although the values of resins VII and VIII with ω -hydroxyalkyl spacers are slightly smaller than those of resins V and VI, no marked changes in surface properties are found on reducing the carbonyl group on the spacer arm. On the other hand, 15% DVB resins

possess greater specific surface area and pore volume than 7.5% DVB resins, which are largely reflected by the surface properties of the resin used as the starting material. The surface characteristics of resins X – XII are similar to each other. We attempted to prepare gel type 7.5% DVB resins which are chemically equivalent to the porous type. The intended resins could not be obtained, as the Friedel-Crafts reaction to introduce the spacer arm did not proceed.

Sorption studies

Typical sorption isotherms are shown in Fig. 2. Within the range of concentrations employed, the curves tend toward a plateau at high concentrations, indicating that the sorbed amount of the anions approaches the exchange capacity of the resins. The sorption isotherms were examined according to the following Langmuir-type equation:⁸

$$\frac{C_{eq}}{x/m} = \frac{C_{eq}}{k_2} + \frac{1}{k_1 k_2},$$

where C_{eq} is the concentration of organic anion remaining in solution, x is the amount of organic anion sorbed to the resin and m is the amount of resin employed. The constant k_1 is the affinity constant, which is related to the magnitude of the binding force. The capacity constant k_2 indicates the apparent maximum amount of organic anion that can be sorbed per gram of resin. Satisfactory linear relationships between $C_{eq}/(x/m)$ and C_{eq} were observed for all isotherms. The obtained Langmuir constants are listed in Table 2.

In the previous work with 2% DVB resins, we reported that bile acids were sorbed by an electrostatic interaction which occurred between the ionic groups involving an additional hydrophobic interaction of the steroid nucleus with the hydrophobic portion of those resins; the strength of the latter interaction was reinforced by increasing the spacer length. With 7.5

Table 2 Langmuir constants for sorption of organic anions to anion-exchange resins at 25°C

Resin	CCA		CA		DCA		SDS
	k_1	k_2	k_1	k_2	k_1	k_2	k_2
Resin I	0.143	2.60	1.290	1.82	3.610	2.10	2.58
Resin II	0.148	2.55	1.630	2.49	4.412	2.41	2.47
Resin III	0.150	3.00	1.086	2.29	3.353	2.41	3.06
Dowex 1-X2	0.136	3.68	0.905	3.01	3.491	3.12	3.72
Resin IV	0.283	0.83	1.344	1.05	5.095	1.11	2.47
Resin V	0.213	1.28	1.756	1.21	6.107	1.19	1.67
Resin VI	0.451	0.98	1.564	0.96	6.325	0.81	1.52
Resin VII	0.167	1.03	1.014	0.90	4.339	0.78	1.33
Resin VIII	0.175	1.09	0.965	0.77	4.843	0.60	1.20
Resin IX	0.255	0.90	0.574	0.37	1.647	0.23	1.45
Amberlyst A-26	0.329	1.08	0.826	1.41	2.733	1.71	1.68
Resin X	0.619	0.89	1.440	0.65	7.442	0.78	1.65
Resin XI	1.427	0.81	1.780	0.74	10.816	0.95	1.57
Resin XII	0.518	0.89	1.000	0.40	1.610	0.53	1.67

CCA, cyclohexanecarboxylic acid; CA, cholic acid; DCA, deoxycholic acid; SDS, sodium dodecyl sulfate. k_1 , affinity constant, $l\ mol^{-1} \times 10^4$; k_2 , capacity constant, $mmol\ g^{-1}$.

Fig. 2 Typical sorption isotherms of organic anions to anion-exchange resins at 25°C. (A), resin VI; (B), resin IX. ●, cholic acid; ■, deoxycholic acid; ▲, cyclohexanecarboxylic acid. The broken lines express the total capacity of each resin.

and 15% DVB resins, the affinity constants for bile acids are, as was true for 2% DVB resins, larger than those for CCA. Furthermore, the values increase with a decrease in the number of hydroxyl groups substituted in the steroid nucleus and with a lengthening of the spacer arm. It can be considered from the results that bile acids are sorbed to the resins by a mode of interaction similar to that mentioned above. In

addition, the affinity constants of resins VII and VIII for bile acids are smaller than those of resins V and VI, respectively. The conversion of the carbonyl group on the spacer arm into a more hydrophilic hydroxyl group results in decrease in the affinity for bile acids. Concurrently, this fact strongly suggests that not only the resin matrix but also the spacer arm plays a part in additional hydrophobic interactions.

Since 2% DVB resins possess a loosely crosslinked polystyrene matrix, it may be thought that bile acids are almost freely accessible to the functional groups on the resins. The capacity constants of resins I and III are somewhat smaller than their total capacities. However, such differences in k_2 values are not observed for SDS possessing a less bulky linear structure. A possible explanation for this fact is that bile acids can not easily be accessible to some functional groups which are in close proximity to each other due to the bulkiness of the steroid nucleus. However, the k_2 values of resin II bearing the long spacer arm are nearly equal to its total capacity. The capacity constants of conventional-type resin IX for bile acids decrease markedly in comparison with those of resin III. A predominant factor for such decreases is the limited accessibility of bile acids to some functional groups owing to the increasing crosslinkage of the matrix resins. Although the total capacities of resins IV–VIII are smaller than that of resin IX, resins IV–VIII possessing the spacer arm have higher capacities for bile acids than resin IX. A similar trend is also observed with more highly crosslinked 15% DVB resins. These favorable effects are ascribed to the spacer arm allowing the functional groups to protrude into some regions accessible to bile acids. The resins possessing the longest spacer arm, however, did not necessarily have the highest capacity constants. Taking into

Fig. 3 Sorption rate of cholic acid to anion-exchange resins under various conditions. (A), resin IV; (B), resin V. ●, agitation intensity 100 min^{-1} , initial concentration of cholic acid 2.0 mmol l^{-1} ; ▲, 60 min^{-1} , 2.0 mmol l^{-1} ; ■, 60 min^{-1} , 1.0 mmol l^{-1} . Reaction temperature, 25°C ; particle diameter of resin, $420 \mu\text{m}$. The broken lines express the exchange capacity for cholic acid of each resin.

account the substantial decrease in the total capacity with an increase in the spacer length, this sorption behavior may indicate the best compromise between the exchange capacity for bile acids tested here and the spacer length.

A sorption rate study was carried out to obtain more detailed information concerning the spacer effect. The amount of cholic acid sorbed to the resins was followed as a function of time with varying agitation intensities and concentrations of cholic acid solution; the obtained results are shown in Fig. 2. The half time required to reach the exchange capacity for cholic acid, $t_{1/2}$, which was estimated by interpolation from Fig. 3 as an index of the sorption rate, is summarized in Table 3 together with the reaction conditions.

The ion-exchange sorption rate, in general, is dependent on various factors, that is, exchange capacity for counter ions, particle diameter, degree of crosslinking and chemical structure of the exchange group.⁹ The exchange capacities of resins IV and V employed in this study for cholic acid are 0.98 and 0.95 mmol/g , respectively; these values are very close to each other. These two resins show almost the same values for the other factors mentioned above, except for the spacer

Table 3 Sorption rate of cholic acid to anion-exchange resins under various conditions

Resin	Agitation intensity/ min^{-1}	Concentration of cholic acid/ mmol l^{-1}	$t_{1/2}^a$ / min
Resin IV	100	2.0	29.5
	60	2.0	94.7
	60	1.0	119.0
Resin V	100	2.0	49.4
	60	2.0	117.8
	60	1.0	159.0

a. Half-time required to reach the exchange capacity for cholic acid.

length. The rate controlling step in this sorption process is presumably diffusion of cholic acid into the resin particles rather than the actual exchange reaction. As can be seen from Table 3, $t_{1/2}$ for resin V having the long spacer arm is delayed in comparison with that for resin IV having the short one, regardless of the reaction conditions. The observed differences in $t_{1/2}$ evidently suggest that cholates sorbable to resin V diffuse to the more inner parts of the resin. Thus, these facts reflect the conclusion that, as a result of the improved accessibility for counter ions, the functional groups which are introduced not only into easily accessible parts of the resin matrix but also into relatively inaccessible parts are effectively available for the sorption.

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