Synthesis of cationic polysaccharide derivatives and their hypocholesterolaemic capacity

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High-capacity bile acid-sequestrating biopolymers with cationic polysaccharide derivatives have been developed. Cholestyramine, the bile acid sequestrant most widely used for treating hypercholesterolaemia, has unpleasant side effects because of the hydrophobic nature of its backbone. Methylan, a high-viscosity polysaccharide produced by Methylobacterium organophilum, was selected as a model polysaccharide for derivatization. Methylan was aminated to add dialkyaminooalkyl and free amino groups at hydroxyl sites in the methylan backbone, and these derivatives were quaternized to produce pH-independent cationic polyelectrolytes. The in vitro bile acid-binding capacity of quaternized DEAE-methylan was ≈ 50% higher than that of cholestyramine. The bile acid-binding capacity of methylan derivatives increased with the number of carbons in the alkyl groups, indicating that hydrophobic interaction is a secondary factor for the sequestration of bile acids.

Introduction

Cardiovascular disease is still the leading cause of death and disability of adults in many countries, despite recent declines in coronary heart disease mortality rates. Cardiovascular disease accounts for more deaths annually than any other disease, including all forms of cancer combined [1]. In the United States, over 1 million heart attacks occur each year and more than half a million people die as a result. A direct relationship between the incidence of heart disease and hypercholesterolaemia has been established [2]. Elevation of the blood cholesterol level, or, more accurately, of the low-density-lipoprotein cholesterol level, is a major risk factor for the development of atherosclerosis in general and ischaemic heart disease in particular. It is now generally accepted that the incidence of coronary heart disease can be decreased by lowering the blood cholesterol level through diet and medication [3]. Several methods are presently used to reduce cholesterol levels. These include diet, exercise, ileal bypass or resection, nicotinic acid (commonly referred to as niacin), 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, such as lovastatin [4], and bile acid-sequestrating resins, such as cholestyramine [5] and colestipol [6,7].

For many years, bile acid sequestrants such as cholestyramine and colestipol have been considered the drugs of choice for patients with hypercholesterolaemia without hypertriglyceridaemia [8–10]. These sequestrants have been used extensively in long-term trials and are effective in lowering plasma cholesterol and reducing the risk of coronary heart disease. However, cholestyramine, the bile acid sequestrant used most widely for treating hypercholesterolaemia [11], has unpleasant side effects, including constipation, flatulence and abdominal pain, probably as a consequence of the hydrophobic nature of its backbone and the large doses required [5]. Some reports suggest that cholestyramine is linked to colon cancer in humans [12] and rats [13,14], although the mechanism for inducing cancer remains unclear. Recent histological studies indicate that cholestyramine feeding causes cellular disruption in the rat colon [15]. Therefore, it is important to develop new, high-capacity bile acid-sequestrating resins that are biocompatible, more palatable and non-toxic.

Products derived from natural polysaccharides play prominent roles in various industrial and biomedical applications. The need to constantly improve the properties of such products for specific end-uses, as well as growing interest in the fundamental relationship between the structure and properties of polysaccharides, have prompted the development of new chemical approaches for modifying natural biopolymers. There is interest in modifying polysaccharides chemically with a view to replacing hydroxyl groups with amino groups while maintaining the polymeric structure. Such a cationic polymer with a positive charge derived from

Key words: amination, bile acid sequestrant, hydrophobic interaction, methylan.

Abbreviations used: GC, glycocholate; GDC, glycodeoxycholate; GCDC, glycochenodeoxycholate; TC, taurocholate; TDC, taurodeoxycholate; TCDC, taurochenodeoxycholate; DME, dimethylaminoethyl; DMAEP, dimethylaminopropyl; DS, degrees of substitution; qDEAE-methylan, quaternized DEAE-methylan.

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Amino groups is expected to possess physical and chemical properties that would make it very useful. The utility of aminated polysaccharides, such as DEAE-cellulose and DEAE-Sephadex, is well appreciated, and they are widely applied.

Recently, *Methylobacterium organophilum* was found to accumulate methylan, a high-viscosity polysaccharide, extracellularly. It produces methylan using methanol as the sole carbon and energy source under specific culture conditions [16–19]. The sugar components of methylan were identified by GC as glucose/galactose/mannose in the ratio 2:3:2. Its molecular mass was estimated to be 2 × 10^5 Da by gel-permeation chromatography and light scattering [16]. Therefore, we modified methylan, with the possible expansion of the end-use of methylan as a secondary aim.

Since the ionic interaction between the bile acid anion and the sequestrants is important in the sequestration of bile acids, we introduced a permanent cationic charge into methylan by dialkylaminoalkylation and tosylation followed by reduction, and then quaternization. The binding kinetics and affinity of conjugated bile salts to the methylan derivatives were also investigated.

Materials and methods

Materials

Except when noted, all the chemicals were of analytical grade and redistilled if necessary. Methylan was produced and purified as in the literature [16–19] and its purity was confirmed by IR spectroscopy and gel-permeation chromatography. The cholestyramine resin, ammonium acetate, 2-(dimethylamino)ethylchloride, 2-(dimethylamino)isopropylchloride, 2-(diethylamino)ethylchloride, HCl, LiAlH₄, CH₃I, p-toluene sulphonyl chloride, NaN₃, NaCl, sodium glycodeoxycholate (GC), sodium glycochenodeoxycholate (GCDC), sodium glycochenodeoxycholate (GDC), sodium taurocholate (TCDC), sodium taurodeoxycholate (TDC), sodium taurochenodeoxycholate (TCDC) and potassium bicarbonate were purchased from Sigma. Water was distilled and deionized with a Millipore distiller. Anhydrous potassium phosphate monobasic (Sigma) and HPLC-grade acetonitrile (Merck) were used to quantify the bile acids by HPLC.

Dialkylaminoalkylation of methylan

Methylan was dialkylaminolylated using the method of Clifford and Naoyuki [20]. Aqueous 3.0 M dialkylaminoalkylchlorte-hydrochloride (15 ml) was added to methylan (0.3 g) with stirring at 65 °C. Pre-warmed 3.0 M NaOH (15 ml) was added dropwise to the reaction mixture. The reaction was stirred continuously with a magnetic stirrer for 18 h. Then, the mixture was diluted with 2 vol. of water and washed sequentially by ultrafiltration (molecular-mass cut-off 100000 Da) with 0.1 M aqueous HCl (100 ml), 0.0001 M aqueous HCl (100 ml), methanol, diethylether and water. The product was then freeze-dried to give the dialkylaminoalkyl methylan [DEAE-methylan, 0.45 g; dimethylaminooethyl (DMAE)-methylan, 0.32 g; dimethylaminoisopropyl (DMAIp)-methylan, 0.39 g].

Preparation of NH₂-methylan [21,22]

**Tosylmethylan**  Methylan (5 g) was dissolved in dry pyridine (100 ml) and shaken with p-toluenesulphonyl chloride (9.6 g) for 12 h. The mixture was poured into ice and methanol and agitated in a Waring blender. The resulting white powder was washed successively with water, methanol and ether and then subjected to ultrafiltration with an Amicon stirred cell (molecular-mass cut-off 100000 Da). The ultrafiltered product was freeze-dried to give tosyl-methylan (4.2 g).

**Azido-methylan**  Tosyl-methylan (2 g) was dissolved in N,N-dimethyformamide (60 ml). The solution was heated at 125 °C in an oil bath, and water (3.2 ml), urea (0.2 g) and sodium azide (1.65 g) were added. Heating was continued for 72 h under reflux at 125 °C in a stream of nitrogen. After cooling in ice water, the slightly brown resultant supernatant liquid was poured into ice water (250 ml) and stirred. The precipitate was collected by centrifugation, suspended in water, and dialysed against distilled water for 3 days. After ultrafiltration (molecular-mass cut-off 100000 Da), the concentrated product was freeze-dried to yield azido-methylan (1.5 g).

**NH₂-methylan**  Azido-methylan (1.3 g) was dissolved in dried tetrahydrofuran (100 ml) and to this solution a suspension of lithium aluminium hydride (0.5 g) in tetrahydrofuran (75 ml) was added carefully with shaking. The mixture was stirred for 18 h at room temperature and was then refluxed for 2 h in a water bath under protection from moisture. After cooling at 0 °C, water was carefully added to hydrolyse the excess lithium aluminium hydride. The solution was then diluted with an equal volume of water. Cold 1 M HCl (100 ml) was added to the cooled mixture, with stirring. The resultant white precipitate was collected by centrifugation, washed with water and then with a mixture of methanol/ether (1:2, v/v), and freeze-dried to yield NH₂-methylan (0.97 g). With IR spectroscopy, this product did not show any absorption by an azido group.

Quaternization of the aminated methylan

DEAE- and NH₂-methylan were quaternized (I° form) using the method of Chen and Benotion [23]. The dried resin (0.5 g) was added to a solution containing an excess of
potassium bicarbonate (3.75 g) in methanol (70 ml). Excess methyl iodide (3.72 ml) was added and the reaction mixture was stirred at room temperature in the absence of light for 1 week. Additional methyl iodide (2 ml) was added every few days. The reaction mixture was ultrafiltered (molecular-mass cut-off 100000 Da) with methanol and water, and dried under reduced pressure at room temperature for at least 24 h.

**Instrumental analyses**

$^1$H-NMR spectra of methylan derivatives were recorded on a Bruker AMX FT500 (magnetic field, 11.74 T; frequency, 500.1 MHz for $^1$H), and the chemical-shift values are given in $\delta$ (p.p.m.). IR spectra were obtained on a Bio-Rad FTS-60MN spectrometer and are reported in cm$^{-1}$. Elemental analysis of methylan derivatives to determine the degree of substitution was performed on a CE EA-1110 elemental analyser.

**Association rates of the bile acids to the methylan derivatives**

A solution of NaGC (5 mM, 50 ml) in aqueous NaCl (15 mM) was warmed to 37 °C in a 100 ml round-bottomed flask with stirring, and the sequestrants (50 mg) were added to the flask at time 0 min. Aliquots of the sequestrant slurry were removed from the flask at 2, 5, 10, 20, 30, 45, 60 and 90 min with syringes fitted with 0.2 $\mu$m filter units to collect 0.5 ml of solution, and filtered with a Centricon (molecular-mass cut-off 10000 Da; Amicon, Danvers, MA, U.S.A.). The amount of bile acid bound by the sequestrants at each point was calculated by subtracting the amount of free bile acid from the total bile acid in the flask (5.0 mmol/g).

**Equilibrium binding studies**

The design of the equilibrium binding studies included varying the concentrations of bile salts (1, 2.5, 5, 7.5 and 12.5 mM), resin (1 mg/ml) and NaCl (15 mM) to cover the range of substitution (DS) of 0.7 (1.85 mmol/g), 0.55 (1.42 mmol/g) and 0.46 (1.2 mmol/g), respectively. The DS of DEAE-methylan was obtained from the elemental analysis and the peak ratios between anomeric protons and the methyl protons of the DEAE groups using the $^1$H-NMR method [24]. In the IR spectra of DEAE-methylan (Figure 1), the absorptions at 2950 cm$^{-1}$ due to C=H stretching, at 1310–1380 cm$^{-1}$ due to C–N stretching and at 1000–1150 cm$^{-1}$ due to C–O–C stretching became stronger, supporting the occurrence of the substitution. Although the $^1$H-NMR spectra in $^2$H$_2$O were complicated, characteristic peaks were observed at $\delta$ 1.04 (m, CH$_3$ of free DEAE groups), 1.29–1.41 (m, CH$_3$ of protonated DEAE groups), 2.61 (m, CH$_2$ of DEAE groups), 3.13–4.03 (m, pyranose H) and 4.56 (br d, pyranose H at C-1). Substitution at C-6 was in accordance with Katsura et al. [24], and this was further confirmed by a model reaction with glucose.

**Preparation of dialkyaminoolkyl-methylan**

Methylan was dialkyaminoolkylated as shown in Scheme 1. The hydroxyl group was replaced by a dialkyaminoolkyl group, while the polymeric structure was maintained to give DEAE-, DMAE- and DMAiP-methylan with degrees of substitution (DS) of 0.7 (1.85 mmol/g), 0.55 (1.42 mmol/g) and 0.46 (1.2 mmol/g), respectively. The DS of DEAE-methylan was obtained from the elemental analysis and the peak ratios between anomeric protons and the methyl protons of the DEAE groups using the $^1$H-NMR method [24]. In the IR spectra of DEAE-methylan (Figure 1), the absorptions at 2950 cm$^{-1}$ due to C=H stretching, at 1310–1380 cm$^{-1}$ due to C–N stretching and at 1000–1150 cm$^{-1}$ due to C–O–C stretching became stronger, supporting the occurrence of the substitution. Although the $^1$H-NMR spectra in $^2$H$_2$O were complicated, characteristic peaks were observed at $\delta$ 1.04 (m, CH$_3$ of free DEAE groups), 1.29–1.41 (m, CH$_3$ of protonated DEAE groups), 2.61 (m, CH$_2$ of DEAE groups), 3.13–4.03 (m, pyranose H) and 4.56 (br d, pyranose H at C-1). Substitution at C-6 was in accordance with Katsura et al. [24], and this was further confirmed by a model reaction with glucose.

**Preparation of NH$_2$-methylan**

NH$_2$-methylan derivatives were prepared as shown in Scheme 2. Tosylation of methylan followed by azidation and then reduction afforded NH$_2$-methylan with a DS of 0.44 from the elemental analysis. IR spectra of methylan derivatives are shown in Figure 2. Tosylated methylan showed absorptions of aromatic C=C at 1732 and 1534 cm$^{-1}$ and S=O stretching vibrations at 1287 and 1176 cm$^{-1}$. Treat-
ment of tosyl-methylan with sodium azide afforded azidated methylan with a DS of 0.46. The IR spectrum of this product showed the characteristic absorption of an azido group (N≡N=) at 2112 cm⁻¹. Reduction of azido-methylan with LiAlH₄ afforded aminated methylan, which did not show any absorption of an azido group by IR spectroscopy. Although the difference observed in the IR spectrum caused by substitution with an NH₂ group was not remarkable, the product had the broad absorption expected of overlapping OH and NH₂ groups, and the absorptions at 1300–1250 cm⁻¹ due to C=N stretching and at 800–700 cm⁻¹ due to out-of-plane N-H wagging became stronger, supporting the occurrence of substitution. The hydroxyl group at C-6 was believed to be replaced by NH₂ as suggested by Whistler and Hirase [25].

Quaternization of the aminated methylan
The functional groups of DEAE-methylan and NH₂-methylan are protonated amine groups, whereas cholestyramine has quaternary ammonium groups. Considering electrostatic interactions, resins with quaternary amine groups have greater binding capacities than their protonated analogues [26,27]. Studies of quaternized colestipol showed that quaternization increases the in vitro bile acid-binding capacity [27]. Therefore, DEAE-methylan and NH₂-methylan were quaternized with methyl iodide.
Bile acid-sequestering polysaccharide derivatives

Figure 2 IR spectra of NH₂-methylan derivatives (KBr pellets)

A, Methylan; B, tosyl-methylan; C, azido-methylan; D, quaternized NH₂-methylan.

Figure 3 Association of glycocholate (NaGC) with the sequestrants

The sequestrants (1 mg/ml) were incubated in the presence of 5 mM NaGC in 15 mM aqueous NaCl at 37°C. •, Quaternized DEAE-methylan; ○, cholestyramine; ▼, quaternized NH₂-methylan; ▽, methylan. Each value represents the mean of triplicate measurements and varied from the mean by not more than 10%.

The completion of quaternization was verified by the ninhydrin test, which is used to detect unquaternized amino groups [28]. Peaks characteristic of quaternary amines between 1070 and 900 cm⁻¹ and at ≈ 3030 cm⁻¹ were observed by Fourier transform IR spectroscopy. These were absent in the unquaternized DEAE-methylan and NH₂-methylan. In the ¹H-NMR spectra, the quaternized DEAE-methylan showed characteristic peaks at δ 1.32–1.43, caused by the methyl protons of the quaternized DEAE groups.

In vitro bile acid-binding tendencies of the methylan derivatives

Rate of bile acid association A sequestrant entering the small intestine has a limited time to associate with bile acids. Therefore, the rate at which anion exchange occurs is important. This is determined by the time taken for the contents of the small intestine to travel as far as the terminal ileum. The total small intestine transit time for pharmaceutical dosages is 3±1 h [29]. Glycocholate associated with the sequestrants very rapidly, and the binding was essentially complete within 15 min (Figure 3). From these association kinetics, we predict that bile acids associate with methylan derivatives rapidly enough that their sequestering potency is not limited by the association rate.

Bile acid binding to methylan derivatives All the adsorption experiments were conducted in 15 mM aqueous NaCl solution. Under these conditions, the pH of the system (pH 5.4–5.6) after equilibration with the sequestrants (1 mg/ml) was essentially independent of the initial adsorbate concentration. Using reported pKₐ values (4.35 for glycocholate and 3.33 for taurocholate [30]), it has been reported [26] that the salts of the bile acids used in this study are primarily present in the ionized form at concentrations below their respective critical micelle concentrations (10.0 mM [31] for NaGC and 1.5–3.5 mM [32] for NaTC). The binding of bile acids to the sequestrants was measured after a 1 h incubation period at concentrations of 1.0–12.5 mM. With an initial bile acid concentration of 5 mM, NaGC reached equilibrium with the sequestrants within 10 min, indicating that the binding kinetics were sufficient for sequestration.

The adsorption isotherms for the binding of bile salt anions to DEAE-methylan derivatives are shown in Figure 4. DEAE-methylan has a greater binding capacity for NaGC than underivatized methylan. At bile salt concentrations > 5.0 mM, the binding capacity of DEAE-methylan is approximately three times that of methylan. Quaternized DEAE-methylan (qDEAE-methylan) bound from 28 to 81% of the NaGC when the bile acid concentration was in the physiological range (1.0–12.5 mM). On a dry-weight basis, the NaGC binding capacities of methylan, DEAE-methylan, qDEAE-methylan and cholestyramine were 0.65, 2.54, 3.46 and 2.63 mmol/g of resin, respectively (Figure 4A). The quaternization of the DEAE group increased the binding
The sequestrants (1 mg/ml) were incubated in the presence of bile salts in 15 mM aqueous NaCl at 37 °C. (A) NaGC; (B) NaTC. Quaternized DEAE-methylan; ▽, cholestyramine; ▼, DEAE-methylan; ▽, methylan. Each value represents the mean of triplicate measurements and varied from the mean by not more than 10%.

The bile acid-sequestering capacity of qDEAE-methylan was about 32% greater than that of cholestyramine, the most commonly used cholesterol-lowering agent [11]. The bile acid-binding capacity of the methylan derivatives increased as the electrostatic interaction was increased via amination, indicating that the ionic interaction between the cationic ammonium group and the bile carboxylate anion is a major factor in determining the bile acid-binding capacity of the sequestrants. In the same manner, the binding capacity for NaTC, another major bile salt, increased with diethylaminoethylation and quaternization of the DEAE group. The binding capacities of methylan, DEAE-methylan, qDEAE-methylan and cholestyramine were 0.62, 2.65, 3.6 and 2.98 mmol of NaTC/g of resin, respectively (Figure 4B).

The bile acid-binding capacity increased by ≈ 80% with reductive amination followed by quaternization, again indicating that ionic interactions are important. On a dry-weight basis, the NaGC- and NaTC-binding capacities of NH₂-methylan, quaternized NH₂-methylan and cholestyramine were 1.02, 1.81 and 2.63 mmol of NaGC/g of resin (Figure 5A), and 1.48, 2.27 and 2.98 mmol of NaTC/g of resin (Figure 5B), respectively. The adsorption isotherms (Figures 4 and 5) describing the binding of the bile anions to the methylan derivatives reached a plateau at high bile acid concentrations, indicating that the system was approaching the limiting unimolecular exchange capacity of the sequestrants for the bile salt anion. Aminated methylan derivatives had a relatively low binding capacity compared with the diethylaminoethylated methylan derivatives. The results support the hypothesis that the cyclopentanophenanthrene or steroid nucleus of the bile acid molecule interacts more strongly with the more hydrophobic diethylaminoethylated methylan than with its aminated counterparts.

Hydrophobic interactions between bile acid anions and sequestrants Figure 6 shows the adsorption isotherms for the
binding of glycocholate anion to methylan derivatives. The bile acid-sequestering capacity increased with the number of carbons in the alkyl group in the order methylan < NH₂-methylan < DMAE-methylan < DMAiP-methylan < DEAE-methylan. When the DS of methylan derivatives was set at 0.7, the glycocholate-binding capacities of methylan, NH₂-methylan, DMAE-methylan, DMAiP-methylan and DEAE-methylan at an initial glycocholate concentration of 12.5 mM were 0.65, 1.62, 2.05, 2.28 and 2.54 mmol/g of resin, respectively. These results indicate that the number of carbons in the alkyl group affects the bile acid-sequestering capacity of dialkylaminoalkyl methylan derivatives. In other words, the bile acid-sequestering capacity of the derivatives increases with their hydrophobicity. The binding capacity of cholestyramine for bile acid anions decreases as the number of hydroxy substituents on the steroid skeleton increases [33,34], confirming the importance of hydrophobic interactions. The hydrophobic interactions may induce the aggregation of organic anions on the polymer surface [35]. Although the electrostatic ionic interaction between the negatively charged carboxyl groups of the bile acid anions and the positively charged amino groups of the sequestrants is the major factor affecting the sequestration of bile acids, the experimental results clearly show the existence of secondary binding forces consisting of hydrophobic interactions between the hydrophobic regions of the adsorbate (bile acid) and the adsorbent (sequestrant) molecules.

Sequestering preference for individual bile acids in a mixture simulating the intestinal tract

Accurate quantitative and qualitative determination of bile acids after prescription of the resin is a very important prognostic indicator of liver and gastrointestinal tract disease in humans and other mammals, since hepatobiliary and intestinal dysfunction are marked by variation in the concentration and relative proportions of the major bile acids and by increased levels of the minor bile acids. It should be noted that relating in vitro binding results to in vivo bile acid-sequestrant data is questionable due to the complexity of this bile acid-sequestrant problem. The resin must bind bile acids under highly variable and dynamic luminal conditions. Many bile acids are present, each differs in its propensity to bind with the resin and various anions are also present in the lumen (e.g. chloride, phosphate, bicarbonate, etc.). In order to better understand the in vivo performance of the methylan derivatives, the preferential sequestration of individual bile acids in a mixture simulating the intestinal tract was examined.

Although human bile contains small amounts of unconjugated bile acids, most are in the form of taurine and glycine conjugates. The ratio of taurine to glycine conjugates is ≈ 1:3, and the cholic/deoxycholic/chenodeoxycholic acid proportions are ≈ 1:1.0:0.6.1 [36]. The concentration of bile acids usually exceeds 30 mM in gall-bladder bile and is about 10 mM in the upper intestinal contents of fasting subjects. After a test meal, the lower normal limit of the bile acid concentration in the duodenal or jejunal contents is about 4 mM [37,38]. To examine the preferential sequestration of individual bile acids in a mixture, synthetic bile acid mixtures that simulated the composition in the intestinal tract were prepared. Their compositions (I–V) are shown in Table 1.

HPLC separation of mixtures of bile acids differing in the number and position of hydroxyl groups was executed on a YMC-Pack ODS-AQ column with 40% aqueous acetonitrile in 0.05 M KH₂PO₄ as the mobile phase. Other columns, such as Ultremex ODS-2, Sphereclone ODS-2 and Inertsil ODS-3, showed poor resolution. Figure 7 shows the separation of a mixture of six major bile salts: NaGC, NaTC, NaGCDC, NaTCDC, NaGDC and NaTDC. Of particular interest was the feasibility of resolving the amino acid conjugates of bile acids, which are isomeric and differ in their extent of

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Table I  Composition of synthetic mixtures of bile acids simulating intestinal tract

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Figure 7  High-performance liquid chromatogram of a synthetic mixture of bile salts

Mobile phase: 0.05 M KH$_2$PO$_4$ in acetonitrile/water (40/60); column, YMC ODS AQ; detection, UV 200 nm; flow rate, 0.4 ml/min. Peak identification: 1, TC; 2, GC; 3, TCDC; 4, GCDC; 5, TDC; 6, GDC.

Figure 8  Bile acid binding in a mixture simulating gastrointestinal tract

qDEAE-methylan (1 mg/ml) was incubated in the respective mixtures at 37 °C. ○, GDC; ▽, TDC; ▼, GCDC; △, TCDC; ■, GC; □, TC. Each value represents the mean of triplicate measurements and varied from the mean by no more than 10%.

hydroxyl substitution. Figure 8 shows the adsorption isotherms for the binding of bile acid anions in a mixture simulating intestinal tract to qDEAE-methylan. Bile acid anions were bound to the resin in the order TDC > GDC > GCDC > TCDC > GC > TC in terms of the ratio of bound to initial bile acids (Figure 8). With the initial bile acid concentrations of mixture III, the ratios of bound to initial TDC, GDC, GCDC, TCDC, GC and TC were 0.44, 0.39, 0.34, 0.31, 0.19 and 0.13, respectively. A large proportion of deoxycholic acid (GDC and TDC) was bound to the resin at physiological concentration; there is usually much less deoxycholic acid in the intestinal tract than chenodeoxycholic acid and cholic acid. Its binding affinity to the resin was higher than that of the other major bile acids.

Bile acid, especially deoxycholic acid, has long been suspected of playing a role in human carcinogenesis, since deoxycholic acid can be converted chemically into the very potent carcinogenic 20-methylcholanthrene via dehydronorcholeene [39]. Intestinal microflora might perform a similar conversion, since intestinal bacteria can convert dehydronorcholeene, which is not carcinogenic, into a carcinogenic metabolite [39,40]. In addition to their hypocholesterolaemic action, cationic methylan derivatives may help decrease the incidence of colon cancer by lowering the level of carcinogenic deoxycholic acid. These results will give better insight into the interpretation of the performance of cationic methylan derivatives and their effect on the liver and intestine after intake.

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