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Study of lipoprotein sorption by some sulfoderivatives of chitosan

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The possibility of deposition of lipoproteins from blood plasma by different chitosan sulfoderivatives has been demonstrated. The influence of structure, substitution degree and molecular weight of chitosan sulfates on lipoprotein sorption was studied as well as the influence of composition of chitosan interpolymer complexes with dextransulfate. Affinic sorbents based on the silica matrixes and sulfoderivatives of chitosan as a ligand can reveal high specificity in relation to low density lipoprotein, but do not cause essential changes in blood count during contact with blood. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In recent years the increasing interest in chitosan and its derivatives has been accounted for by the possibility of expanding its practical application as a biospecific sorbent and a biologically active compound (Muzzarelli, 1983; Olsen *et al.*, 1989).

It is well known that sulfopolysaccharides (SPS) (heparin (Hep), dextransulfates (DS) and others) form biospecific complexes with low density lipoproteins (LDL) (Bernfeld *et al.*, 1960; Cornwell & Kruger, 1961; Nishida & Cogan, 1970). The high level of LDL in the organism leads to atherosclerosis development, coronary heart disease, familial hypercholesterolaemia, etc. (Lopukhin & Molodenkov, 1985). In this connection chitosan sulfates as heparin analogs possessing a anticoagulating activity are of great interest. Thus, it would be interesting to investigate chitosan sulfoderivatives as affinic ligands for the production of sorbents eliminating atherogenic lipoproteins.

EXPERIMENTAL

Materials

Chitosan (Cht) was obtained by deacetylation of chitin with 50% NaOH at 140 + 2°C, for 1 h, in a flow of argon. The intrinsic viscosity of the product was $[\eta] = 4.0$ dl/g (or $\bar{M}_v = 178\,000$ Da) and the deacetylation degree was 87%. Chitosan-O-sulfate was obtained accordingly (Batura *et al.*, 1981); N-succinylated chitosan sulfate was derived by the interaction of chitosan-O-sulfate with succinic anhydride. Chitosan-N-sulfosuccinate was synthesized as reported in Rogozhin *et al.* (1985).

Chitosan sulfoderivatives with different molecular weights were obtained from chitosan samples that had been hydrolyzed by hydrochloric acid in the range 0.2–1.1 M upon boiling (100°C) in argon (Rogozhin *et al.*, 1988).

Interpolymer complexes (IPC) of chitosan hydrochloride (ChtCl) ($[\eta] = 0.57$ dl/g, or $\bar{M}_v = 18\,000$ Da) and sodium DS (NaDS) (Farmacia Fine Chemicals, Sweden) with molecular weight 500 000 Da were prepared by mixing equal volumes in 4 M water–urea at molar ratio ChtCl:NaDS = 1:10 and exposing the mixture to pH 8.0–8.5 followed by dialysis and lyophilization (Gamzazade & Nasibov, 1994a).

The sorbents were prepared by the immobilization of chitosan sulfoderivatives on the previously aminated (or epoxidated) surface of macroporous silica matrixes (Gamzazade & Nasibov, 1994b, c). As a support we utilized silochrome (SCh, $S_{sp} = 25\,m^2/g$, $d = 180$ nm, granule diameter 0.2–0.4 mm) and macroporous glass (MPG, $S_{sp} = 40\,m^2/g$, $d = 130$ nm).

Method

In vitro sorbent testing was carried out in either a static (0.1 g of sorbent incubated with 2 ml of plasma) or a

dynamic regime (perfusion of 6 ml of plasma or 10 ml of blood through a column of 1 ml with a flow rate of 0.3 ml/min for blood).

The technique of LDL precipitation from plasma was as follows: 2 ml of 30 mM $CaCl_2$ and 0.4 ml of 1% (w/v) solution of chitosan sulfate in saline were added to 0.2 ml of plasma in succession; after 10 min the mixture was centrifuged at 3.000×*g* for 30 min.

The cholesterol concentration in the lipoproteins was estimated using an autoanalyser (Centrifichem, USA) and the blood count was evaluated using the Picoscale (Hungary).

RESULTS AND DISCUSSION

The complex-forming ability of DS in relation to LDL depends on a number of factors, particularly on the molecular weight and sulfating degree of the biopolymers (Cornwell & Kruger, 1961). However, the influence of structure or conformation conversion of DS and other SPS on their sorption ability has not been studied in detail. To achieve a better understanding of the problem we investigated the sorption ability of chitosan sulfates and their IPC with DS depending on their structure or composition (Table 1).

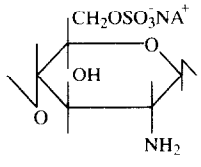
The investigation of the interaction between different structure chitosan sulfates and LDL in solution showed that almost all the substances mentioned above efficiently precipitated blood plasma lipoproteins. It was observed that a decrease in the molecular weight of chitosan sulfates containing a carboxylic group brought about an enhancement of their complex-forming ability (Table 2).

The sample SH-3 is of great interest among the chitosan sulfates investigated because it demonstrates a high complexing ability in spite of low sulfur content (6.5) and high molecular weight. This fact is obviously connected with the specific structure of this derivative. Unlike SH-2, the carboxy groups of SH-3 are located

Table 1. Structure and characteristics of chitosan sulfoderivatives

Run no.	Type of compound (repeated unit)	Symbol	Content (%)			Substitution degree of amino groups
			Sulfur	AmineN	TotalN	
1	Chitosan-O-sulfate (a)	SH-1	16.6	3.2	3.7	14
2	N-succinylated chitosan O-sulfate (b)	SH-2	10.4	0.7	2.5	74
3	Chitosan N-succinyl sulfate (c)	SH-3	6.5	0.8	4.6	83
4	Interpolymer complex	IPC	12.8	—	1.9	50

(a)



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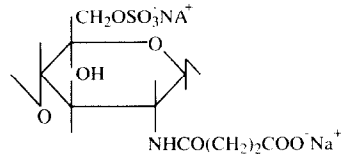

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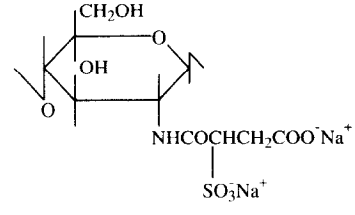

Table 2. LDL–chitosan sulfates interaction

Run no.	Sulfated polysaccharide	Molecular weight ($\bar{M}_v \times 10^{-3}$)	Sulfur content (%)	Precipitation efficiency* (%)
1	SH-1	120	16.6	82.3
2	SH-2	350	10.4	71.4
3	SH-2	80	10.8	85.5
4	SH-2	45	10.0	86.4
5	SH-2	25	10.6	88.6
6	SH-3	270	6.5	82.3
7	DS	500	17.4	92.5

*Concentration of total cholesterol = 229 mg/dl.

Table 3. LDL–IPC interaction

Composite IPC (DS):(Cht) (mole/l)	Concentration of cholesterol in precipitate (mg/dl)	Concentration of cholesterol in supernatant (mg/dl)
3:1	80.0	117.7
6:1	157.2	37.2
9:1	119.0	78.6
DS (in water–urea solution)	132.4	62.0
DS (in water solution)	142.6	53.8
Phosphotungstic acid	—	58.5

Table 4. Sorption characteristics of sorbents with immobilized sulfopolysaccharides

Run no.	Carrier	Ligand	Intrinsic viscosity (dl/g)	Sulfur content (%)	Removal efficiency* (%)	
					Static	Dynamic
1	SCh	SH-1	—	16.6	42	53
2	SCh	SH-2	0.68	10.4	31	60
3	SCh	SH-3	1.50	6.5	36	53
4	MPG	SH-3	1.50	6.5	48	57
5	SCh	IPC	1.24	12.8	51	70
6	SCh	DS	1.80	17.4	36	60
7	MPG	DS	1.80	17.4	55	—
8	SCh	Hep	0.17	13.6	45	66
9	MPG	Hep	0.17	13.6	50	—

*Concentration of total cholesterol = 157.2 mg/dl.

Table 5. Effect of sorbents on blood count and plasma calcium level

Sorbent	Leukocytes $10^3/\mu l \pm 0.5$	Thrombocytes $10^3/\mu l \pm 20$	Erytrocytes $10^6/\mu l \pm 0.5$	Calcium (mmol/l) $10^6/\mu l \pm 0.5$
SCh–Hep	8.2	480	4.3	1.5
SCh–DS	8.0	470	4.4	1.3
SCh–SH-3	8.2	465	4.3	1.9
Control	8.1	520	4.6	2.1

close to its sulfo groups and can form a chelating structure.

The investigation of interaction between IPC and LDL in solution demonstrated that an alteration in IPC composition resulted in the changed complexing ability of polycomplexes (Table 3). This observation can be explained by the conformational features of IPC particles of different composition. In fact, the increasing chitosan portion in the polycomplex leads to electrostatic interaction of chitosan with DS; the latter's macromolecules straighten and the conformation of IPC particles probably alters as a result. The most favourable conditions for LDL interaction with IPC particles evidently are realized at the specific neutralization degree of DS–sulfate groups that corresponds to the IPC:component ratio (in base mol/l), (DS):(Cht) = 6.

It was interesting to compare the results cited above, obtained during LDL–IPC interaction in solution, with the sorption characteristics of sorbents based on silica matrixes with immobilized chitosan sulfoderivatives. As can be seen from Table 4 the sorption capacity of sorbents based on silica matrixes with immobilized chitosan sulfoderivatives is as high as that of sorbents with immobilized DS or heparin. These data, particularly the findings obtained while studying LDL sorption in the dynamic regime, show good correlation with results given in Table 2 for the same chitosan sulfates interacting with LDL in solution.

Such comparative data fully correlate in the case of sorbents with immobilized IPC of different composition (Table 3). The high LDL sorption is observed on the sorbent with immobilized IPC at the component ratio (DS):(Cht) = 6. Such correlation indirectly shows that the features of matrix itself and the method of ligand immobilization do not essentially influence the ligand conformation. This can allow us to evaluate the ligand contribution to the sorption capacity of a sorbent in its net form.

Sorbent prepared by immobilization of sulfated chitosan SH-3 on the silochrome surface demonstrated

sufficiently high capacity characteristics and did not affect the blood count any more than sorbents modified by athrombogeneic substances such as heparin or DS (Table 5). At the same time the calcium ion sorption from blood plasma on this sorbent is minimal in comparison with other sorbents modified with conventional athrombogeneic substances. These data are of great importance for practical hemosorption. The results obtained are of great interest due to the fact that conventional sorbents based on activated coals for 60 min of hemoperfusion can lower the level of blood leukocytes and thrombocytes by 60% and 43%, respectively, and adsorb serum proteins, etc. (Lopukhin & Molodenkov, 1985).

Thus, the possibility that affinic sorbents can be synthesized by the immobilization of chitosan sulfoderivatives on silica matrixes, as well as their functioning in contact with blood during hemosorption, has been shown in this work.

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