HYPOCHOLESTEROLEMIC EFFECT OF AL^{3+} COMPLEXES

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SUMMARY

We are proposing a simple model by which acidic polysaccharides of the critical charge density 1 minus/1 carbohydrate unit can be converted into anion exchangers by complexing with a trivalent cation. We have constructed a complex of alginic acid and Al^{3+} which was shown to bind nucleotides. It was assumed that bile acids would also be bound and, thus, Al- alginate would be hypocholesterolemic in vivo. The feeding of Al- alginate to rats which were fed a high cholesterol diet produced a marked decrease of serum cholesterol. It is proposed that Al^{3+} may have a nutritional role if present in conjunction with acidic polysaccharides. Ternary complexes involving lipids may exist in many biological structures.

INTRODUCTION

Because of its putative linkage to atherosclerosis, the lowering of serum cholesterol levels by dietary manipulation has become a public concern. There is a considerable confusion as to the rationale of why some plant mucilagineous polysaccharides are effective in lowering serum cholesterol in rats while others are not (1). The great differences between pectin preparations from protopectin to pectic acid, alginate (1), gum arabic, carageenan (2) etc. cannot be explained by viscosity differences (3) alone. Little attention was paid to the other common denominator of these products, i.e., they are polyanionic. The mechanism of action seems to involve increased bile salt excretion (4) and it is not obvious how and why a bile salt anion should bind to a polyanion. The most powerful means of lowering serum cholesterol is the binding of its anionic metabolites to a nondigestible synthetic anion exchanger such as cholestyramine (5). No materials of significant anion exchanger capacity have been isolated from plants. Here we report on the development of a simple principle by which some polyanionic materials could be converted to polycations whereby they be-

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come capable of binding a variety of anions. We shall demonstrate the effectiveness of our model to function *in vitro* as an anion exchanger. We shall also present preliminary data which suggest that our theoretical model may be a basis of regulating cholesterol levels *in vivo*.

RATIONALE

The formation of a strong complex between polycarboxylic acids and polyvalent cations is known to occur when the electric charges are suitably distanced and oriented. The neighboring carboxylate groups in polyuronic acids are capable of forming coordination complexes with di- and trivalent metal ions (6,7) which have the appearance of an insoluble gel. It is likely for steric reasons that the positive charge of most Al^{3+} ions is not neutralized by the polyanion, and the free valencies are available to bind external anions. Thus a cation exchanger can be converted to an anion exchanger albeit with a loss of binding efficiency. Of particular interest to us was the binding of phosphate esters and bile acids. While the interaction of a single anion with the Al^{3+} -polyanion complex is relatively inefficient, the binding of large negatively charged structures such as lipid micelles would be very tight and actually require very little Al^{3+}. The model structure of a polyanion-Al^{3+} -micelle complex is depicted in Fig. 1.

MATERIALS AND METHODS

The aluminum salt of pectin (3.3% Al) was donated by Sunkist Growers, Inc. through the courtesy of Dr. D. B. Nelson. Citrus pectin, N. F. (6% CH_3O), alginic acid, sodium taurocholate and bentonite were purchased from Sigma. The Al-pectinate was kept 12 hrs in water for swelling, and the resulting soft gel was used for the diets. Al- alginate was freshly prepared initially but the same results were obtained with the less laborious mixing of the solid alginic acid with aluminum acetate. Aluminum was determined by atomic absorption spectroscopy (8), the uronates were quantitated by the carbazol reaction (9). Total serum cholesterol determination was done by a variation of the Lieberman-Burchard reaction (10). Nucleotides were measured by UV spectroscopy.

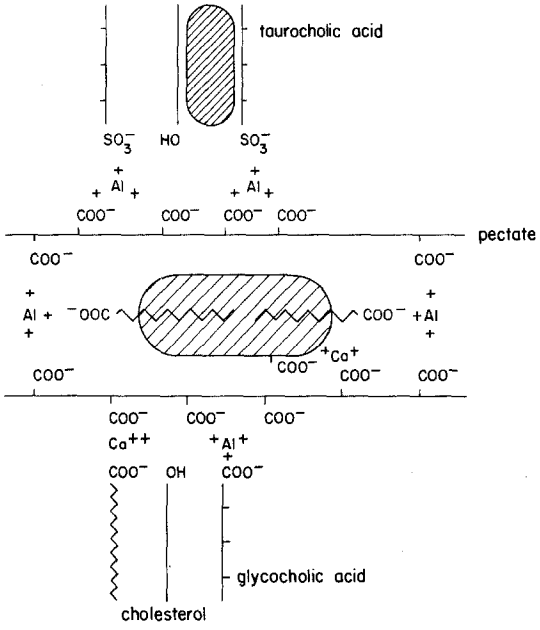

Figure 1. Hypothetical structure of a polyuronate-Al^{3+}-micelle complex.

The basic diet contained per Kg: 200 g beef lard, 220 g casein, 509 g sucrose, 30 g vitamin mix, 30 g salt mix USP XVII, 10 g cholesterol and 1 g bile salts, all from ICN Pharmaceuticals with the exception of locally produced lard and sugar. This basic high-fat, high-cholesterol diet was diluted with 8% non-digestible fiber, one half of which was the test substance, the other half was cellulose. The following test diets were then established according to fiber and Al content. Diet 1, cellulose only (Al < 20 ppm); diet 2, 4% alginic acid - Al < 20 ppm; diet 3, 4% alginic - 0.02% Al; diet 4, 4% alginic acid - 0.2% Al; diet 5, 4% alginic acid - 0.3% Al. Also fed were diet 6 with 4% citrus pectin -20 ppm Al, diet 7 with 4% pectin - 0.13% Al, and diet 8 with 4% bentonite - 0.07% Al.

Sprague-Dawley retired breeder, male rats weighing approximately 500 g each were randomized in groups of eight; they were weighed before and after the 2 weeks feeding period. The rats were starved 2 hr. before killing by decapitation, whereupon their blood and livers were collected.

RESULTS AND DISCUSSION

The theoretical prediction that Al^{3+} salts of some acidic polysaccharides behave as anion exchangers was first verified in vitro by determining the binding of the 5' nucleotides AMP, GMP, CMP and UMP to Al- alginate (6% Al).

Ten μmole-quantities of these nucleotides were incubated with 25 mg portions of alginate containing 50 μmoles of Al^{3+} in 5 ml water, the pH adjusted to 4.8, for 5 hr. Concentrations of the supernatants were determined by UV, and the percentage of bound nucleotides were calculated to be 66, 61, 55 and 20% for AMP, GMP, CMP and UMP, respectively. At the acidic pH chosen, it is unlikely that a significant part of the nucleotides was removed from the solution as a precipitate. The formation of a true ternary complex is indicated by the following observations. Under the above conditions only 10 percent of Al^{3+} and about one half of the nucleotides are interacting. The interaction seems to involve the base moieties in AMP, GMP and CMP and this may account for the stronger retention of these nucleotides over UMP. Upon washing the gel with 0.2 M NH_4OOCCH_3, the nucleotides are released as expected from an ion exchanger.

The ternary complex of Al-pectinate with a lipid combination consisting of ox bile and triolein had the form of a gummy mass and precise ways of its characterization have yet to be found.

The dietary experiments in rats provided results which support our original assumption concerning the physiological relevance of the polyanion-Al^{3+}-lipid complex. As expected (1), the feeding of alginic acid along with high fat-cholesterol diet has slightly elevated serum cholesterol (Table 1) over that found with the same diet in which cellulose was substituted for alginic acid. The high cholesterol level could be substantially lowered even by a small amount of aluminum. There was no significant difference when pre-made Al-alginate was fed or the alginic acid and Al-acetate were individually mixed into the diet. While coordination complexes most likely exist at the acidity of the stomach, the ionic bonds should be promptly established in the small intestines. Under our experimental conditions, the actions of pectin and Al-pectinate were in-

TABLE I

The Effect of Al^{3+} on Serum Cholesterol of Rats

Diet	Fiber	Aluminum	Weight Gain grams ± S. D.	Serum Cholesterol mg/100ml. ± S. D.
1	8% Cellulose	<20ppm	42 ± 13	*371 ± 36
2	4% Alginic Acid 4% Cellulose	<20ppm	55 ± 17	ǂ398 ± 49
3	4% Alginic Acid 4% Cellulose	0.02%	52 ± 14	ǂ280 ± 24
4	4% Alginic Acid 4% Cellulose	0.2%	36 ± 19	ǂ261 ± 30
5	4% Alginic Acid 4% Cellulose	0.3%	42 ± 19	*247 ± 29
6	4% Pectin 4% Cellulose	20ppm	27 ± 9	*103 ± 16
7	4% Pectin 4% Cellulose	0.13%	25 ± 11	ǂ 92 ± 9
8	4% Bentonite 4% Cellulose	0.07%	44 ± 8	*402 ± 59

ǂ Groups of eight rats
* Groups of six rats

Data obtained for diets 3,4,5,6 and 7 are significant at $p < 0.01$ compared to control diet 1. Diets 2 and 8 have no effect in lowering the serum cholesterol level.

High fat-high cholesterol diet containing variable non-digestible fiber and Al^{3+} were fed to Sprague-Dawley adult male rats for 2 weeks. Total serum cholesterol was determined according to Huang et al. (10).

distinguishable both being close to the normal range observed on low fat diet (60-80 mg/100 ml). The numerous OCH_3 groups in pectin may provide adequate hydrophobic binding sites. However, to the extent that these ester linkages may be hydrolyzed by nonspecific esterases, the addition of Al^{3+} may potentiate the hypocholesterolemic action of pectin in man. The addition of 0.07% Al in the form of aluminum acetate to a bentonite containing diet had no effect on serum cholesterol.

The experiments described above, however preliminary, provide a strong indication that our rationale concerning the formation of a polyuronate-Al^{3+}-lipid complex is correct. Cholesterol is expected to partition into the hydrophobic region, the pool of lipid droplets, which may escape digestion and absorption. Our most significant observation is that the inclusion of Al^{3+} in the alginate containing diets provides a marked hypocholesterolemic influence. This action of Al^{3+} is not due to precipitation of bile salts because the amount of aluminum at 0.02% level is insufficient for this purpose. The addition of aluminum acetate to bentonite remained without any effect, thus suggesting that Al^{3+} cannot combine with this polyanion efficiently enough to bind the anionic lipids. The amount of 0.02% Al corresponds to less than one sixth of the alginate binding sites, which means that the lipid micelles can be immobilized by a few distant positive charges.

Further studies will establish the structural requirements and stoichiometry of complex formation for a variety of polysaccharides and lipids. This hitherto unrecognized product of macromolecular architecture may have an important function in human nutrition. Aluminum containing food additives may easily contribute sufficient quantities of Al^{3+} to have a fortuitous effect on serum cholesterol level of consumers. Because of the ubiquitous nature of Al^{3+}, this new principle of cross linking may contribute to the shape and stability of some tissue matrices, especially in aging and atherosclerosis. Possibly, our old notion on the inertness and insignificance of aluminum to nutrition and biochemistry may have to be revised (11).

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