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Interaction of Glycyrrhizic Acid with the Products of Cholesterol Oxidation: a New View of the Problem of Atherosclerosis

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Abstract

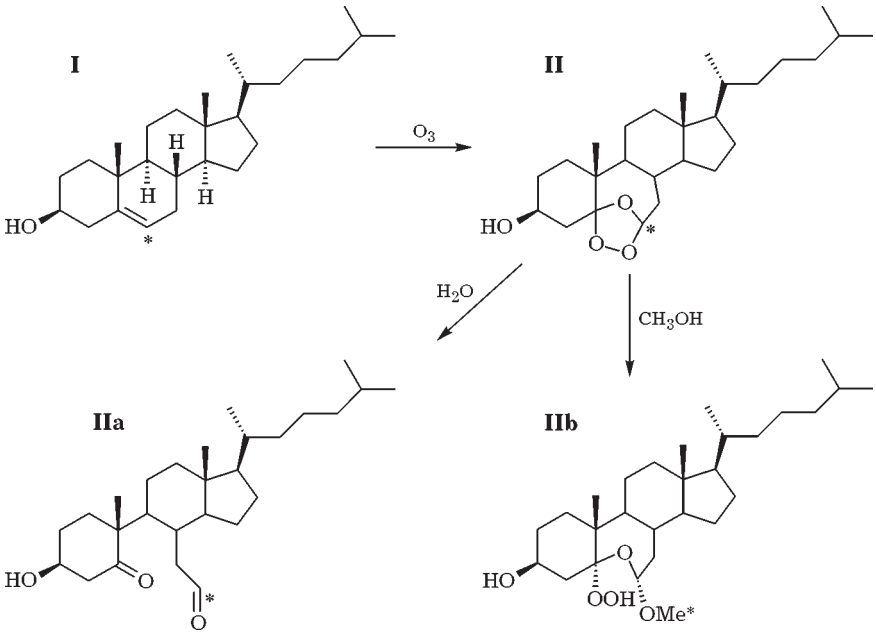
The ability of glycyrrhizic acid to form complexes with the products of cholesterol oxidation was studied. The effect of complexing on the rate of cholesterol oxidation with ozone was studied. It was shown that the formation of complex with glycyrrhizic acid may become an efficient approach to govern the level of cholesterol inside and outside of cell membranes, and to extract the products of cholesterol oxidation.

Key words: cholesterol oxidation, ozone, glycyrrhizic acid, atherosclerosis, cholesterol complex

INTRODUCTION

Cholesterol plays the most important part in many biochemical processes in organism, in particular it provides the stability of cell membranes, it is necessary for the formation of vitamin D and various steroid hormones. Cholesterol enters organism from two basic sources – with food and due to the endogenous synthesis, the prevailing route (about 80 %) being the synthesis by the organism itself. At the same time, cholesterol has fallen into disrepute because of implication in the formation of atherosclerotic plaques. Increased concentration of cholesterol and low-density lipoproteins (LDLP) in blood is considered to be the major risk factor for atherosclerosis. However, a connection of the increased level of cholesterol with atherosclerosis is ambiguous: on the one hand, an increase in cholesterol content in blood plasma is considered as an indisputable risk factor for atherosclerosis; on the other hand, atherosclerosis often develops in persons with normal cholesterol level. In reality, the high level of cholesterol is only one of numerous risk factors for atherosclerosis. The occurrence of these factors in persons with the normal level of cholesterol potentiates the negative effect of free cholesterol on vascular walls and promotes the development of atherosclerosis even in the case of lower cholesterol concentrations in blood. According to the results obtained by the authors of [1], the major hazard to the organism is brought not by cholesterol itself but by the products of its oxidation.

The presence of a double bond in cholesterol molecule determines its susceptibility to oxidation by different active oxygen forms that are present in the organism. When their formation exceeds the possibilities of the antioxidant systems of the organism, oxidative stress arises. It plays an essential part in the pathogenesis of many dangerous diseases, in particular atherosclerosis. The atherogenic action of oxysterols was demonstrated both *in vitro* and *in vivo* [1]. It was discovered that atherosclerotic plaques contain not only cholesterol but also the series of oxysterols [2]. The contribution from oxidized forms of cholesterol and LDLP into the pathogenesis of atherosclerosis is substantial. In addition, the interaction of the products of cholesterol oxidation with β-amyloid leads to the impairment of protein exchange. For example, Alzheimer's disease arises as a result of the accumulation of β-amyloids in brain tissues; their interaction with ox-

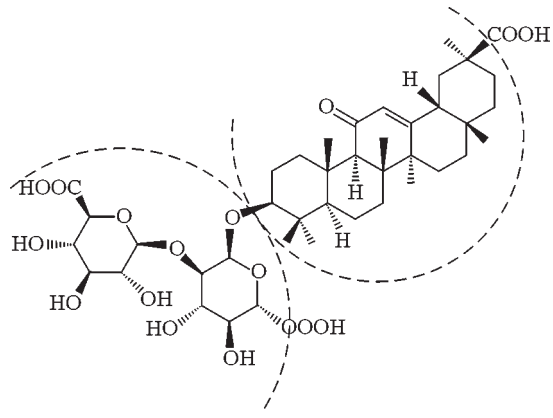


Scheme 1.

ysterols causes their enhanced accumulation and, as a consequence, the acceleration of the disease [3]. In the light of this fact, the development of methods to affect the toxicity of the products of cholesterol oxidation becomes important problem.

Recent studies identified one more active oxygen form in human arteries affected by atherosclerosis. This form is ozone [4]. Ozone is a very strong oxidant. it is often used for disinfection, to remove odours, to purify water, polluted air and food. Since the moment when it was established that the primary targets of ozone are unsaturated lipids in cell membranes and food, chemistry of ozone reactions with lipids has become the subject of more detailed investigation [5]. It was established that ozone is generated in the inflammation focus by antibodies located there and by the cells of the immune system [4]. Among all the forms of active oxygen, only ozone breaks the double bond of cholesterol with the formation of 5,6-secosterol **IIa**, while cholesterol oxidation in methyl alcohol leads to compound **IIb** (Scheme 1). Subsequent experiments showed that atherosclerotic tissues contain the products formed during the oxidation of cholesterol by ozone [4]. So, through the oxidation of cholesterol, ozone makes its contribution into the formation of atherosclerotic plaques. In addition, the products of ozonolysis are toxic for blood cells and thus inflammation may be enhanced [4].

At present, the regulation of cholesterol level is performed using the medicines through inhibition of cholesterol synthesis. An alternative approach may become the use of natural complex-forming agents that are able to bind cholesterol molecules and the products of their oxidation, and affect their properties. At present, only one example of the successful realization of this approach is known – the complexes of cholesterol with cyclodextrins (CD) [6, 7]. It is known that CD extracts cholesterol from membranes efficiently but this is accompanied by the distortion of membrane structure and the pos-



Scheme 2.

sibility of crystallization of CD themselves and their complexes with cholesterol.

The interaction of cholesterol with glycyrrhizic acid (GA) is shown in Scheme 2. It can be interesting for a number of reasons. First of all, GA is a natural complexing agent exhibiting a broad range of biological activity. It forms inclusion compounds with many medical preparations and is widely used in medicine [8]. Second, unlike for CD, the data on the toxicity of GA are absent. Third, according to some data, GA affects the biosynthesis and properties of cholesterol [9]. In particular, it is known that GA possesses the properties of cortisone antagonist, blocks up the anti-granuloma action of glucocorticoids inhibiting the deposition of glycogen in liver and the biosynthesis of cholesterol. This effect is observed in the case of the joint introduction of cortisone and GA. It was demonstrated in experiments on the animals with atherosclerosis that GA and its salts cause a decrease in the concentration of cholesterol, LDLP and triglycerides in blood, and a decrease in the concentration of cholesterol in liver tissues [9]. Fourth, there are data providing evidence that GA decreases the level of cholesterol oxidation [10]. However, molecular mechanisms of these effects have not been studied yet. The formation of a complex of cholesterol with GA can through light on these facts and open a new route to fight against atherosclerosis.

The goal of the present work was to study the possibility of complexation of the products of cholesterol oxidation with GA by means of NMR relaxation, and to study the effect of GA on the oxidation of cholesterol by ozone.

EXPERIMENTAL

Ozonation of cholesterol was carried out with the help of Groza ozonator with the productivity of 300 mg O_3/h. Ozonolysis products were obtained by ozonation of by the dry films and the alcohol solution of cholesterol. Analysis of the NMR spectra of products and measurement of relaxation time (T_2) of the protons of oxidation products and GA was made with a Bruker DPX-200 spectrometer. The formation of complexes was studied with the help of NMR relaxation. It is known that the time of proton relaxation is very sensitive to the mobility of molecules [11]. The formation of complexes is accompanied by a decrease in the mobility of molecules, which causes a substantial decrease in the time of proton relaxation. Generally, the formation of a complex leads to the bi- or even triexponential relaxation kinetics if several different types of aggregates are present in solution. Measurement of pre-exponential coefficients allows us to determine the fraction of molecules present in complex and to calculate, on their basis, the stability constants and the stoichiometry of the complex. In the general form, for reaction nChol + mGA ↔ $\mathrm{Chol}_n\mathrm{GA}_m$, the stability constant is expressed as

$K = [\mathrm{Chol}_n\mathrm{GA}_m]/[\mathrm{Ghol}]^n[\mathrm{GA}]^m$

where [Chol] is the concentration of free cholesterol; [GA] is the concentration of free glycyrrhizic acid. The m and n values were calculated with the help of the program of optimisation from experiments with different $[\mathrm{Chol}]_0/[\mathrm{GA}]_0$ ratios. It was demonstrated that the complex includes one cholesterol molecule and two GA molecules.

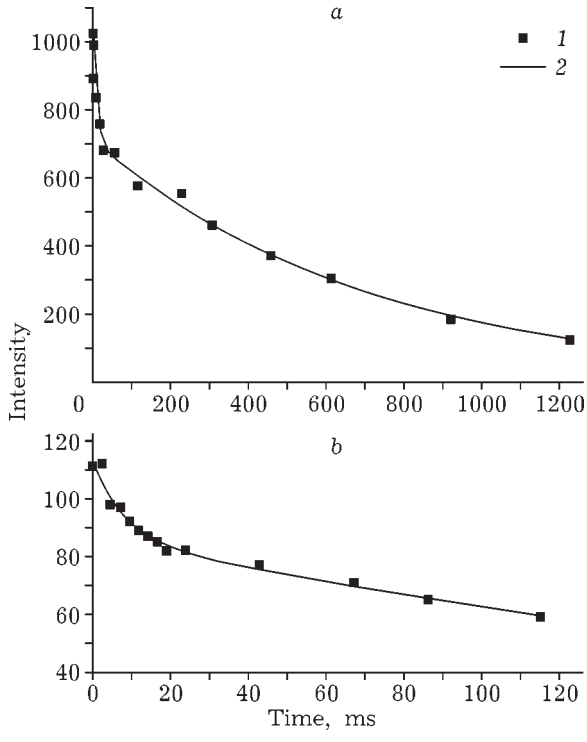


Fig. 1. Kinetics of the decay of proton echo signal for the product **IIb** of ozonation of **I** in methanol (*a*) and ozonide **II** (*b*): *1* – experiment, *2* – biexponential approximation.

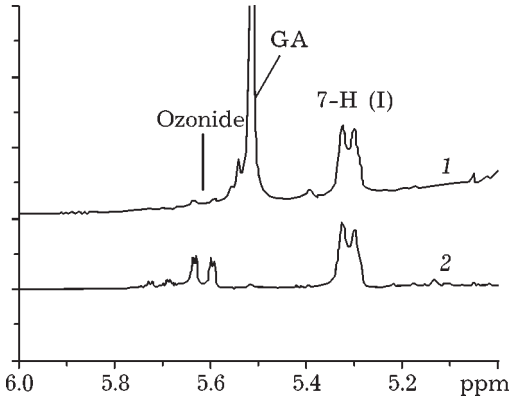


Fig. 2. Fragments of ^{1}H NMR spectra of the products of ozonolysis of dry films of pure cholesterol (*1*) and the complex of cholesterol with GA in CD_3OD (*2*). Ozonolysis time: 60 min.

RESULTS AND DISCUSSION

It is known that ozonation of cholesterol in different solvents leads to different oxidation products [12]. We studied complexation of three products of ozonolysis: ozonide (**II**) arising in the oxidation of dry films; product (**IIb**) arising during the oxidation of cholesterol in methanol solution; secosterol **IIa** which is formed in both cases (see Scheme 1).

The kinetic curves of relaxation of the product **IIb** of cholesterol oxidation in methanol and ozonide (**II**) are presented in Fig. 1. One can see that the curves have the biexponential character; small relaxation time (about 20 ms) corresponds to the bound state. For secosterol, both for the ozonation of dry films and for oxidation in methanol, the relaxation kinetics has the monoexponential character. Therefore, this product does not get bound with GA.

For the ozonide, we calculated the stability constants at room temperature and at 320 K assuming the 1 : 2 stoichiometry. The calculated stability constant for the complex of ozonide with GA was K_{12} (300 K) $\approx 7 \cdot 10^7$ ($\pm 3 \cdot 10^7$) M^{-2}. Thermodynamic parameters of complex formation were determined: $\Delta H \approx (-28 \pm 14)$ kJ/mol, ΔG (300 K) $\approx$ (-45 ± 22) kJ/mol, ΔS (300 K) $\approx$ (57 ± 25) J/(mol · K).

It is known that the stabilization of the complex of cholesterol itself with GA occurs exclusively due to the entropy factor. The negative change of enthalpy observed by us points to the fact that the hydrophobic interaction makes an additional contribution into the formation of the complex. This is exhibited also as the higher stability constant of the complex.

Additionally, we studied the effect of GA on the rate of cholesterol oxidation by ozone. For this purpose, we compared the intensities of NMR signals of the products of ozonolysis of the dry films of pure cholesterol and the complex of cholesterol with GA (Fig. 2). It was established that the yield of oxidation product in the complex with GA decreased by a factor of approximately 4.5. We suppose that this effect is connected with the antioxidant properties of GA itself. Previously we discovered the ability of GA to capture free peroxide radicals in solution [13].

CONCLUSION

Thus, the formation of stable complexes of glycyrrhizic acid with the products of cholesterol oxidation was discovered for the first time. This observation is undoubtedly of interest, taking into account the cytotoxicity of these products and their assumed participation in the development of atherosclerosis. The proposed approach can be the beginning of the new direction of the struggle against one of the most widespread diseases of the 21 century.

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