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The antioxidant effect of β-cyclodextrin nano-sized inclusion compound (clathrate) with benzoic acid *in vitro*

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ABSTRACT

This study provides the information on the synthesis of a novel inclusion complex (clathrate) of β-cyclodextrin with pentaerythritoltetrabenzoate and its antioxidant activity in vitro. On the models of ascorbate-dependent and NADPH lipid peroxidation in rat liver microsomes substrate the antioxidant and antiradical activities of this clathrate were shown. Considering both the obtained data and the structural specificity of the clathrate, it prognostically appears the argument for the application of this complex compound in vivo with minimizing potential side effects, particularly in view of kidney apparatus.

Keywords: clathrate of β-cyclodextrin, benzoic acid, antioxidant activity, lipid peroxidation.

Lipid peroxidation, which proceeds by a free radical chain reaction mechanism, performs a number of functions in a living organism. It is one of the main conditions for the maintenance of homeostasis in the living organism, and is involved in electron transport in the chain of respiratory enzymes, in the synthesis of prostaglandins and leukotrienes, in the cell proliferation and differentiation, in phagocytosis, in the regulation of lipid composition of the membrane and in other processes.

Under physiological conditions, free radical oxidation reactions play an important role in the processes of tissue structures renewal and in the regulation of the biological membranes permeability. However, when the antioxidant system of the organism exhausts its reserves and becomes unable to adequately regulate the course of free radical reactions, the oxidative modification of lipids of biological membranes leads to loss of membrane barrier function, disturbance of ion homeostasis and energy balance of the cell, and to ultimately destruction of membranes and cells death [1-4].

Inclusion compounds of cyclodextrins with known pharmacological substances, showing a variety of biological activity, attract some interest from a practical point of view [5, 6].

Cyclodextrins are cyclic oligosaccharides made of D-glucose repeating units coupled withα-1,4-glycosidic bond (Fig. 1). Today, due to the relative cheapness, non-toxicity and biodegradability, they have been widely used in various fields of chemistry, primarily supramolecular chemistry, fine organic synthesis and in many interdisciplinary fields. Particular interest of cyclodextrinsis caused by both their cyclic structure and the presence of an internal hydrophobic cavity capable of forming clathrates like "guest–host" type with a variety of organic substrates [5, 6].

Cyclodextrins are widely used in pharmacology as a drug delivery system by molecular encapsulation due to their ability to form inclusion complexes with numerous hydrophobic compounds. At the same time, in recent years more

perspective possibility of using cyclodextrins in pharmacology began to develop: namely, creation of medicine preparations based on covalent "linking" (conjugation) of drugs to the cyclodextrin frame. Thus, the synthesis of more effective drugs, having fewer severe side effects, is enabled.

Benzoic acid in a pure form is a natural water-soluble non-enzymatic antioxidant. Some interest concludes in the presence of the same effects of cyclodextrin inclusion compounds with benzoic acid, prognostically possessing physiological antioxidant activity.

In this paper, we present data on the synthesis and pharmacological tests of antioxidant activity of a novel β-cyclodextrin inclusion complex with pentaerythritoltetrabenzoate ([3-benzoyloxy-2,2-bis(benzoyloxymethyl)propyl] benzoate) (Fig. 2).

RESEARCH TECHNIQUE

The induction of lipid peroxidation was performed in the incubation medium, containing rat liver microsomes*in vitro*. Standard procedure was used to obtain microsomes. After slaughtering the animals, the inferior vena cava was cannulated. The liver was perfused with 0.15 M KCl solution containing 5 mMtris-HCl buffer, pH 7.4, to a light yellow color to remove blood *in situ*. The liver was removed, squeezed on the gauze, weighed and minced with scissors. The homogenate was prepared with the ratio of tissue weight to volume of solution as 1:3. All procedures were carried out using solutions cooled to 40 °C. The homogenate was centrifuged at 9500 rev/min for 20 minutes at 30 °C. The supernatant fluid was further centrifuged at 24000 rev/min for 120 minutes at 30 °C. The microsomes precipitate was resuspended in a medium allocation to a protein content of 20–25 mg/mL and used in the test subsequently.

Lipid peroxidation in rat liver microsomal suspensions containing 0.8 mM ascorbate (or 1 mM NADPH); 0.2 mM sodium pyrophosphate; 50 mMtris-HCl buffer, pH 7.4; 0.5 mg of a phospholipid/mL of microsomes initiated by introducing the Fe^{2+} to a final concentration of 12 uM. The total volume of the incubation mixture was 1 mL. The incubation duration was 5 minutes for ascorbate-dependent and 20 minutes for NADPH-dependent lipid peroxidation, the temperature was 37 °C. The reaction was stopped by adding 30% trichloroacetic acid solution of ethylenediaminetetraacetic acid at a concentration of 1.5 mM. After incubation the number of was determined by color reaction with thiobarbituric acid in the samples.

RESULTS

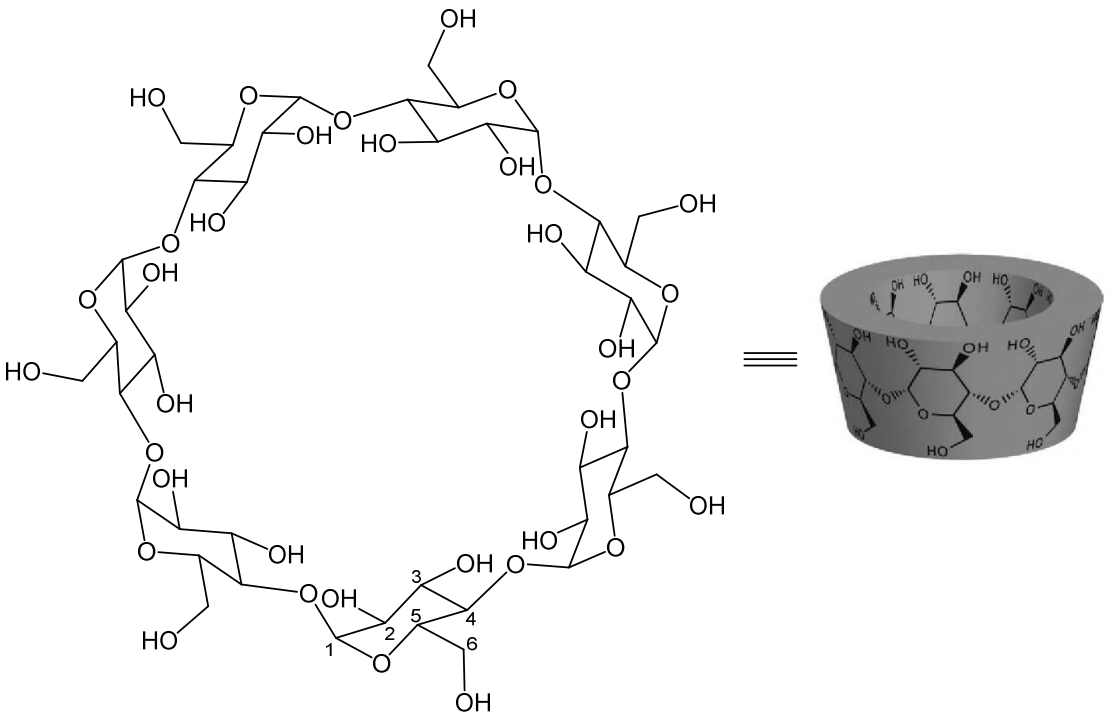
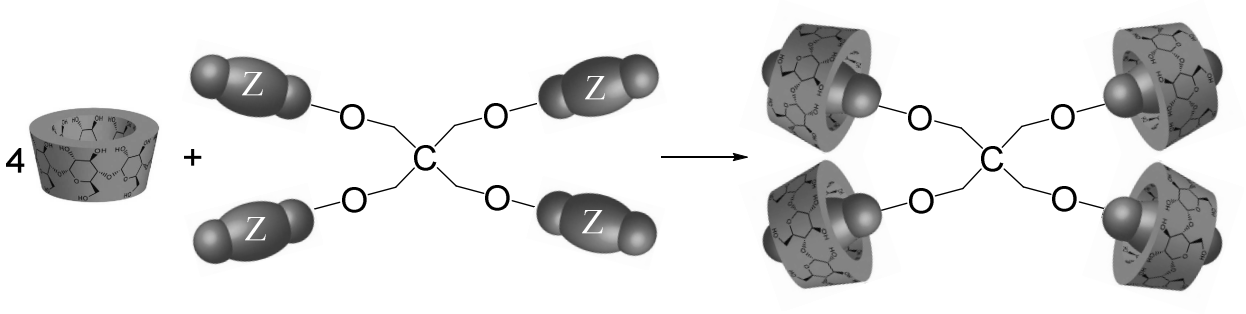


Fig. 1. The structural formula of β-cyclodextrin

Synthesis of β-cyclodextrin inclusion compound with pentaerythritoltetrabenzoatewas carried out in a following way. Pentaerythritoltetrabenzoate(0.03 g, 0.05mmol) was added to a solution of β-cyclodextrin(0.20 g, 0.18mmol) in

4 mL of water at stirring. Themixture was stirred at 70°C for 1 h, then cooled to 20°C, and kept for 20 h. The precipitate was filtered off, washed with acetone (3×3 mL), and dried invacuum (1 mm) for 4 h at 50°C.Yield 0.13 g (58%), mp280–282°C (decomp.), R_f 0.64 (Silufol UV-254, eluent: 6% aqueous ammonia–ethanol–butanol, 5: 5: 4). ^{1}H NMR spectrum (DMSO-d_6), δ,ppm (*J*, Hz):): β-cyclodextrin: 3.27–3.68 m (168H; C^2H–C^5H, C^6H_2), 4.42–4.49 m (28H, C^6OH), 4.76–4.84 br.s (28H, C^1H), 5.63–5.76 m (56H; C^2OH, C^3OH); pentaerythritoltetrabenzoate: 4.67 s (8H, CH_2), 7.41 t (8H, *m*-CH, $^3J_{HH}$ 7.6), 7.59 t (4H, *p*-CH, $^3J_{HH}$ 7.6), 7.91 d (8H, *o*-CH), $^3J_{HH}$ 7.8].Found, %: C 46.92; H 6.29. $C_{201}H_{308}O_{148}$.Calculated, %: C 47.41; H 6.10.



Z = $C_6H_5C(O)$-

Fig. 2. The structural formula of the inclusion complex of β-cyclodextrin with pentaerythritoltetrabenzoate

As a model system for the investigation of the antioxidant activity of the studied clathrate, we used ascorbate-dependent lipid peroxidation in rat liver microsomes substrate. The concentration of the microsomes in the incubation medium was 2 mg protein per mL. The influence of the clathrate on the rate of lipid peroxidation was estimated by the change in the formation of malondialdehyde in microsomessuspension during incubation and expressed as a percentage. Ascorbate-dependent lipid peroxidation in the suspension of the microsomes in standard conditions was initiated. The solution of studied clathrate at a concentration of 10 mg/mL or the solution of tocopherol acetate at the same concentration (as a comparison) was added to the experimental samples prior to incubation.

The clathrate displays the antioxidant properties in ascorbate-dependent lipid peroxidation in rat liver microsomes*in vitro*. Quantitative changes in the level of antioxidant protection (percentage) of the clathrate and tocopherol was calculated based on the average value of the total concentration of conjugated dienes, malondialdehyde and lipid hydroperoxides in the control samples.Its antioxidant activity was 23.1%, that below the antioxidant activity of tocopherol, which equaled58.7% (Fig. 3). Consequently, clathrate exhibits antioxidant activity suggesting the presence of its antiradical properties.

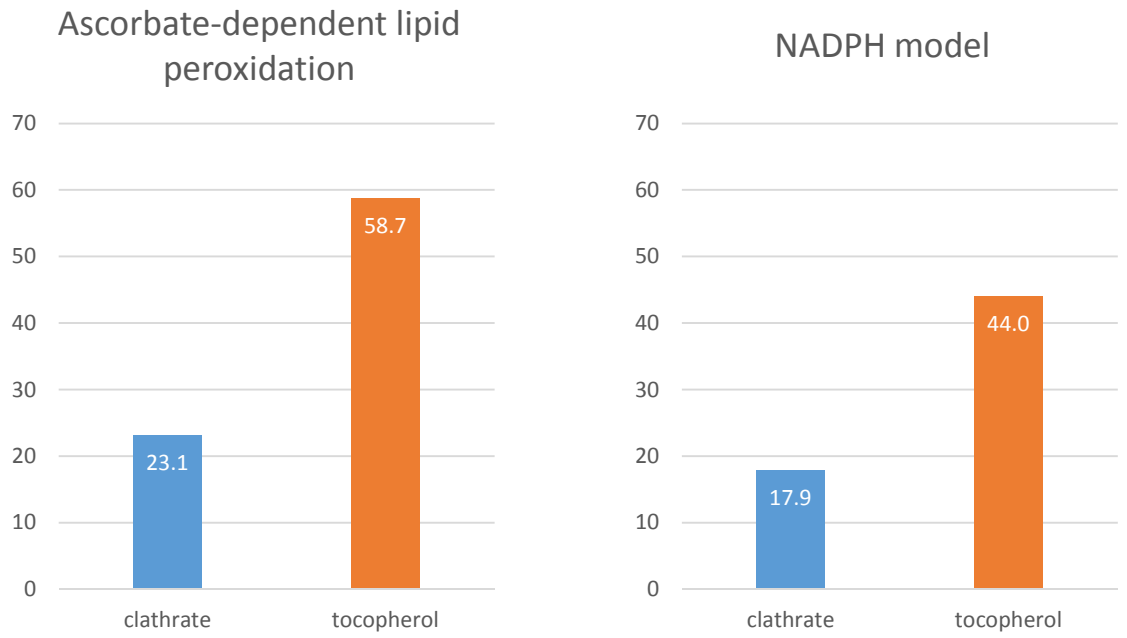


Fig. 3. The antioxidant activity (%) of β-cyclodextrin inclusion compound (clathrate) with pentaerythritoltetrabenzoate compared with the tocopherol under various experimental models *in vitro*

In addition, a model system like NADPH(reduced nicotinamide adenine dinucleotide phosphate) for the evaluation of the antioxidant effect of the clathrate in rat liver microsomes substrate was used. The clathrate exhibits antioxidant properties in the NADPH-dependent system of lipid peroxidation in rat liver microsomes substrate *in vitro*. Its antioxidant activity under these conditions was 17.9%, and the antioxidant activity of tocopherol was significantly higher and equaled 44.0% (Fig. 3).

CONCLUSION

Considering the obtained results we can convincingly saythat the studied clathrate may exhibit the antioxidant activity*in vitro*, thus confirming the presence of its antiradical properties. In view of the structural feature of the test compound, it should be noted that existing in its structure four nanoscale complexes, connected by an aliphatic bridges, will not have a side effect on the type of occlusion of the renal tubules, brain edema, damage to blood cells and other negative effects observing duringthe application of such compounds [7].In this regard, it is of interest the testing of the biological activity of this inclusion complex *in vivo* model for the purpose ofremoving the pathological process during the combined action withthe other drugs.

REFERENCES

[1]Cheeseman K.H. Mechanisms and effects of lipid peroxidation. *Mol. Aspects. Med*.,**1993**, 14 (3), 191-197.
[2]Comporti M. Lipid peroxidation. Biopathological significance. *Mol. Aspects. Med*.,**1993**, 14 (3), P.199-207.
[3]Halliwell B., Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. *Am. J. Clin. Nutr*.**1993**. 57 (5), 715-725.
[4].VladimirovYu.A. Physicochemical Mechanism by whict the Barrier Function of Biomembranes May be Impaired in Disease // Physicochemical Medicine Reviews. / Ed. by Yu.M.Lopukhin. - London, Gordon and Breach Science Publishers, Inc., **1987**. 52-129.
[5]UekamaK., Hirayama F., IrieT.*Chem. Rev*, **1998**, 98 (5), 2045–2076.
[6]Davis M.E., Brewster M.E. Nature Rev. *Drug Discovery*, **2004**, 3 (12), 1023–1035.
[7]Tutelyan V.A., Gmoshinskiy I.V., Glintsburg A.L. Guidelines on the identification of nanomaterials pose apotential danger to human health: guidelines. Moscow, **2009**,35 p. [in Russian].