Dextran as antioxidant's activity carrier

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SUMMARY: Two series of conjugates of dextran and antioxidants from the class of sterically hindered phenols were prepared. The conjugates were characterized by the substitution degree of glycoside units, solubility in different solvents, intrinsic viscosity. The investigation of radical scavenging activity (RSA) of conjugates was carried out in their reactions with two free radicals - 2,2-diphenyl-1-picrylhydrazyl (DPPH) and sodium salt of 2,2-diphenyl-1-picrylhydrazyl sulfonic acid (DPPH-salt). The usage of water soluble DPPH-salt enabled to estimate the RSA of the conjugates in water. It was shown that the rate constants of interaction of the DPPH-salt and the conjugates were 10-30 times higher than this value for low-molecular analogue of phenoxan. High RSA of the conjugates in water can be explained by large solvation shell formed due to high content of hydroxy groups in dextran.

Introduction

The search and investigation of compounds with antioxidative activity is one of the intensively developing trends in modern chemistry of biologically active compounds. This is connected with a wide distribution of diseases, caused by the violation in vivo of the level of free-radical processes. The free radicals play an important role in aging and in the development of a number of pathological states (growth of malignant tumors, radiation injury, mutagenesis, action of some poisons, etc). Free-radical processes regulation in biological objects is carried out by multicomponent antioxidant systems capable of reactions with various radicals [1]. Sterically hindered phenols (SHP) are considered now one of the most promising groups of bioantioxidants. Their main structural feature is the presence of bulky alkyl substituents in *orto*, *orto*'- positions to phenolic hydroxy group.

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High efficiency and non-toxity of SHP have determined the possibility of their application in biology and medicine as analogues of natural inhibitors of radical oxidative processes.[2] However, the poor solubility of SHP in water prevents them from more diverse application in this field. One of the possible way of solving the problem might be the development of polymeric SHP forms on the basis of different hydrophilic polymers. Besides, the application of the polymeric forms of biologically active compounds will allow to vary their biotransport and pharmacokinetic properties, to create their high local concentrations near the target and to use other advantages of macromolecular systems [3].

We suggested earlier the methodology of the creation of polymeric SHP forms with the usage of synthetic hydrophilic polymers, such as polyvinyl alcohol, polyvinylpyrrolidone copolymers with different hydrophilic monomers and etc[4-6].With it the type and the lability of SHP-polymer bond were changed. This made possible to create conjugates with controlled release of the active substance. Antioxidative properties and radical scavenging activity of prepared conjugates were not higher than corresponding characteristics of the low-molecular analogue - phenoxan (potassium salt of β-(4-Hydroxy-3,5-di-*tert*-butylphenyl)propionic acid).

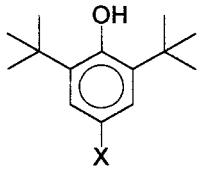
This approach to synthesis of water soluble polymeric SHP derivatives was used for the creation of conjugates of SHP and polysaccharide dextran. Dextran is biodegradable, hydrophilic and non-toxic polymer widely used as a carrier for different biologically active compounds. It was shown that contrary to the conjugates of SHP with synthetic polymers the conjugates with dextran had higher radical scavenging activity in reaction with DPPH in water-organic mixture than low-molecular phenoxan [7].

The present work deals with the study of influence of nature of dextran-SHP bond and molecular weight value of dextran samples on their radical scavenging activity.

EXPERIMENTAL

Reagents

Samples of dextran (Fluka) with molecular weight of 10000,40000,70000 and 200000 were used. Commercial antioxidants β-(4-hydroxy-3,5-di-*tert*-butylphenyl)propionic acid(PA), potassium salt of β-(4-Hydroxy-3,5-di-*tert*-butylphenyl)propionic acid (phenoxan) were used. 4-Hydroxy-3,5-di-*tert*-butylbenzilbromide (BB) was prepared by bromation of 2,6-di-*tert*-butyl-4-methylphenol in acetic acid [2].



X=-CH_2CH_2COOH (PA), -CH_2CH_2COOK (phenoxan),-CH_2Br (BB)

Synthesis of conjugates

Conjugates I (see the scheme) were obtained earlier by the carbodiimide method with the use of PA on the basis of dextran with molecular weight 40000 [7].

Conjugates II on the basis of dextran with varied molecular weight were prepared with the use of BB by the following technique. BB was added in DMSO solution of dextran (10 %wt) at 20° C under stirring. After 5 hours the product of reaction was precipitated in ethanol, then subsequently reprecipitated in ethanol from the ethanol-water solution up to a complete removal of low molecular weight impurities. Purity of conjugates was controlled by GPC on Sephadex LH-20 column with water-ethanol eluent (1:1 v/v).

Chemical structure of the conjugates obtained was proved by NMR and UV spectroscopic data.

The quantity of covalently bounded both PA and BB in the conjugates was determined by UV-spectroscopy in water-alcohol solutions (1:1 v/v) at λ_{max}= 275 nm. The substitution

degree of glycoside units (γ) was calculated as percentage of monosubstituted glycoside units to overall their quantity.

$$\gamma = A/B \bullet 100\%,$$

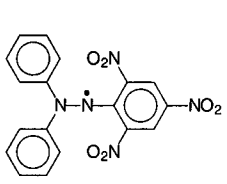
where A = number of monosubstituted glycoside units,

B = total number of glycoside units in a sample.

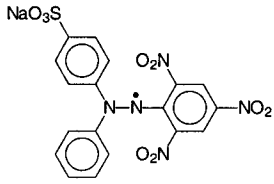
An intrinsic viscosity of the original and modified samples was calculated from viscosity measurements in DMSO solution at 25° C in an Ubbelohde viscometer.

The investigation of radical scavenging activity

The evaluation of RSA of the antioxidants was carried out in their reaction with free radicals diphenylpicrylhydrazyl (DPPH) and water-soluble analogue of DPPH - sodium salt of (DPPH-salt). The latter free radical was received by oxidation with PbO_2 of diphenylpicrylhydrazine sulfonic acid [8].



DPPH

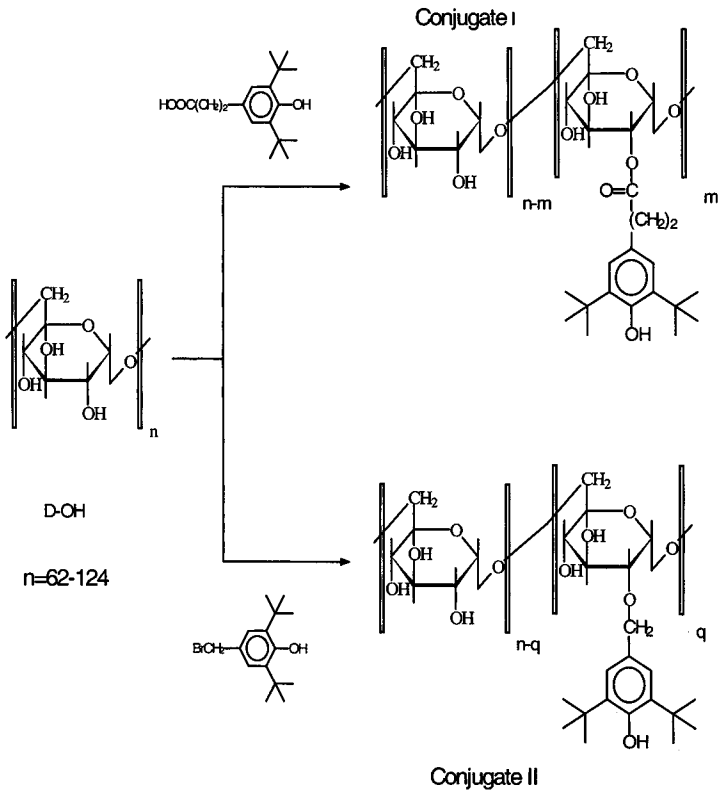


DPPH-salt

Kinetics of the reaction between the antioxidants and the free radicals were studied at 20 ° C, using a 20-fold excess of SHP (for the conjugates taking into account their γ values). The process was followed by the measurement of a decrease of the free radical light absorption at λ = 520 nm vs time in its mixtures with the antioxidant [9]. The concentration of free radical in the solution was $2 \bullet 10^{-5}$ mol/l, and the concentration of SHP was $5 \bullet 10^{-4}$ mol/l. The reaction was carried out up to 20% of free radical conversion.

Results and Discussion

Conjugates of hydrophilic macromolecules dextran and hydrophobic molecules of SHP are macromolecular systems, whose solubility changes in wide range of solvents. Two series of conjugates were obtained with the use of functional derivatives of SHP. The conjugates have different spacers, the substitution degree of glycoside units (γ) and molecular weights.



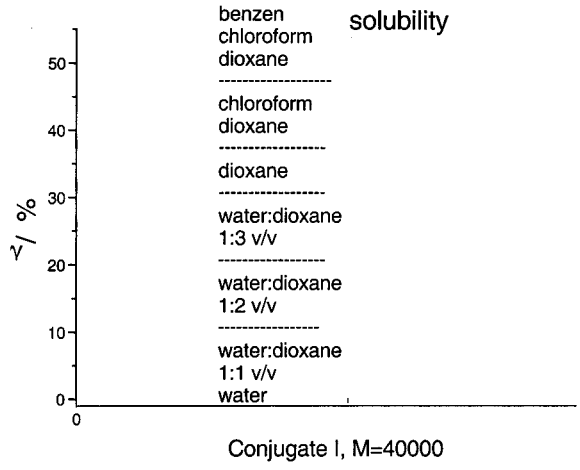


Fig.1: Solubility of conjugates I as a function of the substitution degree of glycoside units γ

Conjugates with substitution degree up to γ 9-10 % are soluble in water. Conjugates with the substitution degree 14-25% are soluble in mixed solvent (dioxane-water). It is interesting to note, that conjugate I with maximum degree of substitution can be dissolved in solvents such as benzene, chloroform, dioxane.

It was found that intrinsic viscosity values depend on the substitution degree of the conjugates.

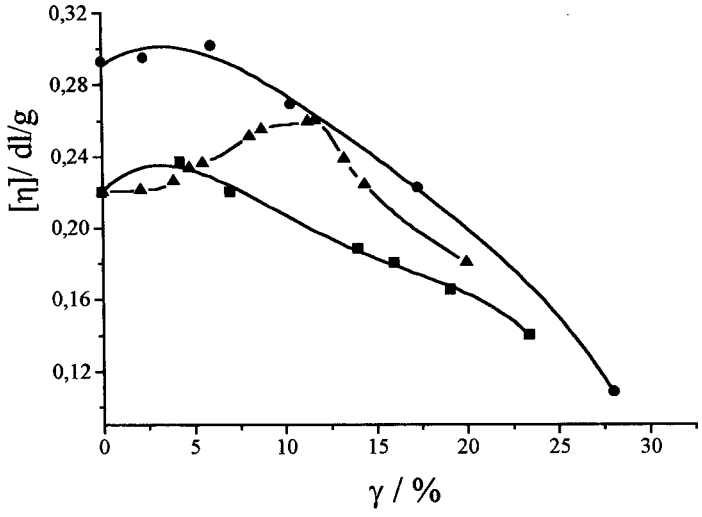


Fig.2: The intrinsic viscosity [η]of conjugates in DMSO solution as a function of substitution degree of glycoside units γ: T=25°C; ▲ = conjugates I, M_n 40000, ■ = conjugates II, M_n 40000, ● = conjugates II, M_n 70000

Figure 2 shows, that there is a tendency to the small growth of intrinsic viscosity at low γ values for all conjugates. However, further increase of γ values results in decrease of intrinsic viscosity. Such relationship can be explained by compacting of modified dextran macromolecules, caused by hydrophobicity increase with growth of SHP fragments number in the chains.

The interaction with free radicals may serve as a test for qualitative estimation of antioxidative properties of SHP and other compounds with antioxidative activity. For this purpose DPPH is used usually, however, as a rule, in organic solvents only, such as benzene, carbon tetrachloride, hexane. For expansion of possible routes to study radical scavenging activity in water-organic mixture and even in water we proposed to use water

soluble free radical - sodium salt of 2,2-diphenyl-1-picrylhydrazyl sulfonic acid (DPPH-salt).

Results of comparative activity study of antioxidants in reaction with free radicals in aqueous dioxane (1:1, v/v) are presented in Table 1.

Tab. 1. Antiradical properties of antioxidants in aqueous dioxane (1:1, v/v)

antioxidant	solubility in water	K, l/mol·s	
		DPPH	DPPH-salt
phenoxan	+	10,0±0,5	3,0±0,2
PA	-	3,4±0,2	1,20±0.05
D-OH+phenoxan (10:1)	+	10,0±0,5	3,0±0,2
D-OH+PA (10:1)	-	3,4±0,2	1,20±0,05
Conjugate I, γ=10,4 %	+	12,5±0,8	3,5±0,2

One can see from the Table 1 that the reaction with the use of water-soluble DPPH proceeds with lower rates for all antioxidants. This may be explained by the fact, that the difference in solvation energies of starting and transition states in case of dissociated in aqueous dioxane for DPPH-salt free radical is substantially less than the same value for undissociated in this media DPPH. It is worth noting that RSA of phenoxan or PA in presence of dextran, in other words their mixture, is the same as that of pure antioxidants. However RSA of conjugate with PA covalently bounded to dextran is a little higher in reactions with both radicals. This comparative study of two free radicals proved the possibility of proposed by us DPPH-salt application for the estimation of radical

scavenging activity in water-organic mixtures as analog of DPPH. On the use of DPPH-salt in aqueous dioxane (1:1, v/v) medium rate constants for conjugates with different molecular weights and degree of glycoside's units substitution were obtained (Fig 3).

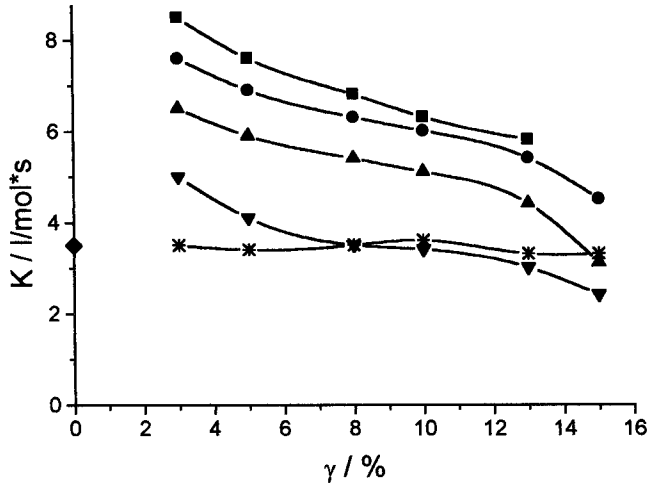


Fig. 3: The rate constant of reaction DPPH-salt K with conjugates as a function of the substitution degree of glucoside units γ; ■= Conjugate II, M_n 10000, ● = Conjugate II, M_n 40000, ▲= Conjugate II, M_n 70000, ▼= Conjugate II, M_n 200000, *= Conjugate I, M_n 40000, ♦= phenoxan

The figure 3 shows that the rate constant of the reaction of conjugates 1 with DPPH-salt does not depend on γ. The values vary within experimental error and are equal to 3.5±0.2. Another situation is observed for conjugates II. Up to γ=12% they have higher reactivity than phenoxan. However with the growth of substitution degree their activity approaches (and even becomes less) to that of phenoxan. The increase of molecular weight of dextran samples from 10000 up to 200000 results in twofold decrease of RSA of the conjugates.

A very interesting and important problem is measurements of RSA of the conjugates in pure water. It was impossible with the use of DPPH because of its insolubility in water. The main advantage of DPPH-salt is the possibility to study radical scavenging activity in water.

The relationship between the rate constants and solvent's mixture composition is given in the Fig.4.

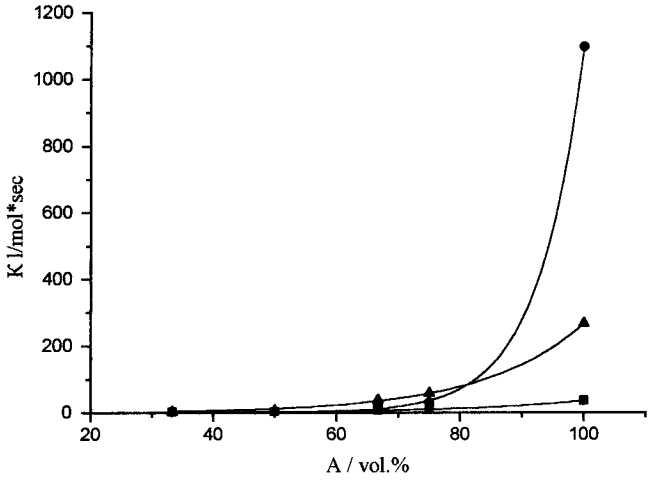
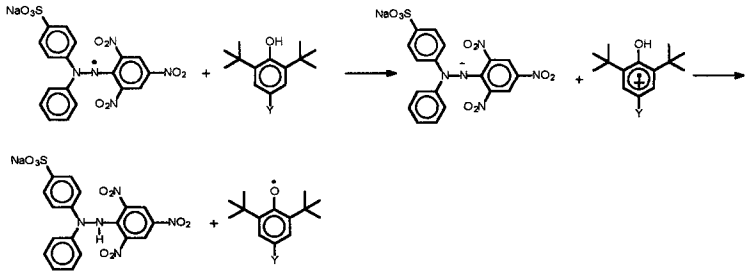


Fig. 4: The rate constant of reaction DPPH-salt *K* with phenoxan and conjugates with M_n 40000 as a function of water contents *A* in a mixture of solvents (water: dioxane); ■= phenoxan, ▲= Conjugate II, γ = 9,4%, ●= Conjugate I, γ = 9,2%

It is evident that at water content 33% the rate constants are almost equal for both phenoxan and conjugates. But in pure water the difference in activities of the conjugates increases in 10-30 times. For conjugate I with more flexible spacer rate constant is higher than that for conjugate II. One of the possible explanations of conjugates high activity in water may be that dextran has large solvation shell in water due to high content of

hydroxy groups. Physical properties of water, which forms the solvation shell, differ from those of usual water. As this reaction proceeds, in our view, through the stage of cation - radical formation[10] medium has to exert strong influence on the rate of this reaction.



Thus, we have obtained SHP polymeric derivatives, whose radical scavenging activity and hence, their efficiency as antioxidants exceeds 10-30 times that of low - molecular phenoxan and also conjugates, obtained on the basis of synthetic polymers.

CONCLUSION

Conjugates of dextran and sterically hindered phenols with different degree of glycoside units substitution, molecular weights, and also types of spacers have been prepared. Solubility of the obtained conjugates was determined in different solvents. For determination of radical scavenging activity in water and water-organic mixture water-soluble free radical - sodium salt of 2,2- diphenyl-1-picrylhydrazyl sulfonic acid (DPPH-salt) was proposed. The relationship between the rate constants of antioxidants interaction with DPPH-salt and the substitution degree of glycoside units, molecular weights and spacers types was established. It was shown that the radical scavenging activity of conjugates in water is about 10-30 times higher than that one of the low-molecular analogue phenoxan.

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