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MACROMOLECULAR DESIGN OF CATIONIC POLYELECTROLYTES ON THE CHITOSAN BASIS FOR ACHIEVEMENT OF HIGH ANTIMUTAGENIC EFFICIENCY AT GAMMA- IRRADIATION

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ABSTRACT

A new approach to the problem of construction of efficient antimutagenic (at γ-irradiation) systems has been developed on the basis of the models- synthetic cationic polyelectrolytes- polyquaternary salts of diallyldimethyl-ammonium type.

Studies designed to reveal correlation between the structure and activity of polymers are of great importance for targetted synthesis of polymers capable to protect cells from γ-radiation. From this point of view a positive contribution of the total charge

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of the macroion and antiradical activity of polycations into their antimutagenic effect were revealed.

Taking into account the dependencies found, the macromolecular design of super efficient antimutagenic systems on the basis of the natural biodegradable polycation-chitosan has been realized. The protective efficiency (up to 93%) of the systems is achieved by combination of an adsorption ability of the polycation matrix and antiradical activity of a hydrophobic derivative of hindered phenol incorporated into the polymer structure. These systems are able to scavenge short-lived radicals formed in water and to some extent to prevent the membrane lipid peroxidation processes responsible for genetic damage at γ - irradiation.

INTRODUCTION

In connection with the deteriorating environmental radiation background caused by technogenic factors a search for compounds able to minimize a risk of genetic damage is very important problem now. Only not far ago it has become obvious that substances able to protect a living organism from a lethal dose of ionizing radiations don't exhibit pronounced protective ability from mutagenic consequencies of γ-irradiation at low and moderate doses.

It makes developement of new approaches to creation of efficient antimutagenic substances absolutely nesessary.

From this point of view a natural biopolymer - chitosan is interesting as an initial polymeric matrix for creation of such protective systems of prolonged action because of possible chitosan ability to exhibit properties of both an adaptogen (as a polysacharide) and a polycation. In this case at such a combination of properties general unspecific resistance of the organism can be increased due to haemo- and immunostimulation[1,2].

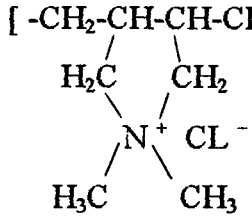
However, for targetted modification of the macromolecule it is nesessary to reveal the factors which make a positive contribution into the antimutagenic effect. We have carried out such investigation using synthetic polycations of a diallyldimethylammonium type as models. A positive contribution of the total charge of the macroion and antiradical activity of polycations into their antimutagenic effect were revealed.

EXPERIMENTAL

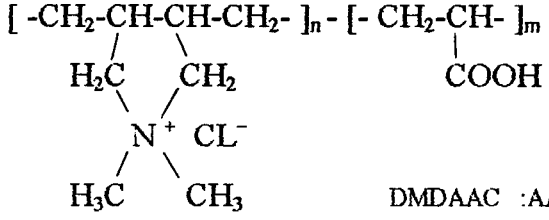
Investigation of the adsorbability of a polyelectrolyte on bilayer lipid membranes (BLM).

Caionic polyelectrolytes of the diallyl series developed at the Institute of Petrochemical Synthesis Russian Academy of Sciences- polycation

poly(diallyldimethilammonium cloride) - PDADMAC and copolymers of diallyldimethylammonium cloride (DADMAC) and acrylic acid (AA)of various composition were taken as objects of study.



PDADMAC



DMDAAC :AA

The samples of PDADMAC and copolymers of DADMAC and AA were thoroughly purified according to the known method [3]. The purity of the substances was controlled using the data of ^{13}C NMR spectra obtained by an MSL-300 spectrophotometer (Brucker, Germany).

The bilayer lipid membranes were formed in a teflon cell on a hole of 0.8 mm in diameter according to Mueller-Rudin's method from solutions of the lipids in decane (50 mg/ml). All experiments were carried out in the 10 mM

phosphate buffer in the presence of 100 mM KCL at pH 6.8. The membranes were formed from soybean L-α-phosphatidylcholine (Sigma, USA).The detailed procedures are described in the previous paper [4]. The value of maximal change in the BLM boundary potential was taken as a criterion of polymer adsorbability. This is such a magnitude at which any further change in the potential at a rise in concentration did not exceed the confidence interval of 2-5 mV. This value is the most correct and independent on the measurement procedure .

Synthesis of ternary copolymers of a diallyldimethilammonium type (TCPs).

Ternary copolymers with a different quantity of the hindered phenol (HP) in the copolymer side chain on the basis of the copolymer DADMAC and AA (30: 70) were synthesized in accordance with the procedure developed by authors [5].

Antiradical activity.

Antiradical properties of TSPs were evaluated by noting of a degree to which a 0.5 % methanol solution of 1,1-diphenyl-2-picrilhidrazil (DPPH) was discolored [6]. The decline in the level of free DPPH was monitored spectrophotometrically at λ=516 nM relative to the solution with completely discolored DPPH (in the presence of a 10- fold excess of α-tocoferol reactive in an equimolar manner with DPPH).

Synthesis of the chitosan derivatives.

The chitosan commercical material (Russia) from shell chitin with 80- 85 % deacetylation was used as an initial polymer matrix for the synthesis. The chitosan derivatives with various ratios of quaternary ammonium and primery amine groups in the polymer chain were obtained by interaction of chitosan with MeI in the presence of $(Et)_3N$ [7]. The chitosan derivatives with the quaternary ammonium groups and the hindered phenol fragments in the polymer side chain were obtained by interaction of partially quaternized chitosan with β-[(4-hydroxy-3,5-ditretbutyl)phenyl]-propionic acid, accoding to the procedure reported in [8].

Testing of the polymers for antimutagenic activity.

The original copolymer, TSPs and chitosan derivatives were tested for their effects on gamma-radiation induced structural changes in plant chromosomes by recording the number of chromosome aberrations at the metaphase of mitosis in the meristematic cells of barley (Moskovskii-121 variety) seedling roots. The experimental prosedure is described in detail elsewhere [9].

RESULTS AND DISCUSSION

It's known that biological activity of cationic polyelectrolytes results mainly from electrostatic interaction of the positively charged macromolecules with the negatively charged cell membranes.

We were interested whether there was any correlation between a polymer structure which determined its adsorbability and efficiency of the polymer as a bioprotector. To answer this question we have investigated the adsorbability of a polycation - PDADMAC and copolymers of DADMAC and AA of various compositions. For this purpose we used the experimental technique of BLM .The data of Fig.1 show that when a part of the cationic groups in the polymer chain goes down the polymer exhibits a decrease of the maximal change in the BLM boundary potential.

Antimutagenic activity of the polymers mentioned above has been investigated using a plant test system (seeds of barley). Comparing results obtained (Fig.1 and Fig.2) one can see that both adsorbability and antimutagenic efficiency of the polymers decrease noticably with the decrease in a part of the cationic groups in the copolymer chain. Hence, there is certain correlation between the total charge of the macroion and antimutagenic efficiency of the polycation.

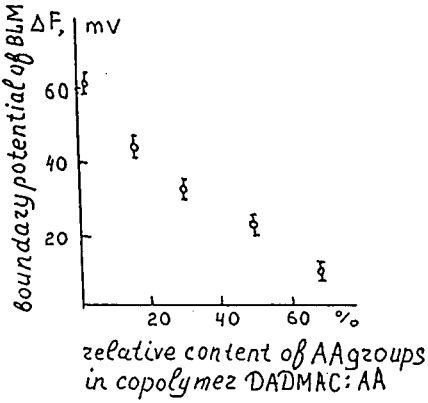


Fig.1 The maximal changes in the boundary potential of BLM from L-a-phosphatidylcholine, measured at the adsorption of polyelectrolytes of the diallyl series with various contents of cationogenic and anionogenic groups in the polymer chain.

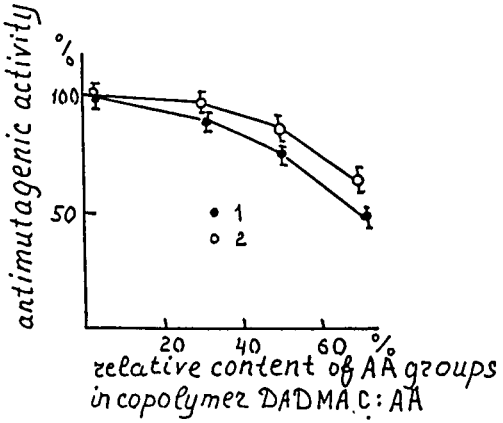


Fig.2 The antimutagenic activity in a series of diallyl copolymers at various contents of the cationogenic and anionogenic groups in the polymer chain : 1, protection before the irradiation ; 2, protection after the radiation.

Taking into account this consideration we supposed that the increase of the total charge of the chitosan macromolecule would result in enchancement of it's antimutagenic properties. To confirm this suggestion chitosan derivatives with the various ratios of quaternary ammonium and primary amine groups in the polymer chain were synthesized. The antimutagenic efficiencies of the polycations obtained were tested on the plant test-system (seeds of barley). The data of Fig.3 demonstrate that the increase in the quantity of quaternary ammonium groups in the structure of the chitosan derivatives results in enchancement of the antimutagenic effect up to 70 %.

Thus, the results obtained confirm that the definite correlation exists indeed between the total charge of the macroion and the efficiency of a polymer as a bioprotector.

However, it should be noted that the strong polycations can disturb membrane architecture at adsorbtion on the surface of cells. Hence, it is undesirable to use strong polycations for creation of effective antimutagenic systems for possible bio-medical application.In connection with said above the question arises whether there is any possibility to enhance the protective efficiency of the weak and moderate polycations with maintaining their low toxicity and the other beneficial properties.To answer this question we tried to increase antiradical activity of synthetic polycations by introducing into the copolymer side chain a derivative of a hindered phenol. It is known that such phenols are able to form

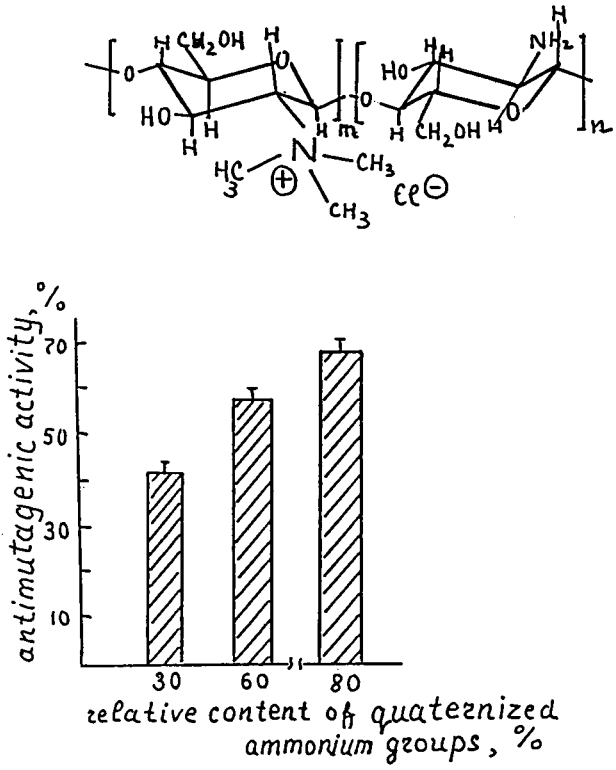


Fig.3 The antimutagenic activity of the chitosan derivatives containing a different number of quaternery ammonium groups. The polycations were tested in a plant system at γ- radiation in a dose of 1500 rad., and their antimutagenic activity is expressed in percent of mutation incidence in the control cells taken as 100 % after this dose.

stable radicals of a phenoxy type, thereby breaking the chains of free-radical reactions.

The ternary copolymers were obtained by interaction of DADMAC and AA (30: 70) - binary copolymer with hydrazide of β-[(4-hydroxy-3,5-ditertbutyl)phenyl] propionic acid [10].

Ternary copolymers (TCPs) differing in the proportion of modified acrylic acid units were synthesized (1 %, 5 % and 15 % in TCP - 1, TCP - 2 and TCP - 3 respectively), Fig. 4. In this investigation we made an attempt to determine correlation between the antiradical activity and antimutagenic efficiency of ternary copolymers.

Antiradical properties of the TCPs were evaluated spectrophotometrically by finding the degree to which a solution of the stable radical DPPH was discolored. The results obtained confirmed that the more number of HP fragments in the macromolecules the greater the capacity of the copolymer to recombine with DPPH.

However. as we can see from the data (Tabl. 1) the proportion of active HP fragments taking part in the homolytic reaction with DPPH was some what lower - 60 % in the test with TCP-3, then that in the test with TCP-1 and TCP-2 - 80 %. Thus, a high relative content of HP fragments is not reasonable for high an antiradical activity achievement.

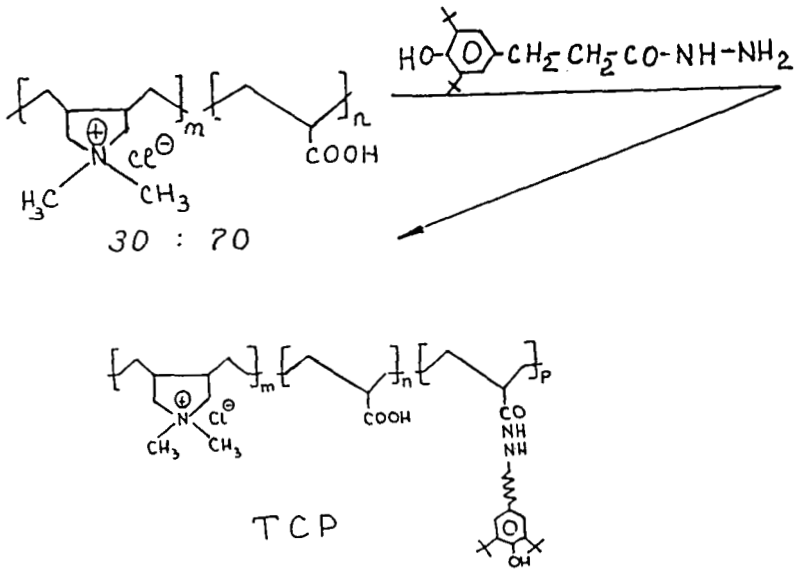


Fig. 4 Ternary copolymers of a diallyldimethylammonium type, containing hindered phenol fragments.

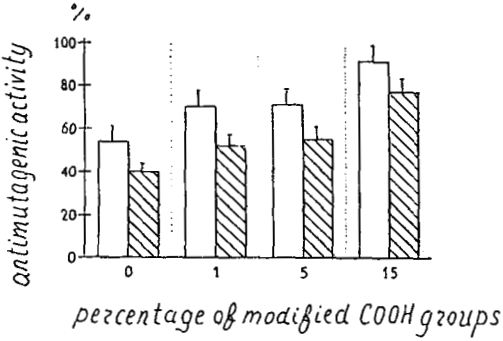


Fig. 5 The antimutagenic activity of cationic polyelectrolytes containig different numbers of hindered phenol fragments. The polyelectrolytes were tested in a plant system before (dark bars) or after (white bars) their exposure at γ- radiation in a dose of 500 rad., and their antimutagenic activity is expressed in percent of mutation incidence in the control cells taken as 100 % after this dose.

Table 1. Dependence of Antiradical Activity of Ternary Copolymers (TCPs) on the Levels of Hindered Phenol Fragments

Copolymer	Percentage of modified COOH groups	$C_{exp.}$, M	$C_{theor.}$, M	$\frac{C_{exp.}}{C_{theor.}}$, %
TCP-1	1	6.8×10^{-7}	8.3×10^{-7}	80
TCP-2	5	1.7×10^{-6}	2.1×10^{-6}	80
TCP-3	15	2.7×10^{-6}	3.8×10^{-6}	60

Note. $C_{exp.}$= concentration of DPPH- reacting HP fragments in the TCPs;

$C_{theor.}$= theoretical concentration of these fragments in the TCPs.

Testing TCPs for antimutagenic activity on the plant test system indicated that the protective efficiency increased with the growth in the number of HP fragments in the copolymers as it can be seen from the data of the Fig.5. However, with the growth in the number of HP - fragments the toxicity of TCPs increased ,and the protective efficiency diminished, and the maximal antimutagenic effect for TCP-3 was equal to only 58 % , as it was shown in the test for micronuclears in mouse bone marrow erythrocytes in the previous paper [1 1]. Thus ,the incorporation in the copolymer side chain a relatively high quantity of the hindered phenol is not favorable for achievement of both an antiradical and antimutagenic high efficiency with maintaining of polymer low toxicity.

Taking into account this consideration we tried to obtain a high efficient antimutagenic polymeric system on the chitosan basis introducing in the structure of partially quaternized chitosan a relatively low quantity of the hindered phenol. For this purpose chitosan in which 50 % of primary amine groups has been converted into the quaternized ammonium groups was chosen as an initial polymer. Hindered phenol fragments in quantity of 4% Mol. were introduced by interaction with chitosan primary amine groups.

The antimutagenic activity of this chitosan derivative was investigated on the plant test - system (barley seeds). The data of Fig.6. confirmed that targetted modification of the chitosan matrix resulted in enchancement of the

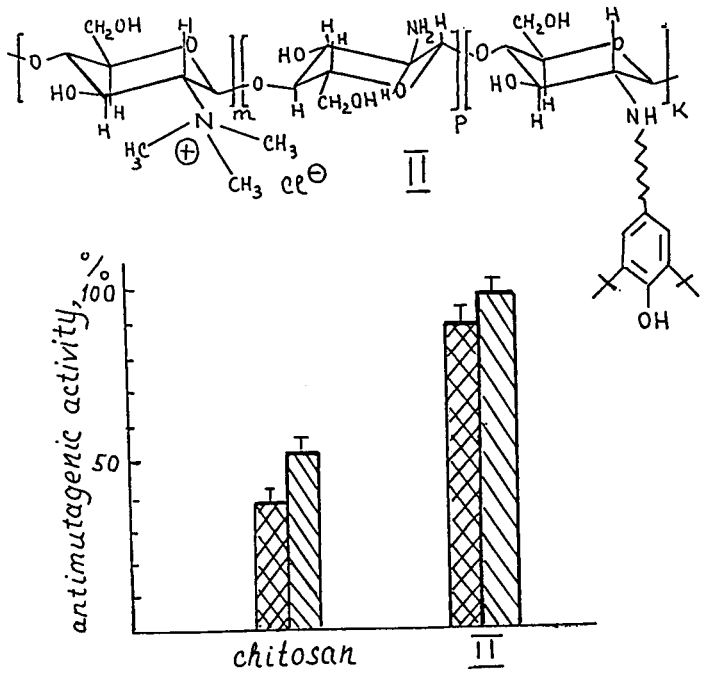


Fig. 6 Antimutagenic activity of chitosan and chitosan derivatives containing quaternary ammonium groups and hindered phenol fragments. The polymers were tested in a plant test system before (left bars) or after (right bars) their exposure at γ- radiation in a dose of 1500 rad. and their antimutagenic activity is expressed in percent of the mutation incidence in the control cells taken as 100 % after this dose.

antimutagenic efficiency of the polymer system up to 89 - 93 %. It should be noted that the high efficiency of such chitosan derivatives was retained in a wide range of polymer concentrations.

CONCLUSIONS

Thus, the new approach to the problem of construction of efficient antimutagenic at γ - irradiation systems has been developed using as models synthetic polycations of a diallyldimethylammonium type. Taking into account the dependencies found the macromolecular design of superefficient antimutagenic systems on the basis of the natural biodegradable nontoxic polycation - chitosan has been realized.

REFERENCES

[1] E. N. Goncharenko, Y. B. Kudryashov, Radiotzionnaya biologiya.Radioekologiya, 36(4) 573-586,(1996)

[2] V.M. Zemskov, A.V.Khramtsov, S.D.Rodionov, A.A. Barsukov, V.N.Paitin, L.P. Alekseev, A.A. Vedernikov, M.I.Mustafaev, Immunologiya, 4, 49-52 (1989)

[3] N.M. Boyarkina, V.V. Kryuchkov, E.S. Parkhamovich, L.A. Amburh, D.A. Topchiev, V.A.Kabanov, Plasticheskiye Massy , 8, 17-20 (1987).

[4] A.P.Odin, V.A.Alexandrova, V.S. Sokolov, D.A. Topchiev, Membrane and Cell Biol., 9, 197-204 (1995)

[5] V.A. Shevchenko, D.A.Topchiev, V.A.Alexandrova, E.B.Kotlyarova, A.P. Odin, N.S.Domnina, RU Pat. N 2043368.

[6] M.S. Blois, Nature, 181 (4617) ,1199-1200 (1958).

[7] L.A.Nud'ga, E.A. Plisko, S.N. Danilov, Zhurnal Obshchey Khimii, 43 (12), 2756-2760 (1973)

[8] D.H. Rich J. Singh, The Peptides: Analysis,Synthesis, Biology, 1, 211-248 (1979).

[9] V.A. Shevchenko, L.M. Bakhitova, L. A. Ostrovskaya, E.B. Kotlyarova, V.A. Alexandrova , D.A. Topchiev, N.A. Plate, RU Pat. N 163204.

[10] V.V. Ershov, K.B. Piotrovskii, N.A. Tupikina, Izv. Akad. Nauk. SSSR, Ser. Khimiya, 5, 1174 (1976).

[11] V.A. Alexandrova, E.B.Kotlyarova, A.P.Odin, N.S. Domnina, V.A. Shevchenko, D.A.Topchiev, Radiotzionnaya biologiya. Radioekologiya, 35 (50),746-752, (1995)