Bulletin of Experimental Biology and Medicine, Vol. 120, №9, September, 1995

Antiradical Activity of Ternary Copolymers from the Diallyl Series in Mediating the Antimutagenic Effect

A. P. Odin, V. A. Aleksandrova, A. V. Lebedev, D. A. Topchiev, E. B. Kotlyarova, and V. A. Shevchenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 9, pp. 265-267, September, 1995
Original article submitted November 30, 1994

Ternary copolymers of the diallyl series were tested for antiradical activity against the stable radical 1,1-diphenyl-2-picrylhydrazyl, and the numbers of active antiradical fragments which are present in their macromolecules and which may participate in the free-radical reactions induced by gamma radiation in living organisms were estimated. A correlation was found between the antiradical activity of ternary copolymers and their ability to protect barley seedling roots from mutagenic effects of gamma radiation.

Key Words: *ternary copolymers; antiradical activity; antimutagenesis; hindered phenols*

Cationic polyelectrolytes are characterized by a broad spectrum of biological activities (antineoplastic, bactericidal, etc.), which mainly result from electrostatic interaction of the positively charged polyelectrolyte macromolecules with negatively charged cell membranes. It remains unknown, however, whether cationic polyelectrolytes can protect cells from the mutagenic action of γ radiation.

Polyelectrolytes of the diallyl series were selected for study as mutagens in view of the potential ability of polycations adsorbed onto the cell surface to participate both in the scavenging of short-lived radicals and particles of ionic nature and in the inactivation of metastable states in the cell during the postirradiation period. Cationic polyelectrolytes of the diallyl series have been found to exhibit pronounced antimutagenic activity in tests with plant cells (barley seeds) and mammalian bone marrow cells [10,11]. Because the mutagenic action of γ radiation is mediated by free radicals generated in extracellular water and biosubstrates [12,13], substances such as α-tocopherol, ionol, and polyphenol, which are capable of scavenging free radicals with the formation of inactive products, can effectively antagonize the mutagenic effects of this radiation [1,2,4,5].

To produce efficient antimutagenic agents with enhanced antiradical activity, ternary copolymers were synthesized on the basis of the dimethyldiallylammonium chloride (DCh): acrylic acid (30:70) copolymer into which a derivative of a hindered phenol was incorporated (Fig. 1). This compound is able to form stable radicals of the phenoxy type, thereby breaking the chains of free-radical reactions [3,7].

Of considerable interest for scientists trying to devise methods for targeted synthesis of polymers capable of protecting cells from γ radiation are studies designed to reveal correlations between the structure and activity of polymers. In this study, we made an attempt to detect correlations between the antiradical activity and antimutagenic efficiency of ternary copolymers in a plant system (barley root seedlings) by treating it with these copolymers before or after exposure to γ radiation.

MATERIALS AND METHODS

We tested cationic polyelectrolytes of the diallyl series produced at the Institute for Petrochemical

Institute of Experimental Cardiology, Cardiological Research Center, Russian Academy of Medical Sciences; A. V. Topchiev Institute of Petrochemical Synthesis, Russian Academy of Sciences; N. I. Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow (Presented by V. N. Smirnov, Member of the Russian Academy of Medical Sciences)

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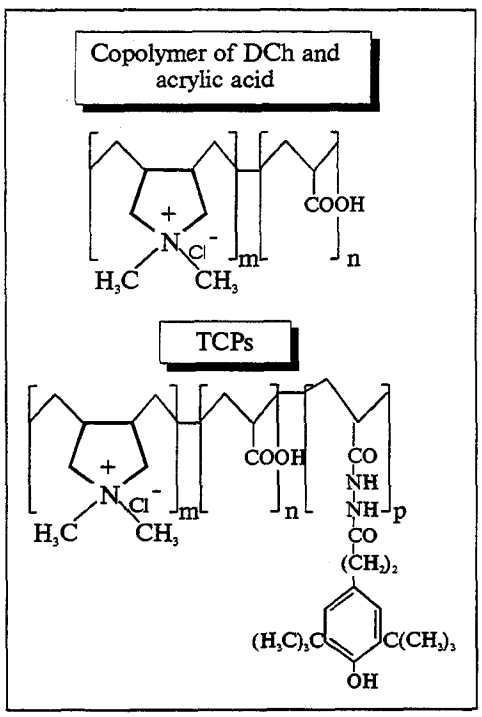

Fig. 1. Polyelectrolytes of the diallyl series: copolymers of dimethyldiallylammonium chloride (DCh) and acrylic acid and ternary copolymers (TCPs) containing fragments of hindered phenols.

Synthesis, namely the polycation polydimethyldiallylammonium chloride and copolymers of DCh and acrylic acid (Fig. 1) differing in composition and having an intrinsic viscosity [η] of 0.21 dl/g (1 N NaCl, 30°C) at a molecular weight distribution of 1.54. All polyelectrolyte samples were thoroughly purified by a conventional procedure [6] and their purity was monitored by recording nuclear magnetic resonance ^{13}C spectra with an MSL-300 apparatus (Brucker).

A ternary copolymer of DCh, acrylic acid, and the β-[(4-hydroxy-3,5-ditert-butylphenyl)ethylcarbonyl]hydrazide of acrylic acid were obtained by reacting the binary copolymer DCh:acrylic acid (30:70) with the hydrazide of β-(4-hydroxy-3,5-ditert-butylphenyl)propionic acid synthesized as previously described [8] (its purity was verified by the melting point). Three 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). The copolymers were modified by the carbodiimide method widely used in peptide synthesis [15] and their structure was confirmed by elemental analysis and ultraviolet spectroscopy by the emergence, in the 240-260 nm region, of a band responsible for the presence of covalently bound benzene rings in the structure.

Antiradical properties of the TCPs were evaluated by noting the degree to which a 0.5% methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was discolored [14]. The model reagent used for plotting the calibration curve was 4-(2-carbamethyl)-2,6-ditert-butylphenol [8]. The decline in the level of free DPPH was monitored spectrophotometrically at λ=516 nm relative to the solution with a completely discolored DPPH (in the presence of a 10-fold excess of α-tocopherol reactive in an equimolar manner with DPPT). The α-tocopherol and DPPH were from Sigma; the dicyclohexyl-carbomiimide we used was of chemical purity.

The original copolymer and TCPs were tested for their effects on γ radiation-induced structural changes in plant chromosomes by recording the number of chromosome aberrations at the metaphase of mitosis in meristematic cells of barley (*Moskovskii-121* variety) seedling roots. The seedlings were treated with the copolymers before or after their exposure to γ radiation in a dose of 500 rad (4.88 Gy) from a ^{137}Cs source at a dose rate of 4.44 Gy/min. The concentration of each copolymer was 1.95×10^{-3} mol/liter. The experimental procedure is described in detail elsewhere [11]. Chromosome rearrangements were analyzed in total seedling root preparations stained by the Feulgen method. For each point, at least 300 metaphases were analyzed, about 50 cells for each root being examined. In addition to the general level of mutability, chromosome and chromatid rearrangements of all types were taken into account. The protective effect of a copolymer was defined by the number of removed mutations and expressed in percent. The significance of differences between the results obtained for the test and control (copolymer-untreated) preparations was estimated by Student's *t* test.

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

Copolymer	Percentage of modified COOH groups	$C_{exp.}$, M	$C_{theor.}$, M	$C_{exp.}/C_{theor.}$, %
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 hindered phenol fragments in the TCP; $C_{theor.}$ = theoretical concentration of these fragments in the TCP.

RESULTS

Our tests of TCPs for antiradical activity confirmed that the more hindered phenol (HP) fragments that are present in their macromolecules, the greater the capacity of the copolymers to recombine with DPPH. That this is so was indicated by decreases in the optical density of the solution, which depends on the concentration of unreacted DPPH. Preliminary tests showed that the antiradical activity (with respect to DPPH) of the polymeric matrices themselves may be disregarded. However, the proportion of active HP fragments participating in the homolytic reaction with DPPH was somewhat lower (60%) in tests with TCP-3, which has a high relative content of HP fragments, than in TCP-1 and TCP-2 (80%), which have a lower content of these fragments (Table 1), probably because some of the HP fragments were screened in TCP-3. This screening phenomenon may be due to hydrophobization of copolymer macromolecules by hydrocarbon radicals in TCPs with increased numbers of HP fragments, leading to substantional conformational changes in their macromolecules in solution, with the formation of a denser globe with hydrophobic HP fragments in the inner part of the macromolecule [9].

Testing the TCPs for antimutagenic activity indicated that their radiation-protective effect increases with an increase in the number of HP fragments they contain (Fig. 2). Indeed, as can be seen in this figure, the enhancement of antimutagenic activity in comparison with the original copolymer (DCh:acrylic acid) correlates with the proportion of HP fragments in the TCPs. Since the homopolymer polydimethyldiallylammonium chloride and the copolymer DCh: acrylic acid failed to exhibit appreciable antiradical activity in the reaction with DPPH, the strong correlation between the antiradical and antimutagenic activities of the TCPs provides convincing evidence that the enhanced antimutagenic effectiveness of the TCPs results from the presence of HP fragments in their structure. This finding augurs well for raising the antimutagenic efficacy of polymeric protective agents by simultaneously incorporating into the macromolecule antiradical fragments differing in chemical structure and capable of interacting in a synergistic manner.

This study received support from the Russian Foundation for Basic Research (Grant No. 94-04-13177a) and from the Scientific Council for the Russian State Research Program Research Priorities in Medicine and Public Health (Topic No. 172: Atherosclerosis).

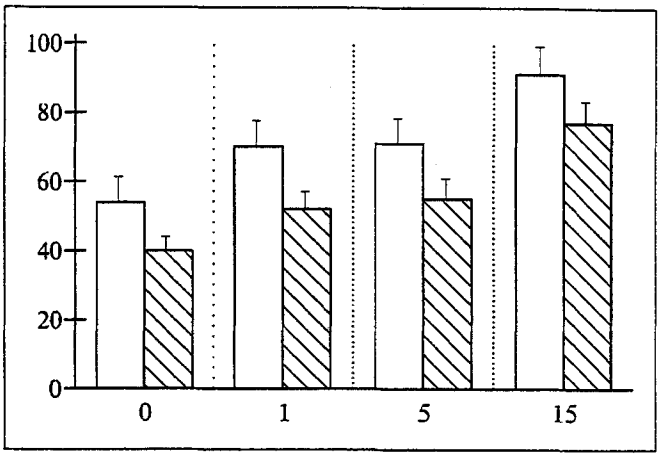

Fig. 2. Antimutagenic activity of cationic polyelectrolytes containing different numbers of hindered phenol (HP) fragments. The polyelectrolytes were tested in a plant system (meristematic cells of barley seedling roots) before (dark bars) or after (white bars) their exposure to γ radiation in a dose of 500 rad, and their antimutagenic activity is expressed in percent of the mutation incidence in the control cells taken as 100% after this dose. Abscissa: percentage of acrylic acid units modified by HP fragments.

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