Effect of Catalin on Radical Production in Aqueous Solutions and Cross-Linking in Deoxyribonucleoprotein Complex

N. B. Polyanskii, V. I. Binyukov, and E. Mil'

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The effect of catalin on cross-linking in the DNA-protein system is studied after illumination with visible and UV light. Catalin is shown to stimulate this process. The use of a hydroxylamine trap reveals that catalin (10-100 μM) induces the accumulation of free radicals in aqueous solutions as soon as 10 minutes after irradiation. A considerable amount of $O_2^{\cdot-}$ is fixed in the medium during illumination of catalin aqueous solution. Superoxide dismutase added to the incubation medium considerably reduced the number of recorded radicals.

Key Words: *catalin; deoxyribonucleoprotein complex; superoxide anion radical; lens; cataract*

UV-induced cataracts comprise a high percentage of all cataract diseases. Disturbance of the nuclear apparatus of the germinal layer of the lens is known to be the main cause of radiation cataracts [2,4].

At present, catalin (Japan) is widely used for conservative treatment of cataract. *In vitro* experiments have demonstrated the ability of catalin to inhibit photooxidation in cultured rat lens and to prevent the 1,2-naphthoquinone-induced formation of covalent bonds in isolated lens proteins. Inhibition of linoleic acid autooxidation has also been reported [5, 7]. Thus, catalin exhibits an antioxidative activity.

Antioxidants are known to inhibit the formation of covalent bonds in a deoxyribonucleoprotein complex (DNP) induced by visible and UV light (reduced number of cross-links in the DNA-protein complex) [3].

Accordingly, the aim of the present study was, by using a simple experimental model, to investigate the effect of catalin on the UV-induced covalent bonding within a DNA-protein complex. It was assumed that catalin, acting as an antioxidant, would inhibit the formation of DNA-protein cross-links.

Laboratory of Physicochemical Regulation of Cell Metabolism, Laboratory of Physicochemical Methods of Study of Nucleic Acids, N. N. Semenov Institute of Chemical Physics, Russian Academy of Sciences; Group of Membrane Biochemistry, A. N. Bakh Institute of Biochemistry, Russian Academy of Sciences, Moscow. (Presented by I. P. Ashmarin, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

DNP was isolated from rat liver as described elsewhere [1]. DNP was UV-illuminated with a BUF-15 mercury lamp (4.0×10.2 erg/sec×mm² power, 10.5 erg/mm²) in the presence of various concentrations of catalin.

TABLE 1. Generation of Radicals in Aqueous Solutions Containing a Hydroxylamine Trap as a Function of the Concentration of Catalin and Duration of Exposure to Visible Light (Intensity of Signal Expressed in Arb. Units with Respect to Mn Standard, $n=3$)

Time, min	Concentration of catalin, M		
	control	10^{-5}	10^{-4}
0	0.50±0.05	0.48±0.05	0.60±0.05
5	0.61±0.05	1.08±0.07	1.12±0.06
10	0.74±0.06	1.95±0.08	6.52±0.14
20	0.78±0.06	3.22±0.10	14.03±0.23

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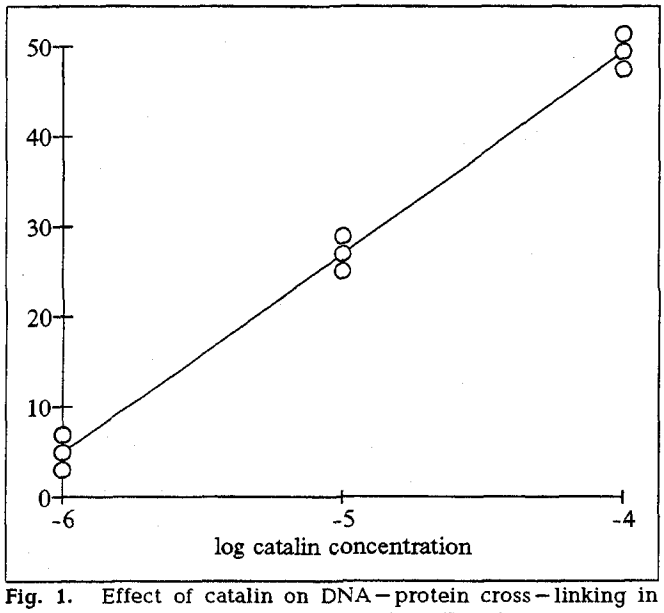

Fig. 1. Effect of catalin on DNA–protein cross–linking in UV–illuminated samples. Here and in Fig. 2: the ordinate depicts the increase of cross–linking in % in comparison with the control (100%).

Illumination with visible light was performed with an LD-20 luminescent daylight lamp (wavelength more than 320 nm) with a piece of glass serving as a filter.

For measurement of the number of DNA-protein cross-links (postillumination) the DNA-protein complex was dissociated in 1.5 M NaCl and 1% SDS-Na and left in the cold for 12 h. The complex was pelleted by 1-hour centrifugation at 8000 rpm. Protein-free DNA was assayed by gel chromatography on LKB AcA-54 ultragel (5-70 kD) by separating high-molecular DNA (first peak) from low-molecular fractions. The column was connected with a flowthrough cuvette of an SF-26 spectrophotometer coupled with a KSP-4 writer. The amount of DNA was determined by the area of the elution peak.

The number of DNA-protein cross-links was calculated from experimental data by the formula:

$$\% \text{ cross-links} = (P_o - P_{ill})/P_o,$$

where P_{ill} is the area of the elution peak after illumination and P_o is the area of the elution peak for a nonilluminated sample of DNA.

Radicals formed in aqueous solutions were recorded by electron paramagnetic resonance (EPR) using an RE 1306 radiospectrometer. Radicals generated in the system were measured using a 1-hydroxy-2,2,4,5,5,-pentamethyl-δ^3-imidazoline spin trap (synthesized at Institute of Chemical Physics and kindly provided by A. B. Volodarskii) and the formation of an iminooxide radical from hydroxylamine was judged from the intensity of the EPR signal (triplet spectrum, g=2.007; K_{CTC}=14.8 G).

$OH^{\cdot}$ and superoxide anion radicals were determined using 5,5-dimethyl-1-pyrroline-N-oxide (DMPO, 10^{-1} M; Sigma). The EPR signal represented a 1 2 2 1 four-component spectrum (K_{CTC}=14.8 G). The intensity of the signal was expressed in arbitrary units with respect to an external standard (Mn).

The effect of superoxide dismutase on light-induced generation of radicals in aqueous solutions of catalin was studied at a final concentration of the enzyme of 0.1 mg/ml.

A medicinal preparation of catalin was used in the experiments; to this end the tablet was dissolved in water and the filler was removed by centrifugation (5000 rpm, 10 min).

RESULTS

The effect of catalin (10^{-4}-10^{-6} M) on UV-induced cross-linking in a DNA-protein system was studied. Increasing the concentration of catalin from 10^{-6} to 10^{-4} M is paralleled by a 10-50% increase in the number of cross-links (Fig. 1). Thus, catalin does not prevent cross-linking in a chromatin complex. UV-induced covalent bonding between DNA and chromatin proteins occurs via a radical mechanism; hence, it is not quite correct to explain the effect of catalin through capture of free radicals derived from tryptophan and tyrosine due to UV irradiation.

Illumination may lead to the generation of free radicals both directly in water and due to the absorption of light quanta by catalin. Taking into account the ability of catalin to absorb light within the visible spectrum, we studied its effect on cross-linking under the action of visible light. The data

TABLE 2. Intensity of Generation of Oxyradicals in Aqueous Solutions of Catalin Exposed to Visible Light in the Presence of DMPO (n=3)

Exposure time, min	Concentration of catalin, M		
	10^{-5}	10^{-4}	control
30	0.74±0.08	0.51±0.06	0.29±0.05
60	0.63±0.06	0.25±0.05	0.27±0.05

TABLE 3. Effect of SOD on Generation of Radicals in an Aqueous Solution of Catalin in the Presence of DMPO (n=3)

Signal, amplitude, min	Signal from trap	Control	In the presence of SOD
10	0.16±0.02	1.38±0.05	1.14±0.05
30	0.29±0.02	1.25±0.07	0.50±0.06

presented in Fig. 2 show that illumination with visible light also increased the number of cross-links in the DNP complex. Thus, generation of free radicals under illumination with visible light is also promoted by catalin.

The generation of free radicals in the presence of catalin was studied using spin traps (Table 1).

Illumination of catalin aqueous solutions enhances the generation of free radicals. Some increase of the EPR signal in the control sample with time results from the accumulation of radicals due to illumination of the spin trap or its photooxidation.

For elucidation of the nature of generated radicals we used a DMPO radical trap, which make it possible to detect the accumulation of OH and superoxide anion radicals. The recorded EPR spectrum of aqueous catalin solutions was characteristic for OH radicals (superoxide anion radicals in aqueous solutions readily transform to OH radicals). The EPR data of catalin aqueous solutions presented in Table 2 show that 30 min after illumination the number of superoxide anion radicals increased by 70% at a concentration of 10^{-4} M and almost 3-fold at a concentration of 10^{-5} M. It is unclear why we observed a higher concentration of radicals at a lower concentration of catalin. This may be partially attributed to photoshielding, an effect which, however, cannot be significant since the samples were agitated. It is more likely that this process was related to phototransformation of the preparation.

In order to elucidate whether the EPR signal arises from the OH or superoxide anion radical, superoxide dismutase (SOD) was added to the incubation medium and then EPR spectra were recorded. Ten and 30 min after the addition of SOD (concentration of catalin 10^{-5} M) the production of radicals was reduced by 20% and 78%, respectively (Table 3).

The difference in the absolute values in Tables 2 and 3 is due to somewhat higher concentration of spin trap in the incubation medium in the experiments with SOD. Thus, the superoxide anion radical makes the maximal contribution to the recorded signal.

Under the action of visible light complex aromatic molecules may actively generate superoxide radicals. The latter give rise to OH^{E} radicals, which attack DNA, proteins, and membranes. Relatively low concentrations of superoxide anion radical are able to induce lysis of cells and cell organelles [6]. The effect of catalin is primarily explained on the basis of the quinoid [11] and "oxidative shock" [8,9] theories. According to the above theories cataract is caused either by peroxidation processes (oxidative shock theory) which affect membranes and induce cross-linking in lens proteins, or by abnormal metabolism of tryptophan and its metabolites (including UV-induced transformations) (quinoid theory), thus yielding quinoid compounds. The latter initiate the formation of covalent bonds between lens proteins and cause membrane damage by inducing peroxidation processes in the membranes [12,13]. In this context it is interesting to note that radiation damage of lens DNA is also mediated through superoxide radicals [10].

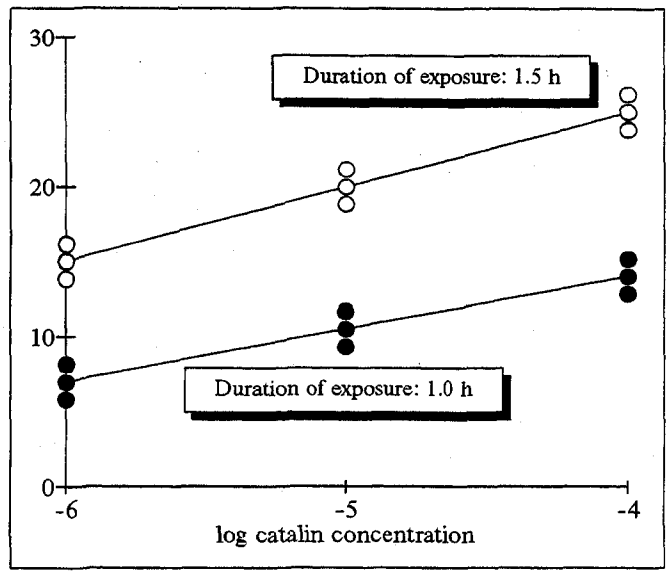

Fig. 2. Effect of catalin on DNA–protein cross–linking in samples illuminated with visible light.

Summarizing our data, we may conclude that in our *in vitro* experiments catalin does not act as an antioxidant but facilitates the generation of superoxide radicals.

This casts some doubt on the explanation based on the antioxidant mechanism of catalin action against cataract.

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Changes in the Serum Antioxidant System and Lipid Peroxidation under the Influence of Asbestos

S. K. Soodaeva, E. A. Ostrakhovich, A. V. Kozlov, and B. T. Velichkovskii

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The iron content, the state of the serum antioxidant system, and their relationship with the changes in lipid peroxidation in rat liver and lungs at the early stages of chrysotile-asbestos action, and the effect of the naturally occurring flavonoid rutin are studied. Intensification of lipid peroxidation in the liver and lungs and an increase in the oxyproline content, which correlates with the rise in serum antioxidant activity, are observed four weeks after a single intratracheal administration of 50 mg asbestos. The total serum iron content remains unchanged. Rutin has a pronounced anti-asbestos effect, inhibits the early stages of fibrosis, and facilitates normalization of the antioxidant system imbalance induced by asbestos.

Key Words: *asbestos; antioxidant activity; ceruloplasmin; transferrin; rutin*

Recently, the pathological effect of asbestos has been attributed to the potentiation of the free-radical processes (FRP) in the organism [4,10]. Intensification of lipid peroxidation (LPO) and changes in some parameters of the antioxidant system [7,12] during prolonged contact with asbestos have been revealed. Ceruloplasmin (Cp) and transferrin (Tf) are the main contributors to the serum antioxidant system (AOS) [3,8]. Their effect is based on oxidation of Fe^{2+} and binding of Fe^{3+} and on the interaction with oxygen radicals. The ratio of Cp and Tf electron paramagnetic resonance (EPR) signals, which reflects antioxidant activity (AOA) is recommended as a parameter displaying the best correlation with the severity of the pathological state [8]. The role of iron ions in FRP induction (these ions are present in asbestos fibers) has been extensively discussed. Of interest in this connection are the preparations with chelating and antioxidant activities, which may prevent the toxic effect of asbestos. For a better evaluation of the role of FRP in the pathogenesis of diseases provoked by asbestos the determination of both the LPO intensity and the assessment of total AOA are necessary. In the present study we investigated the levels of iron ions, the serum AOS, their relationship with changes in LPO in rat liver and lungs at the early stages of asbestos action, and the effect of the naturally occurring flavonoid rutin (the vitamin P group) on these parameters.

Laboratory of Free-Radical Mechanisms of Respiratory Organ Pathology, Institute of Pulmonology, Russian Ministry of Public Health; Laboratory of Pathogenesis and Experimental Therapy of Pneumoconioses, N. I. Pirogov Russian State Medical Institute, Moscow

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