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INHIBITION OF LIPID PEROXIDATION IN ERYTHROCYTE MEMBRANES BY N-PHENYL-1-NAPHTHYLAMINE

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Abstract. It was shown that N-phenyl-1-naphthylamine appears in lipid peroxidation of erythrocyte ghosts in two roles: as an antioxidant it leads to inhibition of lipid peroxidation, and as a fluorescent probe it responds to the lipid peroxidation with a decrease in emission. N-phenyl-1-naphthylamine was more effective as an inhibitor of lipid peroxidation than 1-anilinonaphthalene-8-sulphonate.

Key words: fluorescent probe, N-phenyl-1-naphthylamine, 1-anilinonaphthalene-8-sulphonate, antioxidant, lipid peroxidation.

INTRODUCTION

1-Anilinonaphthalene-8-sulphonate (ANS) is well known as a membranotropic fluorescent probe in biological studies. ANS has been used to establish the influence of many different chemical and physical factors on biological membranes including the changes of membrane properties at lipid peroxidation [1, 2]. The response of ANS to lipid peroxidation consists in a decrease of fluorescence. It has also been shown that ANS is an antioxidant and inhibits lipid peroxidation in biological membranes [3].

N-Phenyl-1-naphthylamine (PNA-1) is a structural analogue of ANS. Owing to their similarity it is likely that also PNA-1 inhibits lipid peroxidation. Lipid peroxidation reactions proceed in the depth of membranes and the action of lipid peroxidation inhibitors depends on their accessibility to the radical forming centres. In membranes, PNA-1 penetrates deeper than ANS [4]. So it may be presumed that the inhibiting activities of PNA-1 and ANS in lipid peroxidation are different due to their different localization in membranes.

In this paper the data on the effect of PNA-1 on lipid peroxidation in irradiated erythrocyte ghosts and the influence of lipid peroxidation on the fluorescence of PNA-1 are presented. The effectiveness of PNA-1 and ANS in the inhibition is compared.

EXPERIMENTAL

In the experiments, PNA-1 and ANS from Merck (Darmstadt, BD) were used. The preparations were purified by recrystallization in cold ethanol.

Erythrocyte ghosts were prepared according to the modification [5] of the description by Dodge et al. [6] and suspended in isotonic phosphate buffer (pH 7.4) at the concentration 2 mg protein/ml (protein was determined by biuret reaction). The ghosts were irradiated with gamma-rays (dose 60 Gy) and incubated 30 min at 37°C in the presence of $FeSO_4$ and ascorbic acid (12 μM and 0.8 mM, respectively).

The intensity of lipid peroxidation was determined by means of the thiobarbituric acid (TBA) test. The components insoluble in 15% trichloroacetic acid were removed, equal volumes of 0.5% TBA were added to the supernatants and heated at 100°C 15 min. Extinction was measured at 532 nm and the results were presented as nmoles of malondialdehyde (MDA) per mg protein using the extinction coefficient $1.56 \times 10^5\ M^{-1} \cdot cm^{-1}$ for the MDA–TBA complex.

Stock solutions of PNA-1 and ANS were prepared in ethanol in concentrations 4 and 40 mM, respectively. PNA-1 and ANS were added to erythrocyte ghosts before or after the irradiation of the ghosts. The concentration of PNA-1 added to the ghosts before the irradiation varied in the range of 0.5–10 μM, that of ANS was in the range of 0.5–50 μM. The concentrations of PNA-1 or ANS added after the irradiation of ghosts were 10 and 50 μM, respectively.

The fluorescence of PNA-1 was measured with a fluorometer Analyz-1 at 411 nm by excitation at 366 nm. The concentration of PNA-1 in the fluorescence measurements was 5 μM.

RESULTS AND DISCUSSION

Data characterizing the formation of MDA in irradiated and nonirradiated erythrocyte ghosts in the presence of Fe^{2+} are presented in Fig. 1. As it can be seen, MDA is not formed in nonirradiated ghosts. In irradiated erythrocyte ghosts, the amount of MDA increased during the whole time of incubation. The addition of PNA-1 to the ghosts before Fe^{2+} inhibited lipid peroxidation completely. PNA-1 when added after Fe^{2+}, stopped further formation of MDA. A similar effect on the inhibition of lipid peroxidation was observed by using 50 μM ANS (data not presented).

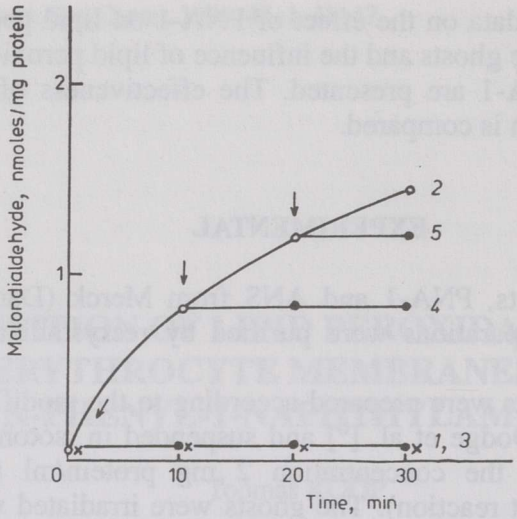

Fig. 1. Effect of N-phenyl-1-naphthylamine on lipid peroxidation in erythrocyte ghosts. *1* – nonirradiated ghosts, *2–5* – irradiated ghosts; *1, 2* – incubation without N-phenyl-1-naphthylamine, *3–5* – incubation in the presence of 10 μM N-phenyl-1-naphthylamine. Arrow points to N-phenyl-1-naphthylamine addition.

The effect of PNA-1 and ANS on lipid peroxidation depending on the concentration of these probes is presented in the Table. PNA-1 and ANS were added to the erythrocyte ghosts before irradiation. The postradiation incubation was carried out with Fe^{2+}. Both PNA-1 and ANS inhibited the formation of MDA completely in the concentrations of 10 and 50 μM, respectively.

The formation of malondialdehyde (MDA) in erythrocyte ghosts irradiated in the presence of N-phenyl-1-naphthylamine (PNA-1) or 1-anilinonaphthalene-8-sulphonate (ANS)[1]

Concentration of PNA-1 or ANS, μM	Concentration of MDA, nmoles/mg protein, after 30 min incubation[2]	
	Irradiation in the presence of PNA-1	Irradiation in the presence of ANS
0.5	1.27	1.34
1	1.01	1.28
5	0.63	1.24
10	0.28	0.65
50	-	0.34

[1] The data are the means of 3–5 measurements. The standard error does not exceed 15%.

[2] The amount of MDA in the initial suspension of erythrocyte ghosts before irradiation was 0.26 nmoles/mg protein and it incresed to 1.38 nmol/mg protein after 30 min incubation of the irradiated mixture at 37 °C in the presence of $FeSO_4$ and ascorbic acid (see Experimental).

For initiating lipid peroxidation we used gamma-irradiation as a producer of free radicals. The first stable products in this case are hydroperoxides of lipids. To continue the lipid peroxidation reactions we used Fe^{2+} which disrupted the hydroperoxides, promoted chain reactions and the formation of end products, including MDA. Lipid peroxidation was inhibited by PNA-1 and ANS both in the phase of initiation (Table) and in the phase of chain reactions (Fig. 1). The formation of free radicals was common for these phases. Thus, PNA-1 and ANS are involved in lipid peroxidation at the level of inactivation of free radicals.

The antiradical actions of PNA-1 and ANS should evidently be identical. Both contain an NH group that can easily exchange proton [7] and therefore inactivate free radicals.

As shown, PNA-1 inhibited lipid peroxidation at lower concentrations than ANS. Since ANS is electrically charged, it should be located in the surface region of biological membranes. Hydrophilic PNA-1 should penetrate deeply into the lipid phase of membranes [4]. Considering that the reactions of lipid peroxidation proceed in the depth of the lipid phase, it is understandable why PNA-1 is a more effective inhibitor of lipid peroxidation in membranes than ANS.

PNA-1 as a fluorescent probe responds to lipid peroxidation with a decrease of emission. Fig. 2 shows that the decrease of PNA-1 fluorescence in erythrocyte ghosts correlated well with the amount of the formed MDA, i.e. with the intensity of lipid peroxidation. It must be emphasized that the addition of PNA-1 to the membranes stopped the progress of lipid peroxidation reactions immediately (Fig. 1). Therefore, continuous testing of lipid peroxidation with PNA-1 is not possible and it is necessary to carry out the measurements in the aliquots taken from the incubation mixture at suitable time intervals.

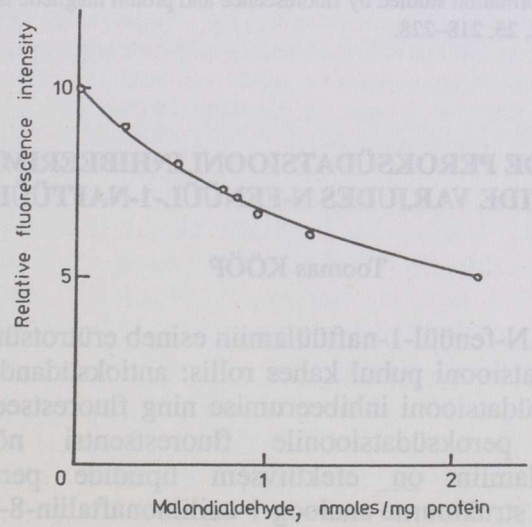

Fig. 2. Dependence of N-phenyl-1-naphthylamine fluorescence on the formation of malondialdehyde in erythrocyte ghosts. The erythrocyte ghosts were incubated without N-phenyl-1-naphthylamine.

In conclusion, when studying membranes with PNA-1 or ANS one has to consider the appearance of their antiradical effect at the concentrations suitable for using them as fluorescent probes.

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LIPIIDIDE PEROKSÜDATSIOONI INHIBEERIMINE ERÜTROTSÜÜTIDE VARJUDES N-FENÜÜL-1-NAFTÜÜLAMIINIGA

Toomas KÖÖP

On näidatud, et N-fenüül-1-naftüülamiin esineb erütrotsüütide varjudes lipiidide peroksüdatsiooni puhul kahes rollis: antioksüdandina põhjustab ta lipiidide peroksüdatsiooni inhibeerumise ning fluorestseeruva sondina vastab lipiidide peroksüdatsioonile fluorestsentsi nõrgenemisega. N-fenüül-1-naftüülamiin on efektiivsem lipiidide peroksüdatsiooni inhibiitor kui tema struktuurne analoog 1-aniliinonaftaliin-8-sulfonaat.

ИНГИБИРОВАНИЕ ПЕРЕКИСНОГО ОКИСЛЕНИЯ ЛИПИДОВ В ТЕНЯХ ЭРИТРОЦИТОВ N-ФЕНИЛ-1-НАФТИЛАМИНОМ

Тоомас КЁЭП

Показано, что N-фенил-1-нафтиламин выступает при перекисном окислении липидов в тенях эритроцитов в двух ролях: в качестве антиокислителя он обусловливает ингибирование перекисного окисления липидов, в качестве флуоресцентного зонда отвечает на перекисное окисление липидов подавлением флуоресценции. N-фенил-1-нафтиламин является более эффективным ингибитором перекисного окисления липидов по сравнению с его структурным аналогом 1-анилинонафталин-8-сульфонатом.