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INFLUENCE OF CARBONYL COMPOUNDS ON THE FLUORESCENCE OF *N*-PHENYL-1-NAPHTHYLAMINE IN ORGANIC SOLVENTS

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Abstract. It is shown that aliphatic aldehydes are effective quenchers of the *N*-phenyl-1-naphthylamine fluorescence in organic solvents and erythrocyte membranes while no chemical reaction between the probe and aldehydes is observed. We suggest that *N*-phenyl-1-naphthylamine can be used in certain cases as a reagent for detecting aldehydes in membrane systems.

Key words: *N*-phenyl-1-naphthylamine, aldehydes, fluorescence quenching.

INTRODUCTION

Fluorescence probes from the arylaminonaphthalene family are widely used in the studies of biological membranes damaged by free radical oxidation of lipids: in comparison with intact membranes, a significant decrease in the fluorescence of the probes has been observed in the damaged membranes [1–3]. Since the fluorescence of these probes decreases with the increasing of the polarity or fluidity of the environment [4–6], the fluorescence quenching in the damaged membranes has usually been considered to be a result of changes in their structure and/or general physical properties.

On the other hand, it has been shown that on the free radical oxidation of membranes a considerable amount of the end-products of the decomposed lipids, aliphatic aldehydes and ketones, appears in the membranes [7]. It may mean that the most important factor of the fluorescence quenching in the damaged membranes is the appearance of carbonyl compounds rather than the changes in the polarity and viscosity of the environment.

In order to evaluate the role of carbonyl compounds in the quenching of the fluorescence of arylaminonaphthalenes in membrane-modelling low-polarity media we have studied the influence of several aliphatic aldehydes and ketones on the fluorescence of *N*-phenyl-1-naphthylamine (PNA) in various organic solvents as well as in erythrocyte membranes.

It has been shown earlier in the literature that the fluorescence of PNA in cyclohexane can be quenched by esters [8], and the fluorescence of *N*-phenyl-2-naphthylamine in heptane, by acetaldehyde [9].

EXPERIMENTAL

In our study we used PNA from Merck. The preparation was purified three times by recrystallization in cold ethanol (—5 to —10 °C). Organic solvents — butanol, 3-methyl-1-butanol, hexane and dioxane were from Reakhim (analytical grade) and were used without further purification. 2-Butanone, butyraldehyde, propionaldehyde, *n*-valeraldehyde, 1-heptaldehyde, butyric acid and epoxybutane from Reakhim (pure grade) were distilled in the nitrogen atmosphere.

The erythrocyte ghosts were prepared by the treatment of erythrocytes with saponins followed by the washing of the ghosts in 0.154 M NaCl solution four times and their being resuspended in the same medium. The erythrocyte ghosts were incubated at 24 °C for 30 min with or without 1-heptaldehyde, thereafter PNA dissolved in ethanol was added. In fluorescence measurements the concentration of the ghosts protein was 0.2 mg/ml; the concentration of PNA, 5 or 10 μM; and that of ethanol, 0.048 per cent. The amount of PNA bound with the erythrocyte ghosts was determined by the measurement of the fluorescence of PNA after the erythrocyte ghost sediment had been treated with Triton X-100.

In typical experiments with organic solvents a carbonyl compound was added to a solvent in appropriate concentrations, then incubated at 24 °C for 60 min. PNA dissolved in the same solvent was then added, and after 30 min the fluorescence was recorded. The final concentration of PNA was 5 μM.

Fluorescence spectra were registered on an ISP-51 spectrograph with excitation at 366 nm. The intensity of fluorescence was recorded at 411 nm with excitation at 360 nm using an Analyser-1 apparatus.

The results were calculated as an average of 3—4 experiments.

RESULTS AND DISCUSSION

Influence of oxygen-containing aliphatic compounds on the PNA fluorescence in butanol. The results of the study of the influence of various oxygen-containing functional groups of aliphatic compounds on the fluorescence of PNA dissolved in butanol are shown in Fig. 1. Epoxide had practically no influence on the fluorescence properties of PNA while other functional groups quenched the probe emission in the following order: carboxyl < ketone < aldehyde.

Fluorescence maximum of PNA in butanol was observed at about 415 nm and the shift in the presence of quenchers did not exceed 13 nm. From that we made the conclusion that the addition of the studied compounds to the butanol solvent did not cause any significant change in the polarity of the medium. As illustrated in Fig. 2, PNA responds to the increase in the medium polarity by a quite remarkable bathochromic shift in λ_{max} of the fluorescence: almost 80 nm when going from dioxane to water.

Since solvent and quenchers with hydrocarbon chains of the same length and flexibility were chosen, we did not expect any significant differences in the medium viscosities of the systems and suggest that the fluorescence quenching by the studied compounds was a result of the influence of their carbonyl oxygens on the PNA probe.

Quenching of the PNA fluorescence in 3-methyl-1-butanol and hexane by aldehydes. Fig. 3 shows that the efficiency of an aldehyde as fluorescence quencher depends upon the length of its chain and on the solvent used. In 3-methyl-1-butanol, the compounds with the longer hydrocarbon chains were the stronger quenchers of the PNA fluorescence.

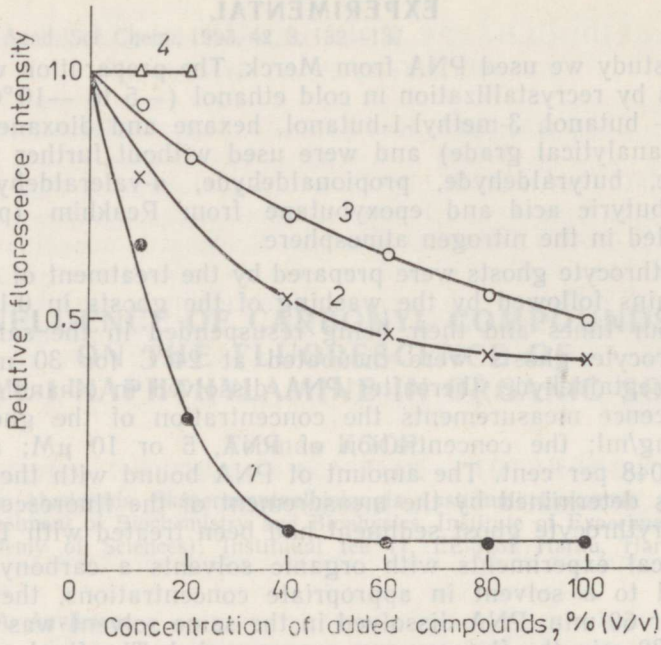

Fig. 1. The effect of butyraldehyde (*1*), 2-butanone (*2*), butyric acid (*3*) and epoxybutane (*4*) on the *N*-phenyl-1-naphthylamine fluorescence intensity.

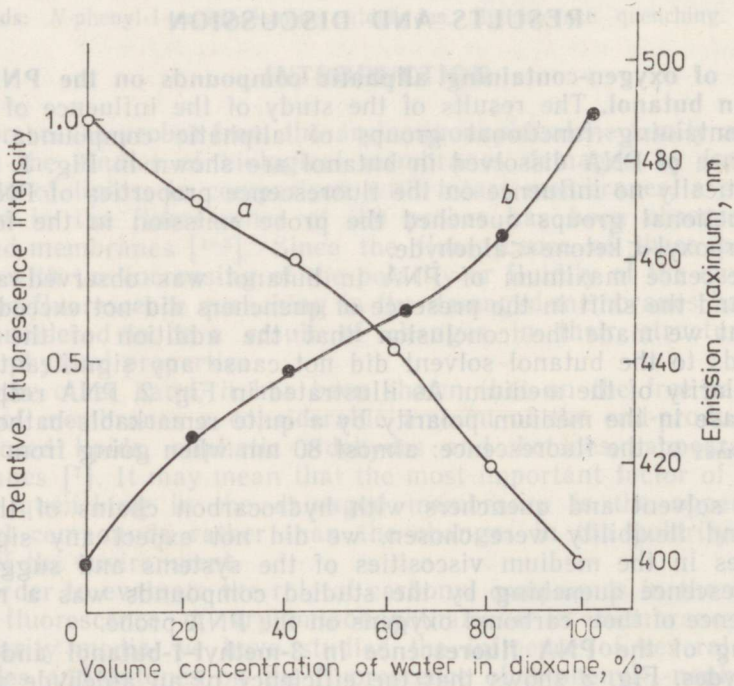

Fig. 2. The dependence of the intensity (*a*) and the position of fluorescence maximum (*b*) of *N*-phenyl-1-naphthylamine on the concentration of water in dioxane.

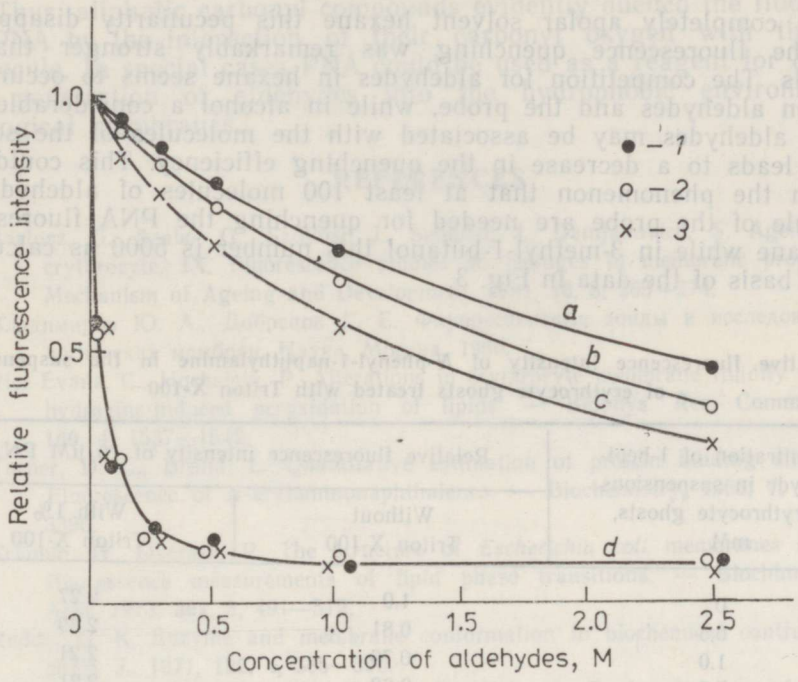

Fig. 3. The effect of aliphatic aldehydes on the fluorescence intensity of *N*-phenyl-1-naphthylamine in 3-methyl-1-butanol (*a*, *b*, *c*) and in hexane (*d*). *1* — propionaldehyde, *2* — valeric aldehyde, *3* — 1-heptaldehyde.

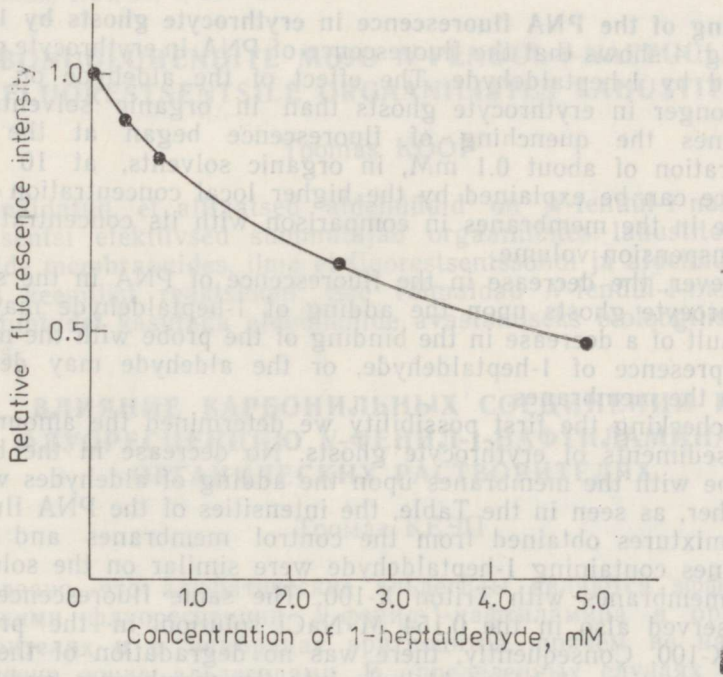

Fig. 4. The effect of 1-heptaldehyde on the fluorescence intensity of *N*-phenyl-1-naphthylamine in suspensions of erythrocyte ghosts.

In the completely apolar solvent hexane this peculiarity disappeared and the fluorescence quenching was remarkably stronger than in alcohols. The competition for aldehydes in hexane seems to occur only between aldehydes and the probe, while in alcohol a considerable part of the aldehydes may be associated with the molecules of the solvent which leads to a decrease in the quenching efficiency. This could also explain the phenomenon that at least 100 molecules of aldehyde per molecule of the probe are needed for quenching the PNA fluorescence in hexane while in 3-methyl-1-butanol this number is 5000 as calculated on the basis of the data in Fig. 3.

Relative fluorescence intensity of *N*-phenyl-1-naphthylamine in the suspensions of erythrocyte ghosts treated with Triton X-100

Concentration of 1-heptaldehyde in suspensions of erythrocyte ghosts, mM	Relative fluorescence intensity of 10 μM PNA*	
	Without Triton X-100	With 1% Triton X-100
0	1.0	2.27
0.5	0.81	2.29
1.0	0.76	2.21
2.5	0.68	2.21
5.0	0.61	2.19

* The values in the Table are referred to the fluorescence intensity of the suspension of pure erythrocyte ghosts without 1-heptaldehyde or Triton X-100 added. The fluorescence intensity of 10 μM PNA in the 0.154 M NaCl solution without Triton X-100 was 0.04 and with 1% Triton X-100, 2.11 relative units.

Quenching of the PNA fluorescence in erythrocyte ghosts by 1-heptaldehyde. Fig. 4 shows that the fluorescence of PNA in erythrocyte ghosts was quenched by 1-heptaldehyde. The effect of the aldehyde on the probe was stronger in erythrocyte ghosts than in organic solvents: in the membranes the quenching of fluorescence began at the aldehyde concentration of about 0.1 mM, in organic solvents, at 10 mM. The difference can be explained by the higher local concentration of 1-heptaldehyde in the membranes in comparison with its concentration in the whole suspension volume.

However, the decrease in the fluorescence of PNA in the suspension of erythrocyte ghosts upon the adding of 1-heptaldehyde may possibly be a result of a decrease in the binding of the probe with the membranes in the presence of 1-heptaldehyde, or the aldehyde may degrade the probe in the membranes.

For checking the first possibility we determined the amount of PNA in the sediments of erythrocyte ghosts. No decrease in the binding of the probe with the membranes upon the adding of aldehydes was found.

Further, as seen in the Table, the intensities of the PNA fluorescence in the mixtures obtained from the control membranes and from the membranes containing 1-heptaldehyde were similar on the solubilization of the membranes with Triton X-100. The same fluorescence intensity was observed also in the 0.154 M NaCl solution in the presence of Triton X-100. Consequently, there was no degradation of the probe at the incorporation of 1-heptaldehyde into the membranes and the fluorescence quenching by aldehydes in organic solvents and membranes proceeded by the same mechanism of collision.

Thus, aliphatic carbonyl compounds evidently quench the fluorescence of PNA by the interaction of their carbonyl oxygen with the probe molecule. In special cases PNA could be used as a reagent for detecting the penetration of aldehydes into the hydrophobic environment of biological membranes.

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KARBONÜÜLÜHENDITE MÕJU *N*-FENÜÜL-1-NAFTÜÜLAMIINI FLUORESTSENTSILE ORGAANILISTES LAHUSTITES

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On näidatud, et alifaatsed aldehüüdid on *N*-fenüül-1-naftüülamiini fluorestsentsi efektiivsed summutajad orgaanilistes lahustites ja erütrotsüütide membraanides, ilma et fluorestsentssondi ja aldehüüdide vahel toimuks keemilist reaktsiooni. See võimaldab *N*-fenüül-1-naftüülamiini teatud juhtudel kasutada aldehüüdide avastamiseks bioloogilistes membraanides.

ВЛИЯНИЕ КАРБОНИЛЬНЫХ СОЕДИНЕНИЙ НА ФЛУОРЕСЦЕНЦИЮ *N*-ФЕНИЛ-1-НАФТИЛАМИНА В ОРГАНИЧЕСКИХ РАСТВОРИТЕЛЯХ

Тоомас КЁЭП

Показано, что алифатические альдегиды являются эффективными тушителями флуоресценции *N*-фенил-1-нафтиламина в органических растворителях и в мембранах эритроцитов, причем не наблюдается разрушения зонда альдегидами. В определенных случаях *N*-фенил-1-нафтиламин можно использовать в качестве реагента для установления альдегидов в биологических мембранах.