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Abstract

Full Text

PHYSICAL CHEMISTRY

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THE STIMULATING ACTION OF CERTAIN COMPOUNDS ON THE QUENCHING OF CHLOROPHYLL FLUORESCENCE BY NITRO COMPOUNDS

(Presented by Academician A. N. Terenin, 13 XII 1963)

In studying the nature of the quenching of fluorescence of pigments of the chlorophyll series, it was found ([1]) that the quenching action of aromatic nitro compounds on the fluorescence of pheophytin is manifested noticeably only in alcoholic solutions. In more inert solvents, such as carbon tetrachloride, hexane, benzene, toluene, acetone, pyridine, etc., the quenching efficiency of nitro compounds is expressed to a much lesser extent. To explain this effect, the supposition was advanced that alcohols exert a stimulating action on the quenching process, which is connected with their electron-donor properties. It was assumed that the presence of alcohol molecules, potential electron donors, in the environment of the pigment molecule leads to the localization of a certain excess of electron density at the latter. As a result of this, the electron-donor activity of the pigment molecule is increased, which should, upon subsequent action of a light quantum, promote electron transfer to the oxidant. We assume here that the fluorescence-quenching act is based on reversible electron transfer ([2], [3]).

Fig. 1. *a*–Quenching of the fluorescence of pheophytin *a* by *m*-dinitrobenzene in hexane; *b*–the same in the presence of 0.085 mol/l aniline

In the present work an attempt was made to verify the correctness of the above hypothesis. In doing so we proceeded from the assumption that, besides alcohols, other compounds having an electron-donor nature should also exert a stimulating action on the degree of fluorescence quenching of pigments. As the latter, aromatic amines and ascorbic acid were used, which, as is known ([4]), by themselves do not quench the fluorescence of chlorophyll and its derivatives.

Fig. 2

Figure 2: Fig. 2

The quenching of the fluorescence of chlorophyll *a* and pheophytin *a* by nitro compounds was studied in solutions of hexane, toluene, acetone, and pyridine in the presence of additions of aniline, benzidine, toluidine, and diphenylamine. The experimental material obtained shows that all the above-listed additives, to one degree or another, increase the degree of fluorescence quenching of chlorophyll *a* and pheophytin *a* by aromatic nitro compounds.

Chlorophyll *a* and pheophytin *a* were obtained and chromatographically separated according to the known procedure described in (^{5}). Commercial *m*-dinitrobenzene, dinitrotoluene, *m*-nitrophenol, and dinitrophenol were used as quenchers; they were additionally purified by repeated recrystallization. Purification of aniline and toluidine was carried out by boiling them

over KOH and by double vacuum distillation. Commercial diphenylamine was recrystallized from alcohol. Ascorbic acid and benzidine (analytical grade) were used without additional purification. Toluene, hexane, acetone, and pyridine (analytical and chemically pure grades) were used after additional purification and distillation.

Our investigations consisted in a comparative study of the dependence of the fluorescence intensity of pigments on the concentration of nitro compounds in the presence of definite amounts of "stimulators" and in their absence. In doing so, the procedure described earlier (^{1}) was used. In all experiments the concentration of pigment in solution was of the order of 10^{-5} mole/l.

Fig. 2. Quenching of the fluorescence of pheophytin a by *m*-dinitrobenzene in toluene:
a—without additions of aniline, *b*—in the presence of 0.0085 mole/l aniline, *v*—in the presence of 0.085 mole/l aniline, *g*—in the presence of 0.17 mole/l aniline

Fig. 1 illustrates the stimulating action of 0.085 mole/l aniline on the quenching of the fluorescence of a pheophytin a solution by *m*-dinitrobenzene in hexane. From the data presented it is evident that the presence of aniline at this concentration in the solution increases the degree of quenching of the pigment fluorescence by 14%. A completely analogous picture is also observed when diphenylamine, toluidine, and benzidine are used as stimulating additives. The effect of enhanced fluorescence quenching also appears in the presence of ascorbic acid in pyridine solution.

An increase in the degree of quenching of pigment fluorescence in the presence of the listed additives also takes place when other nitro compounds (nitrotoluenes and nitrophenols) are used as quenchers.

The "stimulator" additives studied by us intensify the effect of quenching of chlorophyll a fluorescence as well. This is seen from the data on the amounts of

Fig. 3

Figure 3: Fig. 3

m-dinitrobenzene required to reduce the fluorescence intensity of chlorophyll a by one half [$Q_{1/2}$], without additives and with various additives.

	Without stimulator	With 0.01 mole/l aniline	With 0.01 mole/l toluidine	With 0.01 mole/l benzidine	With 0.01 mole/l diphenylamine
$[Q_{1/2}] \cdot 10^{-2}$	1.19	1.00	0.99	0.96	1.02

In all cases [$Q_{1/2}$] is smaller in the presence of additives.

It turned out that the magnitude of the stimulating effect of the various additives depends on their concentration. Fig. 2 presents data on the quenching of the fluorescence of pheophytin a by *m*-dinitrobenzene in toluene solution in the presence of 0.0085, 0.085, and 0.17 mole/l aniline. It is clearly seen that the degree of quenching is the higher, the more aniline the pigment solution contains. A completely analogous picture is found in the case of chlorophyll a.

It might have been supposed that the molecules of the quencher and the "stimulator" are bound in a complex and that this increases the electron-acceptor capacity of the nitro compounds and enhances the quenching effect. Such an assumption is natural, since the tendency of nitro compounds to form complexes with amines is known (^{6}). Nevertheless, a number of data obtained by us provide grounds for another interpretation of the phenomenon.

In one series of experiments, solutions of pigments, the "stimulator," and the quencher were introduced not separately, as in the experiments described earlier, but in the form of their mixtures prepared in advance. It turned out that, in the case when *m*-dinitrobenzene is introduced into a pigment solution already containing aniline, the degree of fluorescence quenching is appreciably greater than when a ready-made mixture of the nitro compound with aniline is used as the quencher. This is clearly demonstrated by

the data presented in Fig. 3. We therefore consider it more reliable that the observed stimulating action of amines on fluorescence quenching is the result of a shift in the electron density of the pigment molecule under the action of molecules of an electron-donor nature. This should facilitate the transfer of an electron to the oxidant.

Fig. 3. *a*—quenching of pheophytin fluorescence by *m*-dinitrobenzene; —the same in the presence of 0.17 mole/liter aniline; —quenching by ready-

made mixtures containing 0.17 mole/liter aniline and different amounts of *m*-dinitrobenzene.

It is characteristic that the effect of "stimulators" (amines) on the process of fluorescence quenching appears only at comparatively high concentrations of them, exceeding the pigment concentration by three to four orders of magnitude. Under these conditions each pigment molecule is surrounded by stimulator molecules. The fact that the bond established between them in this case is not manifested spectrally is not unexpected. Such cases have been noted and discussed earlier (7). The presence of this bond leads to a shift of electron density from the amine to the pigment, which subsequently ensures electron transfer in the quenching event.

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