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ON THE NATURE OF THE QUENCHING OF CHLOROPHYLL FLUORESCENCE BY NITRO COMPOUNDS

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Although at the present time a fairly substantial body of material has already been accumulated on the quenching of chlorophyll fluorescence, making it possible, for example, to derive the principal quantitative regularities of this phenomenon (^{1}), there is only very scant information about its primary act. Proceeding from the fact that quenchers of chlorophyll fluorescence are, as a rule, compounds possessing more or less clearly expressed oxidizing or reducing properties, E. Rabinowitch (^{2}) suggested that the quenching in this case is based on a chemical interaction. The "chemical" concept of quenching was, as is known, formulated earlier in a more general form by Weiss (^{3}). According to this concept, the elementary act of fluorescence quenching in a number of organic and inorganic compounds is associated with electron transfer between the fluorescing molecule and the quencher molecule. As far as chlorophyll is concerned, apart from considerations of a general nature, there are no data on the basis of which conclusions could be drawn about the quenching mechanism.

V. B. Evstigneev and V. A. Gavrilova (^{4}), studying the kinetics of photochemical oxidation and reduction of pigments of the chlorophyll series, showed that their relative ability to undergo oxidation reactions increases in the series pheophytin a < chlorophyll b < chlorophyll a. The reverse series was observed for reduction processes. We therefore believe that, by studying in parallel the fluorescence quenching of all these pigments under comparable conditions, it will be possible to establish a connection between their oxidation-reduction properties and the degree of quenching. On the other hand, the corresponding dependence of the effect on the potential of the quencher can be obtained by using oxidants of one and the same series.

In the present work, aromatic nitro compounds were used for this purpose; it is known for them that an increase in the number of nitro groups in the ring leads to a simultaneous increase in their oxidizing potential (^{5}). The quenchers were nitrobenzene, 1,3-dinitrobenzene, 1,4-dinitrobenzene, *m*-nitrophenol, 2,4-dinitrophenol, and 2,4,6-trinitrophenol. Commercial preparations of these compounds (chemically pure grade) were subjected to an additional double recrystallization. Chlorophyll and pheophytin were extracted from dry nettle leaves. Separation of the pigments into components a and b was carried out by the

Fig. 1. Quenching by nitrobenzene of the fluorescence: pheophytin a (1) $0.86 \cdot 10^{-5}$ mole/l; chlorophyll b (2) $0.7 \cdot 10^{-5}$ mole/l; chlorophyll a (3) $0.8 \cdot 10^{-5}$ mole/l

Figure 1: Fig. 1. Quenching by nitrobenzene of the fluorescence: pheophytin a (1) $0.86 \cdot 10^{-5}$ mole/l; chlorophyll b (2) $0.7 \cdot 10^{-5}$ mole/l; chlorophyll a (3) $0.8 \cdot 10^{-5}$ mole/l

Fig. 2. Quenching of the fluorescence of chlorophyll a by m-nitrophenol (1), 2,4-dinitrophenol (2), 2,4,6-trinitrophenol (3)

Figure 2: Fig. 2. Quenching of the fluorescence of chlorophyll a by m-nitrophenol (1), 2,4-dinitrophenol (2), 2,4,6-trinitrophenol (3)

methods described in (^{6}). Carefully purified and dried benzene was used as the solvent.

In the fluorescence study, an FEU-22 photomultiplier was used. The magnitude of the photocurrent was determined in relative units from the readings of a microvoltmeter with a 28 tube amplifier. The fluorescence of the solutions was excited by the blue light of a PRK-4 mercury-quartz lamp, filtered with an SZS-10 light filter. The quenching effect of the corresponding additives was judged on the basis of changes—

···of the fluorescence intensity for λ670 mμ upon introduction of definite amounts of nitro compounds into the pigment solutions. This wavelength corresponds to the emission maximum of chlorophyll a and is located close to the corresponding maxima of chlorophyll b and pheophytin a. It was isolated with a universal UM-2 monochromator.

Figure 1 presents data on the quenching of the fluorescence of chlorophyll a, chlorophyll b, and pheophytin a by nitrobenzene. From the data presented it is clearly seen that nitrobenzene quenches the fluorescence of chlorophyll a most strongly; its effect is somewhat weaker in the case of chlorophyll b. The intensity of the luminescence of pheophytin a under these conditions changes only very slightly.* An entirely analogous regularity is observed for all the other nitro compounds.

Fig. 1. Quenching by nitrobenzene of the fluorescence: pheophytin a (1) $0.86 \cdot 10^{-5}$ mole/l; chlorophyll b (2) $0.7 \cdot 10^{-5}$ mole/l; chlorophyll a (3) $0.8 \cdot 10^{-5}$ mole/l

Fig. 2. Quenching of the fluorescence of chlorophyll a by *m*-nitrophenol (1), 2,4-dinitrophenol (2), 2,4,6-trinitrophenol (3)

Thus, the effect of quenching of the pigment fluorescence is the greater, the more strongly its electron-donor activity is expressed.

Fig. 3. Quenching of the fluorescence of chlorophyll a by *m*-nitrobenzene (1),

Fig. 3. Quenching of the fluorescence of chlorophyll a by m-nitrobenzene (1), p-nitrobenzene (2)

Figure 3: Fig. 3. Quenching of the fluorescence of chlorophyll a by m-nitrobenzene (1), p-nitrobenzene (2)

Fig. 4. Photochemical bleaching of benzene solutions of chlorophyll a with the participation of nitrobenzene (1), m-dinitrobenzene (2)

Figure 4: Fig. 4. Photochemical bleaching of benzene solutions of chlorophyll a with the participation of nitrobenzene (1), m-dinitrobenzene (2)

p-nitrobenzene (2)

Fig. 4. Photochemical bleaching of benzene solutions of chlorophyll a with the participation of nitrobenzene (1), *m*-dinitrobenzene (2);

Figure 2 presents data on the quenching of the fluorescence of a solution of chlorophyll a by *m*-nitrophenol, 2,4-dinitrophenol, and 2,4,6-trinitrophenol. From these data it is seen that the degree of quenching increases with an increase in the number of nitro groups in the aromatic nucleus. An analogous picture is also observed upon additions to a solution of chlorophyll a of nitro- and dinitrobenzene. The latter quenches fluorescence several times more strongly than nitrobenzene. As was to be expected, the action of *m*-dinitrobenzene proved to be somewhat less—

* The same was obtained for pheophytin b.

than for its *n*-derivative, in which the mutual influence of the substituents, transmitted along the conjugation chain, should have a greater effect on the oxidation potential. The corresponding data are presented in Fig. 3. Thus, the results obtained show that the effect of quenching the fluorescence of one and the same pigment by different quenchers from the series of nitro compounds is the stronger, the more nitro groups there are in the molecule and, consequently, the more pronounced its electron-acceptor activity.

The dependences we have noted can be explained if it is assumed that the quenching process in the case under consideration is based on an act of electron transfer from the pigment to the nitro compound, which in general form may be written as follows:

$$\mathrm{Chl}^* + \mathrm{RNO_2} \rightleftarrows \mathrm{Chl}^+ + \mathrm{RNO_2^-}.$$

It should be assumed that the electron transfer responsible for the act of quenching is reversible. The ion-radicals formed in this process remain for a certain interval of time in close proximity to one another, in one "cage" of the solution. This creates favorable conditions for their easy recombination and for an almost complete reversal of the process. Indeed, during the measurement of fluorescence the pigment concentration does not change. Nevertheless, under very prolonged irradiation of thoroughly deoxygenated solutions of chlorophyll *a* containing nitro compounds, a slow photoreaction is observed, leading to destructive degradation of the pigment. As was to be expected, the rate of bleaching of chlorophyll in this process is also greater in the presence of dinitrobenzene than of nitrobenzene (see Fig. 4). It is possible that electron transfer in this photochemical reaction likewise takes place between the singlet-excited pigment molecule and the molecule of the nitro compound located in the same "cage" of the solution.

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Note: Figure translations are in progress. See original paper for figures.