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Abstract

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DURATION OF THE EXCITED STATE AND QUANTUM YIELD OF CHLOROPHYLL FLUORESCENCE IN VITRO AND IN VIVO

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It is known that the quantum yield of chlorophyll fluorescence in its natural state in plant cells is approximately an order of magnitude smaller than in solutions. According to data in ([1]), for example, in cells of various algae the fluorescence yield has values of 1.5-3%, whereas in solutions it reaches 30%. Thus, in plant cells there occur certain very effective processes of fluorescence quenching, connected, evidently, with the mechanism of the primary photochemical reaction and with the plant' s use of the excitation energy of chlorophyll molecules for photosynthesis.

To elucidate the nature of these processes, we carried out parallel measurements of the relative quantum yield and the duration of chlorophyll fluorescence in vitro and in vivo. The yield measurements were made in an integrating sphere; the duration of the excited state was measured with a phase fluorometer ([2]), the amplifier and phase-measuring part of which was constructed according to the scheme proposed by A. M. Bonch-Bruevich ([3]). In vitro, solutions of a preparation kindly supplied to us by A. A. Krasnovskii were studied; the preparation contained an unseparated mixture of chlorophylls a and b, purified of carotenoids, as well as extracts from sugar-beet leaves. Chlorophyll fluorescence in the cell was studied on suspensions of the unicellular alga *Scenedesmus acuminatus*, a culture of which was kindly provided to us by N. S. Gaevskaya*. Excitation was produced by the Hg 436 mμ line, isolated by light filters from the radiation of a superhigh-pressure mercury lamp.

Table 1

Object	ρ_{rel}	$\tau \cdot 10^9$ sec.
Solutions of chlorophylls a + b in acetone	1.00	5.4
„ in ethyl alcohol	1.05	5.3
„ in ethyl ether	1.0	–
Extract from leaves in acetone	–	5.7
„ in ethyl alcohol	–	5.5
„ in ethyl ether	0.98	5.1
Scenedesmus acuminatus	0.08	0.8–0.9

The results obtained by us are summarized in Table 1. The fluorescence yield

of the chlorophyll solution in acetone is conventionally taken as unity. The root-mean-square error in determining τ, calculated by averaging 15–20 fairly extensive series of measurements performed on different days by three observers, was about $(0.1–0.2) \cdot 10^{-9}$ sec., but the scatter of mean results from individual days in the case of the alga exceeded this value by a factor of 2–3.

* *Note added in proof.* For a suspension of chloroplasts and their fragments isolated by the usual method from leaves of various plants (sugar beet, tobacco, etc.) in an aqueous medium, we obtained values of τ of $(1.0–1.2) \cdot 10^{-9}$ sec., close to the values of τ in vivo. Upon addition of alcohol, acetone, or pyridine to the medium, the values of τ increase, approaching the value of this quantity in vitro. This increase proceeds in parallel with the increase in ρ, noted in work ([9]) and confirmed by us.

It is possible that this is connected with the physiological state of the alga and the intensity of photosynthetic processes in it, but a systematic study of the influence of physiological conditions on the yield and duration of fluorescence has not yet been carried out. In absolute magnitude, the values of ( ) obtained by us for solutions and algae agree, within the accuracy of the measurement errors, with the data obtained by another method by E. Rabinowitch ((ˆ4)).

Table 2

Values of (_0 10ˆ8) (sec.)

	According to Pringsheim (1934)	According to Livingston (1952)	According to Jacobs (1954)
Chlorophyll a	8.2	1.8	1.27
» b	8.9	4.4	1.62

Comparison of the cited values of the "actual" duration of the excited state of chlorophyll in solutions (( )) with the absolute values of the fluorescence quantum yield (( )) and the values of the "natural" duration of this state ((_0)), calculated by various authors ((ˆ5)) from the integral of the first absorption band of chlorophyll (T. P. Kravts' s integral), shows that the relation (= _0) is justified with sufficient accuracy. Especially good agreement is obtained if, for (_0), one adopts Livingston' s value (Table 2) or the value (_0 = 1.52 10ˆ{-8}) sec. cited in Rabinowitch' s work.

This shows that the factor limiting the fluorescence yield of chlorophyll in solutions is the competition, occurring throughout the entire excited state of the molecule, between the process of spontaneous emission by the excited molecule and the processes of its radiationless deactivation. In solution, in the absence of conditions for chlorophyll-sensitized photochemical reactions, these processes—regardless of whether they represent internal conversion, i.e., a direct transition of the energy of electronic excitation into vibrational energy, or are associated

with the transition of the molecule into another tautomeric form and the subsequent restoration of thermodynamic equilibrium—always represent conversion of the excitation energy into heat. The value of the yield (= 0.25)-(0.30) indicates that the probability of fluorescence in solution is approximately three times smaller than the probability of these processes of conversion of excitation energy into heat, and we have no grounds to think that in the cell the relationship between these probabilities is different.

However, in the cell, to the two possible pathways of deactivation of excited chlorophyll molecules indicated above, a third possibility is added—the use of the excitation energy for the primary photochemical reaction. The decrease in the fluorescence yield in the cell is obviously due to the presence of this third pathway of deactivation, and the sharp shortening of the fluorescence duration accompanying the decrease in yield indicates that the competition among these three processes occurs throughout the entire time of the excited state of the chlorophyll molecule. In other words, it may be said that the specific processes of fluorescence quenching occurring in the living cell are, using the terminology of S. I. Vavilov ((⁀6)), mainly processes of the "second kind."

It is important to note, however, that in our case there is no strict proportionality between the quantities ( ) and ( ) for the solution and the cell: a decrease in yield by approximately 12 times corresponds to a decrease in yield of only 6-7 times. For comparison with the simplest case of quenching of the second kind —the quenching of fluorescence of solutions by foreign substances—we measured ( ) and ( ) during quenching of acetone solutions of chlorophyll by quinone and of aqueous solutions of uranin by potassium iodide. The measurements showed that in both of these cases there is strict proportionality between ( ) and ( ).

The absence of such proportionality when comparing ( ) and ( ) for chlorophyll in solutions and in cells can be explained in two ways. One may suppose that, along with quenching processes of the second kind, in the cell···

there occur also processes of "instantaneous quenching" (quenching of the first kind according to Vavilov), which do not shorten the duration of fluorescence. This is equivalent to the assumption expressed by Rabinovich that some of the molecules in the cell are in a nonfluorescing state.

Another possible explanation of these facts consists in the assumption that in the cell we are dealing with pure quenching of the second kind, but that, unlike the quenching of the fluorescence of solutions by extraneous substances, in the present case the probability of the photochemical reaction leading to the quenching of fluorescence does not remain constant throughout the entire time of the excited state, but gradually decreases. Such a case, as M. D. Galanin (7) showed, occurs in the concentration quenching of the fluorescence of a number of dyes in viscous solutions.

It may be thought that in the cell the processes of fluorescence quenching likewise have the character of such complicated concentration quenching, i.e., are

associated with processes of migration of the excitation energy and its transfer to "centers" of photosynthetic activity.

As applied to our case, we would have to assume that the probability of photochemical utilization of the excitation energy depends on the distance between the excited chlorophyll molecule and other molecules participating in the reaction, or on other statistically distributed factors determining the state of the reacting system. Since the process takes place in a very viscous medium, where the diffusional displacement of molecules during the time τ is very small and the process of "mixing" does not have time to restore the statistically equilibrium distribution of molecules, there must occur, in the region near the excited chlorophyll molecule, a gradual "depletion" of easily reacting molecules, i.e., the probability of the photochemical reaction during the excited state must gradually decrease. Predominantly short-lived molecules will be quenched, and the duration of the excited state will decrease more slowly than the yield.

At present we have no unambiguous experimental evidence for the correctness of either of the proposed explanations. However, in favor of the second is the fact that, from this point of view, one can simply and without special assumptions explain the possibility of a very high quantum yield of the primary photochemical reactions. Indeed, if throughout the entire time of the excited state the process of photochemical utilization of the excitation energy competes both with the process of fluorescence and with the process of conversion of this energy into heat, then the plant has the possibility of using for photosynthesis a considerable part not only of that energy which in solution is emitted in the form of fluorescence, but also of that which in solution is converted into heat. The decrease in fluorescence yield *in vivo* by a factor of 12 in comparison with the yield in solution shows that the probability of photochemical utilization exceeds by the same factor the total probability of the other two processes, i.e., for photosynthesis there can be used the energy of more than 90% of the excited molecules.

On the other hand, if, in order to explain the discrepancies between ρ and τ, we were to admit the presence of "instantaneous" quenching processes of the first kind, then we would have to attribute to them the quenching of approximately 40% of the excited molecules. Thus, in order to explain a high, nearly unit, quantum yield of the primary photochemical reactions in the cell, we would have to introduce some special ideas about the mechanism of such "instantaneous" capture by the plant of the energy of these 40% of excited molecules. Since there are as yet insufficient grounds for constructing such models, we believe that one should accept, at least as a working hypothesis, the second of the indicated assumptions.

Attention is further drawn to the fact that, according to our measurements, the quantities ρ and τ have practically identical values for the fluorescence of a preparation purified of carotenoids and of an extract from leaves, which contains them,

apparently, did contain. This contradicts the data of (8), according to which migration of energy from carotenoids to chlorophyll takes place in the living cell, but does not occur in solutions. This entire set of questions requires further, more detailed investigation.

The present work was reported at the 2nd All-Union Conference on Photosynthesis in January 1957 and will be published in more complete form in the proceedings of this conference.

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