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pH CONTROL OF THE CHLOROPHYLL *a* FLUORESCENCE IN ALGAE

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SUMMARY

The pH of the suspension medium was found to have a remarkable influence on the "slow" (min) time course of Chlorophyll *a* fluorescence yield in the green alga *Chlorella pyrenoidosa* and in the blue-green alga *Anacystis nidulans*. In Chlorella, the decay of fluorescence yield, in the 1- to 5-min region, is strongly retarded at alkaline pH; this decay rate shows an optimum at pH 6–7. In Anacystis, the rise of fluorescence yield, in the same time range, is decreased optimally at pH 6–7; poisoning with 3(3,4-dichlorophenyl)-1,1-dimethylurea reverses the direction of this pH effect. These observations suggest a correlation of the H^+ status (or the processes associated with it such as photophosphorylation and resulting conformational changes) of the chloroplast to the yield of chlorophyll *a* fluorescence *in vivo*.

Light-dependent proton uptake by cloroplasts is related to the overall process of photosynthesis[1,2]. Similar movements of protons and of other cations have also been demonstrated in suspensions of whole algal cells[3–7]. In the latter case, the observed pH change is the result both of a light-induced proton pump, and of the migration of undissociated carbonic acid from the medium to the CO_2-depleted photosynthetic sites of the interior. Electron transport inhibitors, and uncouplers of photophosphorylation that affect the normal course of photosynthesis suppress the light-induced ion fluxes.

The ionic composition of the algal cell interior exerts a 2-fold influence on the conformation of the chloroplasts and thylakoids. It may bring about the osmotic shrinkage or distention of these entities, or it may modify the fine structure of the lamellae by regulating the charge of its ionizable groups (*e.g.* phospholipids, polar amino acid residues). In either case it affects various optical properties of the cell, and especially the fluorescence of chlorophyll *a*. Changes in the fluorescence spectrum, the fluorescence yield, and the kinetics of the fluorescence yield on the addition of electrolytes to the medium have been observed by several investigators. In Euglena chloroplast fragments, BRODY *et al.*[8] demonstrated a suppression of the far-red (715–736 nm) chlorophyll *a* fluorescence band, at low temperature, in the presence of concentrated salts, or at pH values away from neutrality. MURATA[9] and MURATA *et al.*[10] have shown that this suppression can be caused by dilute Mg^{2+} salts, and that,

Abbreviation: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

at 77°K, it is accompanied by a relative increase of chlorophyll *a* fluorescence bands at 684 nm and at 695 nm with respect to that at 715–736 nm. In chloroplasts of *Phytolacca americana*, HOMANN[11] observed an increase of the P level of the fast fluorescence transient on the addition of dilute Mg^{2+}; this increase was also present in preparations treated with 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and sodium dithionite. On the other hand, P. MOHANTY (personal communication) and DE KOUCHKOVSKY[12] showed independently that concentrated salts suppress the amplitude of the fast fluorescence transient (*i.e.* the P level) in suspensions of whole Chlorella cells, and influence in general, the kinetic course of the chlorophyll *a* fluorescence yield. In this communication we report the effect of the proton concentration of the suspension medium on the slow kinetics of chlorophyll *a* fluorescence in *Chlorella pyrenoidosa* (unicellular chlorophyte) and *Anacystis nidulans* (unicellular cyanophyte).

The algae were grown in inorganic medium, in continuous illumination with white light of low intensity, as described elsewhere[13]. 3- to 6-day-old cultures were transferred into a buffered solution (0.02 M Tris–HCl, 0.04 M NaCl, or 0.01 M phosphate buffer), and the absorbance of the suspension at the red Chl *a* absorption maximum was adjusted to 0.5 for 1-cm light path. The total chlorophyll content of the Chlorella samples was 18 μg/ml, while that of the Anacystis samples was 8μg/ml. These values were determined with the procedure of ARNON[14]. The pH of the samples was adjusted by adding HCl or NaOH to the suspension. In spite of the poor buffering capacity of Tris below pH 6.5, the pH of the samples, assayed before and after the fluorescence measurements, did not change. The instrument and the technique used in recording the slow change of the chlorophyll *a* fluorescence yield in these algae has been detailed elsewhere[15–17]. The reproducibility of the kinetic pattern was ensured by a 15-min adaptation of the cells to darkness that preceded all measurements. The fluorescence time course data are presented in terms of the ratio $f_t = F_t/F_3''$ (relative fluorescence yield), where F_t and F_3'' denote the fluorescence intensity at t sec and

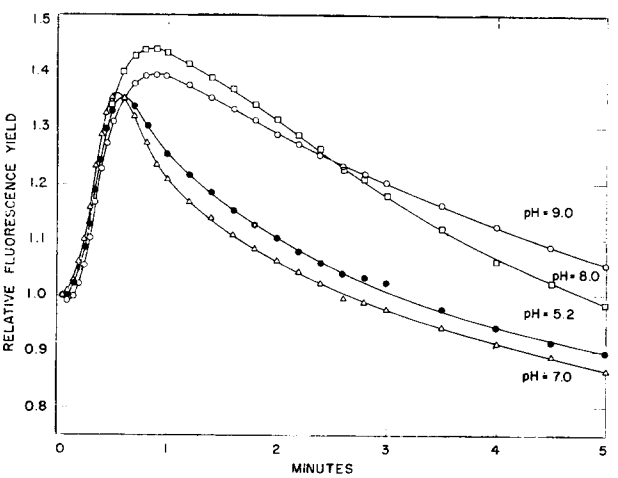

Fig. 1. Time course of the relative fluorescence yield ($f_t = F_t/F_3''$) of chlorophyll *a* in *Chlorella pyrenoidosa* at different pH values of the suspension medium. The fluorescence kinetics are recorded with continuous exciting illumination; 15-min dark adaptation preceded these measurements. Fluorescence excitation: λ = 480 nm; half-band width, 6.6 nm; incident intensity, 650 ergs·cm^{-2}·sec^{-1}. Fluorescence observation: λ = 685 nm; half-band width 16.5 nm; Corning sharp cut off filter, C.S. 2-62.

at 3 sec, respectively. F_3'' corresponds to the "S level" at the end of the fast fluorescence transient. Further details are given in the legends to the figures.

Fig. 1 shows the time course of the relative fluorescence yield of chlorophyll *a* in Chlorella at various proton concentrations of the suspending phase. The rise portion of the depicted fluorescence transient appears to be insensitive to the proton concentration of the medium, while the decay portion is markedly retarded in the alkaline region. The greater amplitude of the transient and the displacement of the maximum toward longer times at high pH values can be traced to the different sensitivities of the fluorescence rise and decay processes to the pH of the medium. A decelerated decay allows the forward changes to proceed further resulting in transients of greater amplitudes and with maxima displaced to longer times. These results suggest that different physico-chemical causes underly the rise and the decay portions of the slow fluorescence change in Chlorella.

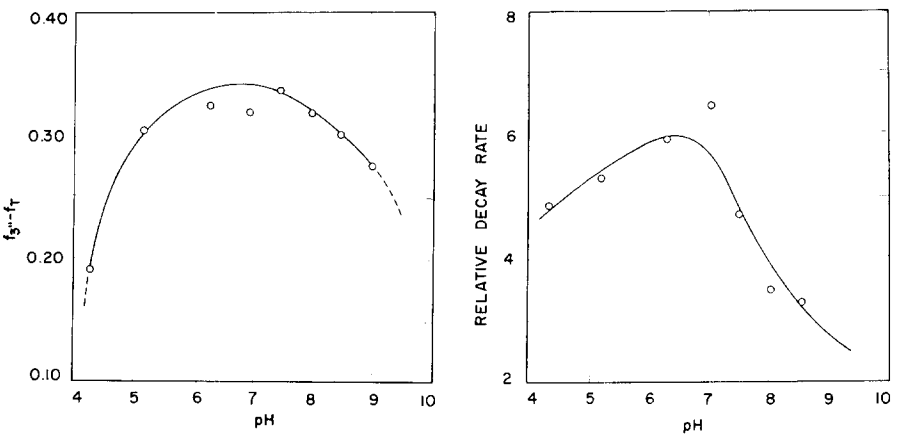

Fig. 2. The fluorescence change $f_3''-f_T$ (left) and the decay rate of the fluorescence time course (right) of chlorophyll *a* in *Chlorella pyrenoidosa* as a function of the pH of the suspension medium; details as in Fig. 1.

The difference $f_3''-f_T$ (Fig. 2, left) and the rate of fluorescence decay after the induction maximum (Fig. 2, right) exhibit optima in the pH range of 6–7. f_T is lower than f_3'', and denotes the final level of the relative fluorescence yield at the end of the induction process. The inverse of the time interval at which f_t crosses the level 1.0 (the level equal to that at S) of the ordinate is taken as a measure of the fluorescence decay rate. This is justifiable as the rise portion of the transient appears to be independent of the pH (Fig. 1).

In the blue-green alga *Anacystis nidulans*, the chlorophyll *a* fluorescence yield rises from the level S to a higher plateau (M), and then decays very slowly to final level T (ref. 18). In Anacystis, in contrast to Chlorella, the oxygen evolution inhibitor DCMU does not prevent the slow fluorescence change, but instead, it enhances it. The phenomenology and the implications of these effects have been discussed elsewhere[16]. In the present study, we found the fluorescence yields at the "S" levels of both normal and DCMU-poisoned Anacystis to be pH insensitive, and the observed pH effects were limited to the light induced increments (S to M rise) only. (See Fig. 3 for DCMU-poisoned Anacystis.) The pH curves of the maximum amplitude f_M-f_3''

(f_M is the plateau level) are given in Fig. 4 for a normal (left) and a DCMU-poisoned sample (right). The fluorescence induction amplitude of normal Anacystis has a minimum at pH 6–7, while the DCMU-poisoned cells exhibit a maximum in the same pH range.

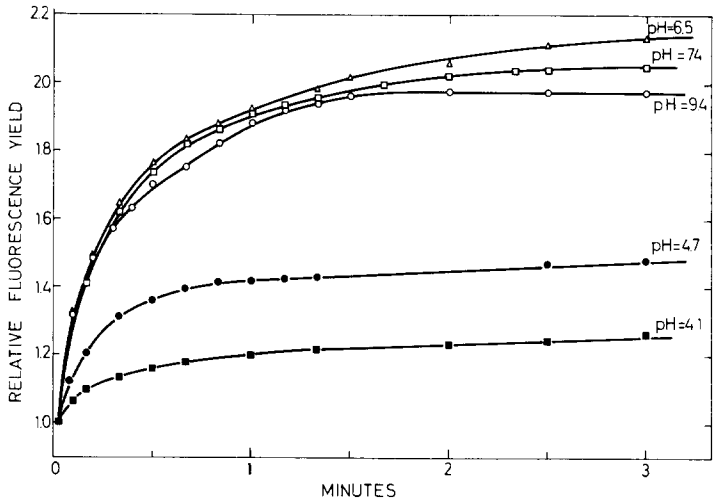

Fig. 3. Time course of the relative fluorescence yield ($f_t = F_t/F_3'$) of chlorophyll *a* in DCMU-poisoned *Anacystis nidulans* at different pH values of the suspension medium. The fluorescence kinetics are recorded with continuous exciting illumination; 15-min dark adaption preceded these measurements. DCMU, $5 \cdot 10^{-5}$ M. Fluorescence excitation; λ = 590 nm; half-band width, 16.5 nm; incident intensity, $4.1 \cdot 10^3$ ergs.cm^{-2}·sec^{-1}. Fluorescence observation : λ = 685 nm; half-band width, 6.6 nm; Corning sharp cut off filter, C.S. 2-58.

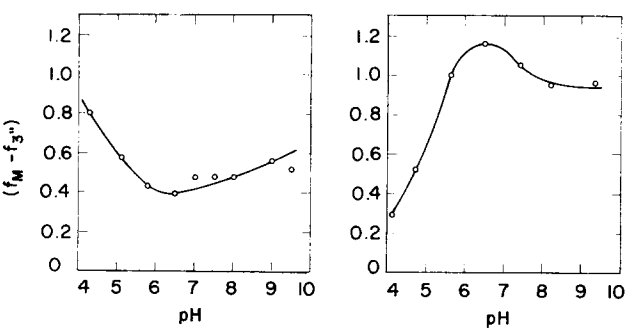

Fig. 4. The fluorescence change f_M–f_3'' of chlorophyll *a* in normal *Anacystis nidulans* as a function of the pH of the suspension medium (left); the same in DCMU-poisoned Anacystis (right); details as in Fig. 3.

These results underscore a direct or an indirect importance of the proton concentration of the medium for the slow kinetics of chlorophyll *a* fluorescence in whole cells of unicellular algae. Although we do not know the pH at the photosynthetic sites in the interior of the cell, we consider that the proton concentration of the suspension medium exerts an effect both by the passive diffusion of undissociated carbonic acid and by the operation of a light-dependent proton pump[6,7].

In the algae studied here, as well as in isolated higher plant chloroplasts[19,20], phenomena associated with light-induced shifts of ion concentrations have optima in the physiological pH range of 6–7. The results presented here illustrate the sensitivity of the fluorescence induction to pH (see review on fluorescence induction[21]). The limitation of the pH effect in Chlorella to the decay portion of the slow fluorescence change resembles similar limitations in the action of carbonyl cyanide *p*-trifluoromethoxy phenylhydrazone[17] and KCN[22]. Evidently, the physical causes that lead to the rise and the decay of chlorophyll *a* fluorescence in Chlorella are distinguishable. According to previous data[16,21], only the cyclic electron transport contributes to the slow fluorescence change of DCMU-poisoned Anacystis, while in normal samples both the non-cyclic and the cyclic transports are effective. This differentiation may be the basis of the opposite trends in the pH curves of the fluorescence induction amplitude in normal and poisoned Anacystis.

In conclusion, the above results show clearly that the pH of the suspension medium has a remarkable effect on the slow time course of chlorophyll *a* fluorescence yield in algae, and thus suggest a correlation of the H^+ status of the chloroplast to chlorophyll fluorescence *in vivo*. We do not yet know whether this correlation is direct or indirect *via* the associated processes such as phosphorylation and the resulting conformational changes (see ref. 22).

ACKNOWLEDGEMENT

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