BBA 43240

Enhancement of the dark reconstitution of photosynthetic reaction center 2 by nigericin and CCCP

It is a well-known phenomenon that in intact algae the fluorescence yield of the chlorophyll *a* excited by Pigment System 2 (chlorophyll a_2) changes upon illumination[1,2].

Different phases can be distinguished during the so-called induction of the light-induced fluorescence change. In this communication the nomenclature used by BANNISTER AND RICE[3] will be adopted. The induction curve generally shows an immediate biphasic increase in the yield of the emission from a level, F_0, at O to a maximum at P_2. A first fast phase, OP_1, is followed by a slower-starting second phase, P_1P_2 or M_1P_2, depending upon whether or not there is a small decreasing phase, P_1M_1. Subsequently the yield decreases from P_2 towards a lower level, M_2, which can be as low as P_1 or M_1 (Fig. 1). In Porphyra, the transition between OP_1 and P_1P_2 is seen as an inflection in the rise curve (see Fig. 1d). At the intensity used P_2 is reached after about 1–2 sec; P_2M_2 takes about 8–15 sec. It has been shown[1] that the increase in yield is due to the System 2 driven photoreduction of a fluorescence-quencher Q of chlorophyll a_2 of the reaction center of System 2. The decrease in yield has been thought to be due to a conversion of non-quenching reduced Q (QH) into a quenching form, Q′ (ref. 1). Significant characteristics of the induction phenomenon are: (1) After the completion of induction in the light (*i.e.* after QH has been converted to Q′), a dark-time of certain length, or pre-illumination with light of System 1, is needed before a subsequent illumination will cause complete induction, $P_1P_2M_2$ (*i.e.* complete reduction of Q maximally reducible at the intensity used)[1,4]. (2) A relationship between the conversion of QH to Q′, and a photochemical reaction causing a decrease in pH at the surface of a Porphyra thallus, has been demonstrated[4]. (3) The P_2M_2 phase (conversion of QH into Q′) can only be observed in intact cells; in isolated chloroplasts[5], in disrupted cells of fresh plant leaves and in a broken characean internodal cell (Nitella), the fluorescence yield, after completion of the biphasic increase, OP_1P_2, was found to remain at the maximum, P_2, upon prolonged illumination (VREDENBERG, to be published). It has been suggested[4], as a possible alternative explanation, that the conversion of QH into Q′ is due to, or associated with, intracellular ion transport. Restoration in the dark of Q′ to Q, would then be accomplished or presumably regulated, by a redistribution in the dark of the transported ions.

This communication gives the results of experiments, carried out with Porphyra, which show that dark restoration of Q′ to Q is strongly enhanced by compounds that selectively alter the membrane permeability to ions.

Fig. 1a shows the kinetics of the changes in fluorescence yield during illumination periods of about 12 sec, after preceeding dark-times ranging from 1 to 10 min. It appears that a dark-time of about 7 min is needed before the complete induction of change in fluorescence yield occurs. The effect of pre-illumination with blue light is also shown (Fig. 1c). Pre-illumination for 2 min causes an induction which is as

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone.

large as would have been observed after 5–7 min of darkness (Fig. 1c). The rates of increase and decrease in the fluorescence yield during the P_1P_2 and P_2M_2 phases, respectively, appear to be lower than the comparable rates after darkness. Addition of 2.5 μM nigericin causes a considerable shortening of the dark-time needed to restore complete induction (Fig. 1b). The antibiotic did not affect the rate of the changes in yield during any of the separate induction phases, nor did it change the "dark" fluorescence yield, F_0. In the presence of nigericin, pre-illumination with blue light also causes a lowering in the rate of the changes during the P_1P_2 and P_2M_2 phases (Fig. 1d). The slower rise in the P_1P_2 phase after pre-illumination is due to a rapid reoxidation of reduced QH by constituents of a pool[6–8], reacting between

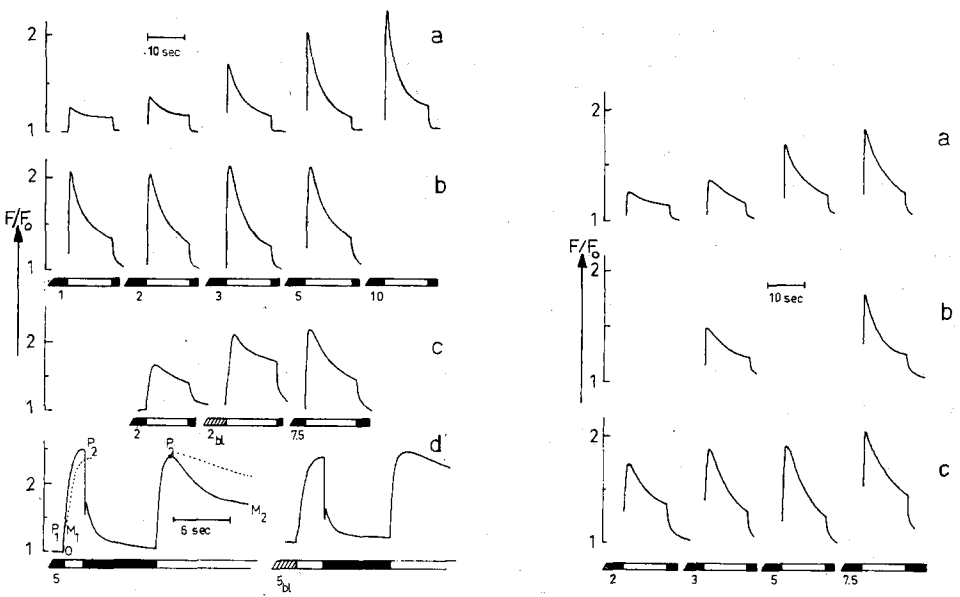

Fig. 1. Kinetics of the light-induced changes in fluorescence yield, F/F_0, of chlorophyll *a* in Porphyra, occurring after dark periods of varying length, or after pre-illumination with blue light, in the absence (traces a and c), and in the presence of, 2.5 μM nigericin (b and d). F_0 is the fluorescence emitted by the weak 400 cycles/sec modulated exciting light (wavelength 558 nm, intensity approx. 0.02 nEinstein·cm^{-2}·sec^{-1}), without actinic illumination. Non-modulated actinic light (wavelength 558 nm, intensity approx. 4 nEinstein·cm^{-2}·sec^{-1}) causes changes in the amount of fluorescence, F, emitted by the exciting light. The apparatus does not respond to the (continuous) strong fluorescence emitted by the actinic light. The response time of the apparatus was 60 msec. Further details of the equipment are given in ref. 4. Dark and light periods are indicated by the black (darkness) and white areas drawn under the curves. The numbers indicate the duration of the dark-time (in min) preceeding the illumination. A pre-illumination period with blue light (wavelength 430 nm, intensity approx. 2 nEinstein·cm^{-2}·sec^{-1}) is indicated by the shaded area, the time (in min) by the number, marked with "bl". The curves arranged under (a) and (b) are measured with the same thallus: (a) without additions, (b) with 2.5 μM nigericin and aqueous ethanol (0.6% by vol.). The curves in (c) and (d) are those measured with another thallus: (c) in the absence, and (d) in the presence of 2.5 μM nigericin and aqueous ethanol (2% by vol.). Parts of the induction curve after pre-illumination (right hand part of d) have been drawn (dotted curves) in the induction curve, measured after darkness.

Fig. 2. Kinetics of light-induced changes in fluorescence yield of chlorophyll *a* in Porphyra after dark periods of varying length, (a) without additions, (b) in the presence of aqueous ethanol (0.6% by vol.), and (c) in the presence of 1 μM CCCP and aqueous ethanol (0.7% by vol.). Other conditions are the same as in Fig. 1.

the two light reactions, which has become oxidized in the blue light. The lower rate of decrease during the P_2M_2 phase, however, must have a different cause: after shutting off the light at the maximum level of induction, P_2, a second illumination after about 7 sec, causes the same enhanced P_1P_2 phase (because the pool is now in a more reduced form), whereas the decrease in the P_2M_2 phase is lower in the pre-illuminated sample than in the dark-pretreated sample (Fig. 1d). A documented explanation of this phenomenon cannot as yet be given. The effect of 1 μM carbonylcyanide *m*-chlorophenylhydrazone (CCCP) can be seen from Figs. 2a and 2c. The uncoupler considerably shortens the dark-time needed to restore complete induction without affecting F_0 and the rates of change during any of the induction phases. In the presence of 5 μM CCCP, the rate of change in the P_2M_2 phase was found to be lowered. At concentrations of 10 to 30 μM, the rate of decrease in the P_2M_2 phase was found to be almost completely inhibited, if not totally. A similar effect is brought about by 50 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) in Chlorella[9]. From Fig. 2b it can be concluded that at the concentrations used, the alcohol, in which nigericin and CCCP were dissolved, did not cause an effect of the kind induced by these chemicals.

Valinomycin (1 μM) and Dio-9 (7.5 μg/ml) neither influenced the rate of the dark process leading to the restoration of complete induction, nor induced changes in F_0 and the kinetics of any of the phases of the induction curve.

The results reported here indicate that the rate of the dark reaction in which Q′ is restored to Q, is substantially enhanced by the antibiotic nigericin and by CCCP, whereas the antibiotic valinomycin and the energy transfer inhibitor Dio-9, do not affect this reaction. It has been observed, and Figs. 1b and 1c show, that the enhancement caused by nigericin and CCCP is much stronger than can be induced by pre-illumination with blue light. Evidence has accumulated that uncouplers[10] like CCCP and members of a special class of antibiotics[11], interact with the ion-translocating system of the membranes of cellular organelles. Dio-9 has been shown to be an inhibitor of coupled electron flow and photophosphorylation in isolated chloroplasts[12,13]. The ineffectiveness of Dio-9 and of valinomycin, and the pronounced effectiveness of CCCP and nigericin to enhance the dark reaction in which Q′ is converted to Q, indicate that the rate of the reaction responsible is not determined by the ADP–ATP ratio, and suggest that the reaction is associated with, or probably reflects, a cellular dark process in which proton movements and proton–cation exchange reactions across cellular membranes are involved. These reactions have been studied in isolated chloroplasts[14,15] and in chromatophores[16], and it has been shown that valinomycin, in contrast to nigericin and CCCP, does not cause a dissipation of energy stored in a proton gradient across the membranes. It has been shown that FCCP and nigericin inhibit light-induced chloroplast shrinkage *in vivo*[17]. Possibly the emission yield of chlorophyll a_2 associated with the thylakoid-bound reaction centers, is dependent on the ionic concentration gradient across the chloroplast membrane, or alternatively, on the conformational state of the chloroplasts *in vivo*. This would mean that Q′ reflects the reduced state of the reaction center in a specifically altered energy state of the membrane(s). The fact that this state is not reached in isolated systems would point to a regulating function of transport mechanisms across cytoplasmic membranes. Rapid reversible changes in light emission of chlorophyll *a*, brought about by rapid changes in ionic gradients, have been observed in isolated

chloroplasts[18,19]. The stimulatory effect of pre-illumination with light of System I on the dark restoration of Q in this concept, may be due to a System I driven reconstitution of ionic concentrations by active transport. It has been shown for several algae (for a survey of literature see refs. 20 and 21), and recently for intact leaves[22], that alkali ions are transported across cytoplasmic and chloroplast membranes, respectively, at the expense of light absorbed by System I. However, the present stage of knowledge of the interrelationship between the several light-dependent energy processes *in vivo* (*i.e.* electron transport and ion transport), does not allow definite conclusions to be reached. Nigericin and Dio-9 were kindly given by Dr. R. L. Harned, Commercial Solvent Corporation, Terre Haute, U.S.A., and Dr. P. L. Hoogland, Koninklijke Nederlandse Gist- en Spititusfabriek, Delft, the Netherlands, respectively.

Centre for Plant Physiological Research, W. J. VREDENBERG
Wageningen, P.O. Box 52 (The Netherlands)

1 L. N. M. DUYSENS AND H. E. SWEERS, in H. TAMIYA, *Studies on Microalgae and Photosynthetic Bacteria*, University of Tokyo Press, Tokyo, 1963, p. 353.
2 W. L. BUTLER, in D. R. SANADI, *Current Topics in Bioenergetics*, Vol. 1, Academic Press, New York, 1966, p. 49.
3 T. T. BANNISTER AND G. RICE, *Biochim. Biophys. Acta*, 162 (1968) 555.
4 W. J. VREDENBERG, in H. METZNER, *Progress in Photosynthesis Research*, in the press.
5 W. J. VREDENBERG AND L. SLOOTEN, *Biochim. Biophys. Acta*, 143 (1967) 583.
6 B. FORBUSH AND B. KOK, *Biochim. Biophys. Acta*, 162 (1968) 243.
7 J. AMESZ AND W. J. VREDENBERG, in T. W. GOODWIN, *Biochemistry of Chloroplasts*, Vol. II, Academic Press, New York, 1967, p. 593.
8 L. N. M. DUYSENS AND A. TALENS, in H. METZNER, *Progress in Photosynthesis Research*, in the press.
9 G. PAPAGEORGIOU AND GOVINDJEE, *Biophys. J.*, 8 (1968) 1316.
10 P. MITCHELL, *Biol. Rev.*, 41 (1966) 144.
11 H. A. LARDY, S. N. GRAVEN AND S. ESTRADA-O, *Federation Proc.*, 26 (1967) 1355.
12 R. E. MCCARTY, R. J. GUILLORY AND E. RACKER, *J. Biol. Chem.*, 240 (1965) PC 4822.
13 S. J. D. KARLISH AND M. AVRON, *Biochim. Biophys. Acta*, 153 (1968) 878.
14 N. SHAVIT, R. A. DILLEY AND A. SAN PIETRO, in K. SHIBATA *et al.*, *Comparative Biochemistry and Biophysics of Photosynthesis*, University of Tokyo Press, Tokyo, 1968, p. 253.
15 L. PACKER, J. M. ALLEN AND M. STARKS, *Arch. Biochem. Biophys.*, 128 (1968) 142.
16 J. B. JACKSON, A. R. CROFTS AND L.-V. VON STEDINK, *European J. Biochem.*, 6 (1968) 41.
17 P. S. NOBEL, *Plant Cell Physiol.*, 9 (1968) 499.
18 B. C. MAYNE AND R. K. CLAYTON, *Proc. Natl. Acad. Sci. U.S.*, 55 (1966) 494.
19 D. MILES AND A. T. JAGENDORF, *Plant Physiol.*, 43 Suppl. (1968) 28.
20 J. A. RAVEN, *J. Gen. Physiol.*, 50 (1967) 1627.
21 J. BARBER, *Biochim. Biophys. Acta*, 163 (1968) 141.
22 P. S. NOBEL, *Biochim. Biophys. Acta*, 172 (1969) 134.

Received February 24th, 1969