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DISTRIBUTION OF EXCITATION ENERGY AMONG PHOTOSYSTEM I AND PHOTOSYSTEM II IN RED ALGAE

I. ACTION SPECTRA OF LIGHT REACTIONS I AND II

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SUMMARY

Action spectra of light reaction I and light reaction II from red algae (marine members of Florideae and Bangiales) were measured with 550 nm (light 2) or 699 nm (light 1) background light, using a Teflon-covered platinum electrode for O_2 measurement. Care was taken to ensure that maximum enhancement was reached by the background light.

The action spectra of light reaction I, we found under these conditions, are very similar to the thallus absorption, whilst the action spectra of light reaction II show, besides strong bands of the phycobilins, only minor bands of chlorophyll *a*, which account for only 10–20 % of the total chlorophyll.

The spectra are discussed on the basis of two main types of models of energy distribution over both photosynthetic systems. If this distribution is considered to be invariable (models 1a and b), one has to assume that almost exactly half of the total chlorophyll is not involved in the supply of the non-cyclic electron transport with excitation energy. This part, however, has to be thought of as incorporated in the thylakoid membrane in a similar manner to the chlorophyll in photosystem I. However, if one supposes an almost complete equilibration in the energy distribution over both systems as long as the primary absorption in photosystem II prevails (models 2a and b), there is no need for the assumption of such photosynthetically 'inactive' or less active chlorophyll. Some evidence is shown that strongly supports model 2.

INTRODUCTION

Red algae appear most suitable objects for in vivo experiments on the regula-

Abbreviations: PS I, PS II, photosystems I and II respectively; light-1, light-2, light of wavelengths predominantly absorbed by photosystems I and II respectively; LR I, LR II, light reaction I and II respectively; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; NADP, nicotinamide adenine dinucleotide phosphate.

tion of the distribution of excitation energy among the photosystems I and II, because of the distinct inequality of the distribution of the collector-pigments to both photosystems. However, the proper distribution of the collector-pigments is by no means clear. First, there exists no reliable action spectrum for photosystem II (early workers being mainly interested in photosystem I, to find out why chlorophyll seemingly works so inefficiently in red algae); secondly, there exist two completely different types of action spectra for light reactions attributable to photosystem I. French and Fork [1] measured action spectra of two transient phenomena in the oxygen exchange of *Porphyridium*, called "negative spike" and "photostimulation of respiration", which may be attributed to photosystem I and which show only the bands of chlorophyll *a* and of carotenoids. On the other hand, there are action spectra, obtained with completely different methods, which closely correspond to the absorption spectra of the algae, involving strong bands of phycoerythrin and phycocyanin. This type is represented by the spectra for oxygen evolution in the presence of strong light-2 background [2], for photoreduction in DCMU-inhibited *Porphyridium* [3] and for the light-dependent change of the membrane potential of *Griffithsia* after inhibition of photosynthesis with DCMU [4].

In this first paper action spectra of light reacton I and light reaction II of some red algae will be compared with the thallus absorption and the consequences of different models of excitation energy distribution, applied to these spectra, will be discussed.

MATERIALS AND METHODS

Thallus absorption was measured with a Shimadzu UV 200 spectrophotometer using an integrating sphere. A stationary, Teflon-covered platinum electrode of the type described by Fork [5] was used for oxygen measurements. Measuring light came from a recalibrated Bausch and Lomb monochromator with a 0.75-mm exit slit equipped with a 250 W/24 V iodine lamp. For background illumination the light of a 250 W/24 V iodine lamp was filtered by interference filters (Schott u. Gen. Mainz, half-band width 10–11 nm). If not mentioned otherwise, the light intensities, measured by a calibrated silicon cell or by a calibrated photodiode, were corrected for reflection of light from the platinum surface, as described by Haxo and Blinks [6]. This reflection would tend to fill up the minima in the action spectra by extending the path-length of the light in the thallus, especially in the regions of low thallus absorption. Our measurements of the reflection of the Teflon-covered platinum surface (62.1 % at 400 nm, 66.9 % at 700 nm), obtained with the integrating sphere, were fairly similar to the data given by Haxo and Blinks [6] for the bare electrode.

Fluorescence was measured at an angle of about 45° above the platinum electrode with a R666 photomultiplier from Hamamatsu equipped with interference- and cut-off filters (λ_{max} 684 nm). In this case, the measuring beam and the background light were further filtered by a set of interference- and cut-off filters.

The red algae, laminate marine species of a thickness of only one or exceptionally a few cell layers, were collected at different points in the environment of Roscoff (France) and used for experiments either immediately or after a stay of a few days in rinsing sea water.

Action spectra were measured with a background of 550 or 699 nm. Light intensities of the background were chosen to give maximum enhancement. In some

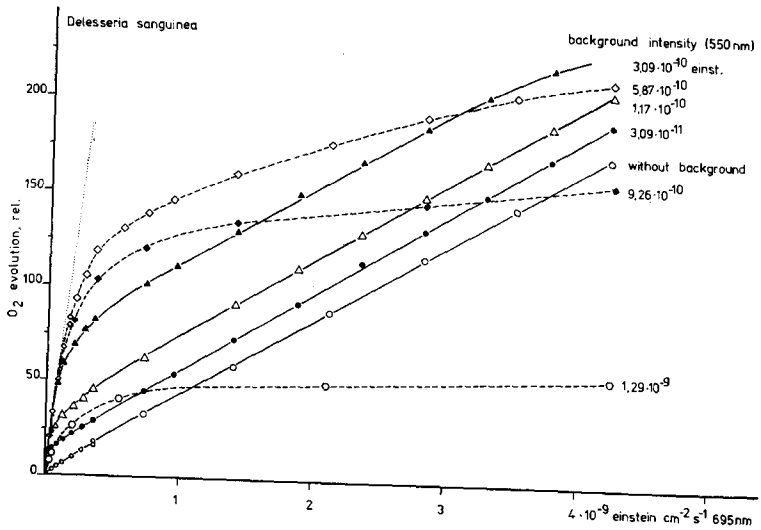


Fig. 1. Rate of oxygen evolution of *Delesseria sanguinea* as a function of the intensity of 695-nm light at different intensities of 550-nm background light. The values of the rate of oxygen evolution in measuring light plus background minus the rate in background light ($V_{695+550}-V_{550}$) are plotted. The dotted line gives the slope of the maximum enhanced rate of oxygen evolution.

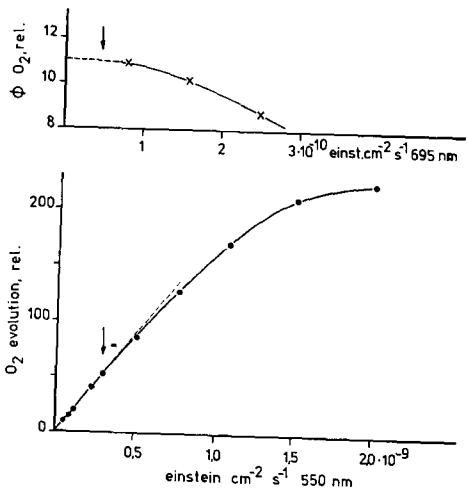


Fig. 2. Conditions of measurement of the action spectra for light reaction I. Bottom: O_2 evolution as a function of the intensity of 550-nm light. The intensity used as background light for measuring the light reaction I spectrum is marked by an arrow, the range of total oxygen evolution at the individual wavelengths is marked by a bar beside the arrow. Above: Relative O_2 yield as a function of the intensity of the 695-nm light at a constant background of $2.98 \cdot 10^{-10}$ Einstein $\cdot$ $cm^{-2} \cdot s^{-1}$ 550-nm light. The arrow marks the intensity of measuring light used in the spectrum. (Object: *Cryptopleura ramosa*).

cases additionally an action spectrum of oxygen evolution was measured without background light. All action spectra of one species were made with the same piece of the thallus, assuring a direct quantitative comparability of the individual spectra. The design of the experiments and their interpretation are based on the premises of the Z-scheme of electron transport, a 1 : 1 stoichiometry of the photoacts 1 and 2, and on an equal quantum yield of the two photoacts. The background light should excite one system in excess to such an extent, that the effect of the low-intensity measuring beam would depend only on that portion of additional quanta that is trapped from the reaction centers of the other system [7, 8]. The total intensity of background and measuring light had, of course, to lie within the range of linear correlation to response. It was, however, sometimes difficult to fulfil these conditions completely. As Fig. 1 shows, the intensity range of measuring light of 695 nm, within which maximum enhancement can be obtained, is very small (down to less than $1 \cdot 10^{-10}$ Einstein $\cdot$ $cm^{-2} \cdot s^{-1}$). The intensity of the 550-nm background light, which gives best values ($2.07 \cdot 10^{-10}$ Einstein $\cdot$ $cm^{-2} \cdot s^{-1}$), lies, for *Delesseria sanguinea* already at the upper limit of the linear part of the response/intensity curve. Usually, the maximum tolerable background intensity was determined and then the useful range of intensities for the measuring beam at 695 nm estimated as in Fig. 2. Deviations up to 3 % from maximum enhancement sometimes had to be tolerated to get sufficiently high measuring values. At each wavelength the intensity of the measuring beam was adjusted to get roughly equal response in all spectra from one species. During the long-term experiments (lasting up to 48 h), the constancy of the photosynthetic capacity of the alga was tested by a standard illumination at intervals of about one hour and normally proved very good. When smaller deviations occured, the standard values were used as a reference (cf. ref. 9).

RESULTS AND DISCUSSION

Fig. 3 gives an example of the type of action spectra obtained with minor differences with all (eight) members of the Florideae examined and, with some greater deviations, with 3 species of the Bangiales. The action spectra obtained with 550-or 699-nm background light are called henceforth light reaction I or light reaction II spectra respectively. They certainly indicate fairly exactly the fraction of measuring light available under the given conditions of measurement, for light reaction I or light reaction II respectively. This light energy may be collected by the system's own pigment antenna or may come from a more or less sophisticated spill-over mechanism [10] from a collector unit of the other system or from collector pigments belonging to both systems [11]. Therefore, they do not necessarily simply reflect the composition of the collector pigment units of both systems.

The light reaction II spectra show always the expected extremely unequal provision of the reaction centers of photosystem II by the individual plastid pigments. Besides the predominant bands of phycoerythrin and the lesser bands of R-phycocyanin and allophycocyanin, the spectra show only very small shoulders in the region of the red and the blue band of chlorophyll *a*.

In contrast to this, despite most careful selection of the intensities of background and measuring light to ensure that the oxygen evolution caused by the measuring beam depends exclusively on that part of the energy of the beam, transmitted to

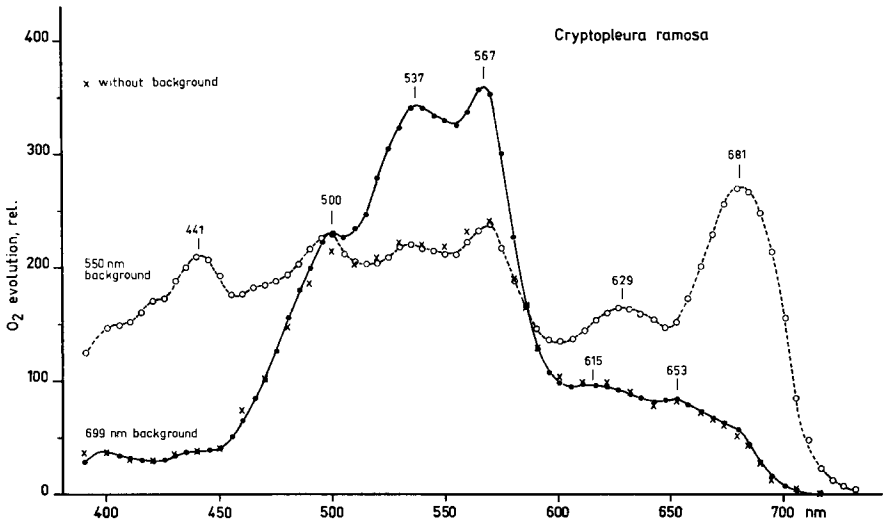


Fig. 3. Action spectrum for O_2 evolution with 550-nm background ("light reaction I spectrum"), with 699-nm background (light reaction II spectrum) and without background light. (Spectra not corrected for reflection from the platinum surface).

the reaction centers of photosystem I, the action spectra with 550 nm background light, the light reaction I spectra, never did indicate a similar (but complementary) inequality in the supply of the reaction centers of photosystem I with excitation energy from the plastid pigments, as the action spectra from French and Fork seem to denote [1]. Rather, as in the absorption spectrum, the three peaks of phycoerythrin attain a similar height to the chlorophyll *a* bands. These spectra coincide largely with those obtained by Fork [2], Gingras [3] and Throm [4].

The action spectrum of oxygen evolution without background light (marked by crosses) follows exactly the lower curve section of both light reaction spectra, as Jones and Myers [8] found for Anacystis. This merely shows the perfection with which the spectra can be reproduced even after 24 or 48 h, and does not allow any far-reaching conclusion about the mechanism of the distribution of excitation energy. It also demonstrates a strict linear intensity-response relationship at all wavelengths. In many of our other spectra the values obtained without background light lie well above the lower curve of the action spectra obtained with background, especially at wavelengths between 490 and 590 nm, as a consequence of a weak Kok effect. This effect, however, should not impair the reliability of the light reaction spectra, the Kok effect being saturated in these cases by the fairly strong background light.

The most intriguing feature of the light reaction I spectra, their close resemblance to the thallus absorption spectra is shown for *Cryptopleura ramosa* in Fig. 4 and for *Phycodrys rubens*, as a still more striking example, in Fig. 5. Aside from the range of carotenoid absorption below 520 nm (cf. also the spectra of *Porphyra umbilicalis*, Fig. 10), where the action spectrum (after normalisation at 680 nm) lies well below the absorption, even with *Cryptopleura ramosa* the difference between both spectra is not significant. Absorption and action spectra are made from different thallus pieces and they do not differ by more than adjacent parts of the thallus often

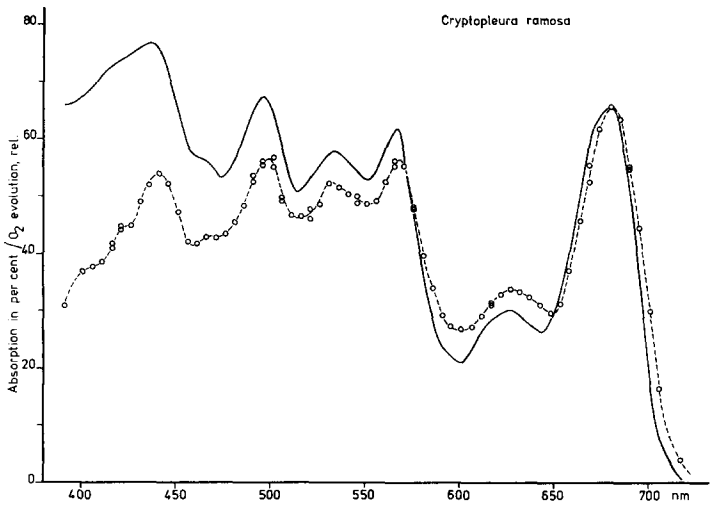


Fig. 4. The light reaction I spectrum (-○-) corresponds with the absorption in percent (—). The spectra are equalized at 680 nm.

differ in absorption. This similarity of both spectra means that for all pigments aside from some carotenoids, an equal part of the absorbed energy is diverted to the reaction centers of photosystem I. Together with the action spectra of light reaction II, it further indicates that either (the partition of excitation energy among photosystem I and photosystem II assumed to be constant) an appreciable part of chlorophyll *a* is inactive in the sense that it does not deliver excitation energy for non-cyclic electron transport, or that, at prevailing excitation of photosystem II and only in this case (this is the condition under which our light reaction I spectra are measured), there is an almost complete equilibration of the distribution of excitation energy between both systems.

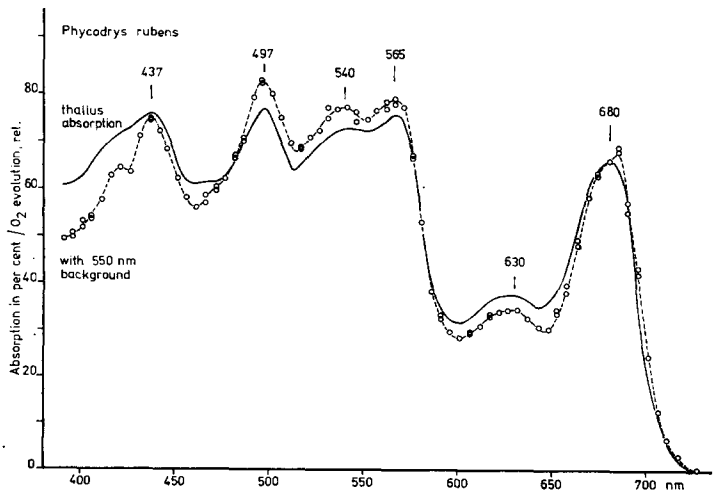
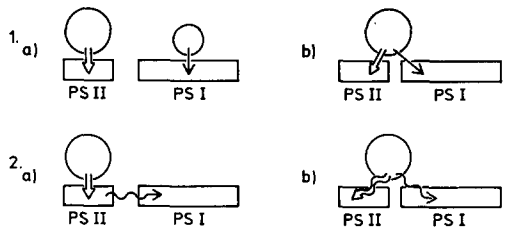


Fig. 5. As Fig. 4.



Scheme 1. Models of distribution of excitation energy absorbed by biliproteids located in phycobilisomes over the chlorophyll beds of photosystem II and photosystem I: 1. Models which provide an invariable distribution among both systems. 2. Models which assume variability of the proportion of energy finally obtainable to each of both types of reaction centers (for details see text). Straight arrows, constant energy transfer wavy arrows, variable energy transfer.

The highly simplified Scheme 1, which makes allowance for the localisation of the biliproteids in phycobilisomes [12] discriminates four main possibilities of energy transfer from the phycobilins to the reaction centers of photosystem I. In this scheme the proportions are arbitrary and the membrane fragments labeled photosystem I or photosystem II represent roughly four units of photosystem I or photosystem II (cf. ref. 13). Models 1a and 1b provide for no variability in the distribution of excitation energy between photosystem I and photosystem II. Model 1a, nearest to the classical separate package model [10] is highly improbable, as the phycobilisomes of one object are always found to be of uniform size and shape (cf. ref. 14). In both models, the light reaction I and light reaction II spectra can be regarded as identical with the photosystem I and photosystem II spectra. In both cases therefore it should be possible to add together the light reaction I and light reaction II spectra to get the total absorption of the pigments which deliver energy for non-cyclic electron transport. A comparison of this sum of both spectra with the absorption spectrum as is made in Fig. 6 for *Porphyra umbilicalis* should reveal that part of pigments that does not supply or only inefficiently supplies the reaction centers with excitation energy. It should be emphasized that to get the sum spectrum the original values of the light reaction I and light reaction II spectra which are added together are obtained from the same piece of thallus and are therefore directly comparable. To get the difference spectrum the sum of the light reaction I and light reaction II spectra and the absorption spectrum are adjusted in such a way that they do not cross, but are at a tangent to another at one point. The resulting spectrum is remarkable in two respects: First, it shows almost the same shoulders in the region of the red band of chlorophyll *a*, and the same peak wavelength as the light reaction I spectrum does. According to the models 1a and b this would mean that the part of chlorophyll not involved in energy supply of the non-cyclic electron transport is fitted into the thylakoid membrane in a similar complex manner to the functional chlorophyll in photosystem I. Secondly, the red peaks of the difference and of the sum spectrum have exactly the same height. This would signify that only half of the total chlorophyll takes part in the supply of the non-cyclic electron transport with light energy. The same features are found in the corresponding curves of members of the Florideae, as in *Cryptopleura* (Fig. 7), which differ only in the region of principal carotenoid absorption, the thallus of *Cryptopleura* possessing a lower concentration of carotenoids.

The consequences of accepting the first two models do not seem too likely.

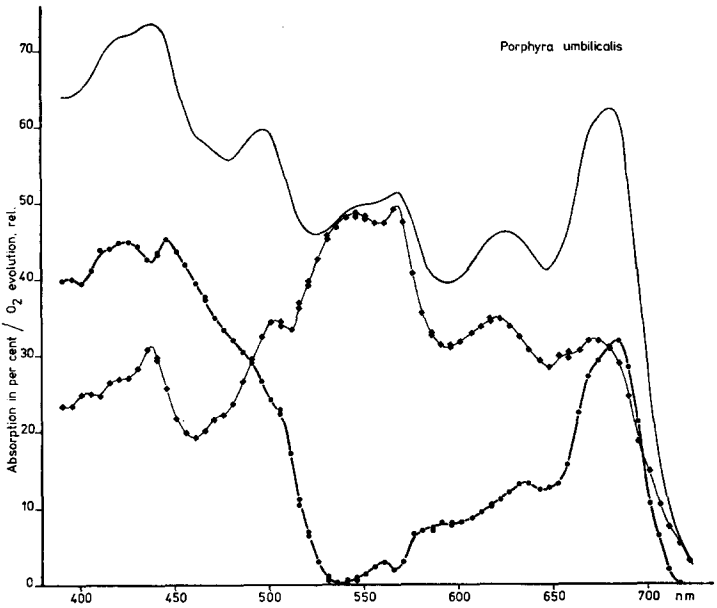


Fig. 6. Sum of the light reaction I and light reaction II spectra (– ♦ –) (the original values of the light reaction I and light reaction II spectra, obtained from the same piece of the thallus, are added), absorption of the thallus in percent (—), adjusted in such a way that both curves do not cross each other, and the difference between both (–●–).

Certainly the chlorophyll not involved in the energy supply of the non-cyclic electron transport has not to be completely functionless. It might belong to a third system which drives a cyclic electron flow. This system would have a collector pigment unit completely different from the two other systems, composed only from chlorophyll *a*

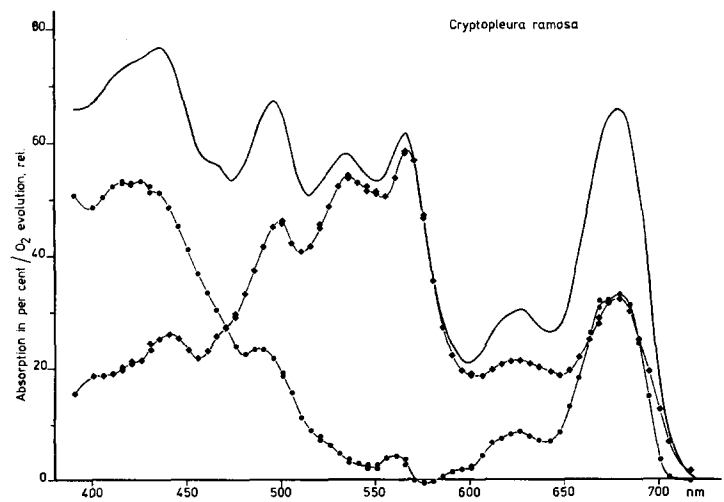


Fig. 7. As Fig. 6.

and carotenoids. However, until now there are no indications of the existence of such a third system. Since the work of Bonaventura and Myers [15] and of Murata [16] much evidence has accumulated in favour of a variability of the distribution of energy among both photosynthetic systems dependent on irradiation of whole cells with light 1 or light 2 as is provided for in the last two models 2a and b of Scheme 1. The two models differ in the way in which a variable portion of excitation energy absorbed in the phycobilisomes is diverted to photosystem I. Model 2a provides for a tight exclusive association of the phycobilisomes to photosystem II, a transfer of excitation energy to photosystem I occuring only at the level of the chlorophyll beds (or of the reaction centers) of both systems. In contrast, in model 2b a direct transfer of energy from the phycobilisomes to both systems in variable proportions is assumed. This last model is homologous to that proposed by Butler and Kitajima [11] for green plants. To make allowance for the approximate identity of the light reaction I and the absorption spectra (Figs. 4 and 5), both models, 2a and 2b, have to provide an almost equal distribution of energy between both systems at all wavelengths, as long as there is a surplus of light 2 (e.g. as background) of sufficient intensity to maintain the material in state 2 [15, 17]. This implies that there remains almost no residue of unused excitation energy in photosystem II when light 2 background is given, whilst in photosystem I any excess energy will be wasted or possibly used in cyclic electron transport. Therefore, the minimum apparent quantum requirement for the transport of 1 electron from H_2O to NADP will approach 2 at 550 nm background, but approaches 1 at 699 nm background. This would explain why in Figs. 6 and 7 the red band of the light reaction I spectra has exactly the same height as in the difference spectra.

We have observed with our materials the same state 1-state 2 effects as described for *Porphyridium* [16] and *Chlorella* (eg. ref. 15), especially the parallel rise of fluorescence intensity and rate of oxygen evolution in light 1 at the transition from state 2 to state 1 and the strong stimulation of fluorescence emission immediately after return into light 2. A transition from state 2 to state 1 and back to state 2 is shown in the trace of chlorophyll fluorescence of *Porphyra umbilicalis* (Fig. 8). More results on fluorescence phenomena will be presented in a forthcoming paper. The existence of such a clear state 1-state 2 phenomenon suggests the calculation of spectra of photosystem I on the basis of model 2a (which is equal in effect with model 2b). These spectra should match the light reaction I spectra if measured in state 1 that means under conditions of complete absence of energy transfer from photosystem II or immediately from phycobilisomes to photosystem I.

A basic assumption for the calculation of these spectra is that the light reaction II spectra are made in a complete state 1. In this case, when no energy transfer occurs between photosystem II and photosystem I, the light reaction II spectrum should be identical to the photosystem II spectrum. It is further assumed that the light reaction I spectra were obtained in an absolute state 2, resulting in complete equilibration of energy between the two systems. The first condition may not always, and the second possibly never be fulfilled completely. In this case the correction of the light reaction I spectrum to get the true photosystem I spectrum as proposed below will lead to an overcorrection. However, we have convinced ourselves by means of fluorescence measurements, that a satisfactory approximation to both conditions was reached in most cases. The photosystem I spectra therefore were calculated as follows: When x is the part of incident light transfered to the reaction centers of photosystem I (given by

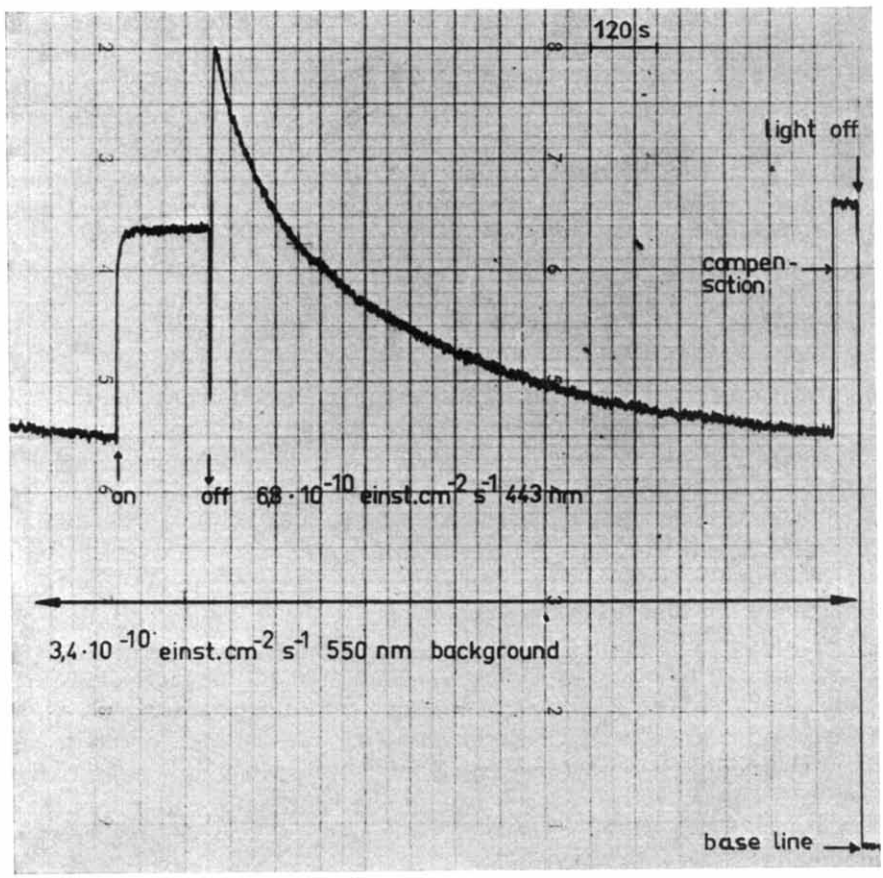


Fig. 8. State 2 → state 1 → state 2 transition in *Porphyra umbilicalis*. Time course of chlorophyll *a* fluorescence in 550-nm background light before, during and following an additional illumination with 443-nm light. In a (submaximum) state 1 the fluorescence intensity in light 2 is enhanced by more than 65 % above the stationary fluorescence intensity in state 2.

the values of the light reaction I spectrum) and a_I and a_{II} are the absorption fractions of incident light by the collector units of photosystem I and photosystem II, (where a_{II} is given by the light reaction II spectrum), then according to our premise:

$x = \frac{1}{2}(a_I + a_{II})$ and $a_I = 2x - a_{II}$.

The spectra obtained from all members of the Florideae and of the Bangiales were nearly identical (Fig. 9). They show only bands from chlorophyll *a* and from carotenoids. They resemble the light reaction I spectrum of *Chlorella* [9] as well as the spectrum for the "negative spike" of *Porphyridium* [1].

This result suggests a simple explanation for the difference between the published action spectra, which are or may be interpreted as action spectra of light reaction I. One type [1] may be obtained while the object was in state 1 (which is very probable) the other type [2–4] while the object was in in state 2. This is surely true for the spectra obtained with light 2 background [2], but is most dubious for the spectra

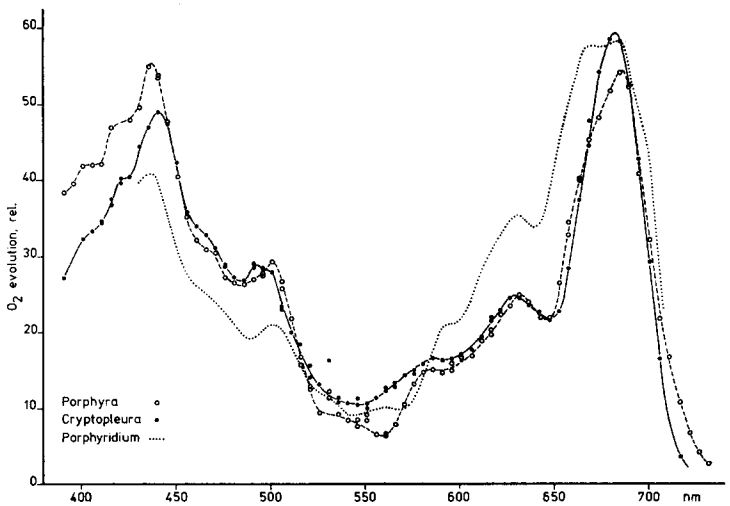


Fig. 9. Computed photosystem I spectra, compared with the action spectrum for the "negative spike" from *Porphyridium cruentum* (French and Fork [1]).

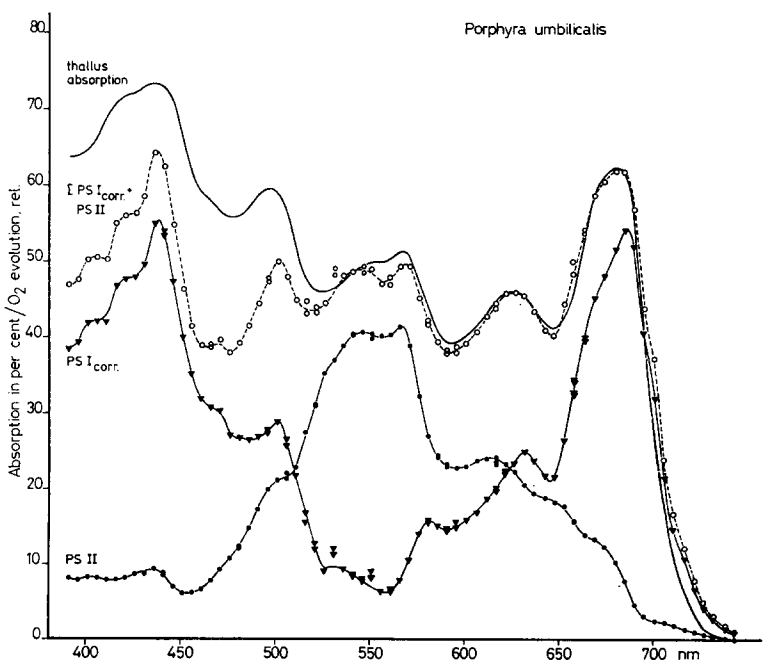


Fig. 10. Photosystem II (●) and computed photosystem I (▼) spectra of *Porphyra umbilicalis*, the sum of both (○) (equal to the measured light reaction I spectrum multiplied by 2) compared with the absorption spectrum of the thallus (——). Point of adjustment: 680 nm.

of Gingras [3] and of Throm [4], obtained in the presence of DCMU (compare for instance refs 16 and 18). Further work seems necessary to clarify the effect of DCMU on the state 1-state 2 phenomenon under different marginal conditions.

The whole set of spectra: photosystem I, photosystem II and sum Σ of both (which is identical with the measured light reaction I spectrum multiplied by 2), obtained in the manner described, is shown for *Porphyra umbilicalis* in Fig. 10. With these spectra the thallus absorption is compared after adjustment with Σ at 680 nm. Although Porphyra possesses an unusually high portion of chlorophyll a in photosystem II together with high concentrations of R-phycocyanin and of allophycocyanin, the overlapping between the spectra of photosystem I and photosystem II is fairly small. Equal efficiency of chlorophyll and phycobilins is shown, only the carotenoids absorbing at wavelengths below 530 nm have to be assumed to be less effective as collector pigments.

There is much indirect proof favoring such an interpretation of our spectra on the basis of model 2a or 2b, which will be presented in forthcoming papers. Further work is in progress to give direct evidence by means of measurements in modulated light.

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