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DETAILED ACTION SPECTRA OF PHOTOPHOSPHORYLATION CATALYZED BY PHOTOSYSTEM I

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SUMMARY

Action spectra of PS-1 photophosphorylation show absorption bands or shoulders with peak locations around 640, 647–650, 660, 672–674, 680, 690, 701–703, 710–712 and 721 nm. Phosphorylation activity is reduced around 665–667 and 676 nm suggesting decreased absorption bands of pigments involved in the light absorption process of PS-2.

Abbreviations: ATP: adenosine-5'-triphosphate, BSA: bovine serum albumin Chl_a- or Chl_b-: Chlorophyll *a* or *b*, DCMU: 3(3-4) dichlorophenyl-1,1-dimethyl urea, DCPIP: 2,6-dichlorophenol-indophenol, MV: methylviologen, NADP: nicotinamide adenine dinucleotide phosphate, ^{32}P: the radioactive isotope phosphorus-32, PS-1 (2): photosystem 1 (2), PMS: phenazine methosulphate.

1. INTRODUCTION

Information about the pigments that participate in reactions of PS-1 has been given in literature in different studies relating, for example, to

(a) absorption and fluorescence spectra of chloroplast fragments enriched by chemical or mechanical treatment with PS-1 activities, cf. BOARDMAN et al. (1966), BRIANTAIS (1967, 1969), CRAMER & BUTLER (1968), FRENCH et al. (1972) and OGAWA & VERNON (1970).

(b) action spectra of light-induced reactions catalyzed by PS-1, cf. BROWN et al. (1972), FORK & AMESZ (1969), GOEDHEER (1972), HALLDAL et al. (1974), RIED (1972) and WANG & MYERS (1976).

Generally absorption spectra of PS-1 enriched fractions show a much smaller absorption band of Chl_b-650 than PS-2 enriched fractions or nontreated chloroplast preparations, or they show no chlorophyll-*b* absorption band at all. Absorption spectra also show that long wavelengths chlorophyll forms with peak locations near 693 and 704 nm are more common in PS-1 than in PS-2 preparations and that PS-1 preparations generally contain relatively more Chl_a 684 cf. BROWN & GASANOV (1974), FRENCH et al. (1972), FRENCH & BROWN

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(1972) and GASANOV & FRENCH (1973). FRENCH & BROWN (1972) have reported that the amount of *Ca* 662 and *Ca* 670 in PS-1 fractions is about the same as in PS-2 fractions. However, BRIANTAIS (1969) observed Chl_a-665 only in PS-1 fractions. Action spectra of different PS-1 reactions also sometimes show conflicting results e.g. some action spectra do not show a chlorophyll *b* absorption band, cf. JOLIOT et al. (1968) BROWN et al. (1972) and LOOS (1974), whereas other PS-1 action spectra clearly show the presence of this band, cf. BROWN et al. (1972), FORK (1963), HALLDAL et al. (1974), MÜLLER et al. (1963), VERNOTTE et al. (1963) and WANG & MYERS (1976).

The majority of PS-1 action spectra published do not show any structure in the longwavelength region ranging from 685–740 nm. More detailed PS-1 action spectra show absorption bands with peak locations around 690, 705 and 720 nm, cf. BROWN et al. (1972), HALLDAL et al. (1974) and RIED (1972). Until now only a few photophosphorylation action spectra have been published, and it is not possible to draw from these data any definite conclusions about the pigments involved in the process of ATP formation. The aim of the present study is to present as accurate information as possible about the pigments involved in the ATP formation process of PS-1.

2. MATERIAL AND METHODS

Isolation of spinach chloroplasts, measurement of light intensity, irradiation of the samples and extraction and measurement of the ATP formed were carried out as described earlier, cf. VAN GINKEL (1975, 1977). As it takes 10–20 hours to establish an action spectrum the photochemical activity of the chloroplast preparation used may decrease due to ageing, cf. VAN GINKEL (1975, 1977). If necessary the action spectra presented are corrected for activity losses due to ageing as described by VAN GINKEL (1975). Each plot in the given action spectra represents the average of five experiments. The standard deviations are indicated by bars.

3. RESULTS

To obtain detailed action spectra that are quantitatively comparable with absorption spectra, the photochemical response should be linear with light intensity, cf. FORK & AMESZ (1969). For that reason the rate of ATP formation was measured as a function of light intensity in a medium containing PMS chemically reduced by ascorbate, in the presence of 8,5 μM DCMU. Under these conditions one is able to measure only the activity of cyclic photophosphorylation of photosystem I, cf. HAUSKA et al. (1970), JAGENDORF & MARGULIES (1960) and TREBST (1974).

Fig. 1 shows PMS catalyzed ATP formation as a function of light intensity measured with monochromatic light of 680 nm, halfwidth 6.4 nm. *Fig. 2* shows

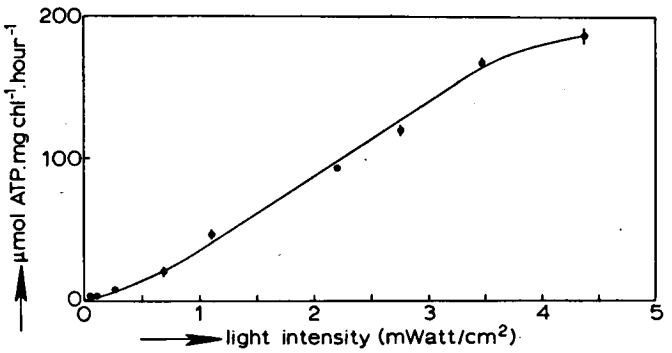

Fig. 1. Cyclic photophosphorylation as a function of light intensity. Illumination time was 2 min. with monochromatic light of 680 nm, halfwidth 6.4 nm. The 0,525 ml reaction mixture consisted of: 2.5 mM Na_2HPO_4 (containing approx. 1×10^7 desintegrations per minute of ^{32}P); 1.9 mM ADP; 50 mM Tricine/NaOH pH 8,5; 1 mg/ml BSA; 37.5 mM sucrose; 4.5 mM $MgCl_2$; 0.9 mM NaCl; 39 µM PMS; 3,6 mM ascorbate; 8.5 µM DCMU; 4.6 µg chl/ml. Reactions were run in a thermostated cuvette regulated at 25 ± 0°C.

an action spectrum of cyclic photophosphorylation catalyzed by PMS. The estimated position of peaks and shoulders is given and the reaction conditions are indicated in the captions for the figure.

Activity minima are located at 665–667 and 676 nm, suggesting strongly decreased activity of (PS-2) pigments. Another PS-1 action spectrum is shown

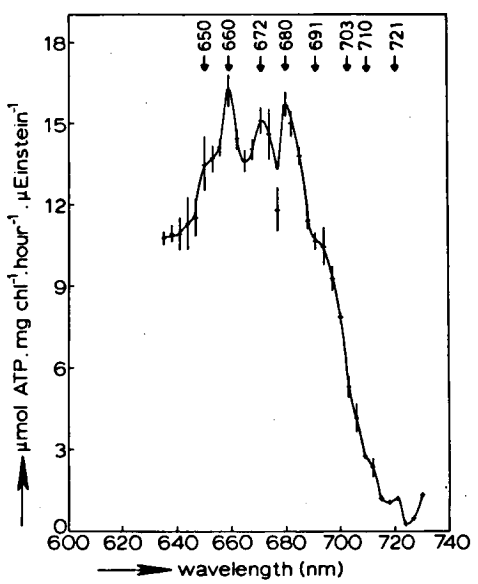

Fig. 2. Action spectrum of cyclic photophosphorylation catalyzed by reduced PMS. Light intensities ranged from 16,4 (at 635 nm) to 11,8 (at 730 nm) n Einstein·cm^{-2}·sec^{-1}. The concentration of chlorophyll was 4.7 µg/ml. All other reaction conditions were as in *fig. 1.*

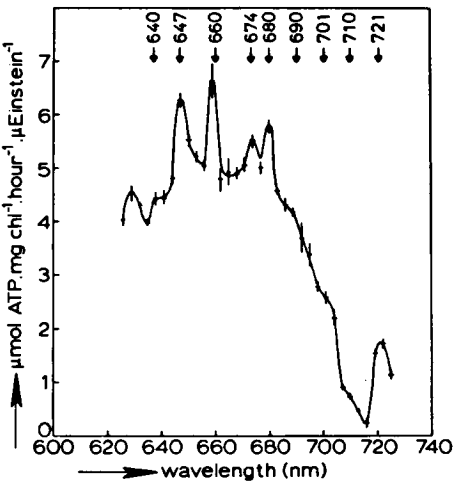

Fig. 3. Action spectrum of photophosphorylation with reduced DCPIP as electron donor for PS-1 and MV as electron acceptor.
The reaction conditions employed were identical to those in *fig. 1* except the light intensity, that ranged from 17.4 (at 626 nm) to 10.5 (at 725 nm) n Einstein·cm^{-1}·sec^{-1} and the concentrations of the following components: 0.2 mM DCPIP; 1.8 mM ascorbate; 0.2 mM MV; 8.5 µm DCMU and 6.7 µg chl/ml.

in *fig. 3*. In this case reduced DCPIP was used as electron donor and methylviologen as electron acceptor. A high concentration of DCMU (8.5 µM) was added to inhibit electron flow from PS-2. In the wavelength range 606–724 nm this action spectrum shows the same maxima and minima as the action spectrum of PMS-catalyzed photophosphorylation.

Deviations are observed in the chlorophyll *b* region ranging from 645–655 nm. The spectrum in *fig. 3* shows a pronounced chlorophyll *b* absorption band peaking at 647 nm and an activity minimum around 655 nm. In *fig. 2*, however, only a shoulder around 648–650 nm can be seen. In *fig. 3* the 640 nm shoulder is rather pronounced, but it is very weak in *fig. 2*.

4. DISCUSSION

On the basis of data from literature we shall discuss the maxima and minima observed in our action spectra and their significance, but we feel it is rather premature to give definite conclusions about the pigments participating in ATP formation of PS-1 before the spectra have been treated mathematically and deconvoluted in the way described by VAN GINKEL (1975). However, we feel justified in drawing some conclusions. It is fairly certain that, in the presence of high concentrations of DCMU, light absorbed by Chl_b-650 is transferred to P_{700}, the PS-1 reaction centre, and induces photochemical activity of PS-1.

The occurrence of activity maxima around 640, 660 and 690 nm in PS-1 action spectra was predicted by VAN GINKEL (1975) on the grounds of studies of other photophosphorylation action spectra. Although not indicated numerically in his figure 14 FORK (1963) too has observed these maxima in an action spectrum of O_2 uptake for aged Swiss chard chloroplasts that had been treated with DCMU. An action spectrum for cytochrome c oxidation by PS-1 enriched fractions from spinach, measured by BROWN et al. (1972), also shows these maxima. As mentioned already in the introduction, BRIANTAIS (1969) and HALLDAL et al. (1974) have suggested that chlorophyll *a*-665 is involved in the light absorption process of PS-1. If they mean the same pigment as we observed in our action spectra at 660 nm their results agree with ours.

BEN-HAYYIM & AVRON (1971) have shown that 640 nm light is involved in the light absorption process of PS-1 or of both photosystems, depending on the electron acceptor used. The 690 nm absorption band observed in our action spectra is obvious in many PS-1 absorption and action spectra. The interpretation of minima and maxima in the 660–685 nm wavelength region is difficult.

Derivative spectroscopy suggests the occurrence of chlorophyll-*a* forms with absorption peaks at 662, 669, 672 and 678 nm in this spectral region, cf. THOMAS et al. (1970). However, fourth derivative low temperature absorption spectra, deconvoluted low temperature absorption spectra and deconvoluted photophosphorylation action spectra suggest the occurrence of only four chlorophyll absorption bands peaking at 661 (662), 669 (670), 677 and 684 nm, cf. BUTLER & HOPKINS (1970), FRENCH et al. (1972) and VAN GINKEL (1975).

As mentioned already, deconvolution of low temperature absorption spectra shows that in PS-1 fractions the proportions of the 662 and the 670 forms are about the same. In PS-2 fractions the total amount of Chl_a-662, Chl_a-670 and Chl_a-677 is greater than in PS-1 fractions. However, there is about twice as much of the 683 nm component in PS-1 fractions, cf. FRENCH & BROWN (1972) and FRENCH et al. (1972). The number of maxima and minima observed in our action spectra suggests the occurrence of five pigment forms with peak locations around 660, 665–667, 672–674, 676 and 680 nm. The minima observed in our action spectra at 665–667 and 676 nm might indicate decreased absorption bands of pigments involved in the light absorption process of PS-2.

This hypothesis relating to Chl_a–676 gains support as a result of absorption spectra of PS-2 enriched fractions which show the main absorption band around 676 nm, cf. FRENCH et al. (1972), FRENCH & BROWN (1972) and BOARDMAN (1972).

The occurrence of five pigment forms in the spectral region 660–685 nm does not gain support from other PS-1 action spectra, for the most detailed PS-1 action spectra we know show only two or three absorption bands peaking. around 665, 672 and 680–685 nm, cf. BROWN et al. (1972), HALLDAL et al. (1974), RIED (1972), VERNOTTE et al. (1973) and WANG & MYERS (1976).

As mentioned already we want to obtain more experimental information before we come to any definite conclusions about the number of absorption bands occurring in this spectral region. In the spectral region ranging from

690–730 nm derivative spectroscopy suggests the occurrence of chlorophyll *a* forms with absorption peaks around 725–730, 716, 709–712, 702–705, 696 and 690–693 nm, cf. GULYAYEV & LITVIN (1970) and THOMAS et al. (1970). A number of absorption spectra and action spectra show the presence of an absorption band at 700–705 nm cf. BRIANTAIS (1967, 1969), BROWN et al. (1972), FRENCH et al. (1972), VAN GINKEL (1975), HALLDAL et al. (1974) and RIED (1972). Fluorescence spectra and derivative absorption spectra provide evidence for the occurrence of an absorption band at 720 nm cf. GOEDHEER (1972) and THOMAS et al. (1970). The only action spectra we know that show an absorption band around 720 nm are the ones mentioned by LITVIN & SINESHCHEKOV (1975) and those published by VAN GINKEL (1975) and HALLDAL et al. (1974). It is generally considered that Chl_a-705 and Chl_a-720 are involved in the light absorption process of PS- 1 only. The present study also indicates activity of these pigment forms in the ATP formation process of PS-1. However, in previous investigations we have also observed these pigment forms in photophosphorylation action spectra when PS-2 was rate-limiting or was obviously the only sensitizing photosystem, cf. VAN GINKEL (1975).

We are not sure about the significance of the shoulder at 710–712 nm observed in our action spectra. To our knowledge the only indication hitherto of the occurrence of this absorption band comes from derivative absorption spectra.

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