SPECTRAL PROPERTIES OF PROTOCHLOROPHYLL IN MODEL SYSTEM

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Spectral properties of protochlorophyll were investigated in solid films by different spectroscopic methods. Solid films were prepared from diethylaether solution of protochlorophyll on cover glass by evaporation of the solvent. The films showed absorption maximum at 635 or 640 nm after preparation. The 635 nm form had no CD signal but the form of protochlorophyll with 640 nm absorption maximum gives intensive negative band at 640 nm and a small positive one at 668 nm. The 635 and 640 nm forms could be transformed into 650 nm form if the samples were treated with vapour of acetone. In this case the main absorption band appeared at 650—654 nm and there was a shoulder at 635 nm. These films had an absorption spectrum similar to the spectrum of etiolated leaves in the red region of the spectrum. In the CD spectrum the 650 nm form showed an intensive positive band at 654 nm and two small negative ones at 632 and 644 nm.

Introduction

The chloroplasts of green plants contain several forms of chlorophyll [1—3] which build up the two photochemical systems [4, 5]. In the etioplasts the precursors of chlorophyll — protochlorophyllide and protochlorophyll — are accumulated. The protochlrophyllide also exists in three different forms *in vivo* which show absorption maxima at 650, 635 and 628 nm, respectively [6].

It has been suggested that the spectral differences between the different forms of pigments are due to pigment-pigment and/or pigment-protein interactions [7, 8]. For investigation of pigment-pigment interactions different model systems have been used. In our earlier work the spectral effects of aggregation of protochlorophyll were studied in solid films by the method of absorption spectroscopy [9].

In this paper the spectral properties of protochlorophyll solid films were studied by different spectroscopical methods as absorption spectroscopy, fluorescence spectroscopy and CD spectroscopy.

Material and methods

For preparation of solid films the protochlorophyll was isolated from pumpkin seed coats and was purified by column chromatography [10]. Solid films were prepared on cover glass by evaporation of some drops of diethylaether solution of protochlorophyll. The traces of solvent were removed in vacuum and the films produced this

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way were used for the spectroscopic measurements. In several cases the solid films after preparation were treated with vapour of aceton.

Absorption spectra were recorded using a Unicam SP 1800 and an SF 18 spectrophotometers. Fluorescence spectra were measured with a spectrofluorometer consisting of a Hilger and Watts grating monochromator with motor drive, a FEU 38 photomultiplier with power supply and a Kipp and Zonen BD 5 recorder. Excitation was carried out with a high pressure mercury lamp with cut off filters.

The circular dichroism spectra were recorded with a Jasco J 40 spectropolarimeter. Circular dichroism is measured in terms of ellipticity (θ) in units of millidegrees (m°). The anisotropic properties of the samples were measured with a Jasco 5 spectropolarimeter. The samples were fixed on a rotatable cell and the ORD spectra were recorded at three different angles. The results of the ORD measurements showed that the samples were invariant at rotation.

Results

In the absorption spectrum of solid films of protochlorophyll the red maximum can usually be observed at 635 nm immediately after preparation of the sample. The half-band width is about 35 nm indicating the complexity of the band. The solid films which showed the red maximum at 635 nm had no CD signal (Fig. 1).

In some cases the prepared samples showed the red maximum at 640 nm instead of 635 nm (Fig. 2). The form of protochlorophyll with 640 nm maximum gives intensive CD signal. There is a negative band at 640 nm and a small positive one at 668 nm.

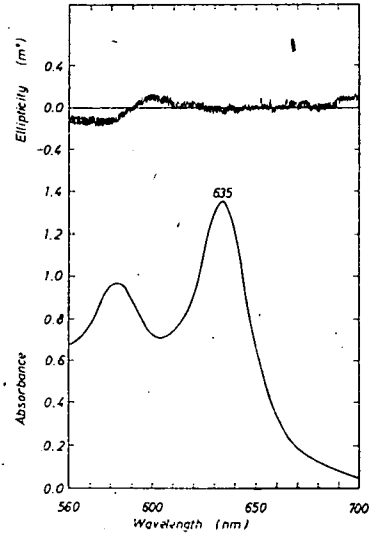

Fig. 1. Absorption and CD spectra of protochlorophyll solid film with absorption maximum at 635 nm

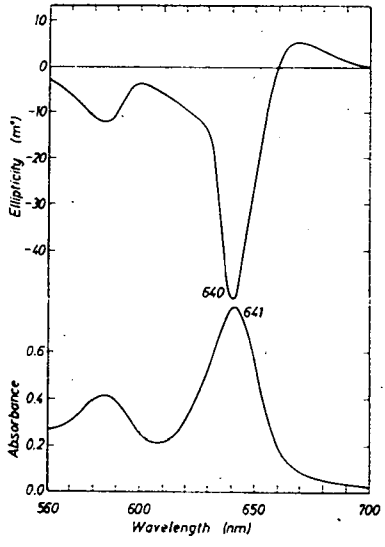

Fig. 2. Absorption and CD spectra of protochlorophyll solid film with absorption maximum at 640 nm

The 635 nm and 640 nm forms could be transformed into 650 nm form in solid films if the samples were treated with vapour of aceton for one hour at room temperature (Fig. 3). After the aceton treatment the main absorption band is found at 650—654 nm and it has a shoulder at about 635 nm. In the CD spectrum an intensive positive signal appeared at 654 nm and two small negative bands at 632 nm and 644 nm.

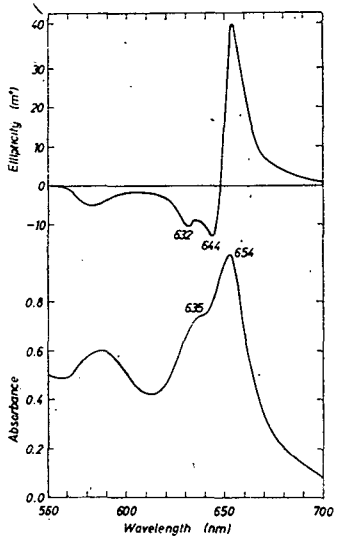

Fig. 3. Absorption and CD spectra of solid film of protochlorophyll treated with vapour of aceton for 1 hour

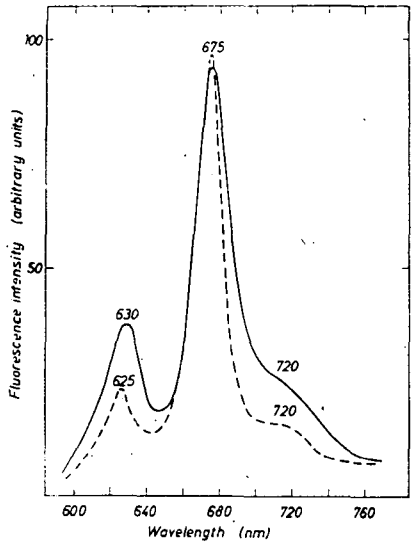

Fig. 4. Fluorescence emission spectra of protochlorophyll solid film with absorption maximum at 635 nm measured in liquid nitrogen (--------) and at room temperature (———)

In solid films with 635 nm maximum kept at room temperature for several days a red shift can be observed from 635 nm toward 640 nm. In these samples a CD signal was found similar to the signal of the sample with absorption maximum at 640 nm. This CD signal appeared only after the beginning of the red shift.

Fluorescence spectra of the sample with 635 nm absorption maximum showed several fluorescence bands. The main maximum appeared at 675 nm but there are bands of low intensity at 625 nm and 720 nm (Fig. 4).

In the fluorescence spectrum of the sample with 640 nm absorption maximum the 625 nm and 675 nm fluorescence bands also are present but the main maximum appeared at 705 nm (Fig. 5).

In the case of 650 nm samples the main fluorescence maximum can be observed at 705 nm but there are small maxima also at 625, 655 and 680 nm (Fig. 6.).

On the basis of fluorescence spectra it may be supposed that the samples with different absorption maxima contain similar forms of protochlorophyll but their

ratio is different. In the 635 nm samples the short wavelength forms are dominating while the 650 nm samples are rich in long wavelength forms. All the samples contain probably a small amount of protochlorophyll with high aggregation degree and as can be judged from the spectra there is an effective energy migration from the short wavelength forms to them.

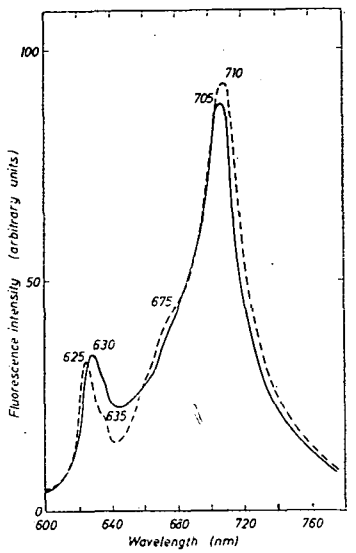

Fig. 5. Fluorescence emission spectra of protochlrophyll solid film with absorption maximum at 640 nm measured in liquid nitrogen (--------) and at room temperature (———)

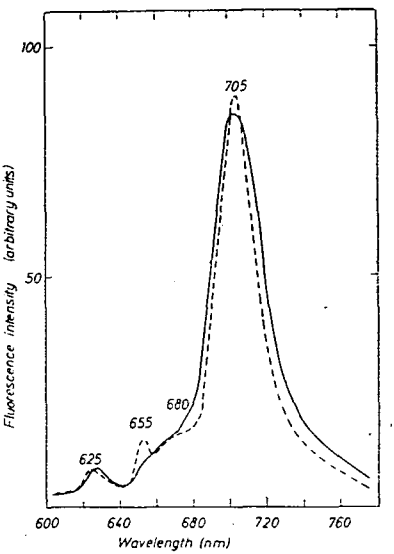

Fig. 6. Fluorescence emission spectra of protochlorophyll solid film treated with vapour of aceton for 1 hour measured in liquid nitrogen (-------) and at room termperature (———)

Discussion

As it could be seen from the absorption spectra of solid films of protochlorophyll in this system several forms of protochlorophyll can be found. Some of them have the absorption maximum at 635 and 650 nm that is at the position as can be observed *in vivo*.

The protochlorophyll in diethylaether solution in the red region of the spectrum shows two distinct absorption bands at 622 and 602 nm respectively [11, 12]. In the CD spectra of the diethylaether solution of protochlorophyll the two electronic transitions give signs of opposite character [11, 12].

In the solid films characterised with maximum at 635 nm the bands corresponding to the two electronic transitions strongly overlap, therefore the absorption band becomes much wider. This may be caused by the interaction of the protochlorophyll molecules [9]. However, when preparing the solid films, due to rapid evaporation of the diethylaether, nonspecific interactions can probably take place between the protochlorophyll molecules, sufficient to shift the absorption maximum to 635 nm.

As a result of the aceton treatment this system "slackens" thus making possible the formation of more specific interactions between the molecules.

In CD spectra of solid films no Cotton effect could be detected. This might have the reason that is a relatively narrow wavelength range several exciton interactions overlap. Apart from this the presence of protochlorophyll forms of different aggregation state with overlapping CD spectra may influence the appearence of the CD signals of the solid films.

The difference between the CD spectra of the different forms is due to the common effect of several factors. The position of the maximum and the shape of the CD signal depend on the number of the molecules involved in the interaction, that is on the degree of the aggregation. The arrangement of protochlorophyll molecules in the aggregates has also an important role in the formation of the CD signals. The water and solvent molecules may also have a role in the arrangement of the molecules of protochlorophyll in the aggregates [13, 14].

It can be suggested that the different forms of protochlorophyll found in our system, have different degree of aggregation and the arrangement of the pigment molecules in the aggregates may also be different.

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СПЕКТРАЛЬНЫЕ СВОЙСТВА ПРОТОХЛОРОФИЛЛА В МОДЕЛЬНОЙ СИСТЕМЕ

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Исследовались спектроскопические свойства протохлорофилла в твёрдых плёнках различными спектроскопическими методами. Твёрдые плёнки были получены на поверхности стекла испарением эфирного раствора протохлорофилла. При получении твёрдых плёнок максимум поглощения был распаложен при 635 или 640 нм. Форма «635» не имел сигнал кругового дихроизма, однако, форма «640» показала интенсивную отрицательную полосу КД при 640 нм а также одну маленькую положительную полосу при 668 нм. Формы «635» и «640» можно превратить в форму «650» если обрабатываем пробы парами ацетона. В этом случае главный максимум поглощения распаложен при 650—654 нм и всегда появилось плечо при 635 нм. Спектр поглощения этих плёнок не отличается от спектра этиолированных листьев в красной области. В спектре кругового дихроизма форма «650» даёт интенсивную положительную полосу при 654 нм и две отрицательных полосы низкой интенсивности при 632 и 644 нм.