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Magnetic Circular Dichroism and FTIR Studies of isolated Cyt b-559

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Introduction

Magnetic circular dichroism (MCD) is the differential absorbance of left and right circularly polarized light under external magnetic field. The MCD signal arises from the Faraday effect induced by magnetic field i.e. the combination of molecular energy splitting and different transitions. It includes three different terms (Briat et al., 1967; Holmquist et al., 1980). The MCD spectra were found to be sensitive to the electronic state of the heme, and the shape and intensity of the spectra could be correlated with the redox state, the spin state and the axial ligation of the central metal atom to proteins. In the previous papers, we used this potent spectroscopy method in investigation of PSII reaction center (Yang et al., 1997). Other researchers reported the MCD studies of series of myoglobin derivatives (Houssier et al., 1970; Frackowiak et al., 1987).

FTIR spectroscopy probes vibrational modes of the pigments, the polypeptide backbone and amino acid side chains without any selectivity and may thus be used to address questions on pigment and protein properties and interactions and so on (Berthomieu et al., 1992). To gain further insight into the heme-protein interactions occurring in Cyt *b*-559 and into the molecular mechanisms of photoprotection, we have studied the redox transitions of the isolated Cyt *b*-559 with FTIR spectroscopy and assigned the vibration modes in Cyt *b*-559.

Here we report investigation of MCD and FTIR spectra of purified Cyt *b*-559 (in oxidized and reduced) isolated from spinach and rice and compared with those of PSII reaction center. The results of FTIR and MCD measurements proved that His residues are ligands of heme in the Cyt *b*-559 and α-helixes are major kind of secondary structures in Cyt *b*-559.

Material and methods

Purification of Cyt *b*-559: Cytochrome b-559 was purified from spinach and rice as before (Xin et al, 2000). The cytochromes were oxidized with potassium ferricyanide and reduced anaerobically with hydroquinone or sodium dithionite. Concentrations of the native proteins were determined according to published extinction coefficients. PSII reaction center isolated by methods of Satoh with little modification (Yu et al, 1992). Crystalline cytochrome *c* was obtained from Sigma.

Magnetic Circular Dichroism: Magnetic circular dichroism (MCD) spectra were recorded with the Jasco J-500CS spectropolarimeter, using a permanent magnet accessory giving a magnetic field of 1.5 T. The propagation direction of the light beam was along the positive direction of the magnetic field. The optical path of

curette was 0.5 cm. The temperature was controlled by water cycle. The bandwidth of the apparatus was set at 0.6 nm in all experiments. The results, expressed in terms of molar absorptivity differences (ε_L-ε_R=ΔE M^{-1} cm^{-1}) are normalized to unit magnetic field. Natural circular dichroism (CD) was measured either with the same apparatus in the absence of magnetic field and was subsequently subtracted from the measured MCD. Results are expressed in molar ellipticity θ=3300 $\Delta\varepsilon$ deg· cm^2 ·$dmol^{-1}$. The signals were collected as averages of 10 summations to improve signal-noise ratio.

Measurement of FTIR spectra: The Fourier transform infrared (FTIR) spectra were measured by a BIO-RAD FT100 FTIR spectrometer. The samples at a concentration of A559=0.1 were dropped on CaF_2 windows, then dried in vacuum to be a layer of semihydrated film in order to eliminate the influence of water vapor. The optical path is 5mm. The signals were collected as averages of 20 summations to improve signal-noise ratio. Identification of band maxima frequencies was performed by inspection of smoothed FTIR absorbance spectra and secondary structure resolution-enhancement analysis using Gaussian curve fitting and second derivative spectra.

Results and Discussion

In the previous research the magnetic circular dichroism (MCD) spectrum of spinach PSII reaction center was reported and a part of primary assignments of chlorophyll components (Yang et al., 1996), but other chromophores not involved. A better understanding of the effects of all chromophores and protein structure on the MCD spectra is necessary if the results of MCD studies of PSII reaction center are to be interpreted (Briat et al., 1967).

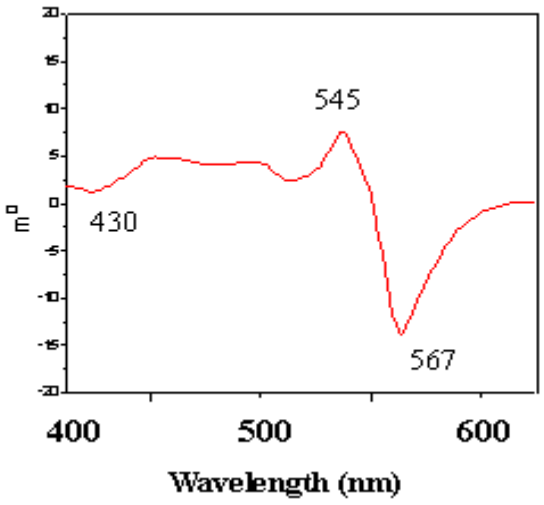

Fig. 1. MCD of Cyt *b*-559 isolated from spinach.

As shown in Fig. 1, in the visible spectral regions the Cyt *b*-559 have MCD signals in two regions: in 540-580 nm α region with a positive peak at 545 nm and a negative peak around 567 nm; and in 400-440 nm region with a small negative 430 nm. The MCD spectrum of the PSII reaction center isolated from rice in the visible region at room temperature is similar to that of our previously reported. The reduced purified Cyt *b*-559 exhibit visible MCD spectra very similar in shape and intensity to those of

PSII reaction center in these regions (Fig. 2). Thus these visible regions can serve as the major contributors for MCD of PSII reaction center in these regions, while the intensity of Cyt *b*-559 in PSII reaction center is smaller than in purified preparations.

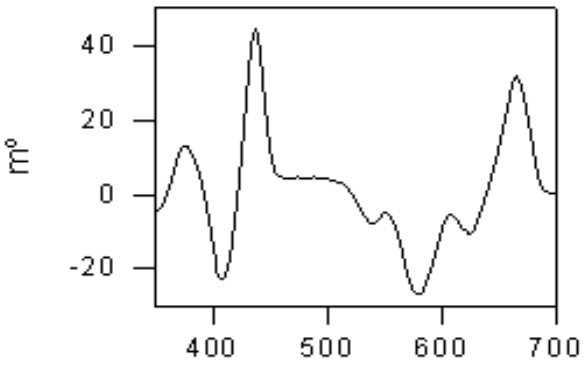

Fig. 2. MCD of PSI

In addition, we observed that the reduced Cyt *b*-559 exhibited visible MCD spectrum similar in shape to cytochrome *c* (date not shown). However, MCD spectra are very different for reduced cytochrome *c* and *b*-559 in the ultraviolet region, this spectral region therefore may be sensitive to the mechanism of heme binding by the polypeptide. From comparison MCD of Cyt *b*-559 and that of cytochrome *c*, it was suggested that the MCD pattern act as a marker for the presence of reduced low-spin heme as the His residue ligand (Cramer et al., 1986). However it is not possible at this stage to give a more precise description of their spatial relationships.

FTIR spectroscopy is a useful tool for elucidating the secondary structure of proteins and is particularly good at monitoring small conformational change occurring within proteins in an aqueous system. However, there are few reports about the application of FTIR method in studying the Cyt *b*-559 (Berthomieu et al., 1992; Garlaschi et al., 1994). We attempt to use the purified Cyt *b*-559 from rice and spinach to determine the vibration mode of Cyt *b*-559 under reduced state. As shown in Fig. 3., it is observed that (a) the modes at 1556, 1535, 1406, 1337 and 1239 cm^{-1} were assigned to the heme vibration by comparing with the published FTIR spectra of model compounds (Palmer, 1985), (b) the mode at 1104 cm^{-1} was assigned to histidine ligands, and (c) the signal between 1600 and 1700 cm^{-1} was belonged to amide I region of Cyt *b*-559 protein crevice, which is associated with the in-plane C=O stretching vibration. Our results are consistent with the proposal that the heme of Cyt *b*-559 was connected with two histidine residue ligands (Babcock et al, 1985). On the basis of the decomposition with Gaussian analysis on the FTIR spectrum of isolated Cyt *b*-559, it was deduced that the peaks in amide I band of FTIR spectrum reflect the secondary structure distribution: the peak at 1657 cm^{-1} is derived from α-helixes, the shoulder peak at 1651 cm^{-1} arises from the stretching transition of ruleless curl, the band between 1620 and 1640 cm^{-1} are assigned to β-sheet, additionally, the presence of absorbance bands over 1660 cm^{-1} are due to β-turn. From calculating, it was found that the percentage of α-helixes in secondary structure of Cyt *b*-559 is 55%, β-sheet 35% ruleless curl 10%.

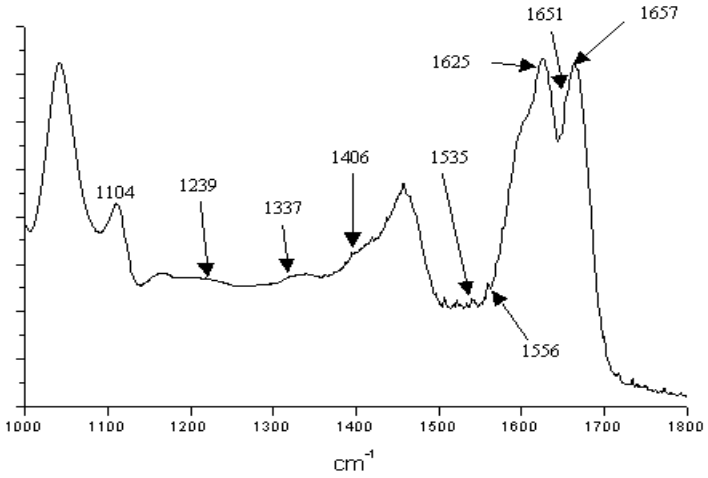

Fig. 3. FTIR spectra of Cyt *b*-559 isolated from rice.

The above properties of Cyt *b*-559 purified from different higher plants are very similar, which suggested that Cyt *b*-559 showed high consistency and genetic conservation in evolution. These research provided the basic structural characteristics for investigation of functions of Cyt *b*-559 and understanding the essential of *b*-type cytochrome in all kinds of organisms.

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