PHOTOSYNTHETICA 34 (1): 141-145, 1997

BRIEF COMMUNICATION

DCMU causes conformational changes of a synthetic fragment of D1 protein: A Fourier transform infrared study

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Abstract

A peptide ranging from residues 229 to 240 (ENESANEGYRFG) of D1 protein was synthesized by stepwise solid-phase method. Resolution enhancement techniques were combined with band curve-fitting procedures to quantitate the FTIR spectra in the amide I' region (1700-1600 cm^{-1}). FTIR analysis showed that DCMU induced drastic structural modification with a relative decrease of the unordered structure and turns, and a substantial increase of α-helix, which indicated that a much more compact structure was formed when DCMU was applied. The results may reflect molecular information for the protective effect of DCMU against photoinhibition.

Additional key words: 3-(3,4-dichlorophenyl)-1,1-dimethylurea; peptide.

The D1 and D2 proteins constitute the photosystem 2 (PS2) reaction centre. Each of these proteins possesses five transmembrane helices with the N- and C-terminal portions at the outer and inner sides of the thylakoid membranes, respectively. The exposed residues incorporate the parallel helix (de) and two connecting loops (D-de and E-de) (Michel and Deisenhofer 1988). The D-E region is involved in PS2 reducing side activity, with various residues interacting with Q_B, herbicides and bicarbonate (Ohad and Hirschberg 1992, Govinjee and van Rensen 1993). A major difference in the amino acid sequence between L-subunit of purple bacteria and D1 protein of higher plants in the area of Q_B site is the lengthy sequence of about 14

Received 3 January 1996, *accepted* 24 May 1996.
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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FSD, Fourier self-deconvolution; FTIR, Fourier transform infrared; HPLC, high performance liquid chromatography; PNO8, N-octyl-3-nitro-2,4,6-trihydroxybenzamide; PS, photosystem.
Acknowledgement: We are grateful to Prof. Hu, X.-L. for peptide synthesis.

additional amino acids from Asn230 to Glu244 in the D1 protein with the extension concentrated mainly in the D-de loop. Current hypotheses imply that this sequence folds atop the Q_B and DCMU binding sites, where Q_B and DCMU function competitively in PS2 (Trebst *et al.* 1988, Kless *et al.* 1994).

The occupation of the Q_B site by DCMU, a PS2 inhibitor, alters the extent of D1 protein degradation during photoinhibition (Giardi *et al.* 1992, Jansen *et al.* 1993, Komenda *et al.* 1993, Kirilovsky *et al.* 1994). These observations seem to be consistent with the proposal that a conformational change around the Q_B site brought about by photoinhibitory irradiation makes D1 protein susceptible to a putative proteinase (Barber and Andersson 1992, Prášil *et al.* 1992, Aro *et al.* 1993). The conformational changes of the Q_B site by the binding of some PS2 inhibitors impair the accessibility of trypsin to the D1 protein at Arg238 located in a vicinity of the Q_B site (Trebst *et al.* 1988). Recently, Nakajima *et al.* (1995) discovered that PNO8, a phenol-type PS2 inhibitor, triggered a selective and specific cleavage of the D1 protein by binding to site without any photoinhibitory treatment, which provided direct evidence that the conformational changes around the Q_B site were required for the putative proteinase.

In this paper, we have synthesized the D-de fragment of D1 protein (ranging from Glu229 to Gly240 as deduced from the spinach *psbA* gene), trying to study the secondary structural alterations induced by DCMU so as to provide insight on the protective effect of DCMU against D1 protein degradation.

The peptide ENESANEGYRFG was synthesized by stepwise solid-phase method and further purified as described in Wang *et al.* (1995). The purity of the synthesized peptide based on analytical HPLC and amino acid analysis was greater than 98 %. Peptide solutions (1-2 mM) were prepared in unbuffered D_2O and the pH value was adjusted to pH 6.5 (uncorrected pH measured in D_2O) with DCl and NaOD. Complete H-D exchange was achieved after incubation of the peptide solution for 12 h at 4 °C. FTIR spectra were recorded on a *Nicolet 170 SX* spectrometer with CaF_2 windows. For each sample 400 scans were collected and Fourier-transformed to produce a spectrum with a nominal resolution of 4 cm^{-1}. The second-derivatives, self-deconvolution and curve-fitting were performed as described in Zhang *et al.* (1995).

The amide I' region of FTIR spectra for the control and DCMU treated synthetic peptides after solvent subtraction is shown in Fig. 1. Fig. 2 shows the FSD deconvoluted amide I' spectra and the second derivatives of the spectra. The agreement of peak positions from these two techniques was good. Deconvolution of the amide I' envelope was performed using the peak position revealed in the second derivative spectra. The peak positions are given along with the secondary structure assignments based on FTIR data of proteins and peptides (Susi and Byler 1986, Holly *et al.* 1993). The band at 1613 cm^{-1} is due to aromatic side frequency and has not been included in the secondary structure calculations. However, it was necessary to include it in curve-fitting in order to avoid distortions of the amide I' bands. The contributions of the different types of the secondary structure for the control and DCMU treated peptide samples were determined from the relative areas of the individual band contributions and expressed in %: they were for the control 8 β-

sheet, 40 turns, and 52 unordered, and for the DCMU treated sample 51 α-helix, 7 β-sheet, 21 turns, and 21 unordered.

Addition of DCMU at a [DCMU]/[peptide] ratio of 0.5 caused dramatic spectral changes suggesting a shift of the conformational equilibrium (Fig. 1). Comparison of the secondary structure contents by FTIR indicated that DCMU titration resulted in a relative loss of unordered structure and turns, and a substantial increase of α-helix, concomitantly. The results indicated that DCMU could bind to the synthetic D1 fragment, and a corresponding conformational alteration would be induced simultaneously. Though this is monitored in synthetic peptide, it may be assumed that the similar event would occur *in vivo* if the fluidity of the thylakoid membranes and the flexibility of D1 protein were taken into consideration. However, other conformation of the synthetic peptide in D1 protein can not be excluded. The fact that DCMU may actually bind to the D1 fragment and cause dramatic conformational changes may provide molecular basis for the protective effects of DCMU against photoinhibition (Giardi *et al.* 1992, Jansen *et al.* 1993, Komenda *et al.* 1993, Kirilovsky *et al.* 1994).

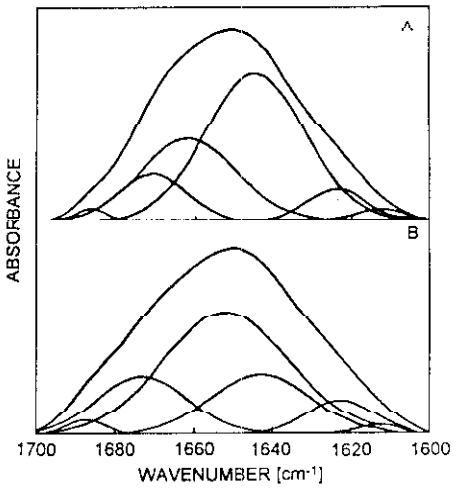

Fig. 1. Comparison of the amide I' spectra of the control and DCMU treated synthetic D1 fragment. The curve-fit amide I' spectra and the contribution of the different secondary structures for the control (*A*) and DCMU titrated (*B*) are shown. Band positions [cm^{-1}] and secondary structure assignments for the amide I' band of the synthetic peptide are shown below. The band positions were determined by second-derivative and fast Fourier self-deconvolution (see text).

	α	β	turns	unordered
control (*A*)		1624	1686, 1671, 1662	1645
DCMU-treated (*B*)	1652	1623	1687, 1673	1643

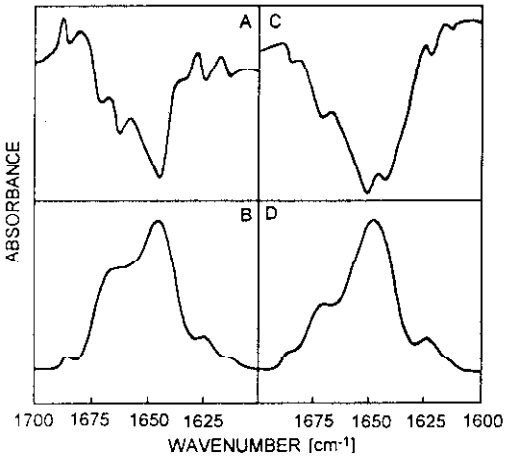

Fig. 2. Resolution-enhanced FTIR spectra of the second-derivative (*A* and *C*) and the FSD (*B* and *D*) deconvoluted spectra for the native (*A* and *B*) and DCMU titrated (*C* and *D*) synthetic D1 fragment.

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