Volume 239, number 2, 207–210 FEB 06446 November 1988

Mapping of two tyrosine residues involved in the quinone- (Q_B) binding site of the D-1 reaction center polypeptide of photosystem II

Ralf Dostatni, Helmut E. Meyer+ and Walter Oettmeier

Lehrstuhl Biochemie der Pflanzen and + Institut für Physiologische Chemie, Ruhr-Universität, Postfach 10 21 48, D-4630 Bochum 1, FRG

Received 19 August 1988

The property of the D-1 subunit of photosystem II in binding herbicides in its quinone-binding niche has provided important approaches to study its structure and function. In the D-1 protein, amino acid residues Tyr-254 and Tyr-237 are labeled by an [^{14}C]azido-urea derivative, as identified by protein sequencing of proteolytic fragments. Whereas Tyr-254 is in a parallel α-helix already indicated to contribute to the herbicide-binding site, Tyr-237 is in a hydrophilic sequence that is partly accessible from the matrix space of the chloroplasts. This area has been implicated to contain a cleavage site for a protease involved in the rapid turnover of the D-1 polypeptide. The photoaffinity labeling results show that some of the amino acids in this cleavage site are actually part of the quinone-binding niche. It allows a refined and extended prediction of the three-dimensional folding of the reaction center of photosystem II.

Photoaffinity label; Herbicide binding; Amino acid sequence; Photosystem II; Protein D-1

1. INTRODUCTION

According to recent evidence two diffuse-staining proteins of apparent molecular masses of 30 to 32 kDa, called D-1 and D-2, constitute the photosystem II reaction center core complex [1,2] (for a review, see [3]). This notion is implemented from the homology of the D-1 and D-2 proteins with the L- and M-subunits, respectively, of the reaction center from photosynthetic bacteria and the structure of the bacterial reaction center as obtained from X-ray data [4]. Both the D-1 and D-2 protein are integral membrane proteins and traverse the thylakoid membrane in five helical spans [1,2]. D-1 and D-2 as an entity carry the pigments necessary for the primary photoconversion and, in addition, the primary and secondary quinone acceptors Q_A and Q_B. Q_A and Q_B as well are specialized plastoquinone molecules.

Correspondence address: W. Oettmeier, Lehrstuhl Biochemie der Pflanzen, Postfach 10 21 48, D-4630 Bochum 1, FRG

The photosystem II reaction center core complex is the primary target for many commercial herbicides. By photoaffinity labeling experiments with radioactive azido-derivatives of common herbicides like triazines [5], triazinones [6] and ureas [7] it could be demonstrated that the herbicides bind to the D-1 protein. They compete with plastoquinone for binding at the Q_B-site and, consequently, photosynthetic electron flow is interrupted.

The D-1 protein is unique amongst the proteins of the thylakoid membrane inasmuch as it is rapidly synthesized and degraded [8]. It is coded by the *psbA* gene in the chloroplast DNA which has been sequenced in spinach [9] and several other higher plants and algae. Mutations in the *psbA* gene and subsequent subtle changes in the amino acid sequence of the D-1 protein (like the replacement of Ser-264 by Gly [10]) lead to weed species which are resistant to triazine herbicides. Several other single amino acid replacements conferring herbicide resistance are known (for reviews, see [2,11]).

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

So far, the D-1 protein has not been sequenced chemically and little is known about the environment of the herbicide-binding site, i.e. the amino acids involved. By comparison of proteolytic and cyanogen bromide digests of D-1 protein labeled with [^{14}C]azido-triazine, Wolber et al. [12] have postulated herbicide binding at Met-214 and a yet unidentified amino acid between His-215 and Arg-225. The situation for D-1 is in contrast to the case in photosynthetic bacteria where inhibitor and protein residues forming the binding pocket are exactly known [13]. Therefore, we have sequenced proteolytic fragments of a D-1 protein labeled by an azido-urea photoaffinity label. This label has been chosen because of the three photoaffinity labels mentioned above it gives the highest yield of radioactivity incorporation [7].

2. MATERIALS AND METHODS

Thylakoids from spinach were prepared according to [14]. They were UV-illuminated for 30 min in the presence of 5 nmol/mg chlorophyll [^{14}C]azido-monuron (spec. act. 57 mCi/mmol) [7]. Samples were subjected to preparative SDS polyacrylamide (10–15%) gel electrophoresis. The D-1 protein was cut out, electroeluted and digested with either trypsin or V8-protease [12]. Proteolytic fragments were visualized by gel electrophoresis and scanning for radioactivity [15]. They were isolated from the gels and their sequence determined by automated gas-phase Edman degradation with on-line identification of the phenylthiohydantoin amino acids [16]. The phenylthiohydantoin amino acids were also analyzed for radioactivity.

3. RESULTS AND DISCUSSION

Upon trypsin digestion of the photosystem II D-1 protein labeled by [^{14}C]azido-monuron and electroeluted from preparative gels three radioactive fragments of apparent molecular masses of 24, 15 and 11 kDa have been obtained. The 24 kDa peptide could not be sequenced because the N-terminus was blocked. The sequence of this proteolytic fragment obviously starts at the amino-terminus of the D-1 protein. According to a most recent report Met-1 of D-1 is split off and the Thr-2 is *O*-phosphorylated and *N*-acetylated during processing [17]. The 15 kDa fragment could be sequenced until the 15th cycle (fig.1). The trypsin cut occurred at Arg-225 (see also fig.3) and this fragment includes the carboxyl-terminus according to its apparent molecular mass. Radioactivity was

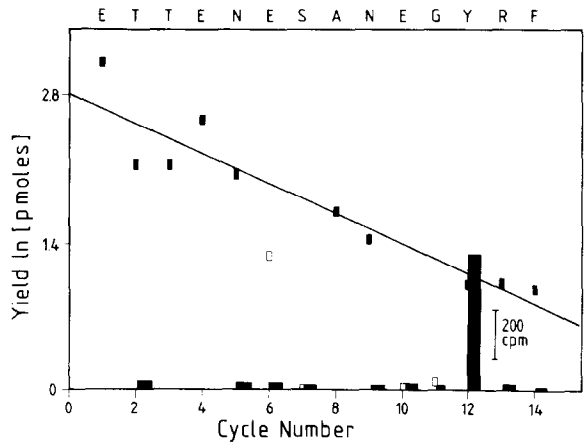

Fig.1. Sequence analysis of a [^{14}C]azido-monuron labeled tryptic peptide. The ln of the yield of the phenylthiohydantoin derivatives in each cycle is plotted against the cycle number. Repetitive yields (86.4%) and initial yield (18 pmol) were calculated from the data points shown in filled squares. The bars indicate the radioactivity count in each cycle.

found in the 12th cycle, which corresponds to Tyr-237 (fig.1). It should be noted that the polybrene support in the sequencing machine after the 15th cycle still contained an appreciable amount of radioactivity. This indicates that another amino acid downstream in the sequence is tagged. The 10 kDa protein fragment proved to be too impure to obtain a readable sequence.

In another series of experiments the labeled D-1 protein was extensively digested with V8 *Staphylococcus aureus* protease. One major radioactively labeled 10 kDa fragment was isolated and sequenced (fig.2). The protease cut took place at Glu-244 and the sequence commenced at Thr-245. It could be identified for 23 cycles. Radioactivity was found in the 10th cycle, which again corresponds to a tyrosine, this time Tyr-254. No noticeable radioactivity was found on the polybrene support, indicating no further labeled amino acid.

Fig.3 depicts the region from transmembrane helix IV to transmembrane helix V (including a parallel α-helix lying on top of the cytoplasmatic side of the membrane) of the D-1 protein which is thought to be involved in Q_B and herbicide binding [1,2]. Several mutations within the sequence of the D-1 protein have been reported to lead to herbicide resistance [2,10,11]. The region where these muta-

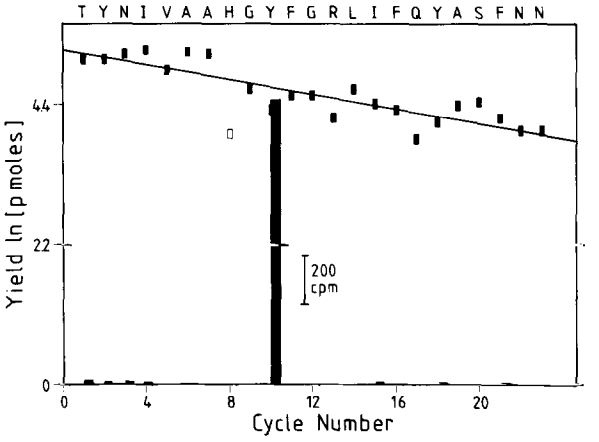

Fig.2. Sequence analysis of a [^{14}C]azido-monuron labeled V8-protease proteolytic peptide. Like in fig.1 the repetitive yield (94.4%) and the initial yield (204 pmol) were calculated from the amounts of all phenylthiohydantoin amino acids shown as filled squares. The bars indicate the radioactivity count in each cycle.

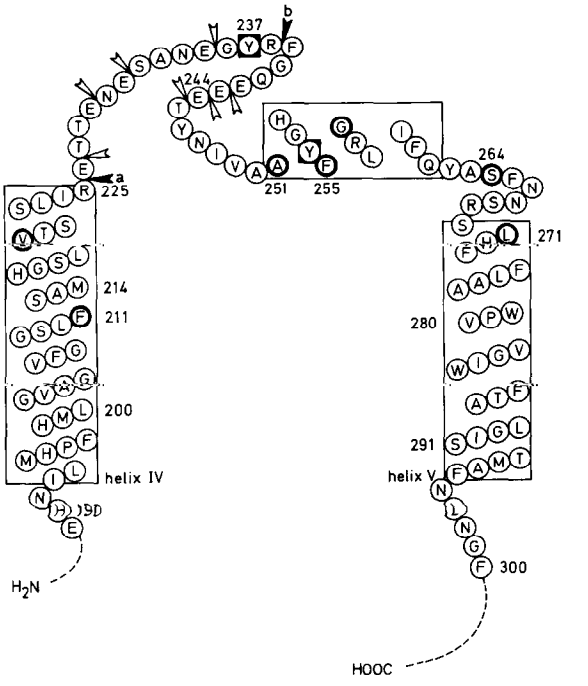

Fig.3. Topography of the herbicide and Q_B-binding site of the D-1 protein of the photosystem II reaction center core complex. The two vertical boxes indicate the two membrane spanning helices IV and V, the horizontal box indicates a helical part lying on top of the cytoplasmatic part of the membrane. Amino acids highlighted by thick circles represent places where mutations had been found which confer herbicide resistance. The two tyrosine residues where [^{14}C]azido-monuron binds, are emphasized by rectangular boxes. Possible cutting sites by V8-protease or trypsin are indicated by arrows and filled arrows, respectively.

tions occur and which consequently is responsible for herbicide binding starts at Phe-211 and ends at Leu-271. An exchange of any amino acid indicated by a thick circle leads to herbicide resistance. Tyrosine residues 237 and 254 which are tagged by azido-monuron fit well into this area. Tyr-254 is located in the parallel α-helix, whereas Tyr-237 is situated in a hydrophilic sequence which is partly accessible from the matrix side of the thylakoid membrane. Tyr-254 is conserved in the L-subunits of *Rhodobacter capsulatus* and *Rhodobacter viridis* [2] but not in *Rhodobacter sphaeroides* [18] and *Chloroflexus aurantiacus* [19]. Its neighbour Phe-255, however, is conserved in all 4 L-subunits sequenced so far [2,18,19]. Phe-255 in D-1 corresponds to Phe-216 in the bacterial L-subunits [2,18,19]. Tyr-254 is close to the mutations Ala-251 [20], Phe-255 [11], and Gly-256 [11]. As concluded from the X-ray data, Phe-216 in the bacterial L-subunit is involved in Q_B binding [21] and binding of the triazine herbicide terbutryn as well [13]. Labeling of Tyr-254 in D-1 indicates that Q_B and herbicide binding in D-1 take place in a similar environment as in the L-subunit.

As already stressed, the D-1 protein is rapidly degraded in vitro. In *Spirodela oligorrhiza* a 23.5 kDa protein was identified as the primary breakdown product [22]. By fingerprint mapping of papain digests, it was concluded that the 23.5 kDa breakdown product is the amino-terminal portion of the D-1 protein. Hence, the cleavage site should be located between membrane spans IV and V at either Arg-255 (a) or Arg-238 (b). The sequence between these arginines is rich in serine, glutamate and threonine and might be considered as a PEST-sequence [22]. Such sequences have been proposed as the primary determinants for rapid degradation of proteins [23]. Furthermore, Trebst et al. [24] have recently reported that the D-1 protein is stable to attack by trypsin in the presence of diuron. Therefore, the native cleavage site of the D-1 protein is Arg-238 for the following reasons. Tyr-237, the direct neighbour of Arg-238, is labeled by azido-monuron and consequently involved in the binding of urea type herbicides. By

binding of the herbicide the environment of Arg-238 is modified in a way that is no longer susceptible to protease digestion. We note that even with isolated radiolabeled D-1 protein no digestion occurs at Arg-238 due to the covalent modification of the neighbouring Tyr-237.

In herbicide-resistant weeds resistance is normally constrained to one certain class of herbicides, like the triazines. Herbicides of other classes, though of identical mechanism of action, like ureas, triazinones or uracils are unimpaired in their activity [25]. Consequently, other amino acids in the binding niche of the D-1 protein must be involved in, for instance, triazine binding as compared to urea binding. It will be additional and important information on the mechanism of herbicide action to sequence D-1 proteins tagged by other photolabile derivatives of herbicides.

Acknowledgements: We thank Dr A. Boschetti, University of Bern, Switzerland for the generous gift of [^{14}C]azido-monuron, Ms K. Angel, Mr J. Höhfeld and Mr H. Korte for experimental help, and the Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

[1] Trebst, A. (1987) Z. Naturforsch. 42c, 742–750.
[2] Michel, H. and Deisenhofer (1988) J. Biochem. 27, 1–7.
[3] Barber, J. (1987) Trends Biochem. Sci. 12, 321–326.
[4] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
[5] Pfister, K., Steinback, K.E., Gardner, G. and Arntzen, C.J. (1981) Proc. Natl. Acad. Sci. USA 78, 981–985.
[6] Oettmeier, W., Masson, K., Soll, H.J. and Draber, W. (1984) Biochim. Biophys. Acta 767, 590–595.
[7] Boschetti, A., Tellenbach, M. and Gerber, A. (1985) Biochim. Biophys. Acta 810, 12–19.
[8] Mattoo, A.K., Pick, U., Hoffman-Falk, H. and Edelman, M. (1981) Proc. Natl. Acad. Sci. USA 78, 1572–1576.
[9] Zurawski, G., Bohnert, H.J., Whitfeld, P.R. and Bottomley, W. (1982) Proc. Natl. Acad. Sci. USA 79, 7699–7703.
[10] Hirschberg, J. and McIntosh, L. (1983) Science 222, 1346–1349.
[11] Rochaix, J.D. and Erickson, J. (1988) Trends Biochem. Sci. 13, 56–59.
[12] Wolber, P.K., Eilmann, M. and Steinback, K.E. (1986) Arch. Biochem. Biophys. 248, 224–233.
[13] Michel, H., Epp, O. and Deisenhofer, J. (1986) EMBO J. 5, 2445–2451.
[14] Nelson, N., Drechsler, Z. and Neumann, J. (1970) J. Biol. Chem. 245, 143–151.
[15] Oettmeier, W., Masson, K. and Johanningmeier, U. (1980) FEBS Lett. 118, 267–270.
[16] Hewick, R.M., Hunkapiller, M.W., Hood, L.E. and Dreyer, W.J. (1981) J. Biol. Chem. 256, 7990–7997.
[17] Michel, H., Hunt, D.F., Shabanowitz, J. and Bennett, J.J. (1988) J. Biol. Chem. 263, 1123–1130.
[18] Williams, J.C., Steiner, L.A., Feher, G. and Simon, M.I. (1984) Proc. Natl. Acad. Sci. USA 81, 7303–7307.
[19] Ovchinnikov, Y.A., Abdulaev, N.G., Zolotarev, A.S., Shmukler, B.E., Zargarov, A.A., Kutuzov, M.A., Telezhinskaya, I.N. and Levina, N.B. (1988) FEBS Lett. 231, 237–242.
[20] Johanningmeier, U., Bodner, U. and Wildner, G.F. (1987) FEBS Lett. 211, 221–224.
[21] Allen, J.P., Feher, G., Yeates, T.O., Rees, D.C., Deisenhofer, J., Michel, H. and Huber, R. (1986) Proc. Natl. Acad. Sci. USA 83, 8589–8593.
[22] Greenberg, B.M., Gaba, V., Mattoo, A.K. and Edelman, M. (1987) EMBO J. 6, 2865–2869.
[23] Rogers, S., Wells, R. and Rechsteiner, M. (1986) Science 234, 364–368.
[24] Trebst, A., Depka, B., Kraft, B. and Johanningmeier, U. (1988) Photosyn. Res. 15, in press.
[25] Oettmeier, W., Masson, K., Fedtke, C., Konze, J. and Schmidt, R.R. (1982) Pestic. Biochem. Physiol. 18, 357–367.