The Topology of the 32 kDa Herbicide Binding Protein of Photosystem II in the Thylakoid Membrane

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32 kDa Herbicide Binding Protein, Hydrophobic Moments

The 32 kDa herbicide binding protein is a membrane bound protein which is implicated in the binding of many photosystem II herbicides as well as in the binding of the endogenous quinone QB which serves as the secondary electron acceptor on the reducing side of photosystem II. The topology of the 32 kDa protein has been predicted using a combination of hydrophobic moment analysis, membrane propensity analysis and empirical secondary structure predictions. Our model consists of five transmembrane helices. The loop connecting the fourth and fifth transmembrane helices is thought to form part of the herbicide binding site. Our analysis suggests that this loop also contains a helical segment which may seek the surface of the membrane by virtue of its relatively high hydrophobic moment. Our topology is compared with several others which have been proposed in the literature as well as with the topology of the L and M proteins of the bacterial reaction center of *R. viridis*. The significance of mutagenesis and photo-affinity labeling experiments is also discussed in terms of our model.

Introduction

The reaction center of photosystem II (PS II) is a multicomponent complex of several proteins. Light absorbed by accessory chlorophyll is transduced into an electrochemical potential when the primary electron acceptor of the reaction center is photo-reduced. The primary electron acceptor, labeled QA, then transfers a pair of electrons, one at a time, to a secondary acceptor QB [1]. The prosthetic groups, QA and QB, are both quinones. QA is more strongly bound than QB, and the latter quinone is displaced by herbicides such as diuron and atrazine [2]. Photoaffinity labeling experiments indicate that azido-atrazine, and by implication QB and other photosystem II herbicides, bind to a 32 kDa protein sometimes referred to as the herbicide or QB binding protein [3]. The primary sequence of the 32 kDa protein has recently been inferred from the gene sequence [4], and it is the structure of this protein that is the subject of this paper.

Our goal in this work has been the prediction of the topology of the protein with respect to its membrane environment. In particular, which regions of the protein are likely to be membrane bound and which are likely to be outside the membrane? For those regions which are outside the membrane, what is their secondary structure? We are especially interested in the structure of the protein in that region implicated as the binding site of both the endogenous quinone QB and herbicides. Comparison will be made to several other models which have recently appeared in the literature [5, 6].

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Methods

In order to predict which regions of the 32 kDa protein are likely to be membrane spanning, a hydrophobic moment analysis was performed on the amino acid sequence [7]. The hydrophobic moment analysis employed here calculates hydrophobic moments using a period of 3.7 residues. A region with a high hydrophobic moment by this criterion will, when twisted into an alpha helix, have most hydrophobic residues on one side of the helix and most hydrophilic residues on the opposite side. The hydrophobic properties of a protein can be represented as a collection of points on a hydrophobic moment plot, on which the vertical axis is the hydrophobic moment per residue and the horizontal axis is the hydrophobicity per residue. Hydrophobic moment plots like that for the 32 kDa protein in Fig. 1, are obtained by moving an 11 amino acid window through a protein, calculating the average hydrophobicity and hydrophobic moment for each such window, assigning those values to the central residue in the window, and plotting the values for each such window.

Intuitively, one expects regions of a protein with high hydrophobicity and low hydrophobic moment to be candidates for transmembrane helices. Regions with unusually high hydrophobic moments are expected to be surface seeking. These intuitive ideas about how hydrophobicity and hydrophobic moment can determine the interaction of a protein with a membrane find support in a systematic study of the topologies of known transmembrane and surface seeking proteins. On the basis of such a study, Eisenberg divides hydrophobicity-hydrophobic moment space into four domains as depicted in Fig. 1. Analyzing a set proteins with known topologies it was determined that membrane spanning helices such as those in the seven helical bundle of bacteriorhodopsin plot in the triangle labeled multimeric. Points corresponding to residues in monomeric membrane anchors were found to fall into the wedge labeled monomeric. Surface seeking proteins, generally have a large number of points falling above the long negatively sloping diagonal. Typical globular proteins have most of their points falling in the region labeled globular.

At the bottom of Fig. 1 we indicate as a function of residue number the regional placement of each window in the scatter plot above by placing a hash mark in one of four lanes labeled monomeric, multimeric, surface of globular.

We have also performed a membrane propensity analysis [8] on the 32 kDa protein. Membrane propensity analysis is a statistical technique based upon the known frequency of occurrence of amino acids in a number of membrane bound proteins. In this analysis we have used a window size of seven.

In addition to the hydrophobic moment and membrane propensity analyses, secondary structure predictions were performed on the 32 kDa protein. The Garnier algorithm was used for this purpose [9]. Since the Garnier algorithm has been parameterized on the basis of the known structures of globular proteins, the predictions are expected to be relevant only to those regions of the protein which are thought to reside outside the membrane.

Results

The hydrophobic moment analysis presented in Fig. 1 clearly indicates the presence of five and perhaps six transmembrane helices. Taking into account that each helix is expected to extend five amino acids to either side of the cluster of hash marks which indicate its presence, the positions of the six helices and their lengths were determined and are presented in Table I. There is a very weak indication of a transmembrane helix in the region 175–189. However, its length of 15 amino acids is short by about five amino acids of the number of residues required to span a membrane. Hence, we dismiss it from further consideration. The putative transmembrane helix labeled "?" is also a little short and will be dismissed based upon additional evidence presented below. In addition to predicting five or six

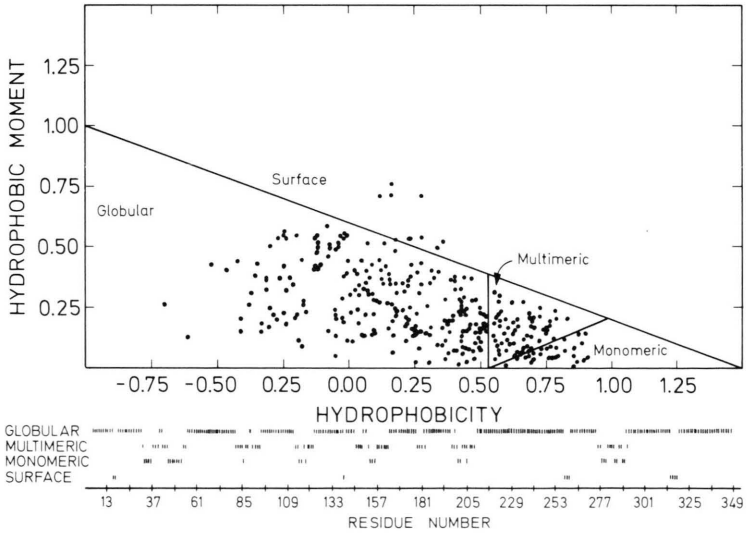

Fig. I. Hydrophobic moment plot for the 32 kDa protein. See text for an explanation of this plot.

Table I. Features of 32 kDa protein predicted on basis of hydrophobic moment analysis.

Residues	Length	Type and Index
13– 24	12	surface seeking helix #1
27– 60	34	transmembrane helix #1
77–100	24	transmembrane helix #2
109–129	20	transmembrane helix (?)
136–146	11	surface seeking helix #2
141–169	29	transmembrane helix #3
193–216	24	transmembrane helix #4
254–266	13	surface seeking helix #3
271–298	28	transmembrane helix #5
311–324	14	surface seeking helix #4

transmembrane helices, the hydrophobic moment analysis indicates four regions which may be membrane surface seeking. Surface seeking regions have high hydrophobic moments when present in alpha-helical conformations and hence may be capable of lying on the surface of a membrane, hydrophilic side out, hydrophobic side in.

The membrane propensity analysis also exhibits six distinct peaks (Fig. 2) located at similar positions in the sequence. However, the width of the third peak is shy of the recommended 19 amino acids required for acceptance as a transmembrane segment [9].

Thus, our model for the topology of the 32 kDa protein based on the hydrophobic moment analysis and supported by the membrane propensity analysis is a five-helix bundle. This topology is depicted in Fig. 3.

Secondary structure predictions for extra-membrane portions of the 32 kDa protein are listed in Table II. Those extra-membrane regions predicted to be helical by the Garnier algorithm are so indicated in Fig. 3.

The above predictions were used in constructing the computergraphic model shown in Fig. 4. In this model, program PSSHOW and its enhancements were used to fold up amino acids 193–216 and 271–298 into two separate helices corresponding to H4 and H5. These helices were then oriented parallel to each other and brought into van der Waals contact. An imaginary membrane forms two parallel planes that are oriented perpendicular to the helices. The linking segment comprising amino acids 217–270 was folded so that amino acids 254–266

Table II. Extra-membrane alpha-helical regions predicted by the Garnier algorithm.

Residues	Length	Index
1– 10	10	1
130–136	7	2
186–190	5	3
246–252	7	4
328–334	7	5
341–347	8	6

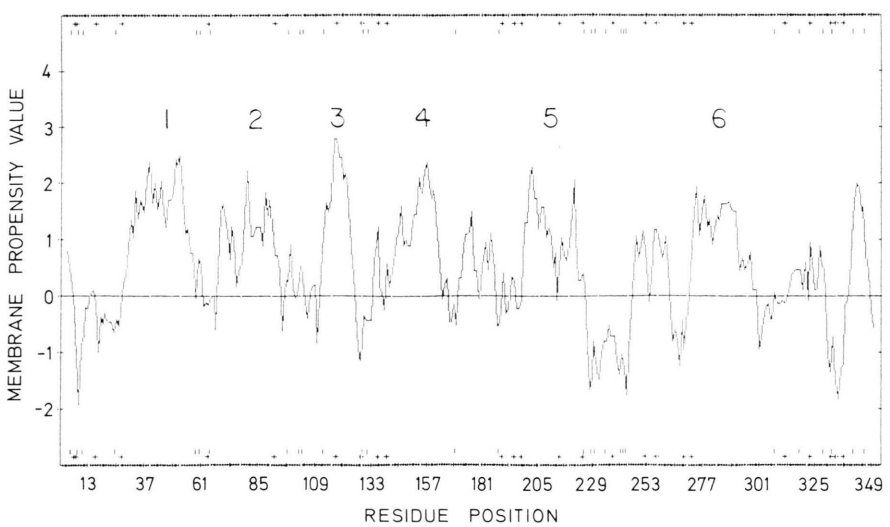

Fig. 2. Membrane propensity plot for the 32 kDa protein.

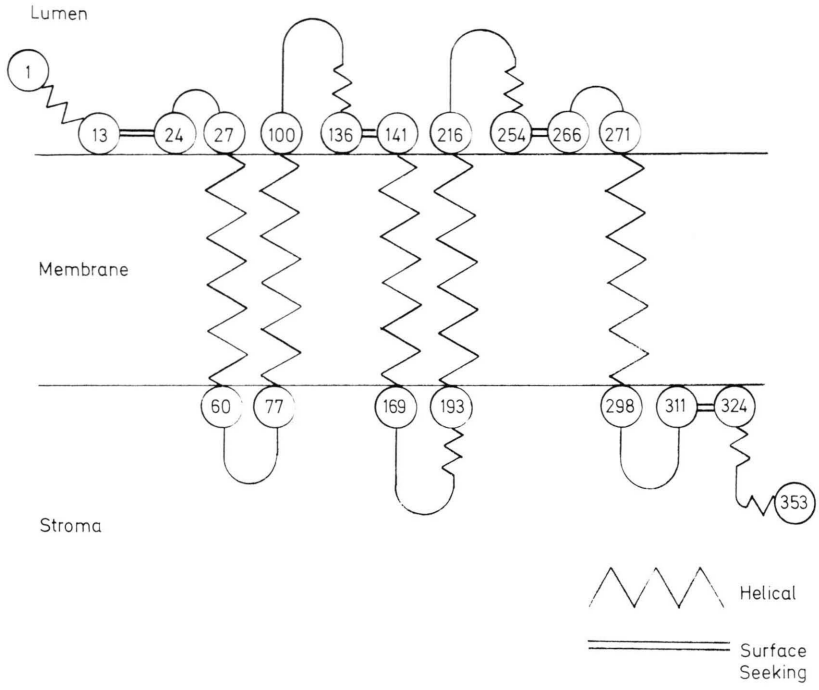

Fig. 3. Predicted topology of 32 kDa protein. Sequence numbers of residues at the termini of key structural features are indicated in circles.

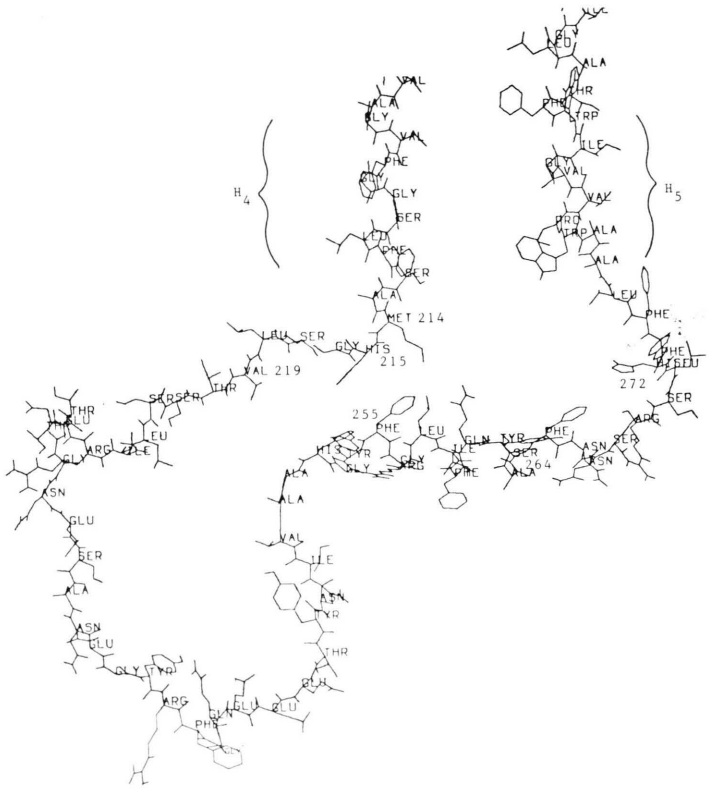

Fig. 4. Detail of loop joining the fourth to the fifth transmembrane helix.

form a helix oriented parallel to the imaginary membrane surface as required by the surface seeking nature of this segment predicted by the hydrophobic moment analysis. Moreover, residues 217–270 were further manipulated graphically so that residues 217 and 270 come in contact with residues 216 and 269 respectively while maintaining residues 254–266 in a helical conformation parallel to the membrane surface. This model constituted the graphical embodiment of the above predictions.

Discussion

Perhaps, the most relevant piece of experimental data with which our theoretical model should be compared is the recently reported five-helix model for the L and M proteins of the bacterial reaction center of *R. viridis* [10]. There are a number of remarkable similarities between the crystal structure of the bacterial protein and the predicted structure of the higher plant protein. For example, both structures place a pair of histidines in the critical region near the putative herbicide binding site. In our theoretical model for the 32 kDa protein of higher plants these are histidines 215 and 272. In the bacterial reaction center these histidines are located at positions 190 and 230. The latter histidines are ligands of a non-heme iron in the bacterial reaction center. An iron has also been proposed to reside at the herbicide binding site of the plant reaction center [11, 12].

It is also interesting to observe that the L and M subunits of the bacterial reaction center possess a number of extra-membrane helical regions which are roughly parallel to the putative membrane surface. These may correspond to the surface seeking regions determined by the hydrophobic moment analysis of the 32 kDa protein. Of particular importance is the surface seeking region predicted to occur in the loop between the H4 and H5. This surface seeking segment (254–266) is preceeded by a region (246–252) which is predicted to be alpha-helical by the Garnier algorithm. The entire segment (246–266) may correspond to the loop observed between the D and E transmembrane helices of the L subunit of the bacterial reaction center. This loop forms part of the herbicide binding site.

That this extra-membrane loop plays an important role in herbicide binding in the 32 kDa protein is indicated by at least four separate mutations which are known to affect herbicide binding [13]. All three of the mutated residues (Val 219, Phe 255 and Ser 264) are in the loop between the last pair of transmembrane helices (Table III). The latter two are in the predicted surface seeking region.

Table III. Effect of mutations of the 32 kDa protein in Chlamydomonas reinhardii on resistance to atrazine and DCMU (Ref. [13]).

Mutation	Resistance	
	Atrazine	DCMU
Val 219 → Ile	2×	15 ×
Phe 255 → Tyr	15×	0.5×
Ser 264 → Ala	100×	10 ×
Ser 264 → Gly	1000×	1 ×

A fourth residue of the 32 kDa protein has been implicated in the binding of herbicides. Photoaffinity labeling using azidoatrazine labels a residue in the region 212–225, most probably Met 214 [14, 15]. This experiment suggests that the substituent at the 2-position of the triazine class of herbicides (chlorine in atrazine) is probably in proximity to Met 214. This residue is near the starting point of the extra-membrane loop which separates the last two transmembrane helices.

Our model for the 32 kDa protein can be compared with two others which have appeared in the literature. Argos, *et al.* have used an algorithm based on 5 physical parameters to detect hydrophobic helical spans within the 32 kDa sequence [5]. Our model differs from theirs in that we reject their third and sixth helices. There is rough agreement on placement of the remaining five helices. The third helix of the Argos model corresponds to the one labeled "?" in Table I. This putative helix has been rejected by us because of its short length.

Trebst has proposed a five-helix model on the basis of amino sequence and hydropathy index plot homologies with the bacterial system [6]. Our model agrees roughly with that of Trebst save that our second helix is earlier in the sequence than his. Trebst (see this issue) accepts the segment labeled "?" in Table I, but does not accept our second transmembrane helix which runs from residues 77 to 100.

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