Protein Sequence Homologies between Portions of the L and M Subunits of Reaction Centers of *Rhodopseudomonas capsulata* and the Q_B-Protein of Chloroplast Thylakoid Membranes: a Proposed Relation to Quinone-Binding Sites

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Sequence homology has been found between the L and M subunits of *Rhodopseudomonas capsulata* reaction centers and the Q_B-protein from all species thus far sequenced. The homology between the L subunit and the Q_B-protein is contained in the sequence PFHMLG---F---- AMHG-LV-S and is believed to have survived three billion years of evolution. A model is presented which associates quinone binding with this highly conserved sequence.

Introduction

The sequences of the reaction center polypeptides of *Rhodopseudomonas capsulata* have been determined [1]. We have discovered that there is a highly conserved pattern of sequences of amino acids which is common to the L and M subunits of *R. capsulata* and the Q_B-protein of chloroplast thylakoid membranes in spinach and tobacco. This conservation of sequence has survived since the divergence of these organisms which is estimated to have taken place 3 billion years ago [2]. Such a striking conservation of amino acid sequence suggests that these portions of all three proteins, which are approximately 60% in from the amino terminus in each case, are at the functional centers of these proteins. Because the *R. capsulata* reaction centers are known to contain two bound quinones [3], and herbicide binding to the Q_B-protein is known to block quinone binding to photosystem 2 [4, 5], and because quinones are known to have unique importance to the primary chemical events in bacterial reaction centers as well as in photosystem 2 of chloroplasts, it is our hypothesis that this highly conserved sequence of amino acids is involved in quinone binding and function.

Abbreviations: Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, photosystem; Q_A, primary quinone electron acceptor of photosystem II; Q_B, secondary quinone electron acceptor.

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A common feature of the photosynthetic reaction centers of photosystem 2 of chloroplasts and cyanobacteria and of purple photosynthetic bacteria is the presence of a quinone-iron complex as the electron acceptor [6]. Photo-induced charge separation in the reaction center results in the transfer of an electron first to a tightly bound quinone (Q_A) and then, by mediation of the intervening Fe(II), to a loosely bound quinone (Q_B), where the electron is stabilized in the semiquinone. The species $Q_B^-\cdot$ is assumed to remain unprotonated, on the basis of the absence of concomitant uptake of protons from the medium [7] and based on difference absorption spectra [8]. Upon the arrival of a second electron by the same route, the species $Q_B^=$ or $Q_A^-\cdot\, Q_B^-\cdot$ picks up a pair of protons and enters the quinone pool. It is at this point that PS 2 inhibitors (herbicides) like DCMU and atrazine are thought to act, by blocking access to the loose quinone binding site [4, 5].

The implication of specific binding sites for quinones is supported by studies of the competition with azido analogs of herbicides, which can be attached to the associated polypeptides as photoaffinity labels [9, 10]. In addition, there must be a strong interaction of the quinone with the binding site to cause a significant modification of its chemical properties. In the common protic solvents, including water, the semiquinone anion produced upon 1-electron reduction of the quinone rapidly picks up a proton in an essentially diffusion-controlled reaction. Furthermore, the neutral semiquinone is unstable against disproportionation to give hydroquinone and quinone. It has been estimated

that the reaction

$$Q + QH_2 \rightleftharpoons 2 \cdot QH$$

has an equilibrium constant of 10^{-12} in a phospholipid medium [11]. Thus, both the unusual stability of the 1-electron reduced quinones in *in vivo* electron transport chains, and their apparent resistance to protonation are properties that require explanation. Related phenomena occur for electrons transferred through the ubiquinone site of mitochondrial membranes [12]. In this case the three quinone-binding complexes that have been identified appear to be the loose-binding type and do not involve direct association with Fe(II).

Results

This manuscript follows one presented at the Sixth International Congress on Photosynthesis which was held from August 1 to 6, 1983 in Brussels. Because the date of submission of this work is three months later than our submission to the Brussels meeting, the DNA sequences of the genes which code for the H, L, and M subunits of the reaction centers of *R. capsulata* have been completed, and we are far more confident that most point errors and frame shift errors in the data have been eliminated. The discrepancies in the data here and the data in the earlier presentation result from the fact that we found one large region of the M subunit sequence which was in error because of a frame shift which has been corrected here. A visual comparison of the translated amino acid sequence of L subunit gene and the amino acid sequence Q_B-protein [13] reveals a sequence PFHMLG---F----AMHG-LV-S which is common to both sequences and which starts at the 172nd amino acid from the N terminus of the 282 amino acid long L subunit and which starts at the 196th amino acid from the N terminus of the 353 amino acid long Q_B-protein. A more careful search of the M subunit translated sequence also reveals a weaker homology with the Q_B-protein at position 198 of 306 amino acids where the homology reads PFH-L--A---G--L--AMHG----. In order to establish the significance of this homology the data bank associated with the Atlas of Protein Sequences at Georgetown University was searched for two tripeptides, AMH and FHM. The search found 27 proteins containing AMH, of which 6 were cytochrome b's, one a cytochrome c′ from *R. sphaeroides*, one a cytochrome c3 from *D. vulgaris*, and of course the Q_B-protein. The search found only 9 proteins containing FHM, including fumarate reductase, cytochrome oxidase and of course the Q_B-protein. No protein other than the Q_B-protein contained both sequences. A more detailed computer analysis performed by Doolittle [14] of the University of California in San Diego has revealed highly significant homologies between the L subunit and the Q_B-protein, between the M subunit and the Q_B-protein, and between the L and M subunits. A much weaker homology was also found between the H subunit sequence and the L and M subunit sequences. Table 1 contains a summary of this data. Figure 1 presents a plausible although not

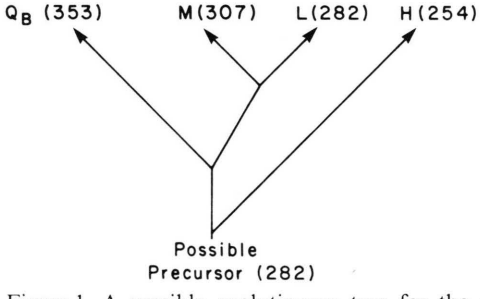

Figure 1. A possible evolutionary tree for the photosynthesis proteins. The approximate number of amino acids in each protein is provided in parenthesis.

Table I. Homology analysis of photosynthesis proteins

Protein 1: Protein 2	# of matches	% Match/ unit length	NAS*	# Gaps
H : L	48	18.9%	130	3 + 3
L : M	86	30.5%		4 + 4
L : Q_B	71	25.2%	199	4 + 2
M : Q_B	68	22.1%		6 + 2

* NAS = normalized alignment scores. See R. F. Doolittle, 1981 [14].

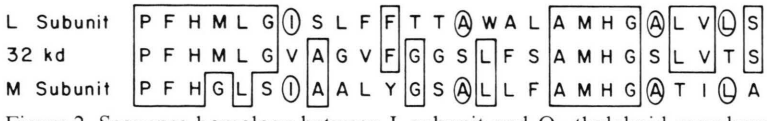

Figure 2. Sequence homology between L subunit and Q_B thylakoid membrane protein and between M subunit and Q_B-protein of the thylakoid membrane in the most highly conserved and presumptive quinone-binding regions.

unique picture of the relationship between these proteins in evolution. Figure 2 shows the strong homology regions of the L subunit, the Q_B-protein, and the M subunit.

Discussion

The data strongly indicate that the L and M subunits and the Q_B herbicide binding protein arose from a common precursor. It is also likely that the H subunit arose from the same precursor, although statistical validity of this statement is marginal. The fact that selection pressure preserved such a precise amino acid sequence over 3 billion years suggests that this region is functionally very significant and that it is interacting with a small and well defined immutable structure. The quinones are the logical candidates. This strong homology is found in a very hydrophobic background of amino acids making the logical amino acid residues to focus on, in order to explain the nature of the interaction between the quinone and its binding site, the histidine-methionine and the methionine-histidine dipeptides. We are suggesting that a charge transfer complex or a strong dipolar interaction exists between the sulfur atoms of the methionines and the conjugated ring of the quinone. The geometry of this presumptive interaction is such that one of the carbonyl oxygens of the quinone is hydrogen bonded to a proton on the imido nitrogen of the histidine. In water, this site has an acid pK of 6.00. It is this interaction which presumably stabilizes the unprotonated semiquinone, $Q_B^{-}\cdot$, so that there is sufficient time for a second electron to arrive by the same path and form hydroquinone, the two-electron reduced product which is released to the quinone pool. Figure 3 presents a schematic picture of this presumed interaction with the amino acid sequence PFHM. The interaction between a quinone and the sequence AMHG is envisioned to be similar. Presumably both of these sequences are associated with the strong quinone binding site and are simultaneously interacting with a single quinone molecule. The conservation of not only these short amino acid sequences but also the precise length of hydrophobic connector between these two sequences suggests a joint interactive role with a small molecule. It is noteworthy that the M subunit contains only one of the two histidine-methionine dipeptides and might therefore be associated with the weak binding site,

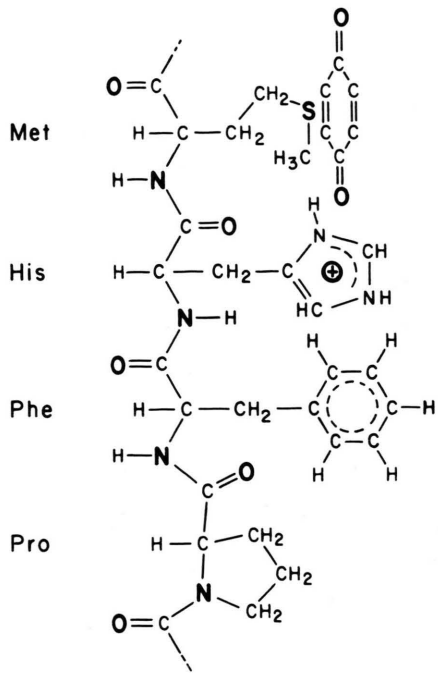

Figure 3. Proposed interaction between a quinone and the highly conserved amino acid sequence: proline – phenylalanine – histidine – methionine.

although such a conclusion is speculative. Clearly there is enough difference between these two sequences to account for the experimental differences between the strong and weak quinone binding sites. It is not possible to know if the presence of the sequence AMH in the cytochrome b's and the presence of the sequence FHM in fumarate reductase (EC 1.3.99.1-succinate dehydrogenase) and in cytochrome oxidase (EC 1.9.3.1) is coincidence or is the consequence of the interaction of these proteins with quinones. The novelty of our model suggests a multitude of new experiments to test such intriguing hypotheses.

During the final stages of preparation of this manuscript we have learned that Wolber and Steinback [10] have proven that the tryptic peptide of the Q_B-protein which contains our presumptive quinone binding site also contains the major site for the covalent linkage between azidoatrazine and the herbicide binding protein in thylakoid membranes. This result suggests that the quinone binding site and the atrazine binding site in this protein are one and the same.

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