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Author

Hearst, J.E.

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UNIVERSITY OF CALIFORNIA

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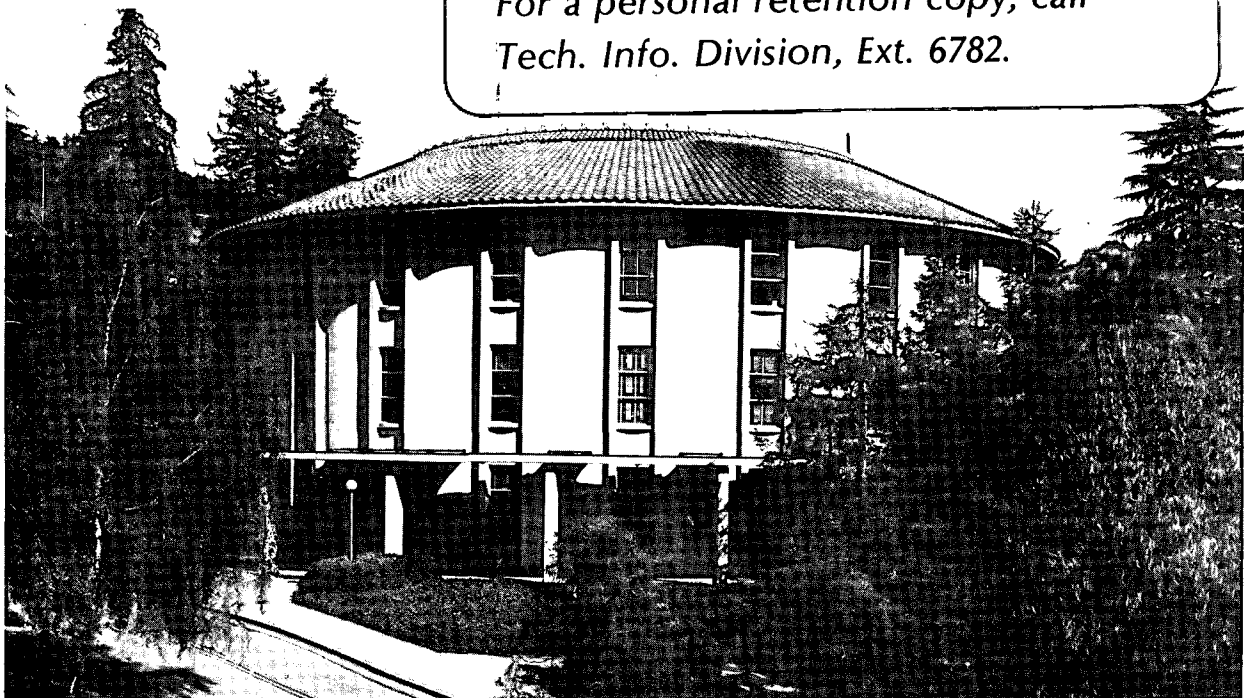J.J.S. Van Rensen, Ed., Springer-Verlag,
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PRIMARY STRUCTURE AND FUNCTION OF THE REACTION
CENTER POLYPEPTIDES OF Rps. capsulata - THE
STRUCTURAL AND FUNCTIONAL ANALOGIES WITH THE
PHOTOSYSTEM II POLYPEPTIDES OF PLANTS

J.E. Hearst

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DISCLAIMER

Primary Structure and Function of the Reaction Center

Polypeptides of <u>Rps. capsulata</u> - The Structural and Functional

Analogies with the Photosystem II Polypeptides of Plants

John E. Hearst

Department of Chemistry

University of California

and

Chemical Biodynamics Division

Lawrence Berkeley Laboratory

Berkeley, California 94720

Youvan et al. (1984) have recently reported the primary sequence of two 4000+ basepair fragments of Rps. capsulata which code for the light harvesting I polypeptides, B870α and B870β, the three reaction center polypeptides H, M, and L, and for some presumed regulatory functions relating to the synthesis of these components of the photosynthetic membrane (Zsebo and Hearst, 1984).   From the deduced amino acid sequences of the reaction center subunits it was concluded that the H subunit is a hydrophilic protein with a very hydrophobic amino terminus of thirty amino acids.   The H subunit is a polypeptide 254 amino acids long with a molecular weight of 28,534 daltons. The L and M subunits are both very hydrophobic proteins with 282 and 307 amino acids respectively.   Their molecular weights calculate to be 31,565 and 34,440 daltons, indicating that they have anomolous SDS PAGE electophoretic mobilities because of their hydrophobic characters.   Hydropathy plots suggest that the L and M subunits are transmembrane proteins which may cross the membrane five times.

Feher and Okamura (1978) have shown that the H subunit is not essential to the electron transfer activities of the reaction centers in Rps. sphaeroides.   While the H, M, and L subunits always occur in equimolar quantities in reaction centers, the H subunit is thirty-five kilobasepairs away from the operon containing the LH B870α, LH B870β, L subunit, and M subunit in Rps. capsulata (Youvan et al., 1983) and so the H subunit gene is clearly under separate transcriptional control from the other structural proteins in the reaction center.   Chory et al. (1984) have suggested that the H subunit serves as the structural anchor in the membrane around which the reaction center forms and evidence is presented suggesting that the H subunit can be found in membrane fractions isolated even from aerobically grown cells.

We are therefore at a point where it is generally accepted that the only

two polypeptides essential for reaction center activity are the L and M sub-units in Rps. sphaeroides. These two subunits must contain binding sites for the strongly bound and weakly bound ubiquinones, Q_A and Q_B, for the Fe(II), and for the bacteriochlorophylls and the bacteriopheophytins. Of these interactions, I consider the sites for binding the quinones most crucial since they serve the roles of preserving or stabilizing very labile anion radicals which are one electron intermediates in the quinone to hydroquinone reduction pathway. This model supposes that pigment binding functions are more variable and therefore less tightly conserved in evolution.

Hearst and Sauer (1983, 1984) have discovered that there is a highly conserved pattern of sequences of amino acids which is common to the L and M subunits of Rps. capsulata and the Q_B-protein of chloroplast thylakoid membranes in spinach and tobacco. The conservation has survived an estimated three billion years of evolution (J. M. Olson, 1981). Such a striking conservation of amino acid sequence suggests that these portions of all three proteins, all of which are approximately 60% in from the amino terminus, are at the functional centers of these proteins. It is their hypothesis that these highly conserved sequences of amino acids are involved in quinone binding and function.

There is a large literature of physical measurements on thylakoid membranes from higher plants which establish the functional and compositional similarities between photosystem II reaction centers in these membranes and the reaction centers of Rps. capsulata and Rps. sphaeroides. Crofts and Wraight (1983) and Vermaas (1984) have written excellent reviews of the subject. Klimov et al. (1977) reported the reduction of pheophytin in the primary light reaction of PS II by the chlorophyll complex P680 (Shuvalov et al. 1980). In a similar fashion after excitation with one photon, the purple

bacteria transfer an electron from a bacteriochlorophyll dimer to bacterio-pheophytin (Urban and Klingenberg, 1969). The semiquinone anion of Q_A was first reported in thylakoid membranes more than ten years ago (Witt, 1973; van Gorkom, 1974). In the green plant Q_A and Q_B are known to be plastoquinones. In purple bacteria, Q_A and Q_B are either ubiquinone or menaquinone. The semiquinone anions in purple bacteria have been reported by Pulles et al. (1976) and Prince and Dutton (1978). Klimov et al. (1980) have reported an Fe(II) atom interacting with the quinones in the PS II reaction centers of chloroplasts. An analogous Fe(II) had been reported earlier in purple bacteria (Tiede et al., 1976 and Okamura et al., 1979).

While a very large literature establishes the physical similarities between purple bacteria reaction centers and photosystem II reaction centers of plants, algae, and cyanobacteria, efforts to isolate reaction centers with similar chemical compositions has led to confusion. Broglie et al. (1980) have demonstrated the successful isolation of purple bacterial reaction centers using lithium dodecyl sulfate treatment of photosynthetic membranes which have well defined chemical composition. In particular, they always show a equimolar composition of the H, M, and L subunits. In higher cells efforts to isolate reaction centers have resulted in far more complex protein gel electrophoresis patterns which suggest many more components. For example, Westhoff et al. (1983) have reported seven nonidentical polypeptides in the watersplitting photosystem II multisubunit structure which has been isolated from spinach. Three of these are termed "peripheral", are located in the thylakoid lumen, have molecular weights 34 kd, 23 kd, and 16 kd, and appear to participate in water photolysis (Akerlund et al., 1982). The remaining four form a membrane spanning core that complexes all photosynthetic pigments of the reaction center. The proteins in this core are the 51 kd and 44 kd

proteins which bind P680 and the pheophytin which is the primary electron acceptor. The core also contains the 32 kd herbicide binding protein which shows homology to the purple bacterial L and M reaction center subunits (Hearst and Sauer, 1983, 1984) and it contains the 10 kd cytochrome b-559. Morris and Herrmann (1983) have sequenced the 51 kd protein and find no sequence corresponding to the hypothetical quinone binding site. The sequence of the 44 kd chlorophyll a-complexed apoprotein of this core has been completed by Morris and Herrmann but is not yet available to me.

If the analogs to the L and M subunits of purple bacteria reaction centers exist in PS II reaction centers, then protein sequence homologies suggest that the 32 kd herbicide binding protein, which binds Q_B, is an analog to the L subunit of the bacteria. The Arntzen laboratory has recently obtained azido atrazine binding to the L subunit of Rps. sphaeroides which also indicates this analogy to be correct (personal communication - 1984). While the 44 kd polypeptide of Westhoff et al.(1983) may be the other member of this pair, the difference in molecular weights suggests that this is not the case. On the physical side there is one additional piece of information which suggests a different solution to this apparent inconsistency. Yamagishi and Katoh (1984) have just developed a new isolation procedure for the PS II reaction center complex of the thermophilic cyanobacterium Synechococcus sp. and found that it resolved into two chlorophyll-protein complexes. The first, which they called CP2-b contained a chlorophyll-binding 47 kd polypeptide (presumably equivalent to the 51 kd protein of higher plants), two polypeptides in the 28-31 kd region, and a 9 kd polypeptide which they attribute to cytochrome b-559. The second complex, which they called CP2-c, contained only a single 40 kd chlorophyll-binding protein (presumably equivalent to the 44 kd protein of higher plants. CP2-b was able to promote the photoreduction of 2,6-

dichlorophenolindophenol with diphenylcarbizide as the electron donor. CP2-c had only weak photoreduction activity in the same reaction leading the authors to conclude that CP2-b is the site of the PS II reaction center and that the 40 kd polypeptide (the 44 kd analog) is not essential for PS II electron transport. In addition to this report Satoh et al. (1983) have reported the following components in isolated reaction center complexes from spinach: two chlorophyll-binding proteins of molecular weight 47 kd and 43 kd, a 30 kd herbicide binding protein, a 6 kd apoprotein of cytochrome b-559 and a 34 kd protein of unknown function. This agrees well with the conclusions of the Hermann lab except for the additional 34 kd protein which is at the reaction center. (Attention should be drawn to the work of Youvan et al., 1984, in which the large underestimation of the molecular weights of these very hydrophobic reaction center proteins from SDS PAGE is clearly documented. One might expect similar low estimates in the works of Yamagishi and Katoh, 1984, and of Satoh et al., 1983 which were mentioned above.)

I would like to suggest here that all PS II reaction centers are likely to contain two similar protein subunits with molecular weights between 30 kd and 40 kd with roles analogous to those of the L and M subunits in the reaction centers of photosynthetic purple bacteria. I am stressing quinone binding here although common features of pigment binding might also prevail. The strongest evidence in favor of this position comes from the recent nucleic acid sequence data of J.-D. Rochaix et al. (1984) and of O.F. Rasmussen et al. (1984). Rochaix et al. (1984) present the nucleic acid sequence of the psbD locus of the Chlamydomonas reinhardii chloroplast genome. The psbD gene codes for the D2 polypeptide which has been associated with photosystem II (Chua and Gillham, 1977) together with its partner, the D1 polypeptide, which is coded by the psbA gene and which corresponds to the 32 kd herbicide binding

protein or Q_B protein of higher plants. Rochaix et al. make the striking observation that while the overall homology between the Q_B protein and the D2 protein of C. reinhardii is only 27%, there are several domains where one can find homology ranging between 33% and 58%. Q_B contains 352 amino acids while D1 contains 339 or possibly 350 amino acids. The putative quinone binding region is highly conserved in both proteins, with Q_B corresponding best with the L subunit of Rps. capsulata and D2 corresponding best with the M subunit of Rps. capsulata.

Rasmussen et al. (1984) have sequenced the corresponding psbD gene from the higher plant chloroplast genome, Pisum sativum or pea. Their D2 protein is 353 amino acids long and shows an 84.7% homology to the D2 protein of C. reinhardii. Again the putative quinone binding region is the most highly conserved region of the protein relative to the M subunit of Rps. capsulata.

Figure 1 shows an alignment of the amino acid sequences of the L subunit of Rps. capsulata (Youvan et al., 1984), the Q_B protein of spinach (Zurawski et al., 1981) and the Q_B protein of C. reinhardii (Erickson et al., 1983). Overall, there is 27% homology between the L subunit and the Q_B protein of spinach, 26.2% homology between the L subunit and the Q_B protein of C. reinhardii and 92.3% homology between the two Q_B proteins. The most striking feature of this figure is the strong homology between all three proteins in the hypothetical quinone binding region: N----PFHMLG----F------AMHG-LV-S.

Figure 2 shows an alignment of the amino acid sequences of the M subunit of Rps. capsulata (Youvan et al., 1984), the D2 protein of pea (Rasmussen et al., 1984) and the D2 protein of C. reinhardii (Rochaix et al., 1984). Overall, there is 20.8% homology between the M subunit and the D2 protein of pea, 19.5% homology between the M subunit and the D2 protein of C. reinhardii and an 84.7% homology between the two D2 proteins. The most striking feature

of this figure is the strong homology between all three proteins in the hypothetical quinone binding region: F---H-N---NPFH----A---G-ALL-A-HGAT.

A comparison of these homologies suggests that in the putative strong site or M subunit site, the histidine-methionine and methionine-histidine pairs of the weak or L subunit site are not the only functionalities of importance (Hearst and Sauer, 1984). Each strong binding site has only one of these two pairs of amino acid sequences, while the weak site in all cases has both pairs.

In conclusion, I am suggesting that the psbA and psbD loci of chloroplast genomes encode for the photosystem II analogs of the L and M reaction center subunits of R. capsulata and other photosynthetic purple bacteria. While the corresponding chloroplast proteins, D1 and D2, appear to have molecular weights of 32 to 34 kd by SDS PAGE, they are very hydrophobic membrane bound proteins which may cross the membrane five or more times. Their true molecular weights will be between 38 and 40 kd (Zurawski et al., 1981 and Rasmussen et al., 1984). Hearst and Sauer, 1983 and 1984 have proposed that the most highly co-conserved sequences between the L subunit, the M subunit and the Q_B protein are involved in quinone binding. While Wolber and Steinback (1984) have proven that the trypic peptide of the Q_B protein which contains the presumptive quinone binding site also contains the major site for the covalent linkage between azidoatrazine and the herbicide binding protein in thylakoid membranes, direct physical evidence relating to quinone binding remains elusive and requires further experimentation. In this spirit, it must be remembered that identification of the Q_B protein with the weak quinone binding site, and the D2 protein with the strong binding site relates to a model and cannot yet be considered experimental fact.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1. An amino acid sequence alignment for the L subunit reaction center polypeptide of <u>Rps. capsulata</u>, the herbicide binding thylakoid membrane protein, Q_B, of spinach and the D1 protein of <u>C. reinhardii</u> which is encoded by the psbA locus of the chloroplast genome. There are 76 matches and a 27.0% homology between L and Q_B, 74 matches and a 26.2% homology between L and D1, and 325 matches and a 92.3% homology between D1 and Q_B.

Figure 2. An amino acid sequence alignment for the M subunit reaction center polypeptide of <u>Rps. capsulata</u>, the D2 protein of pea, and the D2 protein of <u>C. reinhardii</u>. The two D2 proteins are encoded by the psbD loci of the chloroplast genomes of their respective organisms. There are 64 matches and 20.8% homology between the M and D2 pea, 60 matches and 19.5% homology between the M and D2 chlamydomonas, and 287 matches and 84.7% homology between D2 chlamydomonas and D2 pea.

MALLSFERKYRVPGGTLIGGSLFDFWVGPFYVGFFGVTTI   FFATLGFLLILWGAAMQGTWNP
MT AILERRESESLWGRFCNWI TSTENRLYIGWFGVLMIPTLLTATSVF IIAFIAAPPVDIDGIREPVSGSLLYGNNIISGAIIPTSA
MT AILERRENSSLWARFCEWI TSTENRLYIGWFGVIMIPCLLTATSVF IIAFIAAPPVDIDGIREPVSGSLLYGNNIITGAVIPTSN

QLISIFPPPVENGLNVAALDKGGLWQVITVCATGAFCSWALREVEICRKLGIGFHIPVAFSMAIFAYLTLVVIRPMMMGSWGYAFPYGIW
AIGLHFYPIWEAASVDEWLYNGGPYELIVLHFLLGVACYMGREWELSFRLGMRPWIAVAYSAPVAAATAVFLIYPIGQGSFSDGMPLGIS
AIGLHFYPIWEAASLDEWLYNGGPYQLIVCHFLLGVYCYMGREWELSFRLGMRPWIAVAYSAPVAAASAVFLVYPIGQGSFSDGMPLGIS

THLDWVSNTGYTYGNFHYNPFHMLGISLFFTTAWALAMHGALVLS   AANPVKGKTMRTPDHEDT   YFRDLMGYSVGTL
GTFNFMIVFQAEH NILMHPFHMLGVAGVFGGSLFSAMHGSLVTSSLIRETTENESANEGYRFGQEEETYNIVAAHGYFGRLIFQYASFN
GTFNFMIVFQAEH NILMHPFHMLGVAGVFGGSLFSAMHGSLVTSSLIRETTENESANEGYRFGQEEETYNIVAAHGYFGRLIFQYASFN

GIHRLGLLLALNAVFWSACCMLVSGTIYFDLWSDWWYWWV   NMPFWAD   MAGGING
NSRSLHFFLAAWPVVGIWFTALGISTMAFNLNGFNFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAAIEAPSTNG
NSRSLHFFLAAWPVIGIWFTALGLSTMAFNLNGFNFNQSVVDSQDRVLNTWADIINRANLGMEVMHERNAHNFPLDLASTNSSSNN

Amino Acid Sequence Alignments for·

L Subunit   *Rhodopseudomonas capsulata*   (282 amino acids)

Q_B Protein   *Spinacia oleracea*   (353 amino acids)

D1 Protein (psbA) *Chlamydomonas reinhardii*   (352 amino acids)

Fig. 1

— 16 —

MAEYQNFFNQVQVAGAPEMGLKEDVDTFERTPAGMFNILGWMGNAQIGPIYLGIAGTVSLAFGAAWFFTIGVWYWYQAGFDPFIFMRDLF
MTIALGKFTKDQNDLFDIMDDWLRRDRFVFVGWSGLLLFPCAYFAVGGWFTGTTFVTSWYTHGLASSYLEGCNFLTAAVSTP ANSLAH
MTIAIGTYQEKRT WFDDADDWLRQDRFVFVGWSGLLLFPCAYFALGGWLTGTTFVTSWYTHGLATSYLEGCNFLTAAVSTP ANSMAH

FFSLEPPPAEYGLAIAPLKQGGVWQIASLFMAISVIAWWVRVYTRADQLGMGKHMAWAFLSAIWLWSVLGFWRPILMGSWSVAPPYGIFS
SLLLLWGPEAQGDLTRWCQLGGLWTFVALHGAFGLIGFMLRQFELARSVQLRPYNAIAFSGPIAVFVSVFLIYPLGQSGWFFAPSFGVAA
SLLFVWGPEAQGDFTRWCQLGGLWAFVALHGAFGLIGFMLRQFEIARSVNLRPYNAIAFSAPIAVFVSVFLIYPLGQSGWFFAPSFGVAA

HLDWTNQFSLDHGNLFYNPFHGLSIAALYGSALLFAMHGATILAVTRFGGERELEQIVDRGTASERAALFWRWTMGFNATMEG   IHR
IFRFILFFQGFH NWTLNPFHMMGVAGVLGAALLCAIHGATVENTLFEDGDGANTFRAFNPTQAE ETYSMVTANRFWSQIFGVAFSNKR
IFRFILFFQGFH NWTLNPFHMMGVAGVLGAALLCAIHGATVENTLFEDGDGANTFRAFNPTQAE ETYSMVTANRFWSQIFGVAFSNKR

WAIWMAVMVTLT   GGIGILLSGTVVDNWYVWAQVHGYAPVTP
WLHFFMLFVPVTGLWMSALGVVGLALNLRAYDFVSQEIRAAEDPEFETFYTKNILLNEGIRAWMATQDQPHENLIFPEEVLPRGNAL
WLHFFMLLVPVTGLWMSAIGVVGLALNLRAYDFVSQEIRAAEDPEFFFSIFIIPNHIINGSYFFNKSQKQIVYI

Amino Acid Sequence Alignments for :

M Subunit   *Rhodopseudomonas capsulata*   (307 amino acids)

D2 Protein (psbD)   *Pisum sativum*   (353 amino acids)

D2 Protein (psbD)   *Chlamydomonas reinhardii*   (352 amino acids)

Fig. 2