CC/NUMBER 42
OCTOBER 18, 1993

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Kuwabara T & Murata N. Inactivation of photosynthetic oxygen evolution and concomitant release of three polypeptides in the photosystem II particles of spinach chloroplasts. *Plant Cell Physiol.* **23**:533-9, 1982. [Department of Biology, University of Tokyo, Japan]

An oxygen-evolving photosystem II membrane preparation is described, in which the complex functioning in photosynthetic oxygen evolution (water oxidation), located at the inner surface of closed thylakoid membranes in intact thylakoids, is exposed to a suspending medium. This made direct manipulation of the complex feasible, and it was revealed that three extrinsic proteins are involved in the water oxidation. (The *SCI*® indicates that this paper has been cited in more than 280 publications.]

Biochemistry of Photosynthetic Water Oxidation

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Until the early 1980s biochemical research on photosynthetic water oxidation was believed to be difficult since the site of this function is located at the inner surface of closed thylakoid membranes. Although efforts were made to obtain preparations enriched with photosystem (PS) ll-specific electron transport activity, none retained stable oxygen-evolving activity.

At that time, I was a graduate student to N. Murata at the University of Tokyo. My research theme was chemical and physicochemical characterization of the33-kDa protein, whose function was unknown, but its involvement in PS II had been suggested. I was trying to get a 33-kDa protein-rich PS II preparation, without thinking much about water oxidation. In retrospect, the attention to the protein was serendipitously essential for the preparation of oxygen-evolving PS II membranes; the protein was later shown to function in stabilizing the Mn atoms of the water-oxidizing center, and named the Mn-stabilizing protein. After trial and error, we treated the thylakoids simply with a combination of moderate concentration (about 150 mM) monovalent cation and concentrated (about 5 percent) Triton X-100; the former induces stacking of thylakoids and the latter solubilizes unstacked thylakoids, thus giving the resultant stacked thylakoids. A survey of procedures to extract proteins from the obtained preparation led to the finding that alkaline treatment specifically solubilizes three proteins of 33 kOa, 23 kOa, and 18 kDa. Although the treatment had been known to inactivate the oxygen evolution, I did not anticipate at first that our preparation retained the oxygen-evolving activity, since this function was believed to be very fragile. Even when I actually detected it, I could not soon believe it; its extraordinarily stable oxygen-evolving activity convinced me that it was different from ordinary thylakoid membranes. Considering that several predecessors tried to get such a preparation by taking PS II activity as an index but failed, it is nothing but luck that my adherence to the 33-kDa protein (a kind of substantialism?) resulted in the attainment of the preparation.

This paper is cited for the preparation method itself as well as for the finding of the involvement of the three proteins in the water oxidation. With the use of the oxygen-evolving PS II preparation, research on the water oxidation progressed spectacularly in the middle of the 1980s. The functions of the three proteins and their relationship to the essential elements Mn, Ca, and Cl were extensively studied,[1,2] leading to the oxygen-evolving PS II core complex.[3]

Purification and cloning of the three thylakoid-lumenal proteins contributed to the research on the mechanism of protein transport across thylakoid membrane. The deduced amino-acid sequence of the precursor of cyanobacterial Mn-stabilizing protein pointed out the N-terminal signal sequenceforthe translocation across the thylakoid membrane.[4] It was also shown that the precursor of higher plant protein possesses the transit sequence for the import into chloroplast, in addition to the thylakoid-traversing signal.[5]

Research on the extrinsic proteins also revealed the existence of thylakoid-bound proteinases.[6] These include a postproline cleaving enzyme which specifically acts on the 18-kDa protein and a proteinase which can recognize denaturation of the 23-kDa protein. These proteinases appear not to be able to digest other chloroplast proteins. The physiological significance of the proteinases remains to be elucidated, but their extremely high substrate specificity presents intriguing questions about the mechanism of molecular recognition in the degradation of the thylakoidal proteins.

1. Murata N & Miyao M. Extrinsic membrane proteins in the photosynthetic oxygen-evolving complex. *Trends Bichem. Sci.* **10**:122-4. 1985. (Cited 105 times.)

2. **Ghanolakis D F & Yocum C F.** Photosystem II and the oxygen-evolving complex. *Annu. Rev. Plant Physiol.* 41:255-76. 1990.

3. **Tang X-S & Satoh K. The** oxygen-evolving photosystem II core complex. *FEBS Lett.* 179:60-4, 1985.

4. Kuwabara T, Reddy K J & Sherman L A. Nucleolide sequence of the gene from the cyanobacterium .Anacystis *nidulans* R2 encoding the Mn-stabilizing protein involved in photosystem II water oxidation. *PROC Nat. Acad. Sci. USA* 84:8230-4, 1987.

5. **Tyagi A, Hermans J, Steppuhn J, Jansson C, Vater F & Herrmann R** G. Nucleotide sequence of cDNA clones encoding the complete "33 kd" precursor protein associated with the photosynthetic oxygen-evolving complex from spinach. *Mol. Gen. Genet.* 207:288-93, 1987.

6. **Kuwabara T.** Characterization of a prolyl endopeptidase from spinach thylakoids. *FEBS Lett.* 300:127-30, 1992.

Received July 22. 1993