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Short Review

Recent advances in the use of mass spectrometry to examine structure/function relationships in photosystem II

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ABSTRACT

Tandem mass spectrometry often coupled with chemical modification techniques, is developing into increasingly important tool in structural biology. These methods can provide important supplementary information concerning the structural organization and subunit make-up of membrane protein complexes, identification of conformational changes occurring during enzymatic reactions, identification of the location of posttranslational modifications, and elucidation of the structure of assembly and repair complexes. In this review, we will present a brief introduction to Photosystem II, tandem mass spectrometry and protein modification techniques that have been used to examine the photosystem. We will then discuss a number of recent case studies that have used these techniques to address open questions concerning PS II. These include the nature of subunit–subunit interactions within the phycobilisome, the interaction of phycobilisomes with Photosystem I and the Orange Carotenoid Protein, the location of CyanoQ, PsbQ and PsbP within Photosystem II, and the identification of phosphorylation and oxidative modification sites within the photosystem. Finally, we will discuss some of the future prospects for the use of these methods in examining other open questions in PS II structural biochemistry.

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1. Introduction

One of the central objectives of modern biochemical investigation is understanding the structural and functional interactions within membrane protein complexes. These complexes provide the basis for cellular interaction with the environment, intercellular interaction and communication, cell adhesion, inter- and intracellular transport of nutrients, and energy transduction processes. In all photosynthetic organisms, the harvesting of light energy and its conversion to biologically useful forms rely uniformly on ensembles of extrinsic and intrinsic membrane proteins. Since the initial report of the structure of the purple bacterial reaction center over thirty years ago [1,2], X-ray crystallography has been extraordinarily successful at providing molecular structures for most of the membrane protein complexes involved in photosynthetic electron transport. These include the aforementioned purple bacterial reaction center, cyanobacterial Photosystem II (PS II, [3–7]), cyanobacterial and higher plant Photosystem I (PS I, [8,9]), cyanobacterial and green algal cytochrome b_6/f complexes [10,11] and the F_1 domain of ATP synthase (although not from a photosynthetic organism [12]). X-ray crystallography has also provided critical information concerning the structure of a variety of both intrinsic [13,14] and extrinsic light-harvesting antennae components [15,16]. Additionally X-ray crystallography and multidimensional nuclear magnetic resonance studies have provided structures for a variety of isolated extrinsic protein subunits of photosynthetic complexes not present in the current crystal structures of PS II. These include CyanoQ, PsbQ [17–20], CyanoP and PsbP [21–24]. The structures of a number of assembly factors for PS II including Psb27 [25], Psb28 [26], Ycf48 [27] and Psb31 [28] have been solved using these methods. Single particle imaging (and cryoelectron microscopy) has also contributed significantly to our understanding of photosynthetic supercomplexes such as the PS II-LHC II [29–33], PS I-LHCII [34], the PS II-phycobilisome [35] and PS I-phycobilisome [36,37] interactions. Atomic force microscopy is also a developing technique which may be useful in this regard [38–40].

Recently, high resolution tandem mass spectrometry, coupled with site-specific chemical modification and/or protein crosslinking methodologies [41–43], is developing into a complementary technique for the structural examination of membrane protein complexes [41,44]. These techniques provide low resolution structural information concerning the exposure of domains of constituent subunits to the bulk solvent and the identification of interacting domains of the protein subunits within a complex. This complementary information can be quite useful. X-ray crystallography provides high-resolution structural information of the protein complex in, necessarily, a single conformational state. Protein complexes, however, are often dynamic, assuming multiple structural states as their reaction mechanism progresses. While it is sometimes possible to use inhibitors to trap the protein complex in a reaction-specific conformational state (for instance, [45,46]), often this is not possible. It is also assumed that the crystallizable form of a protein complex represents the in vivo structure. However, it must be recognized that crystallization conditions seldom replicate in vivo conditions with regard to ionic strength and make-up, pH, constituent lipids, etc., all of which can affect structure. It should additionally be noted that weakly bound subunits may be lost from protein complexes during the crystallization process, which involves the use of detergents, non-physiological buffer conditions, high ionic or osmotic strength, etc. Posttranslational modifications of membrane protein complexes also occur. If these are present in the subset of the protein complexes that are crystallizable, the electron density at the site of modification will be proportionally decreased. It is also possible that the presence of a posttranslational modification could preclude (or even potentiate) the formation of crystals. Finally, proteins that interact transiently (and usually relatively weakly) with membrane protein complexes cannot be identified using X-ray crystallography alone. All of these considerations can be addressed, at various levels, by mass spectrometry.

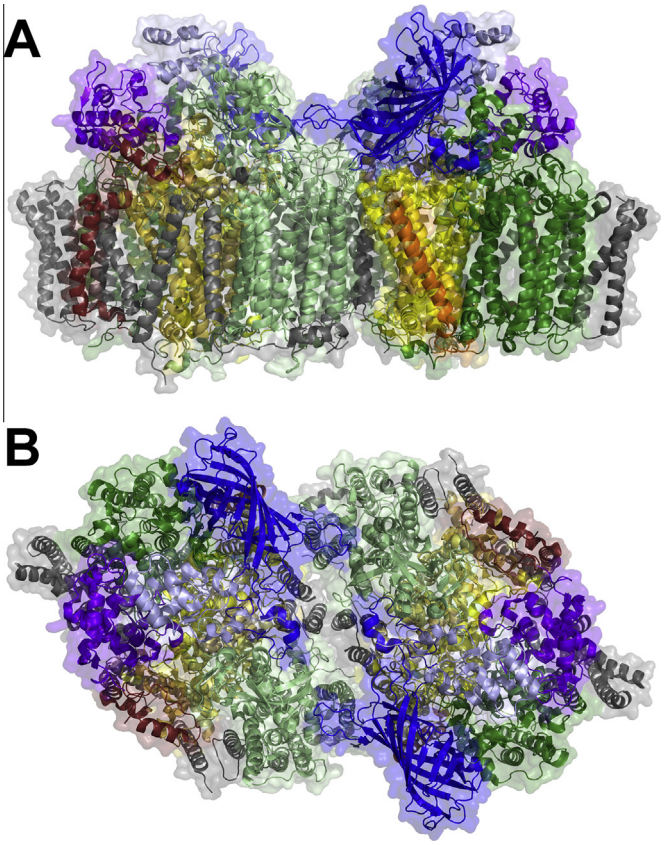

Fig. 1. PS II. Shown is the 1.9 Å crystal structure of the PS II dimer from the thermophilic cyanobacterium *Thermosynechococcus vulcanus* [6]. A. View of the complex from within the plane of the membrane, B. view of the lumenal face of the complex. D1, yellow; D2, pale yellow; PsbI, orange; PsbE, red; CP 43, dark green; CP 47, light green; PsbO, blue; PsbU, pale blue; PsbV, purple; all other subunits are illustrated in gray. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In this review, we will first briefly discuss PS II and some of the techniques that have recently been used to examine this membrane protein complex using mass analysis. This discussion will not be exhaustive and the reader will be directed to more authoritative and complete reviews on these specific topics. We will then discuss a number of recent case studies that have productively used mass spectrometry in the examination of the structure and function of PS II. Finally, we will discuss possible future experiments which can address long-standing problems in the field.

1.1. Photosystem II, Briefly

PS II functions as a light energy-driven water-plastoquinone oxidoreductase. In all oxygenic organisms, light is trapped by and funneled through light-harvesting pigment protein complexes (extrinsic phycobilisomes in cyanobacteria and red algae and light-harvesting chlorophyll (LHC) proteins in green algae, other algae and land plants) to the reaction center of PS II, which contains a special dimeric chlorophyll, P_{680}. After excitation, P_{680} becomes photooxidized and donates an electron to the primary acceptor of PS II, a protein-bound pheophytin. On the reducing side