FUNCTIONAL PROTEOMICS OF PROTEIN PHOSPHORYLATION IN ALGAL PHOTOSYNTHETIC

Pathan Noorsubah Nasir Ullah

Research Scholar, Department of BotanyCMJ university, Shillong, Meghalaya

ABSTRACT

Plants, green algae and cyanobacteria perform photosynthetic conversion of sunlight into chemical energy in the permanently changing natural environment. For successful survival and growth photosynthetic organisms have developed complex sensing and signaling acclimation mechanisms. The environmentally dependent protein phosphorylation in photosynthetic membranes is implied in the adaptive responses; however, the molecular mechanisms of this regulation are still largely unknown. We used a mass spectrometry-based approach to achieve a comprehensive mapping of the in vivo protein phosphorylation sites within photosynthetic membranes from the green alga Chlamydomonas reinhardtii subjected to distinct environmental conditions known to affect the photosynthetic machinery. The state transitions process regulating the energy distribution between two photosystems, involves the temporal functional coupling of phosphorylated light-harvesting complexes II (LHCII) to photosystem I (PSI). During state transitions several of the thylakoid proteins undergo redox-controlled phosphorylation-dephosphorylation cycles. This work provided evidence suggesting that redox-dependent phosphorylation-induced structural changes of the minor LHCII antenna protein CP29 determine the affinity of LHCII for either of the two photosystems. In state 1 the doubly phosphorylated CP29 acts as a linker between the photosystem II (PSII) core and the trimeric LHCII whereas in state 2 this quadruply phosphorylated CP29 would migrate to PSI on the PsaH side and provide the docking of LHCII trimers to the PSI complex. Moreover, this study revealed that exposure of Chlamydomonas cells to high light stress caused hyperphosphorylation of CP29 at seven distinct residues and suggested that high light- induced hyperphosphorylation of CP29 may uncouple this protein together with LHCII from both photosystems to minimize the damaging effects of excess light. Reversible phosphorylation of the PSII reaction center proteins was shown to be essential for the maintenance of active PSII under high light stress. Particularly dephosphorylation of the light damaged D1 protein, a central functional subunit of the PSII reaction center, is required for its degradation and replacement. We found in the alga the reversible D1 protein phosphorylation, which until our work, has been considered as plantspecific. We also discovered specific induction of thylakoid protein phosphorylation during adaptation of alga to limiting environmental CO2. One of the phosphorylated proteins has five II phosphorylation sites at both serine and treonine residues. The discovered specific low-CO2- and redox-dependent protein phosphorylation may be an early adaptive and signaling response of the green alga to limitation in inorganic carbon. This work provides the first comprehensive insight into the network of environmentally regulated protein phosphorylation in algal photosynthetic membranes.

INTRODUCTION

Oxygenic photosynthesis is a process of capture and conversion of sunlight into chemical energy by photoautotrophic organisms. For the efficient energy conversion plants, green algae and cyanobacteria have developed a complex molecular machinery. Most of the reactions of photosynthesis occur in chloroplasts. Chloroplasts are plastid organelles surrounded by two separate membranes: the outer chloroplast envelope and the inner chloroplast envelop. Inside chloroplasts there is a third membrane system which is called thylakoid and which forms a continuous three-dimensional network enclosing an aqueous space called the lumen. The fluid compartment that surrounds the thylakoids is known as the stroma (Nelson and Ben-Shem, 2004). Thylakoids are differentiated into two distinct morphological domains: cylindrical stacked structures called grana and unstacked membrane regions called stroma lamellae, which interconnect the grana (Anderson and Andersson, 1988; Mustardy and Garab, 2003). Algal thylakoids have a loose organization with less amount of the stacked domains comparing to green plants (Aro and Ohad, 2003; Steinback and Goodenough, 1975). Thylakoid membrane provides light-dependent water oxidation, NADP+ reduction and ATP formation. These reactions are catalyzed by four multi-subunit membrane-protein complexes: photosystem I (PSI), photosystem II (PSII), the cytochrome b6f complex, and ATP-synthase. Two photosystems are working in the photosynthetic membrane in parallel. PSII absorbs light, oxidizes H2O to O2 and extracts electrons from water; the electrons are then transported via electron transport chains in the thylakoid membrane to PSI. The electrons from PSII are used for the reduction of plastoquinone (PQ) pool to plastoquinol (PQH2). The cytochrome b6f protein complex then accepts electrons from PQH2. The cytochrome-b6f complex mediates electron transport to PSI via plastocyanin (PC). PSI transfers the electrons across the membrane and reduces NADP+ to form NADPH. NADPH is then used as reducing power for the biosynthetic reactions. The proton-motive force generated by linear electron flow from PSII to PSI powers ATP synthesis by F1F0-complex. To synthesize ATP, photosynthesis provides an alternative route through which light energy can be used to generate a proton gradient across the thylakoid membrane of chloroplasts. This second electron path, driven by PSI only, is the cyclic electron flow, and it produces neither O2 nor NADPH. Electrons from PSI can be recycled to plastoquinone, and subsequently to the cytochrome b6f complex (Heber and Walker, 1992; Joliot and Joliot, 2002). Such a cyclic flow generates pH and thus ATP without the accumulation of reduced species. The role of cyclic electron flow is less clear than linear, while it is proposed, that the linear flow itself cannot maintain the correct ratio of ATP/NADPH production. The absence of cyclic flow will ultimately lead to excessive accumulation of NADPH in the stroma and thereby, its over- reduction (Munekage et al., 2004).

REVIEW OF LITERATURE

In 1906, Phoebus Levene at the Rockefeller Institute for Medical Research identified phosphate in the protein vitellin (phosvitin), and by 1933 had detected phosphoserine in casein, with Fritz Lipmann However, it took another 20 years before Eugene P. Kennedy described the first 'enzymatic phosphorylation of proteins' Reversible phosphorylation of proteins is an important regulatory mechanism that occurs in both prokaryotic and eukaryotic organisms. Kinases phosphorylate proteins and phosphatases dephosphorylate proteins. Many enzymes and receptors are switched "on" or "off" by phosphorylation and dephosphorylation. Reversible phosphorylation results in a conformational change in the structure in many enzymes and receptors, causing them to become activated or deactivated. Phosphorylation usually occurs on serine, threonine, tyrosine and histidine residues in eukaryotic proteins. Histidine phosphorylation of eukaryotic proteins appears to be much more frequent than tyrosine phosphorylation. In prokaryotic proteins phosphorylation occurs on the serine, threonine, tyrosine, histidine or arginine or lysine residues. The addition of a phosphate (PO_4) molecule to a polar R group of an amino acid residue can turn a hydrophobic portion of a protein into a polar and extremely hydrophilic portion of molecule. In this way it can introduce a conformational change in the structure of the protein via interaction with other hydrophobic and hydrophilic residues in the protein. One such example of the regulatory role that phosphorylation plays is the p53 tumor suppressor protein. The p53 protein is heavily regulated and contains more than 18 different phosphorylation sites. Activation of p53 can lead to cell cycle arrest, which can be reversed under some circumstances, or apoptotic cell death. This activity occurs only in situations wherein the cell is damaged or physiology is disturbed in normal healthy individuals.Upon the deactivating signal, the protein becomes dephosphorylated again and stops working. This is the mechanism in many forms of signal transduction, for example the way in which incoming light is processed in the light-sensitive cells of the retina.

Regulatory roles of phosphorylation include

- Biological thermodynamics of energy-requiring reactions
- Phosphorylation of Na^+/K^+-ATPase during the transport of sodium (Na^+) and potassium (K^+) ions across the cell membrane in osmoregulation to maintain homeostasis of the body's water content.
- Mediates enzyme inhibition
- Phosphorylation of the enzyme GSK-3 by AKT (Protein kinase B) as part of the insulin signaling pathway.
- Phosphorylation of src tyrosine kinase (pronounced "sarc") by C-terminal Src kinase (Csk) induces a conformational change in the enzyme, resulting in a fold in the structure, which masks its kinase domain, and is thus shut "off".

RESEARCH METHODOLOGY

The aim of this project was to systematically identify and characterize phosphoproteins and *in vivo* protein phosphorylation events involved in adaptive responses in unicellular green alga *Chlamydomonas reinhardtii*. The mass spectrometry approach was used to resolve the regulatory and signaling network of protein phosphorylation, involved in acclimation of algal cells to different environmental conditions including stress. It should be noted that before our study there were only two phosphorylation sites identified in the proteins from photosynthetic membranes of *Chlamydomonas reinhardtii* (Dedner et al., 1988; Fleischmann and Rochaix, 1999). Reversible protein phosphorylation is a fundamental regulatory cellular mechanism and a crucial part of signaling pathways (Cohen, 2000; Huber, 2007; Pawson and Scott, 2005). Approximately one- third off all proteins are phosphorylated *in vivo* at any given time (Cohen, 2000; Knight et al., 2003; Manning et al., 2002). Phosphorylation at specific serine, threonine and tyrosine residues is the most ubiquitous specific post translational modification that occurs in complex eucaryotic systems (Mann and Jensen, 2003). These modifications are able to change many properties of protein, such as interaction with other proteins, stability, localization and activity. Reversible protein phosphorylation plays an important role in the regulation of many different processes, such as cell growth, differentiation, migration, metabolism, apoptosis and stress responses (Baena-Gonzalez et al., 2007; Huber et al., 1989; Huber, 2007; Hunter, 2000; Tran et al., 2004). Phosphorylation of thylakoid proteins in plants has been implicated in adaptive responses to a number of environmental stress factors (Vener, 2007), such as high light (Ebbert et al., 2001; Xu et al., 1999), cold stress (Bergantino et al., 1995), combined high light and cold treatment (Pursiheimo et al., 2001), heat shock (Rokka et al., 2000), combined magnesium and sulfur deficiency (Dannehl et al., 1995), and waterdeficient conditions (Giardi et al., 1996). Phosphoproteomics have as its object the comprehensive study of protein phosphorylation by identification of phosphoproteins, mapping of phosphorylation sites, quantitation of phosphorylation and, finally revealing the role of protein phosphorylation in signaling/regulatory networks. The analysis of the entire *phosphorylome*, *i.e.* the complete set of all phosphorylated proteins in a cell, is challenging (Cox and Mann, 2007; Goshe, 2006; Olsen et al., 2006) despite of the optimization of enrichment protocols for phosphoproteins and phosphopeptides, improved fractionation techniques and development of methods to selectively visualize phosphorylated residues using mass spectrometry. Phosphoproteomics is a powerful tool for understanding various biological problems (Mann, 2006; Rossignol, 2006; Rossignol et al., 2006; Stern, 2005) but, as any other technique, it has limitations. Classical proteomic approaches imply first proteins dissolving (extraction) and digestion before submission to mass spectrometry analysis (or analysis directly from the digest). In many cases relevant proteins were missed from the analyses since no extraction condition is suitable for extraction of all proteins (especially membrane proteins) from complex samples.

RESULT & CONCLUSION

This paper presents the first systematic identification and characterization of phosphoproteins and in vivo protein phosphorylation events involved in adaptive responses in unicellular green photosynthetic alga Chlamydomonas reinhardtii. The results and conclusions of the research published in papers I-IV are summarized as follows:

- 31 in vivo protein phosphorylation sites were identified within photosynthetic membranes from the alga subjected to distinct environmental conditions known to affect the photosynthetic machinery.
- Acclimation of the alga to limiting environmental CO2 induced specific phosphorylation of Lci5 and UEP proteins. Lci5 is associated with the stromal side of chloroplast thylakoids. The low-CO2-dependent phosphorylation acts as an early adaptive response of alga to limitation in inorganic carbon.
- The reversible phosphorylation of D1 protein is discovered in the alga, which until this work has been considered as plant specific.
- Multiple phosphorylations occur in the minor light harvesting protein CP29 under different light and redox conditions. Two sites (Thr-7 and Thr-33) are phosphorylated under state 1 condition, two additional sites (Thr-17 and Ser-103) are phosphorylated under state 2 conditions and three more sites (Thr-11, Thr-18 and Thr-20) are phosphorylated under high light.
- In contrast to all known nuclear-encoded thylakoid proteins, the transit peptide in the mature algal CP29 is not removed but processed by methionine excision, N-terminal acetylation and phosphorylation on Thr-7. The importance of this phosphorylation site is a probable reason for the transit peptide retention.
- CP29 does not belong exclusively to PSII, as it was postulated before this work, but shuttling between PSII and PSI during state transitions. The LHCI-PSI supercomplex isolated from the alga in State 2 contains strongly associated CP29 in phosphorylated form in the vicinity of the PsaH protein region. Structural changes of CP29, induced by reversible phosphorylation, determine the affinity of LHCII trimers for either of the two photosystems.
- Environmentally induced dynamic changes in protein phosphorylation at the interface between the PSII core and its associated LHCII may regulate photosynthetic light harvesting and PSII dynamics in green algae as well as facilitate State 1-to-State 2 transitions.
- High light-induced hyperphosphorylation of CP29 may uncouple this protein together with LHCII from both photosystems to minimize the damaging effects of excess light.
- This work provides the first comprehensive insight into the network of environmentally regulated protein phosphorylation in algal photosynthetic membranes and explains molecular differences in photosynthetic adaptive responses between green algae and higher plants

- This paper results provide basis for the future mutagenesis and reverse genetic studies aimed at dissecting the exact role of thylakoid protein phosphorylation in regulation of photosynthetic machinery.

REFERENCES

1. *Aboulaich, N., Vainonen, J.P., Stralfors, P. and Vener, A.V. (2004) Vectorial proteomics reveal targeting, phosphorylation and specific fragmentation of polymerase I and transcript release factor (PTRF) at the surface of caveolae in human adipocytes. Biochem J, 383, 237-248.*

2. *Allen, J.F. (1992a) How does protein phosphorylation regulate photosynthesis? Trends Biochem Sci, 17, 12-17.*

3. *Allen, J.F. (1992b) Protein phosphorylation in regulation of photosynthesis. Biochim Biophys Acta, 1098, 275-335.*

4. *Allen, J.F. (2003) Botany. State transitions--a question of balance. Science, 299, 1530-1532.* ***5.****Allen, J.F. (2005) Photosynthesis: the processing of redox signals in chloroplasts. Curr Biol, 15, R929-932.*

6. *Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981) Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. Nature, 291, 25-29.*

7. *Allen, J.F. and Forsberg, J. (2001) Molecular recognition in thylakoid structure and function. Trends Plant Sci, 6, 317-326.*

8. *Allen, K.D. and Staehelin, L.A. (1994) Polypeptide composition, assembly, and phosphorylation patterns of the photosystem II antenna system of Chlamydomonas reinhardtii. Planta, 194, 42-54. Anderson, J.M. (2002) Changing concepts about the distribution of Photosystems I and II between grana-appressed and stroma-exposed thylakoid membranes. Photosynth Res, 73, 157-164.*

9. *Anderson, J.M. and Andersson, B. (1988) The dynamic photosynthetic membrane and regulation of solar energy conversion. Trends Biochem Sci, 13, 351-355.*

10. *Andersson, B. and Anderson, J.M. (1980) Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts. Biochim Biophys Acta, 593, 427-440.*

11. *Aro, E.M., Kettunen, R. and Tyystjarvi, E. (1992) ATP and light regulate D1 protein modification and degradation. Role of D1* in photoinhibition. FEBS Lett, 297, 29-33.*

12. *Aro, E.-M. and Ohad, I. (2003) Redox regulation of thylakoid protein phosphorylation. Antioxid Redox Signal, 5, 55-67.*