LOCALISATION OF A THYLAKOID PROTEIN KINASE AND ITS RELATION TO THE 64 kDa LHC-II KINASE AND REVERSE REDOX TITRATOR.

Paul N Davies, Jens Forsberg and John F Allen.
Department of Plant Cell Biology, Lund University, Box 7007, S-220 07 Lund, Sweden.

INTRODUCTION

The phosphorylation of the light harvesting chlorophyll a/b binding protein associated with PS II (LHC-II) has a major regulatory role in the distribution of excitation energy in thylakoid membranes. The identification and localisation of the LHC-II kinase has therefore attracted significant interest [1].

In t read :-ed to localise and identify the LHC-II kinase. Firstly a method of *in situ* (in SDS-PAGE gel) renaturation and detection of kinase activity is described [2]. Histone III SS was used as a substrate in this procedure, as it has previously been shown to be a substrate for the LHC-II kinase [3]. The second approach was aimed at determining the identity of the reverse redox titrating phosphoproteins described by Silverstein *et al* [4]. The 63 kDa reverse redox titrator has been suggested as a candidate for the LHC-II kinase [5]. This approach concentrated on determining if this characteristic 63 kDa phosphoprotein cross reacts with antibodies to the LHC-II kinase isolated and purified by Gal *et al* [6].

MATERIALS AND METHODS

In situ detection of kinase activity

Pea thylakoid membranes, prepared as described in [7], were analysed on 8 % acrylamide SDS-PAGE gels either with or without the inclusion of 1 mg/ml Histone Type III SS (Sigma). Following electrophoresis the gels were either stained directly with Coomassie Blue or underwent the renaturation and [γ-^{32}P]ATP kinase assay procedure described in [2], with slight modifications.

Redox Titration and Immunodetection

The identity of the reverse redox titrating phosphoproteins were determined using the method of Silverstein *et al* [4]. All samples were analysed on 4 M Urea, 8 % acrylamide SDS-PAGE gels. For immunodetection proteins were blotted onto Flurotrans PVDF membrane (PALL). The proteins which reacted with the 64 kDa putative kinase antibody [6] were detected using the ECL detection system (Amersham).

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RESULTS AND DISCUSSION

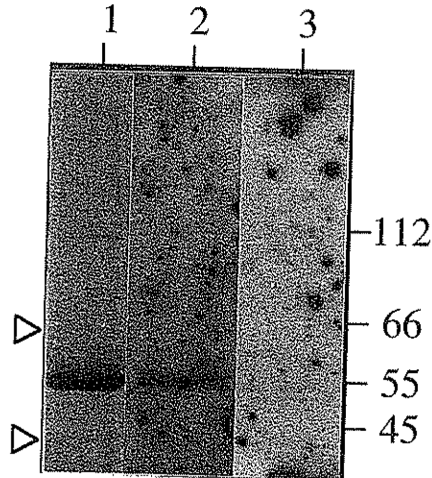

Figure 1. Thylakoid membranes were analysed on 8 % acrylamide SDS-PAGE minigels. Track 1 shows the Coomassie blue stained polypeptides present on the gel, Tracks 2 and 3, show autoradiographs of gels after the *in situ* renaturation and the [γ-^{32}P]ATP kinase assay procedure, with and without the inclusion of Histone III SS respectively. Molecular weights shown are in kDa. The reverse redox titrating polypeptides are indicated with triangles.

The *in situ* kinase assay procedure (Figure 1), reveals the presence of one kinase with an apparent molecular weight of 55 kDa. This kinase phosphorylates Histone III SS, such that a radioactive band can only be seen in track 2 of Figure 1 (when Histone is present) and no band can be seen in track 3 (when Histone is absent). Therefore the polypeptide migrating to this position on the gel is a kinase for the substrate Histone III SS, and as such may be the LHC-II kinase also [3]. It is tentatively suggested from the apparent migration rate on SDS-PAGE that this prominent 55 kDa polypeptide is the C1 ATPase. No kinase activity is detected for the 63 kDa and 46 kDa reverse redox titrating proteins in these experiments.

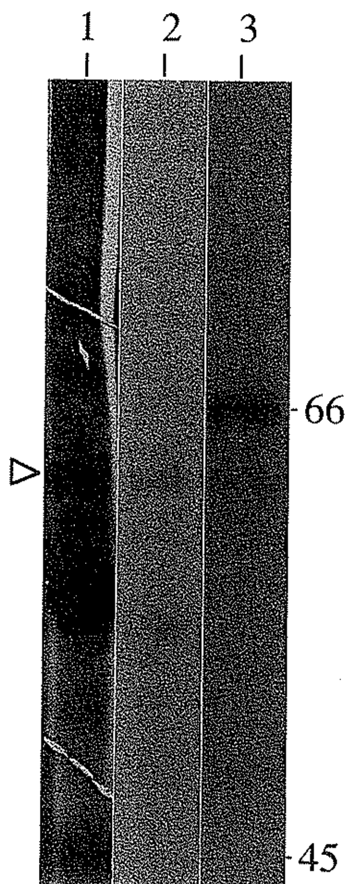

Figure 2 Thylakoid membranes analysed on a 4 M urea 8 % acrylamide SDS-PAGE gel. Track 1 shows the polypeptides present after silver staining. Track 2 shows the autoradiograph of [γ-^{32}P]ATP labelled thylakoid membranes. Track 3 shows the polypeptides which immunoreacted with the antibody to the 64 kDa putative kinase, detected using the ECL detection system. Molecular weights shown are in kDa. The reverse redox titrating 63 kDa polypeptides is indicated with a triangle.

The 4 M Urea, 8 % acrylamide gel system used in this study resolves many polypeptides not normally separated on SDS-PAGE gel systems, see Figure 2, track 1. This high resolution SDS-PAGE system reveals that the antibody to the 64 kDa putative kinase antibody cross reacts with 2 polypeptides of apparent molecular weights 66 kDa and 64 kDa. The multispecificity of this antibody is also reported in [8]. It can also be clearly seen from Figure 2 that the 63 kDa reverse redox titrating protein is one of the polypeptides immunologically detected using this antibody. This strengthens the case for the 63 kDa reverse redox titrating phosphoprotein being the LHC-II kinase [5]. No protein sequence data is available at present for this reverse redox titrating 63 kDa phosphoprotein. However, it is likely to be a different protein from the 64 kDa protein identified and sequenced by Hind and coworkers (personal communication) and Gal *et al* [9]. Furthermore, their identification of a 64 kDa kinase is now in doubt [10, 11].

The polypeptides with which the putative kinase antibody reacts (Figure 2) are clearly not the same protein as that detected using the *in situ* kinase detection procedure (Figure 1). The discrepancy between the mobility of these polypeptides may result from the absence of Mg^{++} ions in the *in situ* kinase detection procedure. $MnCl_2$ ions were included in this kinase assay, whereas it is known that the LHC-II kinase has a specific requirement for Mg^{++} ions [12]. An *in situ* kinase assay designed specifically to visualise the LHC-II kinase is therefore currently in progress.

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