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Discrimination by immunological analysis between two 33–34 kDa polypeptides involved in photosynthetic oxygen evolution

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A 34 kDa protein, which is modified in a mutant of *Scenedesmus obliquus* with impaired oxygen evolution and decreased levels of manganese, has been equated to a hydrophilic spinach 33 kDa protein. In the present study, we show immunological evidence that these two proteins are not identical. *Scenedesmus* wild type, mutant and revertant were analyzed by western blotting using antibodies against the spinach 33 kDa protein. The antibodies did not react with the 34 kDa protein, but with a 30 kDa polypeptide unaltered in the mutant. Thus, in *Scenedesmus*, and probably also in higher plants, there are two distinct proteins with similar molecular weights, both of which are essential for oxygen evolution and possibly also for manganese binding.

In the last few years a number of peptides have been indicated to participate in the photosynthetic water-oxidation process. Their identification has been based primarily on studies of mutants deficient in oxygen evolution and manganese [1] and of everted Photosystem II thylakoids with respect to protein dependent deactivation/reactivation of oxygen evolution [2,3]. Special attention has been directed to thylakoid membrane polypeptides in the molecular weight range of 33–34 kDa. A low fluorescent, non-photosynthetic mutant (LF-1) of *Scenedesmus obliquus*, which was unable to oxidize water and showed a marked decrease in manganese content, but otherwise possessed normal thylakoid structure and function, showed conversion of a 34 kDa polypeptide to a 36 kDa polypeptide. In induced revertants (e.g., LF-1 RVT-1), the recovery of oxygen evolution and normal levels of manganese were accompanied by the reappearance of the 34 kDa polypeptide [1]. A number of biochemical studies, primarily inhibitions [4–7] and reconstitutions [7,8] have suggested a hydrophilic 33 kDa protein as a component of the oxygen evolving complex and possibly also in manganese binding. A general consensus has developed suggesting that the mutational and biochemical studies reveal the same protein in the 33–34 kDa region as an essential component of oxygen evolution [1,3–5,9,10]. However, this equality is based mainly on the apparent molecular weights obtained by gel electrophoresis and recent results have suggested that there may be two different proteins in the 33–34 kDa region both related to oxygen evolution [11,12].

In this study we present direct immunological evidence that the 34 kDa protein with altered mobility in the *Scenedesmus* mutant and the hydrophilic 33 kDa protein of spinach are not related. This was done by Western blotting [13] of the polypeptides of *Scenedesmus* wild type, the mutant LF-1, and the revertant LF-1 RVT-1 [1] using antibodies raised against the 33 kDa protein of spinach. The rationale behind the experiment was to see whether the antibody cross-reacted with the 34 kDa protein with altered apparent molecular weight in the LF-1 mutant or with a different,

but closely migrating polypeptide. The serum against the 33 kDa protein of spinach has previously been shown to be highly monospecific [14], and to show cross-reactivity with other plant and algal species including various cyanobacteria [15].

Fig. 1a shows the polypeptide profile of thylakoids from the various *Scenedesmus* cell lines. The polypeptides were resolved by lithium dodecyl sulphate-polyacrylamide gel electrophoresis according to the procedure of Delepelaire and Chua [16] with 7.5–15% acrylamide gradient gels, run at 4°C and with thylakoid material corresponding to 30 μg chlorophyll per slot. The gel was stained with Coomassie Brilliant blue. Comparison of the polypeptide profiles in lane 1 (wild type) and lane 2 (mutant LF-1) shows that a 34 kDa proteins is missing in the mutant, and instead a 36 kDa protein has appeared. In the revertant LF-1 RVT-1 (lane 3) the polypeptide pattern very much resembles that of the wild type with the 34 kDa polypeptide reappearing at its original position. These observations agree with previous studies on the *Scenedesmus* phenotypes [1,17]. Polypeptides from the same gel in lane 4 (wild type), lane 5 (LF-1) and lane 6 (LF-1 RVT-1) were transferred, without prior staining, to a nitrocellulose sheet by transverse electrophoresis. The nitrocellulose sheet was incubated with the antiserum against the spinach 33 kDa protein (1 : 250 dilution), then with peroxidase-linked secondary antibodies and finally developed with 4-chloro-1-naphtol as substrate [15], according to Bio-Rad's instructions. This gives a stain at the position of the antigenic polypeptide.

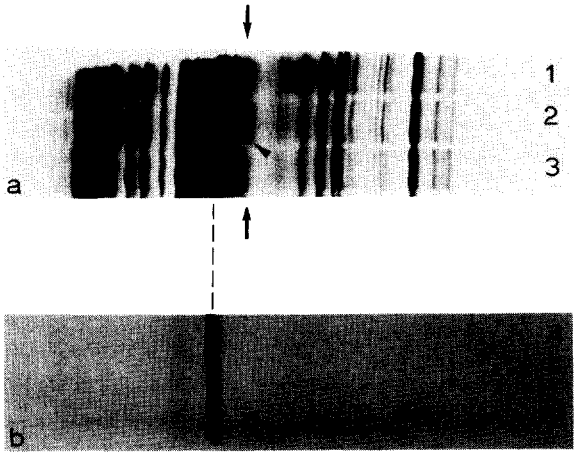

Fig. 1. (a) Polypeptide patterns of *Scenedesmus* wild type (1), mutant LF-1 (2), and revertant LF-1 RVT-1 (3) analyzed by lithium dodecyl sulphate-polyacrylamide gel electrophoresis and Coomassie brilliant blue staining. The 34 kDa protein is marked with arrows in the wild type (1) and the revertant (3) samples. Its modified form in the LF-1 mutant (2) is indicated with an arrow-head. (b) Western blotting of unstained slots corresponding to that of (a) using antibodies against the spinach 33 kDa protein. The *Scenedesmus* polypeptide cross-reacting with antibodies against the spinach 33 kDa protein was visualized by peroxidase-linked secondary antibodies using 4-chloro-1-naphtole as substrate. Notably, this polypeptide shows the same electrophoretic migration in all three samples. The weak band below the main band is chlorophyll, transferred to the nitrocellulose sheet at the position of the main chlorophyll-protein in *Scenedesmus*. The dotted line connects the immuno-stained band with its corresponding polypeptide (30 kDa) in (a).

The immunoblot (Fig. 1b) revealed only one polypeptide to have reactivity with the antibody. Strikingly, neither the amount nor the electrophoretic mobility of this polypeptide was affected in the LF-1 mutant. As is indicated in the legend to Fig. 1a, this protein has an apparent molecular weight of 30 kDa.

This analysis provides direct evidence that the 34 kDa protein affected in the *Scenedesmus* mutant with impaired oxygen evolution is immunologically distinct from the 33 kDa protein released by Tris or $CaCl_2$ extraction of everted spinach Photosystem II thylakoid preparations. Instead, the latter corresponds to a 30 kDa protein in *Scenedesmus*. Thus, the oxygen-evolving complex of this green alga contains two different protein components with similar apparent molecular weights. As will be discussed below, this also appears to be the case in spinach and other higher plants.

Indirect evidence suggesting the existence of two different 33–34 kDa proteins in oxygen evolution in accordance with this study, has recently been presented. Bricker et al. [11] showed by Triton X-114 fractionation of maize Photosystem II particles that the 34 kDa protein affected in the maize hfc-3 mutant, and probably resembling the *Scenedesmus* 34 kDa protein, was intrinsic. This is in contrast to the extrinsic 33 kDa protein of spinach. Recently, Metz and Seibert [12] have shown that Photosystem II core particles contain a 34 kDa polypeptide, while the core particles from LF-1 mutant contained a 36 kDa polypeptide [12]. This protein could not be extracted with $CaCl_2$,

indicating its intrinsic nature. An intrinsic 34 kDa protein, distinct from the herbicide binding protein, can also be resolved in spinach Photosystem II core particles [18]. Thus, spinach Photosystem II also appears to contain one intrinsic and one extrinsic protein in the 33–34 kDa molecular weight region.

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It has recently been shown that the intrinsic 34 kDa protein of *Scenedesmus* is labeled by the azido[^{14}C]atrazine photoaffinity technique (Metz, J.G., Miles, D. and Seibert, M. (1985) FEBS Lett. 185, 191–196), demonstrating that it is not identical to the extrinsic 33 kDa protein, in accordance with this work.

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