Calmodulin-like protein from the fern *Anemia phyllitidis* L. Sw.

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**Abstract.** Spores and prothallia of the fern *Anemia phyllitidis* L. Sw. contain a protein which in its physicochemical properties corresponds largely to calmodulin. It shows immunoreactivity with a calmodulin antiserum and activates bovine brain phosphodiesterase. Its content increases during the early processes of light-induced spore germination, indicating that the Ca**2**+-dependence of these processes may be mediated by this protein.

**Key words:** *Anemia* – Calmodulin – Fern (spore-germination).

**Introduction**

Calmodulin, an acidic, low-molecular-weight protein, was independently discovered as an activator of bovine brain phosphodiesterase by Cheung (1971) and Kakiuchi and Yamazaki (1970). It might exist in all eucaryotic and in some procar- yotic species (Iwasa et al. 1981; Inovye et al. 1981; Fry et al. 1986). The existence of calmodulin and its participation in many Ca**2**+-mediated processes of higher plants has been well documented (for references, see Dieter 1984), although its existence in archegoniate plants has not yet been reported. Since the induction of spore germination in several ferns by red light has been reported to be under the control of Ca**2**+ (Haupt 1985; Wayne and Hepler 1984, 1985), we analyzed the spores and young gametophytes of the schizaeaceous fern *Anemia phyllitidis* and present here the evidence that this fern produces a protein, the major proper-

ties of which match with those of calmodulins isolated from other taxonomic groups.

**Material and methods**

**Chemicals.** Phenyl-Sepharose and PD 10 columns were obtained from Pharmacia (Freiburg, FRG), and ampholine polyacrylamid gel (PAG) plates for electrofocusing from LKB-Instruments (Gräfelfing, FRG). A radioimmunoassay (RIA)-kit from New England Nuclear (Boston, Mass., USA) was used for immunodeterminations. All other chemicals were from Sigma Company (St. Louis, Mo., USA).

**Plant material and culture conditions.** Spores of *A. phyllitidis*, harvested in summer 1985 from plants grown in the greenhouses of the University of Ulm, were stored at 4°C in dark prior to cultivation under continuous white light (10 J·m**-2**·s**-1**; 21 ± 0.2°C) on a medium described by Mohr (1956).

**Isolation, purification and electrophoresis of the calmodulin-like protein from *A. phyllitidis*.** For the preparation of acetone powders, 250 mg spores or light-grown prothallia from 250 mg spores were ground in a glass-bead homogenizer (Braun, Melsungen, FRG), cooled by solid CO**2**, in 6 ml acetone. After washing four times with 10 ml acetone the insoluble material was collected, air dried and extracted with 10 ml buffer (0.05 M 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)-HCl, pH 8.0; 0.05 M KCl; 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N',N'-tetraacetic acid (EGTA); 1 mM 2-mercaptooctanol) containing 100 mg polyvinylpyrrolidone (PVP) to adsorb phenolic compounds. After centrifugation (5000·g, 10 min) the supernatant was heated in a water bath at 96°C for 5 min and the denatured proteins were removed by centrifugation (30000·g; 20 min). For further purification, affinity chromatography on phenyl-Sepharose (Gopalakrishna and Anderson 1982) was used. After lyophilization, samples, desalted by chromatography on PD 10 columns, were separated by electrophoresis on 15% polyacrylamide containing 0.2% sodium dodecyl sulphate (SDS; Laemmli 1970). Isoelectric focusing was performed on LKB Ampholine PAG gels (pH 3.5–9.5) with 1 M H**3**PO**4** as anode solution and 1 M NaOH as cathode solution.

**Calmodulin determination.** Calmodulin activity was determined by phosphodiesterase assay as described by Watterson et al. (1980a) using high-performance liquid chromatography.

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**Abbreviations:** EGTA = ethylene glycol-bis(β-aminoethyl ether)-N,N',N'-tetraacetic acid; RIA = radioimmunoassay
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![Phenyl-Sepharose chromatography of calmodulin from A. phyllitidis.](image)

Results and discussion

Extracts of acetone powders of dry spores and of young, light-grown prothallia of *A. phyllitidis* contained a non-dializable material which stimulated bovine brain phosphodiesterase six-to seven-fold. Comparable rates of stimulation have been obtained in our experiments with bovine brain calmodulin preparations. As described for calmodulin isolated from other sources, this protein fraction proved to be heat stable (5 min; 96°C). For further purification, affinity chromatography on phenothiazine-Sepharose (Caldwell and Haug 1981), modified after Watterson et al. (1980b) as well as hydrophobic interaction chromatography on phenyl-Sepharose (Gopalakrishna and Anderson 1982) were used (Fig. 1). Since the latter method yielded higher purity, it was used for further characterization of the calmodulin-like protein.

Calmodulin from *A. phyllitidis* migrated in SDS-polyacrylamide gels with an apparent molecular weight of ~18000 Da, slightly behind spinach calmodulin (Fig. 2A) and consistent with other plant and protozoan calmodulins (Burgess et al. 1983; Klee and Vanaman 1982). The mobility was calcium-dependent and shifted in the presence of this ion to a lower molecular weight while, simultaneously, double banding was observed (Fig. 2B). The apparent molecular weights in the presence of Ca²⁺ were ~14000 Da and ~15000 Da, respectively. Such a change in mobility, depending on Ca²⁺ ions, is a general feature of calcium-binding proteins (Burgess et al. 1980). Calmodulin from *A. phyllitidis* migrated as a single band in isoelectric-focusing gels (Fig. 3). Its charge corresponded with bovine brain preparations.

![Electrophoretic analysis of calmodulin from A. phyllitidis.](image)

![Isoelectric focusing of bovine brain calmodulin (lane 1), spinach calmodulin (lane 2) and Anemia calmodulin (lane 3).](image)