Photoaffinity labeling and partial purification of the putative plant receptor for the fungal wilt-inducing toxin, fusicoccin

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Abstract. The high-affinity fusicoccin-binding protein (FCBP) was solubilized from plasma-membrane vesicles prepared from leaves of *Vicia faba* L. by aqueous two-phase partitioning. Conditions for the solubilization of intact FCBP-radioligand complexes were worked out. About 60–70% of the complexes can be solubilized with 50–60 mM nonanoyl-N-methylglucamide in the presence of 1 mg ml⁻¹ soybean phosphatidylcholine, type IV S, and 20% (v/v) glycerol at pH 5.5. The slow dissociation of the radioligand, 9'-nor-fusicoccin-8'-alcohol-[^3]H from the FCBP at low temperatures permits the purification of FCBP-radioligand complexes at 4–10 °C by fast protein liquid chromatography on anion-exchange and gel permeation columns. The FCBP, extracted from plasma membranes with cholate and chromatographed in the presence of this detergent, gave an apparent molecular mass (Mₐ) of 80±20 kDa on gel permeation columns under the conditions used. By comparison of the elution profiles of the fraction most enriched in FCBP-radioligand complexes with the new compound 9’-nor-8’[(3,5-[^3]H]-4-azidobenzoyl)ethylenediamine]-fusicoccin ([^3]H)ABE-FC) and subsequent separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis labeled a single band with an Mₐ of 35±1 kDa. Labeling in this band was strongly reduced when the membranes were incubated with[^3]H]ABE-FC in the presence of 0.1–1 μM fusicoccin. From our data, we conclude (i) that the 34–35-kDa polypeptide represents the FCBP and (ii) that in detergent extracts of plasma membranes this polypeptide is probably present as a di- or trimeric structure.

Key words: Fusicoccin – Fusicoccin-binding protein – Photoaffinity labeling – *Vicia*

Introduction

The action of the *Fusicoccum amygdali* Del. toxin, fusicoccin (FC) on cells of higher plants involves a drastic acidification of the apoplastic space concomitant with a strong hyperpolarization of the plasma-membrane potential. As a consequence, ion and metabolite fluxes across the plasma membrane are altered, the osmotic potential of the cell increases and the cell-wall plasticity decreases, leading to increases in growth rate in many plant tissues (for review see Marré 1979). The understanding of the molecular events of FC action will provide insights into such key processes of plant cell function as the regulation of plasma-membrane energization and ion and metabolite transport across the cell membrane. The mechanism of FC action, however, is still unknown, but might involve the stimulation of H⁺-extrusion across the plasma membrane (Rasi-Caldogno et al. 1986) and – or the inhibition of leak currents by the toxin
Kinetic studies have revealed that FC binds rapidly and with high affinity to microsomal sites (Dohrmann et al. 1977; Ballio et al. 1980; Stout and Cleland 1980; De Boer et al. 1987; Feyerabend and Weiler 1988). These sites are associated with the plasma membrane (Ballio 1982; Feyerabend and Weiler 1988), with the FC-binding domain facing the apoplastic space (Feyerabend and Weiler 1988). Chemical and enzymatic studies indicate that the binding sites are glycoproteins (Aducci et al. 1984; Feyerabend and Weiler 1988). There is a significant correlation of the biological properties of FC and related structures and their binding sites are glycoproteins (Dohrmann et al. 1977; Ballio et al. 1980). The sum of all available data indicates that the FCBP is the plant’s toxin receptor. However, the FCBP has not been identified biochemically to date and methods for its isolation are lacking. In this report, we describe the synthesis, properties and use of a tritium-labeled, biologically active azido-analogue of FC. This compound is used to irreversibly target by FCBP and to identify it on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The data are compared with those obtained from the partial purification of the FCBP (as the reversible FCBP-radioligand complex), using 9’-nor-fusicoccin-8’-alcohol-[3H]([3H]FCol) (Feyerabend and Weiler 1988) as the radioligand. In both cases, a polypeptide with an apparent relative molecular mass (Mr) of 34–35 kDa was identified as the FCBP.

Material and methods

Chemicals and equipment. Cholate was purchased form Serva, Heidelberg, FRG, dextran T 500 from Pharmacia, Freiburg, FRG, and 4-azidobenzoic acid N-hydroxysuccinimide ester from Pierce, Rockfield, Illinois, USA. Nonanoyl-N-methyl glucamide (Mega-9) was from Oxyb Chemie, Bobingen, FRG and polyethylene glycol 3350 from Union Carbide, Düsseldorf, FRG. The fast protein liquid chromatography (FPLC) equipment consisted of two pumps P 500, gradient programmer GP 250, monitor UV 1, valve MV-7 with Superloop 50 ml and fraction collector FRAC 100 (all from Pharmacia). Fractogel TSK DEAE 650(S) and Fractogel TSK 55-W(S) were obtained from Merck, Darmstadt, FRG. All other gels were from Pharmacia.

Preparation of membrane vesicles. Microsomal membranes were prepared from leaves of three-week-old Vicia faba L. as described previously (Blum et al. 1988; Feyerabend and Weiler 1988). Plasma-membrane vesicles were enriched from the microsomal fraction by partitioning between dextran T 500 and polyethylene glycol 3350 (Larsson et al. 1984; Blum et al. 1988; Feyerabend and Weiler 1988). Membrane protein was determined by the Bradford assay (Bradford 1976) using bovine serum albumin (BSA) as the standard.