Aminopeptidases of Basidiomycetes

R. BLAICH

Lehrstuhl f. Allgemeine Botanik, Ruhr-Universität Bochum

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Aminopeptidases (AP) are useful tools for the sequence analysis of peptides [1]. Therefore a systematic research for types with narrow substrate specifity seems desirable. These enzymes are known in animals, higher plants and microcorganisms (for lit. see COLOWICK and KAPLAN [2], SCANDALIOS [3], KOHLEHMAINEN and MIKOLA [4]). Whereas there exists some information on AP isozymes in yeast and other Ascomycetes (for lit. see MATILE et al. [5], LEHMANN and UHLIG [6]), our knowledge on their occurence in Basidiomycetes is restricted to the description of two Leu-AP bands in *Schizophyllum commune* [7] and *Trametes versicolor* [8].

To bridge this gap we studied the AP spectra of 30 wood-rotting Basidiomycetes by disc-electrophoresis of partially purified mycelial extracts and concentrated culture media, respectively. The isozymes are easily detectable in electrophoresis gels by their ability to cleave the peptide bonds of artificial substrates (e. g. aminoacid arylamides), the intensity of the staining being dependent on the aminoacid moiety. This facilitated our studies on the substrate specifity of the individual isozymes, where we used 10 different aminoacid-β-naphtylamides. Some enzymes were purified to be tested with natural substrates. Polyporaceae exhibit a complicated AP spectrum consisting of at least 6 bands with partly different substrate specifity as exemplified in *Trametes versicolor* (Fig. 1 a). Other species of this taxon yield similar spectra which are typically composed of 1) a group of bands (Fig. 1a: 1 – 3) with medium R_f-value, representing the common Leu-AP type. The culture filtrate regularly contains one or two isozymes which according to their substrate specifity and electrophoretic mobility correspond to the mycelial bands indicated by arrows; 2) one band or sometimes double band (4) which is more or less specific (depending on the species) for arginine- and lysine-moieties; 3) one band with low electrophoretic mobility (6) which seems to be of high molecular weight and is specific for aspartic and glutamic acid.

In contrast, the spectrum of two typical Agaricaceae *(Coprinus plicatilis* and *Kuehneromyces mutabilis)* is not homologous with the typus of the Polyporaceae (Fig. 1 b) except band 3 (Asp-AP). In the *Lentinus* and *Collybia* group there exist, however, intermediary types of spectra.

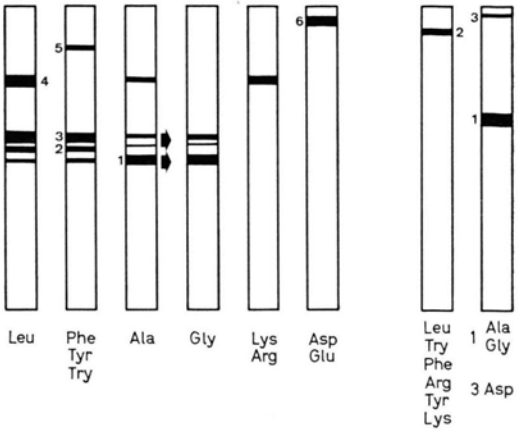

Fig. 1 a. Typical spectrum of the intracellular aminopeptidases of a polypore fungus *(Trametes versicolor)* in standard disc-electrophoresis; gels strained with different aminoacid-naphtylamides (Leu = Leucinenaphtylamide etc.) and Fast Blue B. Anode at the bottom. Band 1 and 3 (arrows) are found also in the culture filtrate.

Fig. 1 b. Spectrum of two Agaricaceae *(Kuehneromyces mutabilis* and *Coprinus plicatilis)*.

The Leu-AP group of *Pleurotus ostreatus* was purified by fractionated precipitation and molecular sieving. As revealed by thin layer chromatography, the enzyme is able to cleave Leu-nitranilinide, the substrates mentioned in Fig. 1, Leu-Ala, native ribonuclease, insuline and RSA, but not Gly-Gly and Gly-Gly-Gly. Its pH-optimum lies around 8 and it is not inhibited by EDTA and DTNB even at concentrations of 0,1 M indicating the absence of metal or SH groups in the active center.

Besides their taxonomic significance, these results indicate that Polyporaceae might be a valuable source of new, highly specific aminopeptidases. A detailed account of our studies will be published elsewhere.

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Requests for reprints should be sent to Dr. R. BLAICH, Lehrstuhl für Allgemeine Botanik, Ruhr-Universität Bochum, *D-4630 Bochum*, Postfach 2148, W.-Germany.

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