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A Comparative Study of a Cationic Peroxidase from Peanut and an Anionic Peroxidase from Petunia

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Summary

A comparative study on a pure cationic and a pure anionic protein from peanut cells and petunia stem tissue respectively, both with peroxidative activity, was made. The cationic protein weighs 44 Kd and the anionic 36 Kd. No immunological cross reactivity could be detected between the two proteins. In assays for peroxidative activity using the substrates 4-aminoantipyrine, guaiacol and eugenol it was noted that the anionic protein had 1.9, 12.7, and 27.7 fold greater enzymatic activity, respectively. For overall peroxidative measurements it is suggested that aminoantipyrine is probably the superior substrate. With regard to IAA oxidase activity of the two protein fractions it was noted that the cationic enzyme possessed optimal activity at pH 3.6 and the anionic protein at pH 7.0. The latter value could only be obtained by the addition of H_2O_2 and dichlorophenol (DCP). Since no additives were needed for the assay of IAA oxidation by the cationic protein it is suggested that this is a true IAA oxidase while the anionic fraction is a peroxidase involved in other reactions such as lignin biosynthesis.

Introduction

Genetic (Tanksley and Rick 1980; van den Berg and Wysman 1981, 1982a,b; van der Berg et al. 1982) and molecular (Ricard 1969; Shih et al. 1971; Mazza and Welinder 1980; Stephan and van Huystee 1981) studies have shown that peroxidase in higher plants is coded by several genes as shown by isozymal expression.

Yet with several functions such as IAA oxidation (Ricard 1969) and lignification (van Huystee and Cairns 1982), no specific activity for individual isozymes has been ascertained. Whether or not the isozyme has a particular function or is an artifact remains a major question until a comparative study on 2 isolated proteins with peroxidative activity can be performed (van Huystee and Cairns 1980).

We now report on a comparative study with a pure cationic peroxidase from peanut (Maldonado and van Huystee 1980) and an anionic peroxidase from petunia (van den Berg and van Huystee 1983). In the study comparisons are made on purity and molecular weight, immunological relatedness, and substrate specificity of the two proteins. Each protein possess the major peroxidative activity in the extra cellular fluid.

Materials and Methods

Peanut cells derived from Virginia 56R type seeds, were maintained routinely in suspension culture in 14 day cycles (Kossatz and van Huystee 1976).

Petunia plants of the F2 progeny (W115 x R51) were grown under standard flower-inducing greenhouse conditions for about 4 months. Line W115 was derived from a cross involving P. axillaris and P. hybrida cv "Rose of Heaven" (Ausubel et al. 1980), and the line R51 was derived from P. hybrida cv "Royal Ruby".

The cationic protein was isolated by acetone precipitation and chromatography on CM cellulose from medium that had supported the growth of peanut cells (Maldonado and van Huystee 1980). The anionic protein was obtained from the intercellular fluid of petunia stems and purified by DEAE cellulose chromatography (van den Berg and van Huystee 1983). Antibodies were raised against either fraction by injection of New Zealand rabbits with 1.5 ml solution containing complete Freund adjuvant and an aliquot of either protein fraction. For the cationic protein 1.5 mg was injected twice over two weeks and for the anionic protein 0.15 mg was injected trice over 3 weeks. Serum was harvested after 4 weeks (van Huystee 1976).

The electrophoresis of the protein fractions was carried out in 12% polyacrylamide gels containing 0.1% SDS as described by Maldonado and van Huystee (1980). Ouchterlony immunodiffusion and one dimensional immuno-electrophoresis was carried out with veronal buffer as described (van Huystee 1976).

Spectrophotometric peroxidase assays were carried out with 100 µl of appropriate protein dilution and one of the following three substrate media in a total volume of 1 ml. For 4-aminoantripyrine 2.5 mM with 0.17 M phenol was used. In the assays with eugenol and guaiacol a solution of 0.01 M and 0.1% respectively were used. Essentially the procedures described in the Worthington enzyme manual (1978) were used for the aminoantipyrine and the procedures by Srivastava and van Huystee (1977) were used for eugenol and guaiacol assays. The assays for IAA oxidase were detailed earlier (Srivastava and van Huystee 1977). The assay medium contained 0.5 mM IAA alone or required additions of 2 mM DCP and 0.015% H_2O_2. Protein determinations were made by the techniques of Lowry et al. (1951) and/or Bradford (1976).

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Results and Discussion

The results of Fig. 1 show the relative purity of both anionic and cationic fractions as well as the differences in molecular weight of 36 Kd and 44 Kd, respectively. Due to the glyco-proteinaceous nature of peroxidase the protein band is not often compact. A slight dissociation of the anionic protein band into 2 with near similar Rm suggested some micro-heterogeneity. Purity of the two proteins is also demonstrated by the high RZ values of each protein (Table 1). Assays for molecular relatedness between the two proteins by immunodiffusion gave negative results (Fig. 2). Serial dilution test for antigen

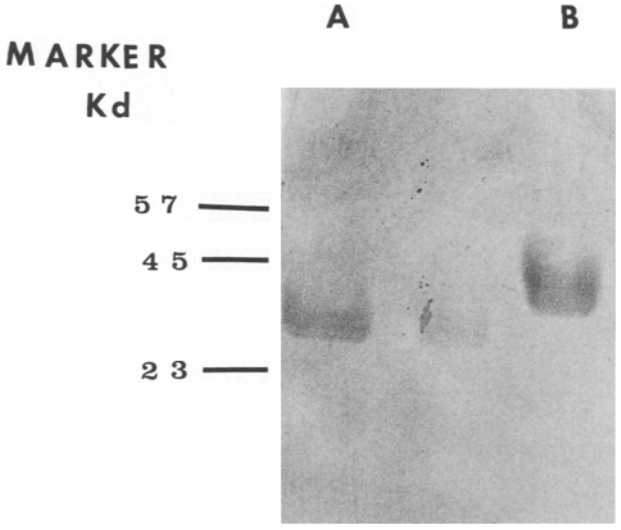

Fig. 1. SDS-PAGE of the anionic petunia protein and the cationic peanut protein. Channel A represents 10 µg of petunia protein isolated from intercellular space of stem tissue. Channel B represents 25 µg of peanut protein isolated from suspension medium. The gel was stained with Coomassie Blue.

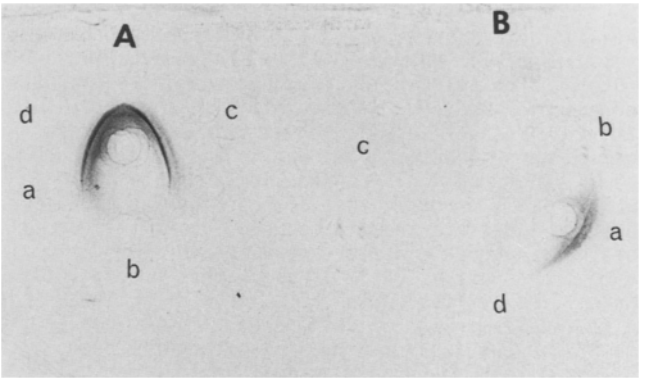

Fig. 2. Ouchterlony immunodiffusion assays with the petunia and peanut proteins and their respective antiserum. A. The antiserum (centre well) against the cationic peanut protein is challenged with a) the anionic petunia protein (7.5 µg); b) crude petunia (56 µg); c) unfractionated peanut peroxidase (200 µg); d) major cationic peanut peroxidase (50 µg). B. Centre well with serum raised against anionic petunia a protein being challenged by the same complement as under A.

Table 1. Peroxidase and IAA-oxidase activities of the cationic protein fraction from peanut cells grown in suspension culture and anionic fraction from petunia stems.

Fraction	RZ[1]	Peroxidase sp. Activity[2]			IAA-oxidase[3] sp. Activity
		Substrate			
		4-amino-antipyrine	eugenol	guaiacol	µg IAA oxidized mg^{-1} protein/ 40 min
Cationic	3.0	61	146	511	88
Anionic	3.6	118	3,879	6,466	nd

[1] RZ = Reinheitzahl value = $\frac{\text{OD at 405}}{\text{OD at 280}}$

[2] 4-aminoantipyrine units were calculated as $\frac{\Delta A\ 510.min^{-1}}{6.58 \times mg\ enzyme^{-1}.ml\ reaction\ mixture^{-1}}$. Eugenol and guaiacol units were calculated as ΔOD at 425 and 480 nm, respectively min^{-1}, mg^{-1} protein.

[3] IAA-oxidase activity in the absence of H_2O_2 and cofactors such as, DCP and $MnCl_2$.

[4] nd = nondetectable

and antiserum reaction arcs also rendered negative results. This result was further corroborated by one dimensional immunoelectrophoresis. While the antigen used to raise antibodies gave positive results with the latter for both proteins, there was no evidence of cross reactivity by reciprocal assays. The lack of relatedness was not unexpected, considering the different charges on the proteins and the significant variation in molecular weight.

Therefore, with such different protein fractions, purified from extracellular proteins of petunia and peanut, a comparison on the basis of peroxidase specificity to various substrates was made. The data in Table 1 show that the anionic protein has a greater specific enzyme activity than the cationic protein. For aminoantipyrine, eugenol and guaiacol the anionic has 1.9, 27.7, and 12.7 fold greater activity, respectively. The cationic protein does possess peroxidase activity but it has much less than the anionic fraction. Considering the data in Table 1 it is proposed that aminoantipyrine is a more suitable substrate than those used formerly since it does not exaggerate differences. Conversely, in terms of IAA oxidation it is plain that proper action can only be attributed to the cationic fraction. While studies on IAA oxidation normally include H_2O_2 and DCP in the assay medium it has recently been suggested that the proper activity measurements should be conducted in the absence of these compounds (Nakajima and Yamazaki 1979). In the presence of these components the pH optima for the reaction of the cationic protein is 3.6 while that for the anionic protein is 7.0 (Fig. 3). Most studies on IAA oxidase have shown that the physiological optimum is closer to pH 3.6 than 7.0 (Hare 1964). This supports further the contention that only the cationic protein possesses true IAA oxidation potential. Conversely, it can be argued that the anionic peroxidase from petunia is probably the enzyme to employ in studies of lignin biosynthesis and concomittant tracheary element differentiation (Fukuda and Komamine 1982).

In conclusion, the comparison between two pure proteins with peroxidase activity indicated specific differences not only on the basis of immunological relatedness but also on the basis of molecular weight and on functional specificity.

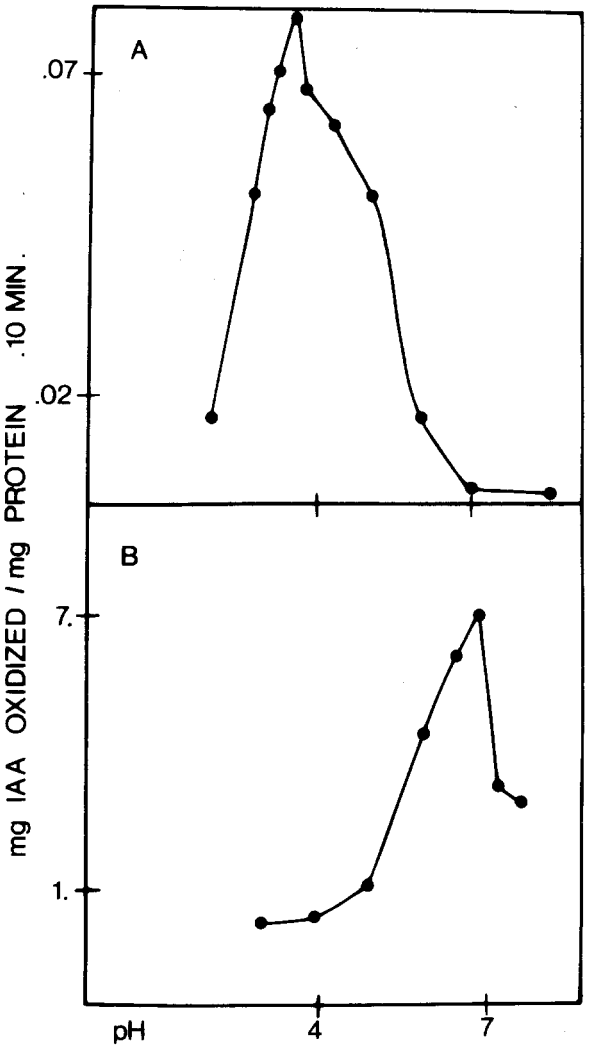

Fig. 3. Comparison of IAA oxidation by the cationic (A) and the anionic protein (B) at various pH values. For the cationic protein (A) reaction no additives were required while for the action of the anionic protein (B) both H_2O_2 and DCP were essential for any reaction.

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