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SPECTRAL AND MOLECULAR PROPERTIES OF PEANUT PEROXIDASE ISOZYMES

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Key Word Index—*Arachis hypogaea*; Leguminosae; peanut peroxidase isozymes; spectral and molecular properties; molecular sizes; Stoke's radius; heat stability; substrate specificity.

Abstract—Properties of four peroxidase isozymes derived from peanut cells were examined. Electrophoresis on various concentrations of polyacrylamide gel indicated that they had the same molecular size. Filtration on Sephadex G–200 gels indicated the same Stoke's radius for all 4 isozymes. They had the same spectral properties in the oxidized, reduced and CO-reduced the pyridine hemochromogen forms, but they differed with regard to heat stability at 50° and 70° and their substrate specificity.

INTRODUCTION

Peroxidase isozymes are released into the medium by cells of several cultures [1–3]. In addition many compounds such as polysaccharides and phenolics are also released by cells of various cell suspensions into the culture media [2, 3]. Such compounds could affect peroxidase and several other enzymes in various ways [4,5]. Several pieces of evidence have suggested that peroxidase isozymes found in the medium of peanut cell suspension are interconvertible, either as a result of phenolic interactions or due to conformational changes [6]. Such interactions might have resulted in changes in spectral and molecular properties of isozymes. In the preceding report [7] peanut peroxidase isozymes were found to differ in their catalytic properties. Thus, an examination of the spectral and molecular properties of these isozymes would be complementary.

Peroxidase isozymes isolated from horseradish roots and from Japanese radish are well characterized in terms of their catalytic and physicochemical properties [8–11]. However, peroxidase isozymes, found in culture medium of various cell suspension are not well characterized. In this report, the spectral and molecular properties of 4 peanut peroxidase isozymes are described.

RESULTS AND DISCUSSION

In the native (oxidized) form, the anionic isozymes (A_1, A_2, A_4 and A_5) had absorption peaks at 403(γ), 445 520β (weak), 550(α) and 650 nm. The difference absorption spectra of sodium dithionite reduced isozyme/native isozyme, CO-reduced isozyme/native isozyme, alkaline isozyme/native isozyme and pyridine hemochromogen form of isozyme/native isozyme revealed a shift in the Soret band (γ-band) from 403 nm to a longer wavelength of 410–415 nm.

The similarities in absorption spectra of the isozymes are an indication of the presence of the same heme moiety. The positions of α, β and γ bands depend on the nature of metal of proteins e.g. the stable chelates with metals such as Ni (II), Co (II) have an order intensities $\alpha > \beta$ whereas in the less stable chelates such as those of Mg (II), Cd (II) the ratio is decreased or reversed [12]. A higher ratio of the α band to the β band in the peanut peroxidase isozymes might be indicative of a stable chelate of protein with iron. The shifts in Soret band of peanut peroxidase isozymes in sodium dithionite reduced form, CO-reduced form, alkaline form and pyridine hemochromogen forms were similar to the shifts reported earlier for tomato peroxidase isozymes [13].

All 4 isozymes had nearly the same molecular sizes and MWs of 60000 as determined by polyacrylamide gel electrophoresis and Sephadex gel filtration techniques. From a Sephadex G-200 column, they all had the same

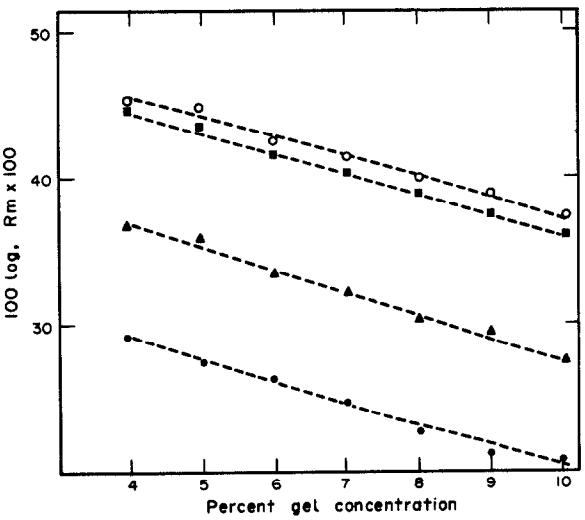

Fig. 1. Molecular size determination of isozymes by polyacrylamide gel electropohresis. —O— isozymes A_1, —■— isozyme A_2, —▲— isozyme A_4 and —●— isozyme A_5.

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Stoke's radius of 3 nm and therefore they probably possessed the same MW of 60000.

During the molecular size determination on polyacrylamide gel electrophoresis, a plot of R_m values of the isozymes as 100 log (R_m × 100) against increasing concentrations of acrylamide in gel (from 4 to 10%) was prepared. Parallel lines were obtained for the isozymes (Fig. 1). According to Hederick and Smith [14] such parallel lines indicate similar molecular sizes but different charges of protein isomers.

A wide range of MWs has been reported in the literature for peroxidase isozymes from various sources, e.g. three ribosome-associated peroxidase isozymes from lentil roots MW 33400, 45000, 57000 [15], turnip peroxidase isozymes MW 33000, 42000 and 51000 [16], kidney bean peroxidase isozymes MW 6000 and 30000 [2], red alga peroxidase isozymes MW 40000 and 50000 [17] and 5 horseradish peroxidase (HRP) isozymes MW 40000 [8]. Thus the MWs of peroxidase isozymes from various sources ranged between 30000 to 60000. In addition, the 4 HRP isozymes possessed the same MW as in the case of peanut peroxidase isozymes.

Although the peanut peroxidase isozymes had similar spectral and molecular properties, they differed in their catalytic properties [7]. Differences were also observed among the isozymes in their substrate specificity and heat stability.

A variety of substrates were utilized by the isozymes during peroxidase activity (Table 1). However, each isozyme differed in their specificity for these substrates. The list of compounds which may be utilized as H-donors during the peroxidative reaction is large [18]. Therefore, it would be important to know if a difference in efficiency of isozymes occurs to utilize the same H-donor. A difference may also reflect differential biological functions of isozymes *in vivo*. There are at least 3 classes of compounds in cells whose formation is linked to the enzyme activity of peroxidase, (a) halogenated compounds, e.g. iodotyrosine and di-iodotyrosine [19], (b) dityrosine [20], (c) lignin [21]. Apparently, peanut peroxidase isozymes can utilize all the 3 classes of compounds as substrates although with different efficiency.

The 4 isozymes behaved differently during heat treatment at 50° and 70°. At 50°, an initial increase in the peroxidase activity of each isozyme was followed by a decline. After 1 hr at 50°, A_4 and A_5 showed a 20–25% decline in their peroxidase activity whereas no appreciable loss in activities of A_1 and A_2 occurred (Table 2). After 2 hr treatment, a decline in the activities of the isozymes was noticed. A_2 was the most stable, showing only a loss of 4% in peroxidase activity, followed by A_1 and A_5 (20% activity decline) and A_4 (66% activity decline). In order to determine if the peroxidase activity could be recovered after 3 hr at 50°, the treated isozyme solutions were immediately transferred to ice. A recovery of 4–7% in peroxidase activities of A_4 and A_5 occurred whereas no such recovery was observed for A_1 and A_2. The treatment of isozymes at 70° for only 15 min caused a decline of 88% and 95% in the peroxidase activity of A_1 and A_2 respectively. A similar decline in activities of A_4 and A_5 occurred after 15 min of treatment. After 3 hr at 70°, the isozymes lost nearly all their activity. Peroxidase activities did not increase on cooling.

Table 2. Effect of treatment at 50° on peroxidase activity of isozymes

Isozymes	Percent peroxidase activity remaining			
	15 min	60 min	120 min	180 min
A_1	108	94	81	78
A_2	103	101	96	70
A_4	125	75	34	26
A_5	96	80	80	72

Peroxidase from various sources has been shown to be relatively heat stable. Misawa and Martin [2] found a loss of 54–57% in peroxidase activity of bush bean isozymes during treatment at 50° for 20 min although HRP isozymes were unaffected. Further, they observed that treatment at 70° for 20 min caused 80–100% loss in the activity of bush bean isozymes and only 52% loss in HRP isozymes. Delincee *et al.* [22] found during the 90° treatment of HRP that molecular aggregation and modification of monomeric enzyme units occurred. However, preliminary results with peanut peroxidase isozymes indicate that a similar phenomenon did not occur during treatment at 50° and 70°.

EXPERIMENTAL

Spectral studies of isozymes. The absorption spectra of peroxidase isozymes and their derivatives, e.g. Na dithionite reduced, CO-reduced, alkaline and Py hemochromogen forms were recorded with a 1 cm light path. The Na dithionite reduced, CO-reduced and alkaline forms of peroxidase were prepared as described in ref. [13]. The reduced Py hemochromogen form of peroxidase was prepared using the technique of ref. [23] with some modifications [13].

The MWs of the isozymes were determined using Sephadex

Table 1. Substrate specificity of peroxidase isozymes

Substrates	λ for assay (nm)	Specific activity of isozymes*			
		A1	A2	A4	A5
Vanillin	425	0.003	0.014	0.17	0.00
Eugenol	425	0.21	0.83	0.03	0.57
p-Coumaric acid	500	0.006	0.002	0.013	0.01
Syringaldehyde	525	0.012	0.006	0.00	0.004
p-Hydroxybenzaldehyde	600	0.009	0.00	0.00	0.00
Benzidine	600	0.29	2.07	2.68	3.5
Potassium iodine	420	0.818	1.15	0.6	1.72

* Sp. act. is defined as e.u. per mg of protein. One e.u. was defined as change in one *A* per min. The values reported are the mean of 3 replicates.

G-200 gel-filtration column [24]. A gel bed (2.5 × 86 cm) was used and cytochrome *c*, alcohol dehydrogenase, jackbean urease and fibrinogen were used to calibrate the column. Molecular sizes of isozymes were determined by polyacrylamide gel electrophoresis with a variable concentrations of acrylamide (4–10%). The isozymes were stained for peroxidase activity on gels using Schrauwen's technique [25]. A plot of 100 log (R_m × 100) against polyacrylamide concentrations was prepared for each isozyme.

Substrate specificity determination. The compounds used are listed in Table 1. The reaction mixture contained 0.5 ml of an isozyme prepn, 1.5 ml of 0.05 M Pi buffer, pH 7, 0.5 ml of 0.01 M H-donor, and 0.5 ml of 0.3% H_2O_2. The change in *A* per min for 5 min was recorded (Table 1).

Effect of heat treatment on isozymes. The isozyme prepns were incubated at 50° and 70° in 0.05 M Pi buffer pH 7. Samples were withdrawn at various intervals and peroxidase activity was determined immediately using guaiacol as H-donor. From peroxidase activity remaining after treatment, the % loss in peroxidase activity of isozymes was calculated.

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