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Production and Preliminary Characterization of Monoclonal Antibodies against Cationic Peanut Peroxidase[1]

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ABSTRACT

Ten monoclonal antibodies (McAbs) have been produced against the cationic peroxidase from peanut suspension cell culture. Eight of these antibodies were found to be of the immunoglobulin (Ig)G_1 subclass and two were of IgA subclass. A combination of competitive enzyme-linked immunosorbent assay, Western blotting analysis, and direct antigen-binding assay revealed that the antibodies are directed against four different epitopes on the cationic peroxidase and the McAbs can be subdivided into four groups. Only group A inhibits peroxidase activity. Group B and D bind equally well to the native and the denatured form of cationic peroxidase, whereas the remaining McAbs react with more or less reduced affinity to the denatured antigen. Group C probably recognizes a conformation-dependent epitope. All the McAbs cross react weakly with the anionic peanut peroxidase, suggesting a structural nonidentity as well as some similarity between these two peroxidase isozymes. Cross reactivities of these McAbs with peroxidases of various plant species were also demonstrated.

Anionic and cationic peroxidases occur in peanut suspension cell culture (16), with little differences in function (4) but probably in compartmentation. Nearly ¾ of all peroxidase activity recovered in culture medium is associated with the cationic peroxidase (12). Consequently the question arises as to the reason(s) for the differential secretion of peroxidases and the structural relationship between the two isozymes. Peptide mapping (13, 16, 23) and immunochemical comparisons (5, 6) of isoperoxidases have revealed that the cationic isozymes are distinct from the anionic ones. However, specific structural differences have yet to be identified. This may be done with the use of McAbs[3] (9, 14).

In this report, we describe the production and preliminary characterization of 10 McAbs raised against cationic peanut peroxidase and discuss their potential usefulness in identifying structural and functional domains within the isozymes.

MATERIALS AND METHODS

Derivation of the Peanut Peroxidase. Peanut peroxidase was obtained and isolated from the medium of cultured peanut cells as reported (4).

Production of Monoclonal Antibodies. Two separate fusions of the nonsecreting mouse myeloma cells P3-NS1-Ag4-1 with spleen cells from BALB/c mice immunized with the cationic peanut peroxidase were carried out. Purified peroxidase ((RZ = 3), 100 μg) mixed with an equal volume of complete Freund's adjuvant was injected intraperitoneally in each mouse, followed by a booster containing 200 μg of peroxidase 5 months later. The mice with the highest titers to the antigen, 10^{-4} dilution in the first trial and 10^{-6} in the second trial, were boosted with 200 μg peroxidase in sterile PBS 3 d prior to the removal of the spleen. The spleen cells were fused with the myeloma cells in 10:1 ratio according to the protocol of Goding (8). The fused cells were distributed in 96 microwell plates (Vanguard Intl.) in Iscoves Modified Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum (FCS) (Gibco Lab.) and containing penicillin (50 units/ml), streptomycin (50 μg/ml), aminopterin (17.6 μg/ml), thymidine (3.8 μg/ml) and hypoxanthine (136 μg/ml). The hybridomas were cultured at 37°C in a humidified CO_2 (5%) incubator. The ability of the secreted antibodies to bind to peroxidase was screened by ELISA (see below). Monoclonal lines were derived by application of the limiting dilution technique. Cell lines positive to the peroxidase were frozen in IMDM containing 10% FCS and 10% DMSO to −70°C and stored. McAbs were derived either by further cell cultures or by eliciting ascites tumours in mice (3). In brief, pristane (Sigma Co.) primed BALB/c mice were injected with 10^7 hybridoma cells/mouse. The ascites fluid from 500 g supernatant was stored at −20°C. Monoclonal antibodies from culture medium were concentrated through precipitation with 50% saturated $(NH_4)_2SO_4$ and resuspended in water and then dialyzed against 20 mM Tris-15 mM NaCl (pH 7.2) containing 0.02% NaN_3. McAbs partially purified by $(NH_4)_2SO_4$ precipitation were diluted according to their titers and used in the following experiments except where otherwise indicated.

ELISA. Each well of a polystyrene microtiter plate (Dynatech) was coated overnight at 4°C with 50 μl (10 μg/ml) cationic peroxidase (RZ = 3.0) in 0.05 M sodium carbonate buffer (pH 9.6). The nonspecific protein binding sites of the wells were blocked by incubation with 2% gelatin in PBS at 37°C for 30 min. The plate was washed 3 times with PBS containing 0.05% Tween 20 and 0.1% gelatin (washing buffer), then 50 μl of the culture supernatant were added to each well. After 2 h incubation at 37°C, the plate was washed as above. The alkaline phosphatase conjugated-goat anti-mouse immunoglobulin (Ig)G antibody (Sigma) was added next in a 1:1000 dilution in washing buffer and incubated for another 2 h at 37°C. Following washing, 50 μl substrate solution (1 mg/ml nitrophenyl phosphate in 9.7% diethanolamine, 0.5 mM $MgCl_2$ [pH 9.8]) was added to each well. After about 30 min incubation at 37°C the reaction was stopped with the addition of 50 μl of 3 N NaOH. The color development was read at A_{405nm} on an EIA ELISA reader (Bio-Rad).

Immunoglobulin Classes and Subclasses. The assay was carried

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[3] Abbreviation: McAbs, monoclonal antibodies.

out by ELISA using mouse subclass specific rabbit antisera from Biorad according to the manufacturer's protocol.

Direct Antigen-Binding Assay. According to Suresh and Milstein (17), three μl of hybridoma medium was spotted on nitrocellulose membrane (0.45 μm, Scheicher & Schell). The further binding of the membrane was blocked with 0.05% Tween 20 in PBS (PBST) and the sheet was incubated in 10 μg/ml purified peroxidase in PBST. The sheet was washed thrice in PBS and the bound enzyme assayed with 2.5 μg/ml *o*-dianisidine and 0.01% H_2O_2 in PBS according to Towbin *et al.* (19).

Competitive ELISA. The competitive indirect ELISA technique of Shimazaki and Pratt (14) was adapted with minor modifications for the determination of epitopes of the peroxidase molecule recognized by the McAbs. Aliquots of 50 μl culture supernatant from each hybridoma line or nonimmune mouse IgGs were immobilized in the wells of microtiter plates precoated with 2 μg/ml goat antibodies to mouse immunoglobulins. Simultaneously, each antibody (approximately 15-fold concentrated by $(NH_4)_2SO_4$) was incubated at 4°C for 3 h with 4.8 μg/ml of purified peroxidase. The plates were incubated as for ELISA and the remaining binding sites of goat antibodies were saturated by incubation with 50 μl of 20 μg/ml nonimmune mouse IgGs for 1 h at 37°C. Then 50 μl free antibody and peroxidase mixture were added to each well and incubated at 4°C for 3 h. A 1:2000 dilution of polyclonal rabbit antiperoxidase serum was added to the wells following washings. The remaining steps were similar to those described for the ELISA above, except that alkaline phosphatase linked antibodies were goat antirabbit IgGs (BioCan) at 1:2000 dilution.

The loss of color development indicated competition between free and bound Abs for the same or overlapping epitope.

Western Blots. SDS-PAGE was performed by the technique of Laemmli (11). Samples were denatured and electrophoresed on 7.5 to 15% polyacrylamide gels. The proteins were blotted electrophoretically onto nitrocellulose membrane as described by Szewczyk and Kozloff (18). Following blotting the nitrocellulose membrane was incubated with 3% BSA in PBS for 1 h to block nonspecific binding. The blots were then exposed to the appropriate dilutions of McAbs in PBS with 1% BSA for 2 h at room temperature. The blots were washed four times with PBST and incubated with goat anti-mouse IgG diluted 1:1000 in PBST for 2 h at room temperature. Following washing, the alkaline phosphatase activity was assayed with 5 bromo-4-chloro-3-indolyl-phosphate and nitro blue tetrazolium according to the method of Blake *et al.* (2).

Inhibition of Peroxidase Activity. Appropriate dilutions of $(NH_4)_2SO_4$ concentrated antibodies were incubated with 0.5 μg of cationic peanut peroxidase at 4°C for 4 h. The peroxidase activity in the solution was then measured with guaiacol as substrate (21).

Cross Reactivity with Peroxidases Extracted from Different Plant Species. Peanut cells (2 weeks), calli (16 d after subculture) or leaves from 4-week-old peanut (*Arachis hypogaea*), broad bean (*Vicia faba*), and cowpeas (*Vigna unquiculata*) were ground and extracted as described (10). The 2000*g* extracts were precipitated by acetone brought to 80% and the pellets resuspended in PBS. Horse radish (*Armoracia lapathifolia*) peroxidase (RZ = 0.4) was purchased from Sigma.

Assays for cross reactivity were done by ELISA with crude extracts at four protein dilutions. These assays were compared to reactions obtained with purified cationic peanut peroxidase (RZ = 3.0). The solutions were applied (50 μl/well) to ELISA plates precoated with rabbit and anticationic peroxidase serum (1:2000) and incubated for 8 h at 4°C. After washing, McAbs were added to the plates and incubated overnight at 4°C. The secondary antibody incubation and color development were carried out as for the ELISA above.

RESULTS

Isolation of Hybridoma Lines. Hybridoma cultures were screened for the production of antibodies specific to purified cationic peroxidase by ELISA. In the first fusion, 4 out of 300 hybridomas and in the second fusion 6 out of 230 were selected (Table I). In the ELISA test, nonimmune mouse IgG gave low background readings. Positive hybridomas typically yielded activities several times higher than that of background. Six monoclonal hybridomas (1M, 1B, 2B, 2F, 2G, and 2H) were chosen for expansion and passages into mice for ascites fluid production. Peroxidase binding was also examined using a direct antigen-binding assay. In this experiment, the antibodies were spotted onto a nitrocellulose membrane, then exposed to the antigen, peanut peroxidase. Bound peroxidase activity was detected using a color development method. McAbs-1F, -2A, -2B, -2F, -2G, -2H, and -2J gave a strong reaction indicating that peroxidase was bound (Fig. 1). However, McAbs-1B, -1S, and -1M were no more effective than nonimmune mouse IgGs (column 6; Fig. 1).

Competitive Binding of McAbs to Cationic Peroxidase. This was carried out to determine the number of epitopes on cationic peroxidase which are recognized by the 10 McAbs. An excess amount of each McAb was incubated with the peroxidase. The ability of the preincubated McAb to block the subsequent binding of immobilized McAb to peroxidase was tested. A summary of the results appears in Table II. McAb-1M was able to block its own binding by >80% but was ineffective in blocking the binding of other McAbs to peroxidase, suggesting that the epitopes recognized by other McAbs do not overlap with the 1M epitope. The results with McAb-1F, -2A, -2B, -2G, -2J, and -2H showed that they reciprocally blocked the binding of each other and

Table I. *McAbs to Cationic Peanut Peroxidase*

McAb	Hybridoma Clonal No.[a]	Isotype[b]	Antibody Activity (A_{405})[c]
1B	1B31-6	IgG_1	1.942 ± 0.082
1F	1F5-9	IgA	1.357 ± 0.238
1M	1M3-1	IgA	1.105 ± 0.158
1S	1S12-2	IgG_1	1.310 ± 0.091
2A	2A6	IgG_1	1.772 ± 0.144
2B	2B4-1	IgG_1	1.711 ± 0.274
2F	2F5-2	IgG_1	1.919 ± 0.102
2G	2G3-4	IgG_1	0.905 ± 0.159
2H	2H2-1	IgG_1	0.706 ± 0.220
2J	2J2	IgG_1	1.388 ± 0.139

[a] Derived from the fusion, the clone number from the initial screening, and that of the first cloning and that from recloning. [b] Determined through ELISA by using culture supernatant. [c] Representative ELISA data from culture supernatants employed in subcloning. A_{405} for 10 μg/ml nonimmune mouse IgG was 0.082 ± 0.100.

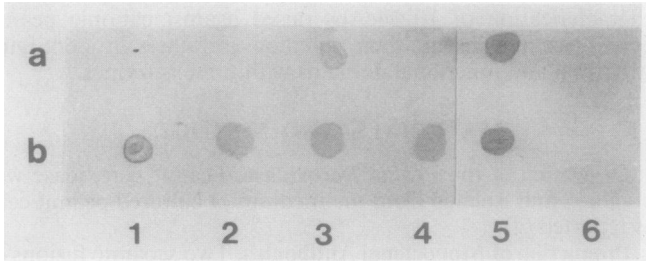

FIG. 1. Direct peroxidase-binding assay of hybridoma supernatants fixed on nitrocellulose membrane. Three μl of hybridoma supernatant was applied to each spot. Row a received McAb-1B, -1S, -1F, -1M, and -2A; and row b received McAb-2B, -2F, -2G, -2H, and -2J from column 1 to 5, respectively. Column 6 received nonimmune mouse IgGs (10 $\mu g \cdot ml^{-1}$).

Table II. *Competition of McAbs for Epitopes on Cationic Peanut Peroxidase as Determined by ELISA*

First antibodies were immobilized onto the microtiter plates. The second antibodies were preincubated with peroxidase and then added to the plates for competition with the first antibodies. The results here are representative readings at A_{405} of 3 trials

Group	First Antibodies	Second Antibodies										
		IgG	1M	1B	1S	1F	2A	2B	2G	2J	2H	2F
	IgG	0.030	0.085	0.022	0.066	0.045	0.020	0.000	0.013	0.013	0.009	0.006
A	1M	1.164	0.095	1.083	0.979	1.323	0.707	0.495	0.927	0.937	0.730	0.736
B	1B	0.023	0.074	0.055	0.049	0.080	0.361	0.378	0.032	0.386	0.053	0.297
	1S	0.028	0.126	0.087	0.099	0.032	0.147	0.094	0.047	0.075	0.042	0.095
C	1F	1.247	1.152	1.432	1.907	0.019	0.081	0.028	0.051	0.032	0.038	1.437
	2A	1.007	1.299	1.678	1.317	0.065	0.001	0.026	0.006	0.062	0.029	0.839
	2B	1.008	1.244	1.276	0.936	0.051	0.043	0.034	0.047	0.043	0.019	0.763
	2G	0.943	1.261	1.533	1.046	0.072	0.063	0.052	0.029	0.034	0.054	0.676
	2J	0.974	1.243	1.026	1.325	0.021	0.019	0.015	0.006	0.026	0.011	0.756
	2H	0.941	1.300	1.560	1.147	0.021	0.025	0.017	0.045	0.129	0.007	0.706
D	2F	0.965	1.031	1.190	0.897	1.034	0.754	0.834	0.707	1.045	0.060	0.070

therefore demonstrate that they are probably directed against a common epitope. Even though preincubation of the cationic peroxidase with 2F had little influence on the binding of other McAbs, the binding of 2F was effectively blocked by 2H. Differences in affinity of the two antibodies for the same epitope do not seem sufficient to explain the pattern (22). This result thus suggests that 2F may define a different epitope which may overlap the epitope recognized by 2H or that the binding of 2H may induce a conformational change in the peroxidase molecule resulting in masking the epitope recognized by 2F.

McAb-1B and -1S preincubated with peroxidase did not inhibit the binding of any other McAbs, implying a spatially distinct epitope recognized by them. However, these two McAbs failed to bind the antigen when they were first used in immobilization on ELISA plates (Table II). Similarly, 1B and 1S spotted on nitrocellulose membrane in the direct antigen-binding assay were not able to fix the cationic peroxidase in solution (Fig. 1). It may be possible that the epitope recognized by them is not exposed in the native peanut peroxidase. The ten McAbs are therefore subdivided into four epitope recognition groups (A, B, C, and D, Table II).

Immunodetection of Peroxidase on Western Blots. The McAbs were also tested for their ability to recognize denatured cationic peroxidase after SDS-PAGE and Western blotting. A single protein band with the mol wt identical to that of purified cationic peroxidase was detected from the crude medium proteins by all of the four groups of McAbs (Fig. 2). The McAb-1B and -2F also reacted with a protein band from peanut leaf extract. McAb-1B (group B) reacted most strongly and quickly to the denatured cationic peroxidase. The intense peroxidase band usually appears within 3 min of incubation with the reagent for McAb-1B while the others gave a weaker and slower response. Group A and C McAbs failed to recognize the peroxidase from the peanut leaf extract. One possible reason for this may be that the peroxidase level in peanut leaves is lower than the detection limit by these antibodies applied to Western blotting.

Inhibition of Peroxidase Activity. McAb-1M (group A) was found to be inhibitory to peroxidase activity when using guaiacol and H_2O_2 as substrate (Table III). This result explains the failure of McAb-1M in detecting peroxidase in direct antigen-binding assays (Fig. 1). Only a slight inhibitory effect was detected for the anionic peanut peroxidase (data not shown).

Cross Reactivity with Anionic Peanut Peroxidase and with Peroxidase from Other Plant Species. The McAbs were examined for cross reactivity with anionic peroxidase and only a weak reaction was revealed in the direct assay with membrane bound McAbs in comparison to those incubated with the cationic isozyme (Fig. 3). McAbs of group A and B were again negative in this assay (not shown), but a weak cross-reaction occurred in

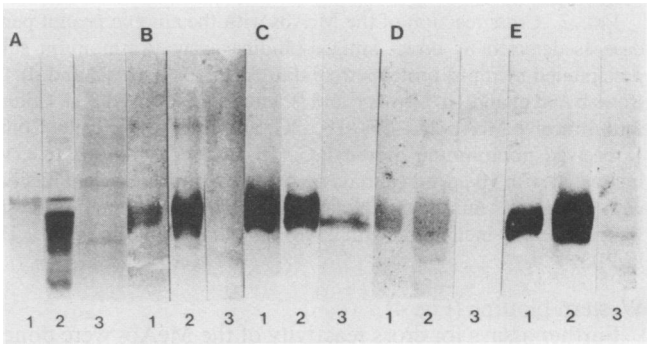

FIG. 2. SDS-PAGE of cationic peanut peroxidase (lane 1), crude medium proteins (2), and peanut leaf extract (3) followed by either protein staining or immunoblotting. The protein amounts used were 1 $\mu g \cdot lane^{-1}$ for purified peroxidase and 20 $\mu g \cdot lane^{-1}$ for crude samples. A, stained with Coomassie blue; B, probed by McAb-1M; C, by McAb-1B; D, by McAb-2H, and E by McAb-2F. The dilutions of culture medium (McAb-1B) or concentrated medium (McAb-1M, -2H, and -2F) were varied according to their titers.

Table III. *Effect of McAbs on Peroxidase Activity*

Cationic peroxidase (0.5 μg) was incubated with various amounts of concentrated McAbs or nonimmune mouse IgG (10 μg/ml) in a final volume of 30 μl of which 5 μl were assayed for peroxidase activity.

McAb	Volume of McAb (μl)				
	0	5	10	20	30
	% of control activity				
1B	100[a]	128	125	101	113
1M	100	37	15	10	0
1F	100	179	101	110	100
2A	100	167	140	122	128
2B	100	134	134	134	170
2H	100	183	166	224	170
2F	100	131	135	146	113
Nonimmune mouse IgG	100	107	107	100	116

[a] Control = 0.838 $A_{470} \cdot \mu g^{-1}$ peroxidase.

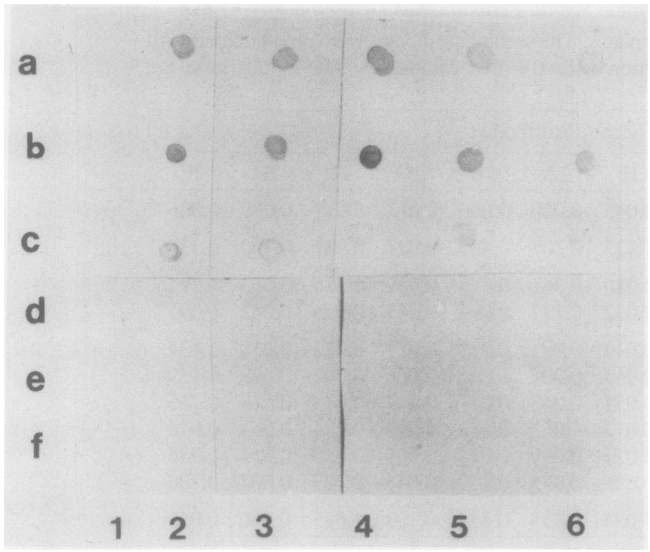

FIG. 3. Cross reaction of the McAbs with the anionic peanut peroxidase as detected by direct antigen-binding assay. Ammonium sulfate precipitated medium proteins were diluted to 10^{-1} (rows a and d), 10^{-2} (rows b and e) and 10^{-3} (rows c and f). Each spot received 3 μl. Columns 2 to 6 receive McAb-2A, -2F, -1F, -2G, and -2H, respectively. Column 1 received nonimmune mouse IgG (10 $\mu g \cdot ml^{-1}$). Rows a to c were incubated with 10 μg/ml cationic peroxidase; rows d to f were incubated with 10 $\mu g \cdot ml^{-1}$ anionic peroxidase. The peroxidase bound to the spots was revealed by incubation with 25 $\mu g \cdot ml^{-1}$ dianisidine and 0.01% H_2O_2 in PBS.

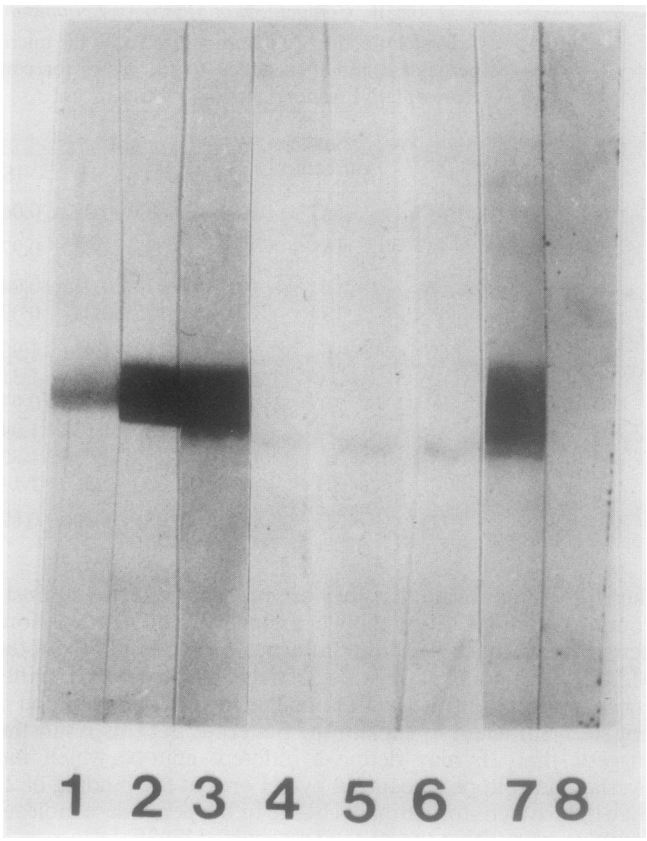

FIG. 4. Cross reaction of McAbs and polyclonal antibodies to cationic peroxidase with the anionic isozyme on Western blots. About 1 μg cationic peroxidase (RZ = 2.9) in lanes 1 to 3 and 1 μg anionic peroxidase (RZ = 2.6) in lanes 4 to 8 were electrophorized, blotted and then detected by McAb-1M (*1*, *4*); -1B (*2*, *5*); -2F (*3*, *6*); rabbit anti-serum to cationic peroxidase (*7*) and nonimmune IgG (*8*), respectively.

Western blotting (Fig. 4).

Further assays for cross reactivity of the McAbs were done on peroxidase derived from other sources than peanut cell culture medium. The representative data for the four McAb groups are presented in Table IV. The baseline for a positive reaction was set as $A_{405} = 0.2$ at a crude protein dilution of 0.25 mg/ml. When this is considered as the minimal criterion for a positive reaction, then all four groups react with peroxidase from the three peanut preparations. A broad cross reaction was observed with group A which recognized peroxidase from all sources tested. Peroxidase from broad bean and horseradish bound to McAbs of groups C and D. Further comparisons with various dilutions of crude protein indicate that group C recognized equally well the peroxidase from broad bean and the purified peroxidase, and to a lesser extent that from horseradish. The cross reactivity of group B seemed more restricted since, even though the McAbs bound to cowpea peroxidase, the reaction is weaker than that of purified peroxidase.

DISCUSSION

The aim of this study is to develop a panel of McAbs which would include probes for individual epitopes (for different molecular structure) of the cationic peanut peroxidase. We describe here the preliminary characterization of ten monoclonal cell line products. Competition studies between pairs of monoclonal antibodies for simultaneous binding to the same peroxidase molecule allowed for four subgroups. Group A (McAb-1M) causes a total inhibition of peanut peroxidase activity and it widely crossreacts with peroxidase of other plant species. This would be expected since antigenic sites involved with enzyme activity are the most conserved (15). In contrast, relatively minor effects were found to occur when McAb-1M was tested for inhibition of anionic peroxidase activity. This agrees with reports on horseradish peroxidase where the antiserum raised against basic (6) or acidic (5) isozymes inhibited only the catalytic activity of the corresponding basic or acidic peroxidase. Our results provide evidence for the existence of structural divergence of the peroxidase isozymes at the catalytic site.

Epitopes recognized by group C and D McAbs exhibit a complex pattern. Although 2H blocked the binding of 2F to the cationic peroxidase, the differences in reactivity demonstrated by 2F and 2H suggest that their epitopes may be different. This is based on the observation that the binding of 2H to peroxidase was not affected by 2F, and that Western blotting of peroxidase from peanut leaves was detected by 2F but not by 2H. Since the properties demonstrated by 2H were otherwise the same as those by 1F, 2A, 2B, 2G, and 2J, we include 2H with them as one group.

Groups B and D McAbs are directed to epitopes that are not affected by denaturation during Western blotting. This suggests that their epitopes are most probably continuous peptide fragments on the peroxidase. In contrast to groups B and D, the staining intensity of Western blots incubated with McAbs of groups A and especially C was much reduced (Fig. 2). This may be explained by the difference between affinity of the McAbs to peroxidase or by partial destruction of the epitopes during SDS electrophoresis and blotting. It is also possible that partial renaturation of the epitopes happened in the post blotting washing process. The latter was observed by Tsang *et al.* (20) who detected peroxidase activity on the nitrocellular membrane after SDS-PAGE and blotting of horse radish peroxidase.

The cross reactivity patterns by the McAbs of groups C and D with peroxidase from other sources were also similar (Table IV). However, group B antibodies only detected cowpea peroxidase.

Table IV. *Cross Reaction of McAbs to Cationic Peanut Peroxidase with Peroxidase from Various Other Sources as Determined by ELISA*

Source of Peroxidase		McAb[a] and Group				
		1M A	1B B	2B C	2F D	2C[b]
	mg/ml	*absorbance at 405 nm*				
Pure PRX_c	0.03	0.748	0.626	1.209	0.782	0.207
Medium (peanut cells)	1.0	0.140	0.201	0.205	0.134	0.028
	0.5	0.341	0.351	0.407	0.424	0.208
	0.25	0.298	0.386	0.294	0.258	0.123
	0.05	0.134	0.186	0.270	0.146	0.009
Peanut cells	1.0	0.284	0.253	0.771	0.344	0.104
	0.5	0.498	0.501	0.786	0.313	0.178
	0.25	0.245	0.245	0.442	0.231	0.012
	0.05	0.161	0.179	0.326	0.216	0.073
Peanut calli	1.0	0.239	0.218	0.680	0.282	0.069
	0.5	0.399	0.281	0.406	0.340	0.271
	0.25	0.261	0.287	0.354	0.377	0.120
	0.05	0.136	0.107	0.222	0.091	0.000
Broad bean (leaves)	1.0	0.364	0.271	1.402	0.267	0.172
	0.5	0.394	0.272	1.215	0.317	0.182
	0.25	0.524	0.156	1.374	0.352	0.115
	0.05	0.244	0.096	1.075	0.077	0.047
Cowpeas (leaves)	1.0	0.178	0.264	0.246	0.226	0.059
	0.5	0.258	0.207	0.300	0.280	0.163
	0.25	0.225	0.253	0.121	0.130	0.087
	0.05	0.063	0.047	0.147	0.083	0.030
Horseradish extract	1.0	0.140	0.173	0.349	0.240	0.015
	0.5	0.341	0.215	0.411	0.377	0.317
	0.25	0.298	0.192	0.351	0.351	0.085
	0.05	0.134	0.074	0.089	0.114	0.034

[a] The McAbs in the medium of 1B and 2C were used as such. McAb-1M, -2B, and -2F were diluted from 50% $(NH_4)_2SO_4$ precipitated culture medium. [b] A nonproducing hybridoma cell line used as control.

The more restricted cross-reactivity with peroxidase from other sources shown by 1B may reflect some amino acid difference or antigenic change of this epitope (1) between the peroxidases of different plants.

Next we examined the McAbs raised against cationic peroxidase as to their cross reactivity with anionic peanut peroxidase. As demonstrated in Figures 3 and 4, the McAbs react only very weakly with the anionic peroxidase. These results again indicate a structural difference as well as similarity between peanut peroxidase isozymes similar to that observed in basic and acidic horse radish peroxidases (5, 6).

In summary, preliminary characterization of 10 McAbs to cationic peanut peroxidase has been performed. A total of four different epitopes are detected in the antigen using these McAbs. One of them (group A) inhibits peroxidase activity. To various degrees these McAbs cross react with peroxidases from sources other than peanut and react very weakly to anionic peanut peroxidase. McAbs of group C probably reacted with a conformation-dependent epitope in the cationic peroxidase. The specific McAb probes along with polyclonal antisera are being employed for further characterization of the epitopes recognized. Since small changes in the primary structure (24) or even minor conformational changes of a protein (7) may affect the rate of transport, it is anticipated that information on the structural similarities and differences between the isoperoxidases may help to elucidate the mechanism of differential secretion.

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