Monoclonal Antibodies to the α- and β-Subunits of the Plant Mitochondrial F_1-ATPase[1]

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We have generated nine monoclonal antibodies against subunits of the maize (*Zea mays* L.) mitochondrial F_1-ATPase. These monoclonal antibodies were generated by immunizing mice against maize mitochondrial fractions and randomly collecting useful hybridomas. To prove that these monoclonal antibodies were directed against ATPase subunits, we tested their cross-reactivity with purified F_1-ATPase from pea cotyledon mitochondria. One of the antibodies (α-ATPaseD) cross-reacted with the pea F_1-ATPase α-subunit and two (β-ATPaseD and β-ATPaseE) cross-reacted with the pea F_1-ATPase β-subunit. This established that, of the nine antibodies, four react with the maize α-ATPase subunit and the other five react with the maize β-ATPase subunit. Most of the monoclonal antibodies cross-react with the F_1-ATPase from a wide range of plant species. Each of the four monoclonal antibodies raised against the α-subunit recognizes a different epitope. Of the five β-subunit antibodies, at least three different epitopes are recognized. Direct incubation of the monoclonal antibodies with the F_1-ATPase failed to inhibit the ATPase activity. The monoclonal antibodies α-ATPaseD and β-ATPaseD were bound to epoxide-glass QuantAffinity beads and incubated with a purified preparation of pea F_1-ATPase. The ATPase activity was not inhibited when the antibodies bound the ATPase. The antibodies were used to help map the pea F_1-ATPase subunits on a two-dimensional map of whole pea cotyledon mitochondrial protein. In addition, the antibodies have revealed antigenic similarities between various isoforms observed for the α- and β-subunits of the purified F_1-ATPase. The specificity of these monoclonal antibodies, along with their cross-species recognition and their ability to bind the F_1-ATPase without inhibiting enzymic function, makes these antibodies useful and invaluable tools for the further purification and characterization of plant mitochondrial F_1-ATPases.

Electrogenic H^+-ATPases have been found in nearly all physiological membrane systems investigated. Each of these membrane-bound ATPases can be placed into one of several groups. The three most common types of proton ATPases are E_1–E_2 type, F_0–F_1 type, and the microsomal type (Al-Awqati, 1986). The E_1–E_2 type ATPases are found in yeast and fungal plasma membranes and the gastric plasma and microsomal membranes. ATPases of the microsomal type are located in the membranes of Golgi apparatus, ER, endosomes, secretory granules, and plasma membranes of many eukaryotes. The F_0–F_1 ATPase is found in bacterial plasma membranes and in the mitochondrial and chloroplast membranes of eukaryotes. The transmembrane portion of the F_0–F_1 ATPase is referred to as the F_0 portion and forms a H^+ channel through the membrane. The catalytic portion is referred to as the F_1 and it is attached to the F_0. Structural and functional comparisons have been repeatedly made between ATPase enzymes of various origins and locations in attempts to draw conclusions concerning the ancestry of these complex enzymes (Senior and Wise, 1983; Vignais and Satre, 1984).

Comparisons between the chloroplast CF_1-ATPase and the plant mitochondrial enzyme have revealed disparity between the relative mol wt of the native enzymes as well as the denatured lower mol wt subunits (Horak and Packer, 1985). Attempts have been made to examine the differences in subunit identity through the use of polyclonal antibodies and MAb's (Spitsberg et al., 1985; Horak et al., 1989a). It has also been noted that F_1 preparations from different plant mitochondrial sources appear to be widely different in apparent subunit mol wt and composition (Spitsberg et al., 1985). Because of the apparent wide variation in F_1 subunit compositions and the need for comparative studies, specific antibodies provide a valuable tool for recognizing antigenic similarities between protein subunits.

We presently describe the production of nine MAb's that have been generated against the α- and β-subunits of the maize mitochondrial F_1-ATPase. Several of these antibodies are widely cross-reactive between mitochondria from various plant species. We have used purified pea mitochondrial F_1-ATPase to prove that the maize MAb's bind to ATPase subunits and to establish that the MAb's bind without inhibiting ATPase activity. These antibodies have been further used to map the F_1-ATPase subunits on a 2D map of pea cotyledon mitochondrial proteins.

MATERIALS AND METHODS

Plant Material

Tissue and mitochondria from *Sauromatum guttatum* Schott and *Arum italicum* Mill. were obtained as described by Elthon et al. (1989). Potato tubers (*Solanum tuberosum* L. cv Russet), red beetroot (*Beta vulgaris* L.), and cauliflower (*Brassica oler-*

Abbreviations: 2D, two-dimensional; Ig, immunoglobulin; MAb, monoclonal antibody.

acea L.) were obtained from a local market. Maize (*Zea mays* L. inbred line B73) and wheat (*Triticum aestivum* cv Arapahoe) seeds were obtained from the Nebraska Seed Foundation (Lincoln, NE). Pea (*Pisum sativum* L. cv Homesteader) seeds were purchased from Apache Seeds Limited (Edmonton, Alberta, Canada). The bean (*Phaseolus vulgaris* cv Sprite) seeds were a gift from Christine Chase, University of Florida Gainesville.

Preparation of Mitochondria and Submitochondrial Particles and Purification of the Mitochondrial Coupling Factor (F_1)

Maize, wheat, and bean seedlings were grown in the dark at approximately 30°C and were typically harvested 3 to 4 d after germination or when the seedlings reached 7 to 8 cm in height. Mitochondria were isolated from the maize, wheat, and bean seedlings and from the red beetroot, potato, and cauliflower tissue as described by Hayes et al. (1991). The growth of the pea seedlings and the preparation of mitochondria and submitochondrial particles was carried out as previously described (Horak and Packer, 1985).

Purification of the mitochondrial F_1 was also performed as previously described (Horak and Packer, 1985) with the following changes. The concentration of Tricine in the low ionic strength Suc solution was 0.5 mM instead of 2 mM. After centrifugation of the submitochondrial particle extraction mixture, the F_1 extract in the supernatant was supplemented with EDTA, ATP, and Tris buffer to match the chromatography buffer of the subsequent purification step. The F_1-ATPase found in the DEAE-cellulose column fractions and the Suc density gradient fractions was not salted out with $(NH_4)_2SO_4$ as previously described but was concentrated with Centricon-10 units (Amicon).

One-Dimensional and 2D SDS-PAGE and Western Blotting

One-dimensional SDS-PAGE was carried out using the Laemmli buffer system (Laemmli, 1970). Slab gels were cast using an acrylamide concentration of 14% (w/v) for the resolving gel and 7% (w/v) for the stacking gel. Gels were electrophoresed under a constant voltage of 21 V/cm of gel until the ion front electrophoresed out of the bottom of the gel.

2D electrophoresis was performed in a Bio-Rad 2D Minigel apparatus according to the manufacturer's instructions with the following modifications. The IEF gels prepared for the first dimension were cast with the protein samples already mixed into the gel solutions. The ampholytes used are the Pharmacia Pharmalyte 3–10 ampholytes. The first dimensions were electrophoresed under a constant voltage of 100 V/cm for 2 h, and no overlay solution was used above the tube gels. The second dimension was carried out according to the procedure for one-dimensional gels.

Western blots were prepared and probed as previously described (Hayes et al., 1991).

Enzyme Assays

The assay for ATPase activity used is a modification of the assay reported by Fisher et al. (1981). The F_1-ATPase activity was monitored using a coupled enzyme system (pyruvate kinase and lactate dehydrogenase). The final assay mixture contained the following, with the final concentrations indicated: 50 mM Tricine, pH 8.0; 5 mM $MgCl_2$; 5 mM ATP; 5 mM phospho*enol*pyruvate; 200 μM NADH; 15 units/mL of pyruvate kinase; and 18 units/mL of lactate dehydrogenase. The activity was measured as a decrease in the NADH concentration at 340 nm ($\epsilon_{340\ nm}$ = 6.22 mM^{-1} cm^{-1}).

MAb Production

MAb's were produced essentially as described by Elthon et al. (1989) from BALB/c female mice immunized against *Z. mays* L. (inbred B73) mitochondrial proteins. Hybridomas that produced antibodies against maize mitochondrial proteins were identified by probing western blots with the spent hybridoma culture media. The hybridomas were cultured as described by Elthon et al. (1989) except that the growth medium contained 20% (v/v) fetal calf serum, 2 mM L-Gln, 25 mg/L of ampicillin, 100 mg/L of streptomycin sulfate, and 0.1% (w/v) amphotericin B in a base culture medium of 1× Dulbecco's modified Eagle's medium (Sigma).

Purification of MAb's

MAb's were purified by applying the spent hybridoma culture supernatant to a protein G-Superose column (Pharmacia) attached in line with a Pharmacia fast protein liquid chromatography system at 4°C. The culture supernatant was diluted with an equal volume of 20 mM phosphate buffer, pH 7.0, and loaded onto the protein-G column, which had been preequilibrated with the same buffer. After the column was washed with several volumes of the phosphate buffer, the MAb's were eluted with 0.1 M glycine-HCl, pH 2.7. The fractions containing purified MAb were neutralized upon collection with 15 μL/mL of 1 M Tris, pH 9.0.

Binding of the F_1-ATPase to Free MAb and to MAb Conjugated to Affinity Beads

Activity inhibition studies were performed by adding purified MAb to purified pea F_1-ATPase in 1:1 and 1:10 ratios (w/w) of ATPase:MAb. The volume was kept to a minimum, and any adjustments to volume were made with PBS (20 mM phosphate buffer [pH 7.2], 150 mM NaCl). The mixtures were allowed to incubate at room temperature for 4 h with gentle shaking. The degree of inhibition was then assessed by measuring ATPase activity. As a control, an equal amount of purified ATPase was allowed to incubate along with the experimental tubes without MAb present.

Antibody-binding experiments were conducted with MAb's covalently bound to the surface of QuantAffinity (Rainin) epoxide-glass beads. Purified antibody was incubated with the beads in 10-fold excess of the manufacturer's calculated binding capacity of each bead for IgGs to ensure saturation of the bead surface. The antibody was diluted in a minimal volume of 0.8 M phosphate buffer, pH 6.6, and incubated with the beads for 14 to 16 h at room temperature with gentle shaking. The beads were then washed three times with several volumes of PBS to remove unbound antibody.

Binding of the pea F₁-ATPase to the derivatized beads was achieved by incubating a 10-fold excess of the pure ATPase with the antibody-affinity beads for 4 h at room temperature with gentle shaking. Unbound enzyme was washed three times from the beads with several volumes of PBS. Determination of the amount of active enzyme bound to the beads was made by incubating the beads with a full complement of the ATPase assay mixture (i.e. all substrates and coupling enzymes present) and measuring the NADH concentrations at various times. The rate of NADH consumption by the coupled enzyme system was then directly related to the amount of ATPase enzyme bound to each bead. Quantities of F₁-ATPase bound to the beads were calculated based on the specific activity of the ATPase control (F₁-ATPase not incubated with QuantAffinity beads). Control beads consisted of underivatized beads that had been incubated without purified ATPase added, affinity beads derivatized with BSA (fraction V, Sigma) and incubated with the ATPase, and beads derivatized with the MAb's but not incubated with the purified ATPase.

MAb Subtyping

Subtyping of the MAb's was done using two commercially available kits: the Sigma ImmunoType Mouse Monoclonal Antibody Subtyping kit (Sigma stock No. ISO-1) and the Serotec Isotyping kit for Mouse Monoclonal Antibodies (Serotec code No. MMT RC1).

RESULTS

Horak and Packer (1985) reported the purification of the F₁-ATPase from pea cotyledon mitochondria. A gentle extraction procedure was used in a special effort to maintain the integrity of the F₁ complex. As reported by Horak and Packer (1985), the pea mitochondrial F₁-ATPase consists of six subunits instead of the typical five-subunit enzyme. The subunits of the F₁ enzyme were determined to be 57 kD (α), 55 kD (β), 36.5 kD (γ), 26.5 kD (δ), 22.5 kD (δ'), and 8 kD (ϵ). We mapped the subunits on 2D gels of pea cotyledon mitochondria and purified pea F₁-ATPase (Fig. 1). The top panel of the figure is a 2D gel of crude pea cotyledon mitochondria. The locations of the F₁-ATPase subunits are indicated. To help place the F₁-ATPase subunits on the whole mitochondrial 2D map, a 2D gel was run in which a light loading of whole mitochondria was spiked with a heavy loading of the purified F₁. An example of such a gel is in the center panel of Figure 1. Several spots can be seen to become more intense upon spiking the mitochondrial protein content with purified ATPase. The lower panel of Figure 1 is a 2D gel of purified pea F₁-ATPase. The combination of these three gels gives us an accurate indication of the location of the ATPase subunits in the whole mitochondrial 2D map. The location of the ϵ-subunit was determined by running the second dimension only to three-fourths completion, allowing for the visualization of the very small proteins on the gels. We determined that the subunit is normally excluded from the 2D profile because it is very basic. The ϵ-subunit is not visible on the 2D maps presented here because it is more basic than the γ- and δ-subunits and is usually located in the
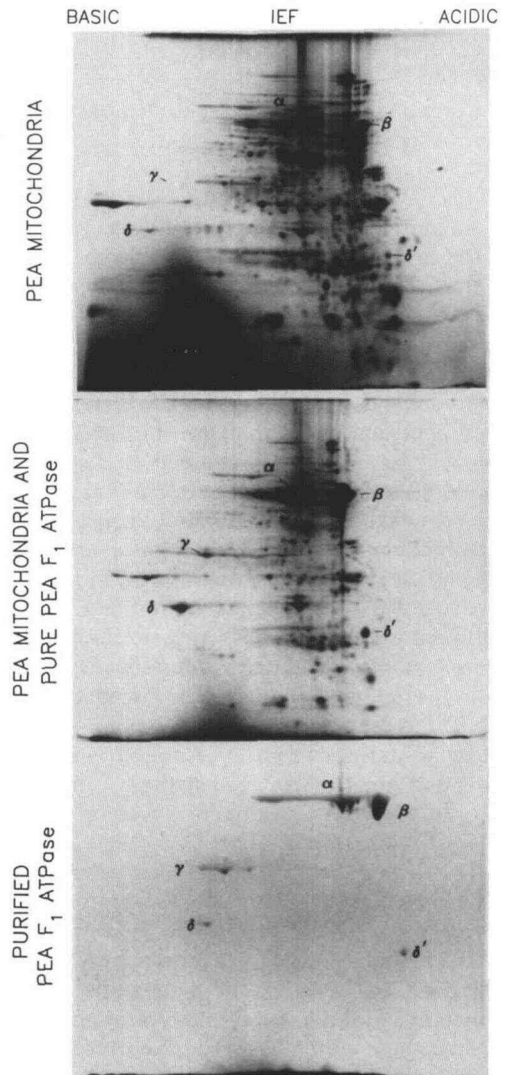
Figure 1. 2D electrophoresis of pea mitochondria and purified pea mitochondrial F₁ ATPase. 2D PAGE gels were prepared and electrophoresed as described in "Materials and Methods." The gel in the top panel contained 250 μg of crude pea cotyledon mitochondria. The center panel depicts a gel that was loaded with approximately 75 μg of crude pea cotyledon mitochondria and approximately 40 μg of purified pea mitochondrial F₁-ATPase. The gel in the bottom panel was loaded with 25 μg of pure pea mitochondrial F₁-ATPase. The protein in these three gels was detected by staining with Coomassie brilliant blue. Five of the six subunits associated with the pea mitochondrial F₁ can be seen on these gels and have been designated with labels.

region where the highest background exists due to the presence of ampholytes.

The γ-subunit is not readily visible in the 2D gel of pea mitochondria, although both the δ- and δ'-subunits can be seen. The γ-subunit is clearly visible in the two gels containing the purified pea F₁-ATPase. A similar situation is observed for the α-subunit, in which the amount of the α-subunit in whole mitochondria is not present in stoichiometric quantities with the β-subunit. However, all of the ATPase subunits

appear to be present in stoichiometric ratios (α:β:γ:δ:δ':ϵ, 3:3:1:1:1:1) when the ATPase is purified. When whole mitochondria or submitochondrial particles are analyzed, an apparent steady-state level of each subunit is observed, which is not stoichiometric relative to each of the other subunits and most likely can be attributed to differences in the rates of protein turnover. In fact, Hack and Leaver (1983) reported a similar situation with maize mitochondria, in which they attribute the loss of the α-subunit to specific proteolysis. Douglas et al. (1977) also observed proteolysis of the α-subunit in yeast F_1-ATPase preparations.

The labels used to indicate the location of the F_1-ATPase subunit spots on the 2D gels in Figure 1 are intended to indicate the general location of the proteins and are not intended to point out every spot seen in the 2D gel of the purified F_1-ATPase. It can easily be seen that several of the ATPase subunits are represented by more than one spot on the 2D gel. For instance, the α-subunit was consistently found to consist predominantly of three major spots and is also represented by the smearing seen at the same molecular mass. We believe that the multiple spots are not necessarily products of different genes but likely represent slight variants of the same protein. The β-subunit is represented primarily by one spot on the 2D gel, although a second spot can be seen slightly shifted to the acidic side of the major β-subunit spot. There are three spots seen for the γ-subunit as well.

We generated nine MAb's against the α- and β-ATPase subunits, collectively. These antibodies were generated randomly against maize mitochondrial proteins. The proteins recognized by each of these antibodies were determined using 2D western blots of maize mitochondrial proteins (data not shown). Four of these MAb's recognize what we believed to be the α-subunit and five recognize the β-subunit. The MAb's are summarized in Table I, and their respective subunit recognition in maize and antibody subtype are indicated. We suspected that these antibodies recognized specific subunits of the ATPase, but we needed to verify this recognition.

To confirm that these antibodies reacted with ATPase

Figure 2. 2D western blot of 5 μg of purified pea mitochondrial F_1 ATPase. The western blot was probed with a 1:25 dilution of the α-ATPaseD MAb. The antibody binding was detected using an alkaline phosphatase-conjugated secondary antibody.

subunits, we assessed their cross-reactivity with the purified pea mitochondrial F_1-ATPase through the use of one-dimensional western blots. We found that only three of the nine MAb's against the F_1 subunits cross-reacted with the pea enzyme (Table I). One of these MAb's is α-ATPaseD, which recognizes the α-subunit of the F_1 enzyme. A 2D western blot of the purified pea F_1-ATPase probed with the α-ATPaseD MAb (Fig. 2) reveals that all of the α-subunit spots and all of the streaking and smearing seen at the top of the 2D gel are recognized by the MAb. This streaking and smearing can also be observed in the Coomassie-stained gel in the lower panel of Figure 1.

The other two cross-reactive MAb's are β-ATPaseD and β-ATPaseE, which recognize the β-subunit of the pea F_1-ATPase. A 2D western blot of purified pea F_1-ATPase that was

Table I. *MAb isotype and cross-reactivity with mitochondria from other sources*

The antibody isotypes were determined as described in "Materials and Methods." Cross-reactivity was tested using western blots of isolated plant and animal mitochondria. A strong cross-reaction is indicated by "+," a weak cross-reaction by "w," and a lack of cross-reaction by "−." *Saurm., S. guttatum* (voodoo lily) mitochondria; *Arum, A. italicum* spadix mitochondria; Caulif., cauliflower (*B. oleracea* L.) mitochondria; Petun., petunia (results provided courtesy of David Ruth and Maureen Hanson, personal communication).

MAb Name	Isotype	Cross-Reactivity										
		Maize	Wheat	*Saurm.*	Arum	Pea	Bean	Beet	Potato	Caulif.	Petun.	Rat
α-ATPaseA	IgG1	+	+	+	+	−	−	−	+	+	+	−
α-ATPaseB	IgG2a	+	+	+	+	−	−	−	+	+	−	−
α-ATPaseC	IgM	+	−	−	−	−	−	+	+	−	−	−
α-ATPaseD	IgG1	+	+	+	+	+	+	+	+	−	+	+
β-ATPaseA	IgG1	+	+	+	+	−	−	+	+	−	+	−
β-ATPaseB	IgG1	+	+	+	+	−	w	+	+	−	+	−
β-ATPaseC	IgG1	+	+	+	+	−	w	+	+	−	+	−
β-ATPaseD	IgG1	+	+	+	+	+	+	+	+	+	+	+
β-ATPaseE	IgG1	+	+	+	+	+	+	+	+	+	+	+

probed with the MAb β-ATPaseE is shown in Figure 3. It easily can be seen that the MAb recognizes both β-subunit spots. Similar results were obtained with western blots probed with β-ATPaseD MAb (data not shown). We assume that the other antibodies in the α and β series also recognize the α- and β-subunits, respectively, because they recognize the same proteins on a maize 2D western blot.

The cross-reactivity of the nine MAb's with mitochondrial F$_1$- ATPases from other sources was also addressed. One-dimensional western blots of various mitochondria were performed with each of the nine MAb's. The results of these western blots have been compiled in Table I. A "+" sign indicates a strong cross-reactivity, a "w" indicates a weak cross-reactivity, and a "−" indicates a lack of cross-reactivity. Several of the MAb's are widely cross-reactive. In fact, most of the β-subunit MAb's cross-react with many different species. The three monocot species (wheat, *S. guttatum*, and *A. italicum*) showed cross-reactivity with eight of the nine MAb's tested. Potato mitochondria cross-reacted with all of the MAb's. Petunia and *B. vulgaris* mitochondria also showed a high degree of cross-reactivity, each being recognized by seven of the nine MAb's. It is surprising that proteins in rat liver mitochondria were recognized by three MAb's. It is clear that several of these MAb's could potentially be very useful as tools in a wide variety of species.

Two of the MAb's that recognize the pea F$_1$-ATPase (α-ATPaseD and β-ATPaseD) were tested for their ability to inhibit the F$_1$-ATPase activity. These two MAb's were incubated with the purified ATPase in two ratios based on a μg of ATPase per μg of MAb basis (1:1 and 1:10, ATPase:MAb). After the MAb's were allowed to bind to the enzyme under the conditions explained in "Materials and Methods," the mixtures were assayed for ATPase activity. Neither of the two MAb's showed any significant signs of inhibition after the 4-h incubation. The specific activity of the control ATPase decreased from 15.2 μmol min^{-1} mg^{-1} to approximately 10.3 μmol min^{-1} mg^{-1} during the 4-h incubation, a decrease

observed for each of the inhibition studies as well. The only inhibition observed was due to incubation of the ATPase with a 10-fold excess (w/w) of the α-ATPaseD MAb, for which the specific activity of the ATPase was 9.42 μmol min^{-1} mg^{-1}, an 8.6% decrease from the control. Therefore, despite strong binding of the MAb's to the pea F$_1$-ATPase, as indicated by western blots, the MAb's do not significantly inhibit ATPase activity.

We also assessed the ability of these two MAb's to bind the F$_1$-ATPase after the MAb's had been immobilized. The MAb's α-ATPaseD and β-ATPaseD were used to coat the surface of epoxide-glass QuantAffinity beads as described in "Materials and Methods." The beads that had been coated with the MAb's were capable of binding a significant amount of the F$_1$-ATPase. Considering the specific activity of the pure ATPase (25.9 μmol min^{-1} mg^{-1}), we calculated the amount of enzyme bound to the bead's surface. The calculated values were also compared with the manufacturer's suggested binding capacity of the beads for IgGs, 0.225 μg per bead. If we assume an equimolar binding of the ATPase to the MAb, we might expect a maximum binding of 0.575 μg of ATPase per bead, taking the differences in the relative molecular masses of the IgG (160 kD) and the ATPase (approximately 409 kD) into consideration. The amount of the F$_1$-ATPase binding to each bead, using the α-ATPaseD MAb, was an average of 0.349 ± 0.032 μg (based on a specific activity of the control enzyme of 25.9 μmol min^{-1} mg^{-1}). The β-ATPaseD beads bound an average of 0.362 ± 0.180 μg of the F$_1$-ATPase per bead. As can be seen from the average deviation, the β-ATPaseD beads did not consistently bind the same amount of the ATPase. The two sets of beads bound approximately 60% of the amount of ATPase we predicted assuming a 1:1 binding. Each IgG molecule has two antigen-binding sites and theoretically would be capable of binding two ATPase molecules if not sterically hindered from doing so. If this were possible, the beads would be capable of binding nearly 1.2 μg of purified ATPase. However, during the derivatization procedure, the IgG molecules can attach to the bead at a number of residues, potentially reducing their protein-binding efficiency.
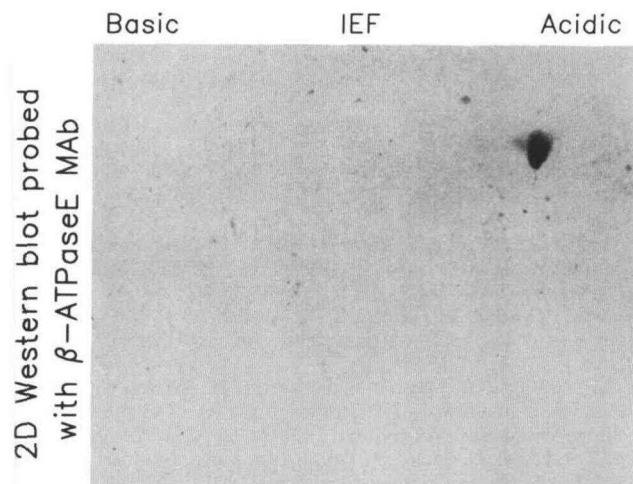
DISCUSSION

The F$_1$-ATPase isolated from pea cotyledon mitochondria has been extensively characterized. Purification of the pea enzyme to homogeneity was initially reported by Horak and Packer (1985). The enzyme was purified 19-fold from submitochondrial particles with a final specific activity of 23 units/mg. This paper was followed by the identification of several of the F$_1$-ATPase subunits (Horak et al., 1987, 1989a, 1990) and by functionality studies performed through membrane reconstitution and stability experiments (Horak et al., 1987, 1988, 1989b). The pea mitochondrial F$_1$-ATPase has six subunits, a characteristic seen in several other preparations from plants (sweet potato root [Iwasaki and Asahi, 1983, 1985] and turnip [O'Rourke, 1988]). However, the majority of plant mitochondrial F$_1$-ATPase preparations indicate that the enzyme is composed of five subunits (maize [Hack and Leaver, 1983, Partridge et al., 1985], fava bean [Boutry et al., 1983], oat root [Randall et al., 1985], cuckoo-pint [Dunn et

Figure 3. 2D western blot of 12.5 μg of purified pea F$_1$-ATPase. The blot was probed with a 1:25 dilution of the β-ATPaseE MAb. Antibody binding was detected as described for Figure 2.

al., 1985], and spinach leaf [Hamasur and Glaser, 1990]). Horak et al. (1989a, 1989b) showed that the sixth subunit (termed the δ-subunit by Horak and Packer, 1985) is the oligomycin sensitivity-conferring protein. This subunit is frequently lost from the enzyme preparation when harsh methods are used to liberate the F_1 portion from the membrane (Horak et al., 1989b).

Using a highly purified preparation of pea mitochondrial F_1-ATPase, we mapped the six subunits using 2D gel electrophoresis. The α-, β-, and γ-subunits were resolved into two or more isoelectric variants. The α- and β-subunits were observed repeatedly to be represented by more than one spot on 2D analyses of mitochondrial F_1-ATPases (Cabral and Schatz, 1979; Boutry et al., 1983; Hack and Leaver, 1983). It is likely that these variants are the result of minor changes or modifications in the protein that lead to subsequent changes in net charge. Cabral and Schatz (1979) speculated that the presence of isoelectric variants may be due to carbamylation of polypeptides by the cyanate that is formed upon hydrolysis of urea. It is also possible that these proteins are the products of different genes. Further investigations are warranted to distinguish among these possibilities. The ε-subunit was found to be an extremely basic protein and typically migrates out of the tube gels during the IEF of the first dimension.

We generated nine MAb's against the maize α- and β-subunits of the F_1-ATPase. Several of these MAb's are widely cross-reactive with mitochondrial protein from a variety of other plant and animal sources. The pattern of cross-reactivity of the four MAb's raised against the α-subunit is unique for each MAb. This indicates that four separate epitopes are being recognized and that these epitopes are not conserved among plant species in an identical manner. If the MAb's were raised against identical epitopes, the patterns of cross-reactivity between the MAb's would be identical. This can be observed for several of the MAb's raised against the β-subunit. For instance, β-ATPaseD and β-ATPaseE have identical patterns of cross-reactivity and either recognize the same epitope or an epitope that is conserved in an identical manner. The same can be said of the β-ATPaseB and β-ATPaseC MAb's, which also display identical cross-reactivity patterns. The β-ATPaseA MAb recognizes a third epitope; it does not recognize the bean β-subunit.

Three of the MAb's cross-react with pea cotyledon mitochondrial protein and the purified pea F_1-ATPase. The MAb's detect all of the isoelectric variants that were observed upon Coomassie staining of the 2D gels of the purified enzyme, indicating that these spots are antigenically related. We found that two of the MAb's that recognize the pea enzyme, α-ATPaseD and β-ATPaseD, are capable of tightly binding the purified F_1 enzyme after the MAb's themselves have been covalently linked to epoxide-glass beads. Our calculations indicate that the amount of enzyme bound to the beads was slightly less than the theoretical binding capacity. A likely explanation for this binding ratio is that some of the IgGs are attached to the beads in a manner that does not allow them to bind the antigen. Again, in spite of the strong binding indicated by the western blots and the QuantAffinity beads, none of the MAb's inhibit the activity of pea F_1-ATPase after

a 4-h incubation, even when present in a 10-fold excess over the enzyme.

We hope to make further use of the strong binding properties of these MAb's in the future. These MAb's make excellent candidates for the production of affinity columns. These columns, in turn, would be quite useful in the purification of the F_1-ATPase from other plant mitochondrial sources. Another potential application of these affinity columns would be to sort submitochondrial particles on the basis of their sidedness. Inside-out vesicles would be retained or slowed by the column, whereas vesicles that are right-side-out would not. Suitable resins that make such an application feasible are available, and the mitochondrial field currently lacks a highly reproducible and reliable method for sorting mitochondrial vesicles. Because at least two of the MAb's bind the ATPase without inhibiting activity, the α- and β-subunits of the ATPase could be immobilized and used for studies concerning assembly of the enzyme and for following activity of the ATPase during various stages of assembly.

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