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Biochemistry

Deletion analysis of the maize mitochondrial superoxide dismutase transit peptide

(protein processing/*Zea mays*)

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ABSTRACT **The maize mitochondrial superoxide dismutase (SOD; EC 1.15.1.1), a nuclear gene product, has been previously shown to be imported into maize mitochondria. The cDNA for maize mitochondrial SOD was subcloned into a vector containing the T7 promoter. Deletions were made in the transit peptide coding region of the cDNA. The undeleted and deleted proteins were synthetically produced by transcription and translation *in vitro*. Undeleted preSOD-3 is translocated into isolated maize mitochondria with an efficiency of ≈30%. Mature SOD-3 subunits are recovered from the matrix but not the membranes of subfractionated mitochondria. These subunits are assembled into the tetrameric holoenzyme. The modified SOD-3 precursors are imported into mitochondria at lower efficiencies than undeleted preSOD-3. The relative import efficiency appears to be dependent upon the deletion size. To our knowledge such analysis of a plant mitochondrial precursor protein has not been reported previously.**

The amino termini of many proteins contain information utilized during compartmentalization (1–4). The amino-terminal signal, or transit, sequence determines the final localization of a polypeptide, although the efficiency of transport is dependent upon other factors—e.g., ATP-dependent unfolding of mitochondrial proteins (5) or the presence of signal recognition particles for secretory proteins (6). Mitochondrial transit sequences vary greatly in size (0.5–10.0 kDa), contain an abundance of basic and hydroxylated amino acid residues, and lack acidic residues (2, 7). In addition, it is hypothesized that mitochondrial transit sequences can form amphiphilic α-helices that allow them to interact directly with the mitochondrial membranes (8, 9).

The amino-terminal presequences (transit sequences) of several mitochondrial precursor proteins have been shown to mediate efficient importation of "passenger" proteins into mitochondria (5, 10–12). Deletion analysis of the cytochrome c_1 transit sequence has shown that it consists of matrix targeting and stop transfer sequences separated by proteolytic cleavage sites (11, 13). The cytochrome oxidase subunit IV transit sequence consists of a matrix targeting sequence and a proteolytic cleavage site (14), although proteolysis also is dependent upon sequences at the amino terminus of the transit sequence.

Site-directed mutagenesis of the preornithine transcarbamoylase transit sequence demonstrated that certain arginine residues are required for its importation (15). Generation of a synthetic transit peptide sequence, containing arginine, serine, and leucine, for cytochrome oxidase subunit IV allows growth of yeast transformed with the gene for this fusion protein on nonfermentable carbon sources. Synthetic transit peptides composed of glutamine, arginine, serine, and leucine were not effective (16). It was suggested (16) that the ratio of basic, hydroxylated, and hydrophobic amino acids in transit sequences allows them to mediate importation.

In contrast, it has been found that the adenine nucleotide translocator, a nonprocessed mitochondrial protein, is still imported even when the amino-terminal 103 (out of 313) amino acid residues are deleted (17). It was shown (18) that the amino-terminal 115 amino acid residues could mediate importation. This protein may have multiple internal targeting sequences (17).

The four superoxide dismutase (SOD; EC 1.15.1.1) isozymes of maize are coded by four nonallelic nuclear genes: *Sod1*, *Sod2*, *Sod3*, and *Sod4* (19). The cytosolic isozymes, SOD-2 and SOD-4, and the chloroplast isozyme, SOD-1, are copper- and zinc-containing homodimeric enzymes. The mitochondrial isozyme, SOD-3, is a manganese-containing homotetrameric enzyme (20, 21). SOD-3 has been shown to be synthesized as a precursor, which translocated into isolated maize mitochondria (22). In this report we present evidence on a deletion analysis of maize mitochondrial SOD. Herein, we report on the *in vitro* production of transit peptide deletions in the maize Mn-SOD and their effects on the importation of these modified proteins into isolated maize mitochondria. Our results confirm the predicted role of presequences for the localization of proteins into the mitochondria of higher plant cells. To our knowledge such analysis of a plant mitochondrial precursor protein has not been reported previously. Our data show that relative importation is dependent on the extent of deletion within the preSOD-3 transit peptide.

MATERIALS AND METHODS

Materials. The cDNA clone for maize SOD-3 encoded in plasmid pSod3.1c was prepared as described (23). The transcription vector, pBS M13, was obtained from Stratagene Cloning Systems. The *Sod4* cDNA clone has been described (24). BAL-31 nuclease was from Boehringer Mannheim Biochemicals. Restriction endonucleases and other DNA modifying enzymes were obtained from various commercial sources. Cesium chloride was from Gallinger-Schlessinger. All other chemicals were of reagent grade or better.

BAL-31 Deletion. *Sod3* cDNA was transferred to pBS M13+ by separately digesting pSod3.1c and pBS M13+ with *Eco*RI. *Eco*RI was heat treated at 70°C for 10 min. The DNAs were mixed and ligated with T4 DNA ligase. *Escherichia coli* strain JM109 was transformed with ligated plasmids according to the $CaCl_2$ procedure (25).

Plasmid pBSSod3 containing the *Sod3* cDNA was linearized with *Xma* III, which cuts this DNA at a unique site in the 21st and 22nd codons of the transit peptide coding region (Fig. 1). After incubation of the DNA with BAL-31 at 37°C for 30 or 60 sec, aliquots were transferred to 20 mM EGTA. The DNA was extracted with phenol/chloroform (1:1) and chlo-

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Abbreviation: SOD, superoxide dismutase.

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roform; each sample was precipitated with ethanol twice. BAL-31-digested DNA was treated with T4 DNA polymerase to produce blunt ends (25). The DNA was extracted and precipitated as above. The plasmid DNAs (<0.01 μg/ml) were treated with T4 DNA ligase and used to transform JM109 as described above.

Colony Screening. Plasmid DNAs were prepared as described (26). DNAs were digested with *Pvu* II and analyzed in 6% polyacrylamide gels (25). Those clones showing deletions of <150 base pairs (bp) were analyzed further by dideoxy chain-termination sequencing of the plasmid DNA (27). The T7 primer (Stratagene) was used to initiate strand synthesis. Plasmids with deletions that maintained the reading frame of *Sod3* were purified by CsCl gradient preparation.

Transcription and Translation. Plasmid DNAs were digested with *Hin*cII or *Bal* I for 2 hr at 37°C. Proteinase K treatment and transcription *in vitro* were carried out as described by Stratagene Cloning Systems. The cap analog, m7GpppG (Pharmacia), was included in all transcriptions (0.5 mM). The reaction mixtures were extracted with phenol/chloroform and chloroform and were precipitated with ethanol twice. *In vitro*-transcribed RNAs were translated in rabbit reticulocyte lysate (Bethesda Research Laboratory) as described by the manufacturer.

Importation Experiments. Isolation of mitochondria and importation experiments were carried out as described (22), with minor modifications. Reticulocyte lysate was diluted with wash buffer, mitochondria, and salts such that a 1:10 dilution was obtained. The final concentrations were 1 mg of mitochondria per ml, 5 mM NADH, 70 mM KCl, 8 mM $MgCl_2$, 0.1 mM L-methionine, 20 mM Hepes-KOH (pH 7.2), 0.1 mM EDTA, 0.1% bovine serum albumin, and 0.4 M mannitol. Valinomycin was added from a 100-fold concentrated ethanol solution to 2.5 μM where indicated.

Subfractionation of Mitochondria. Osmotically shocked mitochondria were prepared as described (11). After importation mitochondria were diluted with 4 vol of 20 mM Hepes-KOH (pH 7.2) and left on ice for 15 min. The mixture was split into two tubes, one of which received 0.2 mg of chymotrypsin per ml. Incubation was continued at 37°C for 15 min, at which time digestion was inhibited with 1 mM phenylmethylsulfonyl fluoride. This fraction is equivalent to isolation of mitoplasts. Mitochondria were recovered from the other tube by centrifugation at 12,000 rpm (Beckman JA-20 rotor). The supernatant is equivalent to the intermembrane space. Matrix and membrane fractions were produced by freezing the mitoplast fraction with liquid nitrogen and then thawing. This was repeated three times. Membranes were recovered by centrifugation at 15,000 rpm in a Hill Microfuge for 20 min. The supernatant was taken as the matrix fraction; the brown pellet was taken as the membrane fraction.

Electrophoresis. SDS/polyacrylamide gels (14%) were prepared (28), run, and treated for fluorography (29). Electrophoresis in nondenaturing polyacrylamide gels (9%) employed the Laemmli procedure, except that SDS and 2-mercaptoethanol were excluded from all buffers. Sequencing gels [7% acrylamide/8 M urea in TBE buffer (Tris-borate/EDTA buffer, ref. 25)] were prepared as described (25). After electrophoresis, sequencing gels were fixed in 10% methanol/acetic acid, dried, and exposed to x-ray film. Autoradiographs were scanned with a Gilford response II spectrophotometer.

RESULTS

The cDNAs for maize mitochondrial SOD, pSod3.1c, and for the cytosolic SOD, pSod4.1c, were transferred into the expression vector pBS M13+ such that the expression of each was under the control of the T7 promoter. The plasmid pBSSod3 was purified by CsCl gradient centrifugation. Plasmid DNA was digested at the unique *Xma* III site located within the transit peptide coding region (Fig. 1 *A* and *B*). Deletions were generated with BAL-31 exonuclease. Clones bearing the recircularized plasmids were selected by restriction endonuclease digestion and plasmid sequencing. The DNA sequences of six modified plasmids retained the pre-SOD-3 reading frame in the transit peptide coding region (Fig.

B) TRANSIT PEPTIDE CODING REGION OF pSod3.1c

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Sod3   -30        -20         -10        1           10          20          30
cDNA CCCAGGAGATACAGCGAGCGAGCGACCAAAGCC ATG GCT CTC CGC ACC CTG GCA TCG AAG AAG
A.A.                                   Met Ala Leu Arg Thr Leu Ala Ser Lys Lys
                                        1                                   10
           40          50          60          70          80          90
GTC CTA TCC TTC CCG TTC GGC GGC GCG GGC CGG CCG TTG GCG GCG GCG GCG TCT GCG AGG
Val Leu Ser Phe Pro Phe Gly Gly Ala Gly Arg Pro Leu Ala Ala Ala Ala Ser Ala Arg
                                    20                                      30
           100         110         120         130         140         150
GGG GTG ACG ACG GTC ACA CTC CCC GAC CTC TCC TAC GAC TTC GGC GCG CTG GAA CCG GCC
Gly Val Thr Thr Val Thr Leu Pro Asp Leu Ser Tyr Asp Phe Gly Ala Leu Glu Pro Ala
                                    40                                      50
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C) BAL 31 DELETION MUTANTS OF THE pSod3.1c TRANSIT PEPTIDE

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     bp  (codon)
                    5         10        15        20        25        30
SOD-3          M A L R T L A S K K V L S F P F G G A G R P L A A A A S A R G|V T
A35 -21 (18/24) M A L R T L A S K K V L S F P F G           A A A S A R G|V T
B10 -30 (14/23) M A L R T L A S K K V L S                 A A A A S A R G|V T
C38 -45 (15/29) M A L R T L A S K K V L S F                         R G|V T
A8  -51 (15/31) M A L R T L A S K K V L S F                           |V T
B3  -60 (9/28)  M A L R T L A S                                   A R G|V T
C37 -96 (16/47) M A L R T L A S K K V L S F P/E P A I S.....
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FIG. 1. (*A*) Restriction enzyme digestion map of *Sod3* cDNA in pBS M13+. Narrow lines indicate nontranslated regions (5′ end to the left) and thick lines at both ends represent vector sequences. The coding regions are the middle thick lines, the dark region denotes the mature polypeptide, and the clear region denotes the transit peptide. Restriction sites are abbreviated: A, *Ava* I; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; N, *Nco* I; P, *Pst* I; Pv, *Pvu* II; X, *Xma* III. (*B*) DNA and deduced amino acid sequences of the pBSSod3 transit peptide coding region. (*C*) Deduced amino acid sequences of deletion clones (only the transit peptide coding region is shown). BAL-31 digestion began at the unique *Xma* III site (underlined in the DNA sequence shown in *B*), encoding residues Arg^{21}-Pro^{22} (underlined). The number of base pairs deleted and the codons deleted are shown. The mature amino-terminal residue (valine) is underlined in *B* and is indicated by the vertical line in *C*.

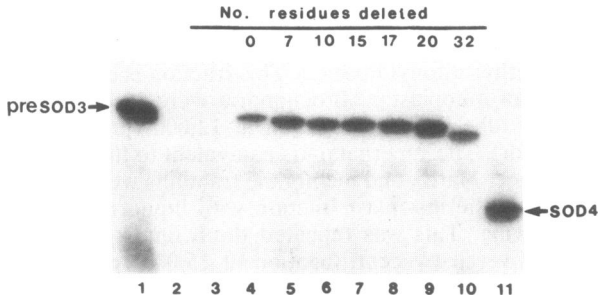

FIG. 2. Synthetically prepared preSOD-3 deletion proteins. *In vitro* translation products of transcribed RNAs prepared from plasmid DNA templates. Lanes 1 and 4, pBSSod3; lane 2, pBS M13+; lane 5, A35; lane 6, B10; lane 7, C38; lane 8, A8; lane 9, B3; lane 10, C37; lane 11, pBS4. Endogenous reticulocyte translation products are shown in lane 3. The number of amino acids deleted in each precursor is indicated above each lane.

1 *B* and *C*). The RNAs prepared from these deletion clones and from undeleted pBSSod3 were translated in the rabbit reticulocyte lysate system (Fig. 2). The major band shown in each lane (except lane 11) was precipitable with SOD-3 antiserum (not shown). RNA prepared from the plasmid vector alone, and translation mixtures not primed with RNA, did not produce any products remotely resembling SOD-3 or SOD-4 (Fig. 2, lanes 2 and 3).

When preSOD-3 is incubated with maize mitochondria, mature SOD-3 is recovered from reisolated mitochondria (Fig. 3*A*, lanes 2, 6–11, 13, and 14). If the mitochondria are lysed with Triton X-100 before addition of protease, mature SOD-3 is still recovered. Our previous results indicated that SOD-3 is resistant to protease digestion (22). The relative proportion of mature SOD-3 in the lysed and nonlysed mitochondrial preparations is ≈50% (Fig. 3*A*, lanes 13 and 14). If valinomycin (an ionophore that inhibits protein importation by mitochondria) is included in the medium (>1 μM), SOD-3 is not recovered from the mitochondria (Fig. 3*A*, lanes 3–5). The addition of ATP to the medium did not stimulate greater importation; in fact, when included at 2 mM, the relative amount of SOD-3 recovered was decreased (Fig. 3*A*, lanes 6–8). Although ATP is necessary for unfolding of polypeptides during importation, it does not appear to

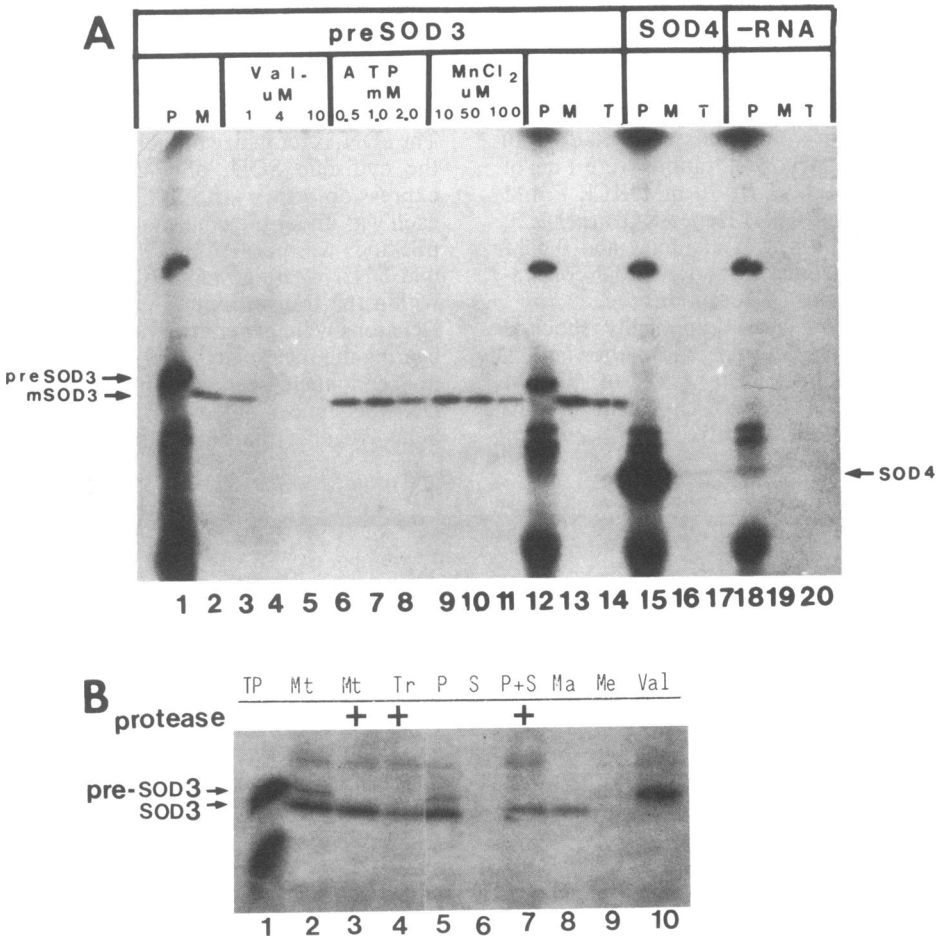

FIG. 3. (*A*) Importation of preSOD-3. Maize mitochondria were incubated with preSOD-3 (lanes 1 and 12), SOD-4 (lane 15), or endogenous reticulocyte (lane 18) translation products. The mitochondria were reisolated and treated with chymotrypsin (1 mg/ml) (lanes 2–11, 13, 16, and 19) or were lysed with 0.1% Triton X-100 and then treated with chymotrypsin (lanes 14, 17, and 20). Prior to importation incubations, mitochondria were treated with nothing (lane 2), 1 μM (lane 3), 4 μM (lane 4), or 10 μM (lane 5) valinomycin (Val), 0.5 mM (lane 6), 1.0 mM (lane 7), or 2.0 mM (lane 8) ATP, or 10 μM (lane 9), 50 μM (lane 10), or 100 μM (lane 11) $MnCl_2$. P, translation products; M, mitochondria; T, Triton X-100-lysed mitochondria. (*B*) Submitochondrial localization of SOD-3. After importation of preSOD-3, mitochondria were fractionated into intermembrane space (lane 6), mitoplast (lane 7), matrix (lane 8), and membrane (lane 9) fractions. They were analyzed in denaturing 14% polyacrylamide gels. Lane 1, translation product of *Sod3*; lane 2, mitochondria (Mt) reisolated after incubation with preSOD-3; lane 3, reisolated mitochondria treated with 0.2 mg of chymotrypsin per ml; lane 4, reisolated mitochondria lysed with 0.2% Triton X-100 (Tr) and treated with chymotrypsin; lane 5, osmotically shocked mitochondria (P); lane 6, supernatant from osmotically shocked mitochondria (S); lane 7, chymotrypsin-treated supernatant and osmotically shocked mitochondria (P+S); lane 8, supernatant from three times frozen and thawed mitoplasts (Ma); lane 9, membrane pellet from three times frozen and thawed mitoplasts (Me); lane 10, mitochondria deenergized with 2.5 μM valinomycin and incubated with preSOD-3.

stimulate translocation *per se* when included at high relative levels. On the contrary, stabilization of the conformation of a precursor inhibits its importation (5, 30). The addition of $MnCl_2$ to the importation medium (10–100 μM) decreased the relative amount of SOD-3 recovered in the mitochondria (Fig. 3*A*, lanes 9–11). When SOD-4 is incubated with mitochondria, none of it is recovered in reisolated mitochondria (Fig. 3*A*, lanes 15–17). Similarly, none of the endogenous reticulocyte translation products become associated with the mitochondria (Fig. 3*A*, lanes 18–20). Subfractionation of mitochondria into matrix, membrane, and intermembrane space fractions indicated that preSOD-3 is translocated into the matrix and processed (Fig. 3*B*, lanes 5–9).

Each of the deleted preSOD-3 proteins was assayed for importation (Fig. 4). The relative proportion of SOD-3 associated with mitochondria was determined by scanning the autoradiographs (Fig. 5). In each case, valinomycin inhibited importation (Fig. 4, lanes 4, 8, 12, 16, 20, 24, and 28). In each case, SOD-3 was detected; however, the SOD-3 associated with mitochondria from proteins Δ9–28 and Δ16–47 is very low; the proportion associated with mitochondria is <6% of the amount of precursor added to the mitochondria (Fig. 4, lanes 22 and 26). All of the remaining deletion proteins were imported into mitochondria, although the efficiency of importation declined as the deletion size increased (Figs. 4 and 5). We observe ≈7% variation in efficiency from repetition of these experiments. It is important to notice that, upon importation of Δ15–29 and Δ15–31, the efficiency of proteolysis by the matrix protease is reduced for these modified precursors. These proteins are imported into the matrix and are accessible to the matrix protease (Fig. 4, lanes 14 and 18). Also, since both imported proteins are further modified by chymotrypsin upon Triton X-100 lysis of the mitochondria, our protease treatments effectively remove nonimported proteins from the outer surface of the mitochondria (Fig. 4, lanes 15 and 19).

Finally, to assess the assembly of mature SOD-3 subunits into tetrameric SOD-3, mitochondria were incubated with or without preSOD-3, subfractionated into membrane/matrix, intermembrane space, and mitoplasts, and analyzed in nondenaturing polyacrylamide gels (Fig. 6). Active SOD-3 is found in osmotically shocked mitochondria and in mitoplasts, as expected for this matrix enzyme (Fig. 6, lanes 2 and 4). Radiolabeled SOD-3 subunits are assembled into tetrameric holoenzyme after importation (Fig. 6, lanes 5 and 7). No radiolabeled SOD-3 is found in the intermembrane space fraction (Fig. 6, lane 6). The radiolabeled preSOD-3 has a much greater relative mobility than tetrameric SOD-3 (Fig. 6, lanes 8–10). In addition, a band of radioactivity appears well above the tetrameric SOD-3 (Fig. 6, lanes 8 and 9). This may be an aggregate of precursor molecules; this has been observed for other mitochondrial precursors (31). Note that none of the precursor bands overlap with the tetrameric SOD-3 band (Fig. 6, lane 8), indicating that preSOD-3 does not simply aggregate into tetramers. These results strongly indicate that preSOD-3 is imported, processed, and assembled into active, tetrameric SOD-3.

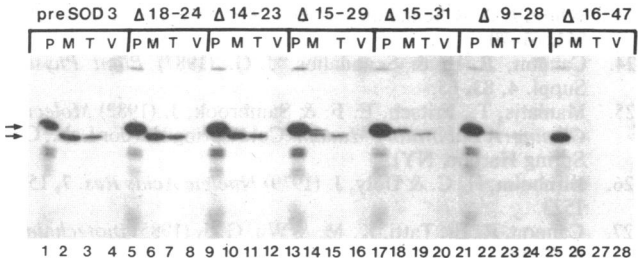

FIG. 4. Importation of preSOD-3 deletion proteins. Maize mitochondria were incubated with preSOD-3 (lane 1), Δ18–24 (lane 5), Δ14–23 (lane 9), Δ15–29 (lane 13), Δ15–31 (lane 17), Δ9–28 (lane 21), and Δ16–47 (lane 25) precursor proteins. Mitochondria were reisolated and treated with 0.5 mg of chymotrypsin per ml (lanes 2, 6, 10, 14, 18, 22, and 26) or were lysed with 0.1% Triton X-100 and then treated with chymotrypsin (lanes 3, 7, 11, 15, 19, 23, and 27). In separate incubations, mitochondria were treated with valinomycin (2.5 μM) prior to incubation with translated proteins (lanes 4, 8, 12, 16, 20, 24, and 28). P, translation products; M, mitochondria; T, Triton X-100-lysed mitochondria; V, valinomycin-treated mitochondria.

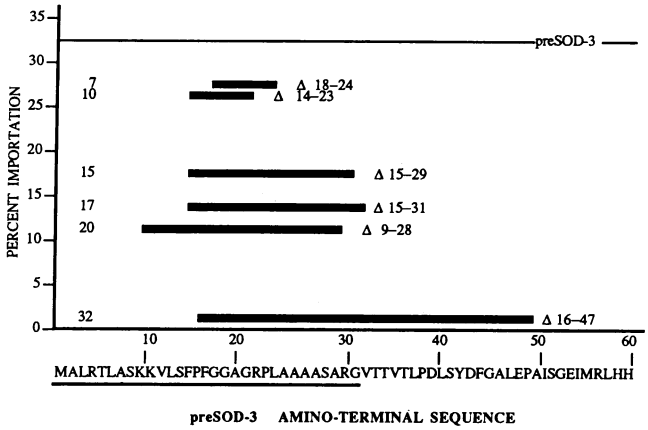

FIG. 5. Histographic representation of importation efficiency. Percent importation is plotted against the preSOD-3 amino-terminal sequence. Each bar represents the percent importation of, and the size of the deletion in, the indicated precursor. Numbers to the left of each bar are the number of amino acids deleted from that precursor. The horizontal line indicates the percent importation of unmodified preSOD-3. The transit peptide sequence is underlined. The values for percent importation are the means (±7%) of two experiments.

DISCUSSION

We have previously shown that the precursor for maize mitochondrial SOD is imported into maize mitochondria (22). In this report, we have shown that a synthetically prepared

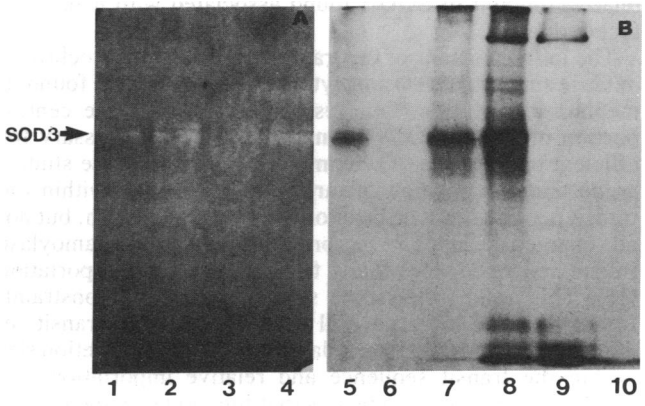

FIG. 6. Assembly of SOD-3 subunits into active holoenzyme. Washed mitochondria were incubated with (lanes 5–8) or without (lanes 2–4) radiolabeled preSOD-3. The mitochondria were osmotically shocked and separated into membrane/matrix (lanes 2 and 5), intermembrane space (lanes 3 and 6), or mitoplast (lanes 4, 7, and 8) fractions. All samples were analyzed in a nondenaturing, 9% polyacrylamide gel. (*A*) Stained for SOD activity. (*B*) Fixed and prepared for fluorography. Equivalent amounts of mitochondrial protein were loaded in each half of the gel. Lane 1, SOD-3 (5 μg) isolated from maize kernels. Lanes 9 and 10, *in vitro*-translated preSOD-3 (equivalent to 1 μl of reticulocyte lysate), nondenatured (lane 9) and heat denatured (lane 10). The mitoplast fractions (lanes 4, 7, and 8) had been incubated with 0.2 mg of chymotrypsin per ml. Lane 8 is a mixture equivalent to lanes 7 and 9.

preSOD-3 from a cDNA clone behaves similarly to preSOD-3 prepared from poly(A)$^+$ RNA. The cytosolic SOD-4, similarly prepared, did not associate with mitochondria under identical conditions. We have attempted to define the minimum sequence required for the importation of preSOD-3 into mitochondria by making deletions in the transit peptide coding region of our cDNA. The results indicate that efficiency of importation is dependent on the extent of deletion (Fig. 5). Importation of undeleted preSOD-3 has the same relative efficiency as importation of other mitochondrial precursors (≈30% of the input precursor).

Analysis of the importation efficiencies of the modified preSOD-3 proteins indicates that five regions of the transit peptide gave distinct effects on importation when deleted (Fig. 5). These are ^{9}Lys-Phe^{14} (compare Δ15–29 to Δ9–28), ^{18}Gly-Ala^{24} (compare undeleted preSOD-3 to Δ18–24), ^{24}Ala-Ala^{29} (compare Δ14–23 to Δ15–29), ^{30}Arg-Gly^{31} (compare Δ15–29 to Δ15–31), and ^{32}Val-Leu^{47} (compare Δ15–31 to Δ16–47). Sequences in the mature amino-terminal region of preSOD-3 may significantly affect importation (compare undeleted preSOD-3 to Δ15–31 and Δ16–47). However, juxtaposition of sequences that interfere with importation might play a role in reducing the importation of Δ16–47. We have not yet made short deletions of the various regions to determine whether ^{9}Lys-Phe^{14}, ^{24}Ala-Ala^{29}, or the mature amino-terminal regions are specifically required for efficient importation.

The limited protease resistance of preSOD-3 may interfere with the determination of importation efficiency. In cases in which ruptured mitochondria were incubated with proteases, ≈50% of the SOD-3 found in mitochondria was degraded (Figs. 3 and 4). SOD-4, a cytosolic protein, was not recovered from mitochondria (Fig. 3), indicating negligible nonspecific association. Fractionation of mitochondria after importation of preSOD-3 indicated that SOD-3 is located in the matrix and is assembled into holoenzyme (Fig. 6). Thus, whatever SOD-3 is found associated with mitochondria after protease treatment appears to be genuinely imported. Our estimation of efficiencies—i.e., percent of input precursor imported and processed into mitochondria—is an accurate measure of the relative amount of SOD-3 found associated with maize mitochondria.

The initial portions of the transit sequences of cytochrome oxidase subunit IV (14) and cytochrome c_1 (11) were found to mediate importation. Our results indicate that the central portion of the preSOD-3 transit sequence is necessary for efficient importation. The combined results of these studies argue that the position of targeting information within the transit peptide is not critical for importation. Certain, but not all, charged residues in the preornithine transcarbamoylase transit sequence were found to be required for importation (16). This argues that some specific functional constraint, rather than the more general basic character of transit sequences, is important. The relationship between deletion size within the transit sequence and relative importation, observed in our results, indicates that functional elements may be spread throughout the transit sequence of preSOD-3. Conventional wisdom, which states that a transit sequence is composed of appropriate ratios of basic, hydroxylated, and hydrophobic residues (17), seems tenuous at best. This hypothesis is certainly not the case for chloroplast transit sequences (4). Since receptors are probably involved in mitochondrial protein importation, one would expect to find some well-defined structural feature recognized by each specific receptor. The amphiphilic α-helix may or may not be that feature. Crystallographic analysis of mitochondrial precursors may be necessary to settle this dilemma.

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