Plant Physiol. (1982) 70, 483–487
0032-0889/82/70/0483/05/$00.50/0

Organelle-Bound Malate Dehydrogenase Isoenzymes Are Synthesized as Higher Molecular Weight Precursors[1]

Received for publication January 19, 1982 and in revised form March 22, 1982

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ABSTRACT

Biosynthesis of malate dehydrogenase isoenzymes was studied in cotyledons of watermelons (*Citrullus vulgaris* Schrad., var. Stone Mountain). The glyoxysomal and mitochondrial isoenzymes are synthesized as higher molecular weight precursors which can be immunoprecipitated by monospecific antibodies from the products of *in vitro* translation in reticulocyte lysates programed with cotyledonary mRNA and with the same size from enzyme extracts of pulse-labeled cotyledons. During translocation from the cytosol into the organelles, processing takes place. An 8 kilodalton extra sequence is cleaved from the glyoxysomal precursor and a 3.3 kilodalton extra sequence from the mitochondrial precursor producing the native subunits of 33 and 38 kilodaltons, respectively. The data support a post-translational translocation of the organelle-destined malate dehydrogenase isoenzymes. The *in vitro* translation of the cytosolic malate dehydrogenase I yields a product which has the same molecular weight as the subunit of the native isoenzyme (39.5 kilodaltons).

The development of cell organelles requires the import of proteins which are synthesized on cytoplasmic ribosomes. The translocation from the site of synthesis to the site of function involves a highly specific recognition mechanism by the organelle envelope which only accepts the organelle-destined proteins for segregation within the organelle.

The use of isoenzyme system for translocation studies requires that individual isoenzymes be located in different organelles within the same cell. Differences in these proteins or their precursors should be related, at least in part, to the pattern of distribution. Only a very few isoenzyme systems have been employed in this way, *e.g.* the aspartate aminotransferase (17) and the malate dehydrogenase (19) from rat liver where the mitochondrial and the cytosolic isoenzymes were compared. *In vitro* transport studies with purified isoenzymes revealed that the high selectivity of the isoenzyme sequestration by the mitochondria could be related to differences in the molecular properties of the mitochondrial and cytosolic isoenzymes.

The malate dehydrogenase (L-malate-NAD-oxidoreductase, EC 1.1.1.37) isoenzyme system in cotyledons from fatty seedlings offers the advantage of a second organelle-housed isoenzyme: in addition to one mitochondrial and three cytosolic isoenzymes, a gMDH[2] can be detected (13, 16). The individual forms are easily distinguished by electrophoretic or serological techniques. Earlier studies on the *in vitro* synthesis of gMDH by a wheat germ system by our group (26) had shown that this isoenzyme is made as a higher mol wt polypeptide compared with the subunit of gMDH isolated from watermelon cotyledons thereby suggesting the need for processing during the importation into the glyoxysomes. The results were confirmed subsequently by Riezman *et al.* (20) for cucumber cotyledons.

We now present a comparative study on the *in vitro* synthesis of gMDH, mMDH, and cMDH I using the heterologous reticulocyte system. Our results indicate that mMDH also is synthesized as a larger *in vitro* product[3] in contrast to the cytosolic isoenzyme. Pulse labeling experiments provided evidence that the higher mol wt MDH polypeptide precursors were also detectable under *in vivo* conditions. The posttranslational implications for the role of such precursors in directing transport into glyoxysomes and mitochondria are discussed.

MATERIALS AND METHODS

Plant Material. Watermelon seed (*Citrullus vulgaris* Schrad., var. Stone Mountain, harvest 1978, supplied by Vaughan's Seed Company, Ovid, MI) were germinated under sterile conditions in the dark at 30°C as described before (12).

***In Vivo* Labeling.** Fifty pairs of 2-d cotyledons were shaken in an Erlenmeyer flask at 80 rpm under sterile conditions in the dark at 30°C in 2 ml H_2O containing 0.5 mCi [^{35}S]methionine (about 1,000 Ci/mmol, New England Nuclear). After 24 h (unless indicated otherwise), the cotyledons were rinsed three times with 20 ml of 10 mM unlabeled methionine at 4°C. For pulse-chase experiments, the 2-d-old cotyledons were incubated under the same conditions except for a 1-h pulse with 1.0 mCi [^{35}S]methionine per 2 ml H_2O. Then, the labeled methionine was removed quickly by three washes with 20 ml of 10 mM unlabeled methionine at 30°C, followed by the chase in 2 ml of 10 mM methionine. After 30 min, 1h, 2h, and 3h, aliquots of 12 pairs of cotyledons were removed and stored under liquid N_2.

Preparation of Enzyme Extracts. The cotyledons were homogenized with a VirTis homogenizer (Cenco) three times 20 s at 40,000 rpm in an ice-cold medium (0.5 ml/pair of cotyledons) containing 0.1 M K-phosphate buffer (pH 7.5), 0.5 M mannitol, 10 mM EDTA (disodium salt), 3 mM $MgCl_2$, and 1 mM PMSF. The crude extract was centrifuged at 4°C for 30 min at 300,000$g_{average}$ (Sorvall OTD-65, T 865 rotor) to yield the clear enzyme extract as supernatant solution.

***In Vitro* Protein Synthesis.** Polyadenylated mRNA was isolated from 2-d cotyledons as described before (26) and translated at 30°C in a rabbit reticulocyte lysate system (New England Nu-

[1] Supported by the Deutsche Forschungsgemeinschaft (Grant Ho 383/19).

[2] Abbreviations: gMDH, glyoxysomal malate dehydrogenase; mMDH, mitochondrial malate dehydrogenase; cMDH, cytoplasmic malate dehydrogenase; PMSF, phenylmethylsulfonylfluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

[3] The *in vitro* synthesis of mMDH and gMDH has already been summarized.

clear). Each 50-μl assay contained 4 μl of mRNA (21.8 A_{260}/ml), 10 μl [^{35}S]methionine (corresponding to 100 μl with 1,000 Ci/mmol), and 36 μl of translation mixture including the energy supply and the reticulocyte lysate. After 60 min, the translation was stopped by a 1:5 dilution with 200 μl of ice-cold, nonradioactive enzyme extract from day 3 cotyledons which proved sufficient to produce coprecipitation of the *in vitro* synthesized MDH isoenzymes by monospecific antibodies. The mixture was centrifuged at 4°C for 30 min at 100,000$g_{average}$ (AH 627 rotor), and the supernatant was used for the immunoprecipitation.

Immunoprecipitation. Monospecific anti-gMDH and anti-mMDH antibodies were produced according to the methods of Walk and Hock (24, 25), while monospecific anti-cMDH I antibodies were developed as described before (22). The immunoprecipitations were carried out with the IgG fractions of the antisera or control sera, respectively, obtained by $(NH_4)_2SO_4$ fractionation.

MDH isoenzymes from the enzyme extract of *in vivo* labeled cotyledons were directly immunoprecipitated in Eppendorf reaction vessels with monospecific antibodies by mixing 200 μl of the enzyme extract with 15 μl of the specific antiserum which had been previously adjusted according to the optimal antigen-antibody ratio. The *in vitro* synthesized MDH isoenzymes were similarly immunoprecipitated directly from the cell-free system after dilution with 200 μl of unlabeled enzyme extract from 3-d cotyledons.

The antigen-antibody mixtures were incubated at 4°C for 16 h. The immunoprecipitates from the *in vivo* labeling experiments were pelleted by centrifugation for 10 min in an Eppendorf centrifuge and washed twice with 100 μl of TKT buffer (10 mM Tris·HCL [pH 7.5], 0.3 M KCl, 1% [v/v] Triton X-100) and three times with 100 μl of TK buffer (10 mM Tris·HCl [pH 7.5], 0.3 M KCl).

The immunoprecipitates of the *in vitro* labeling experiments were layered on stepped sucrose gradients in Eppendorf reaction vessels containing 100 μl of 1.0 M sucrose in 0.15 M NaCl + 1% (v/v) Triton X-100 and 50 μl of 0.5 M sucrose in the same medium, and centrifuged for 10 min in an Eppendorf centrifuge. The precipitates were washed as described before.

SDS Gel Electrophoresis and Fluorography. The immunoprecipitates were dissolved according to Blobel and Dobberstein (4) in 35 μl of sample buffer (90 mM Tris·HCl [pH 8.8], 2.6% [w/v] SDS, 30% [w/v] sucrose, 1.1 mg/ml dithioerythritol, 90 μg/ml [w/v] bromphenol blue). Polyacrylamide gels (10–15% [w/v] acrylamide gradients) and fluorography of the dried gels were carried out as described (4). The exposure time to x-ray films was 10 d.

Limited Proteolysis of the Immunoprecipitates. The immunoprecipitates dissolved in 35 μl of sample buffer were filled into the slots of the SDS gels, overlayered with 10 μl of glycerol medium and 10 μl (corresponding to 5 μg) of protease from *Staphylococcus aureus*, V8 (Miles), and subjected to electrophoresis as described by Cleveland *et al.* (7).

RESULTS

***In Vitro* Translation.** In watermelon cotyledons, five MDH isoenzymes are found. They can be separated by disc-electrophoresis and stained for MDH activity by the reduction of tetrazolium

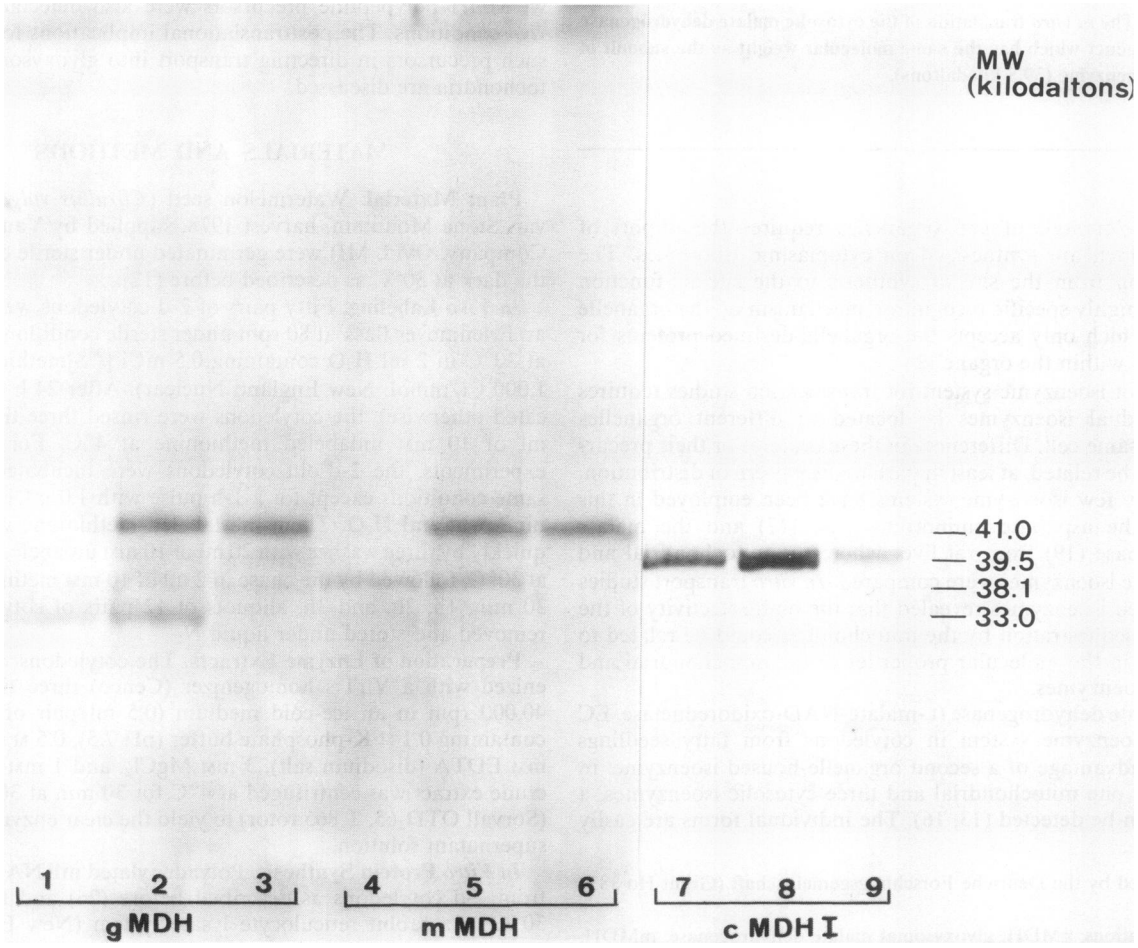

FIG. 1. Immunoprecipitation of MDH isoenzymes by monospecific antibodies and analysis by SDS-PAGE followed by fluorography. Lanes 1–3, gMDH; lanes 4–6, mMDH; lanes 7–9, cMDH I; synthesized *in vivo* in the presence of [^{35}S]methionine (lanes 1, 4, 7) or *in vitro* (lanes 3, 6, 9), respectively. Mixtures of the corresponding *in vivo* and *in vitro* products are shown in lanes 2, 5, and 8.

salts in the presence of NAD and malate. The bands are numbered consecutively from the anode to the cathode. MDH I, II, and IV are cytosolic forms. MDH III is located in the mitochondria, MDH V in the glyoxysomes (13).

The availability of monospecific antibodies against MDH I, III, and V allowed the exclusive immunoprecipitation of the first cytosolic, the mitochondrial, and the glyoxysomal isoenzymes, respectively, from cotyledonary enzyme extracts as well as from cell-free translation products which were obtained in the presence of [^{35}S]methionine and cotyledonary mRNA from 2-d-old dark-grown watermelon seedlings.

Figure 1 shows a representative analysis of the *in vivo* and *in vitro* labeled MDH isoenzymes by SDS-PAGE and fluorography. The native isoenzymes, which are composed of two identical subunits (23), differed significantly with regard to the mol wt of their subunits. The mol wt estimates, which were based on at least 10 independent experiments, were 33,000 ± 200 (mean ± SE) for gMDH (lane 1), 38,000 ± 400 for mMDH (lane 4), and 39,500 ± 300 for cMDH I (lane 7). The *in vitro* products exhibited higher mol wt for both of the organelle-bound MDH isoenzymes (41,000 ± 200 for gMDH [lane 3] and 41,350 ± 100 for mMDH [lane 6]). There were no differences between the *in vitro* product (39,500 ± 300) and the *in vivo* product of cMDH I (lane 9). Mixtures of the corresponding *in vivo* and *in vitro* synthesized isoenzyme yielded the expected electrophoretic pattern: two main bands with gMDH (lane 2) and mMDH (lane 5), and only one band with cMDH I (lane 8). In addition to the main band of *in vitro* synthesized gMDH, two additional faint bands with intermediate mol wt between the *in vitro* and the *in vivo* product were regularly seen. If the antisera were substituted by control sera, no radioactive bands were detected on the fluorograms (not shown). It is concluded from these findings that organelle-bound MDH isoenzymes are normally synthesized *in vivo* in watermelon cotyledons with an extra sequence which is split off after translation, most likely during the importation into glyoxysomes or mitochondria, respectively. On the other hand, cMDH I, which remains in the cytosol, is not synthesized with an extra sequence.

Pulse-Chase Experiments. In order to confirm the existence of higher mol wt precursors of gMDH and mMDH isoenzyme, *in vivo* labeling experiments of 2-d cotyledons were carried out with different labeling times ranging from 5 min to 24 h. Under the stated experimental conditions, radioactive gMDH was detected only after a labeling time of 30 min and as a weak band with the expected mol wt of 41,000. When labeling time was extended from 1 h up to 4 h, both gMDH forms with mol wt of 41,000 and 33,000 became visible with increasing intensities. A 24 h label, however, led to the predominant appearance of the 33,000 mol wt form. The detection of the 41,000 form in contrast to Figure 1, lane 1, was due to the increased concentration of [^{35}S]methionine (1.0 mCi/50 pairs of cotyledons).

The precursor-product relationship between the 41,000 and 33,000 mol wt forms of gMDH was established more firmly by pulse-chase experiments (Fig. 2). After a 1-h pulse label with [^{35}S] methionine, the cotyledons were incubated in 10 mM unlabeled methionine for 30 min (lane 1), 1 h (lane 2), 2 h (lane 3), and 3 h (lane 4). There was a distinct decrease of radioactivity in the 41,000 mol wt form when the chase period was extended beyond 60 min as compared to the 33,000 mol wt form. Under these experimental conditions, the higher mol wt form of gMDH disappeared after a 3-h chase period. During this period, there was a continuous increase of the native form.

Similar results were obtained for the mMDH isoenzyme (not shown). In this case, too, a higher mol wt precursor corresponding to the *in vitro* product was discovered in the crude extract after the 1-h pulse. In comparison with gMDH, the mMDH precursor seemed to disappear more quickly.

No higher mol wt form of cMDH I could be detected during either the pulse or chase experimental periods which seemed to confirm the lack of synthesis of an extra sequence on this isoenzyme.

Limited Proteolysis. If the native subunits of the organelle-bound MDH isoenzymes are produced by the cleavage of an extra sequence from a higher mol wt precursor, the relationship between the precursors and the products should also be detectable by limited proteolysis of the two forms. The similarity of the proteolytic patterns obtained after digestion by *S. aureus*, V8, followed by SDS-PAGE and fluorography (not shown) indicated indeed a

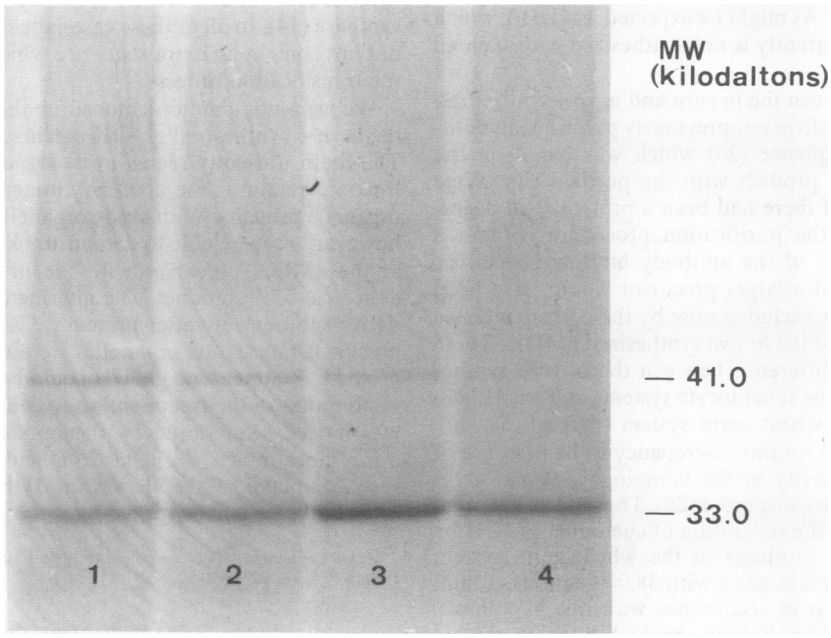

FIG. 2. Pulse-chase labeling of gMDH. Two-d-old cotyledons were pulsed for 1 h with [^{35}S]methionine (1 mCi/50 pairs of cotyledons) and subsequently chased with unlabeled methionine (10 mM) for 30 min (lane 1), 1 h (lane 2), 2 h (lane 3), and 3 h (lane 4). After homogenization in the presence of PMSF, gMDH was immunoprecipitated by monospecific antibodies and analyzed by SDS-PAGE followed by fluorography.

sequence similarity between the larger precursors and the smaller end products.

DISCUSSION

The malate dehydrogenases from watermelon cotyledons offer a unique opportunity to study the simultaneous synthesis and intracellular transport of isoenzymes, which are located ultimately in different cellular compartments and which exhibit different molecular properties even though they catalyze the same enzymic reaction. Biochemical and immunocytochemical data (13, 22) provide evidence that MDH V is located in glyoxysomes, whereas MDH III is confined to the mitochondria. As both isoenzymes are synthesized by cytoplasmic ribosomes (25, 27), there must be a translocation from the cytosol into the organelles. Since the pioneering studies of Blobel and Sabatini (5), Milstein *et al.* (18), and others on signal sequences of secretory proteins and their significance for the transfer across the ER membrane, it has been realized that many but not all cytosol-synthesized organelle proteins are equipped with an extra sequence which is split off during the sequestration by the organelles (6). The occurrence of specific recognition sequences in proteins destined for different organelles would explain their selective import across specific organelle membranes.

For this reason, the MDH isoenzymes were examined initially for the presence of such extra sequences under conditions where processing is lacking, *i.e.* by synthesis in a heterologous cell-free system. As a control, cMDH I was used. This cytosolic isoenzyme is located in the lower epidermis of the cotyledons (22), but is also found in the embryonic axis (13) in contrast to the other two cytosolic MDHs, which are confined to the cotyledons. Two-d cotyledons, which exhibit high rates of isoenzymes syntheses together with high levels of messenger RNA coding for the isoenzymes, were used for the experiments. The data demonstrate that the organelle-destined MDH isoenzymes are synthesized as higher mol wt polypeptides in contrast to the subunits immunoprecipitated from fresh enzyme extracts that were prepared from *in vivo* labeled cotyledons. The mol wt difference between the *in vitro* and *in vivo* products of 8,000 for gMDH and 3,350 for mMDH is in concurrence with the concept of extra sequences serving as topogenic sequences (3) for the correct destination of organelle-directed proteins. As might be expected, cMDH I, which remains in the cytosol, apparently is not synthesized with such an extra sequence.

The size differences between the *in vitro* and *in vivo* synthesized organelle-bound MDHs confirm our previously published hypothesis of a gMDH extra sequence (26) which was based on the comparison of the *in vitro* product with the purified isoenzyme subunit (mol wt, 33,000). If there had been a proteolytical degradation of gMDH during the purification procedure without a concomitant drastic change of the antibody binding properties, the erroneous impression of a larger precursor would have been obtained. This possibility is excluded now by the correspondence of mol wt of the purified and the *in vivo* synthesized gMDH. There is, however, a significant difference between the *in vitro* synthesized gMDH produced by the reticulocyte system (mol wt, 41,000) and the product from the wheat germ system (mol wt, 38,000). The most likely explanation for this discrepancy is the presence of considerable processing activity in the homologous wheat germ system which had been earlier suspected (26). The data of Riezman *et al.* (20; Fig. 3, lane O) in the separation of cucumber gMDH by SDS-PAGE after cell-free synthesis in the wheat germ system include, in addition to the main band with 38,000 daltons, a faint 41,000 dalton band which is in accordance with this hypothesis. Two further, faintly labeled bands between the full length gMDH precursor and the authentic form, which include the 38,000 dalton band, are regularly observed after synthesis in the reticulocyte system as well as under *in vivo* conditions, but absent in the case of mMDH. Possibly, the removal of the large extra sequence during the import into the glyoxysomes might involve a gradual cleavage.

The size of the gMDH extra sequence clearly exceeds the usual length of organelle protein extra sequences, especially the signal sequences of secretory proteins (between 1,500 and 3,000 daltons). There are, however, reports of extra sequences of comparable sizes, *e.g.* NADH Pchlide reductase with 8,000 daltons (1), three subunits of chloroplast ATP synthetase (R. G. Herrmann, personal communication).

The length of the mMDH extra sequence, however, fits into the normal range observed in mitochondrial precursor proteins. A putative precursor for rat liver and bovine heart mMDH has been recently reported (2). The size of the extra sequence is 1,500 to 2,000 daltons, which is somewhat smaller than the extension of the watermelon mMDH. The role of the precursor in import of this enzyme into mitochondria is under discussion (9). It has been reported that the native isoenzyme can be taken up by isolated rat liver mitochondria into the matrix (19). This result completely differs from the normal case where the transport is restricted to the precursor form.

The unexpected relative stability of the gMDH and mMDH precursors established by pulse-chase experiments sheds some light upon the transport mechanism. In a co-translational translocation which was first described for secretory proteins (*e.g.* 4, 5) the transport is strictly coupled to translation. However, the post-translational translocation, first described for the small subunit of ribulose bisphosphate carboxylase (8, 11) and then for several other cytosol-synthesized mitochondrial, chloroplast, and microbody proteins, requires a transport which is uncoupled from translation, *i.e.* takes place after the complete synthesis of the polypeptide chain. Inasmuch as only the respective processed MDH isoenzymes are found in glyoxysomes and mitochondria of *in vivo* labeled cotyledons, the cleavage of the extra sequence must occur during the import into the organelles. The labeling pattern of the organelle-bound MDH isoenzymes during the pulse-chase experiments is therefore only compatible with a posttranslational transport of gMDH and mMDH. Whereas this mode of translocation is generally accepted for mitochondria, direct evidence for glyoxysomes has been obtained only in a few cases, *e.g.* with catalase (10, 15, 21), uricase (10), isocitrate lyase (28), and malate synthase (14). In all of these cases, the *in vitro* synthesized enzymes did not contain an extra sequence which could be split off during the translocation process.

We were not able to demonstrate the uptake and processing of the *in vitro* synthesized gMDH precursor by purified glyoxysomes. The main difficulty seems to be the availability of glyoxysomal fractions retaining the necessary intactness for the importation of organelle proteins. With crude organelle fractions (10,000*g* pellet), however, we were able to demonstrate at least a partial processing of the gMDH precursor with the intermediate form of 38,000 daltons as main product. The intermediate was converted into the native subunit only after proteinase K treatment of the organelle precursor mix. If the organelles were osmotically shocked before protease treatment, no gMDH could be immunoprecipitated, suggesting that in the treatment above, the major part of the gMDH was indeed sequestered by the organelles. Work is in progress using more intact organelles to elucidate the mechanism on the specific uptake of gMDH and mMDH by glyoxysomes and mitochondria, respectively.

Acknowledgment—The competent technical assistance of Miss Irmgard Sperrer is gratefully acknowledged.

LITERATURE CITED

1. APEL K 1981 The protochlorophyllide holochrome of barley (*Hordeum vulgare* L.). Phytochrome-induced decrease of translatable mRNA coding for the NADPH:protochlorophyllide oxidoreductase. Eur J Biochem 120: 89–93

2. AZIZ LE, SM CHIEN, HV PATEL, KB FREEMAN 1981 A putative precursor of rat liver mitochondrial malate dehydrogenase. FEBS Lett 133: 127–130
3. BLOBEL G 1980 Intracellular protein topogenesis. Proc Natl Acad Sci USA 77: 1496–1500
4. BLOBEL G, B DOBBERSTEIN 1975 Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. J Cell Biol 67: 835–851
5. BLOBEL G, DD SABATINI 1971 Ribosome-membrane interaction in eucaryotic cells. *In* LA Manson, ed, Biomembranes, Vol 2. Plenum, New York pp 193–195
6. CAMPBELL PN, G BLOBEL 1976 The role of organelles in the chemical modification of the primary translation products of secretory proteins. FEBS Lett 72: 215–225
7. CLEVELAND DW, S FISCHER, MW KIRSCHNER, UK LAEMMLI 1977 Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J Biol Chem 252: 1102–1106
8. DOBBERSTEIN B, G BLOBEL, NH CHUA 1977 *In vitro* synthesis and processing of a putative precursor for the small subunit of ribulose-1,5-bisphosphate carboxylase of *Chlamydomonas reinhardtii.* Proc Natl Acad Sci USA 74: 1082–1085
9. DOONAN S 1981 How proteins are transported into mitochondria. Trends Biochem Sci 5: X–XI
10. GOLDMAN BM, G BLOBEL 1978 Biogenesis of peroxisomes: intracellular site of synthesis of catalase and uricase. Proc Natl Acad Sci USA 75: 5066–5070
11. HIGHFIELD PE, RJ ELLIS 1978 Synthesis and transport of the small subunit of chloroplast ribulose bisphosphate carboxylase. Nature (Lond) 271: 420–424
12. HOCK B 1969 Die Hemmung der Isocitrat-Lyase bei Wassermelonenkeimlingen durch Weißlicht. Planta 85: 340–350
13. HOCK B 1973 Compartmentation and properties of the MDH isoenzymes from watermelon cotyledons. Planta 112: 137–148
14. KRUSE C, J FREVERT, H KINDL 1981 Selective uptake by glyoxysomes of *in vitro* translated malate synthase. FEBS Lett 129: 36–38
15. LAZAROW PB 1980 Properties of the natural precursor of catalase: implications for peroxisome biogenesis. Ann NY Acad Sci 343: 293–303
16. LIU KDF, ACH HUANG 1976 Developmental studies of NAD-malate dehydrogenase isoenzymes in the cotyledons of cucumber seedlings grown in darkness and in light. Planta 131: 279–284
17. MARRA E, S DOONAN, C SACCONE, E QUAGLIARIELLO 1978 Studies in the selective permeation of radioactively labelled aspartate aminotransferase isoenzymes into mitochondria *in vitro.* Eur J Biochem 83: 427–435
18. MILSTEIN C, GG BROWNLEE, TM HARRISON, MB MATHEWS 1972 A possible precursor of immunoglobulin light chains. Nat New Biol 239: 117–120
19. PASARELLA S, E MARRA, S DOONAN, E QUAGLIARIELLO 1980 Selective permeability of rat liver mitochondria to purified malate dehydrogenase isoenzymes *in vitro.* Biochem J 192: 649–658
20. RIEZMAN H, EM WEIR, CJ LEAVER, DE TITUS, WM BECKER 1980 Regulation of glyoxysomal enzymes during germination of cucumber. 3. *In vitro* translation and characterization of four glyoxysomal enzymes. Plant Physiol 65: 40–46
21. RUIS H 1979 The biosynthesis of catalase. Can J Biochem 57: 1122–1130
22. SAUTTER C, B HOCK 1982 Fluorescence immunohistochemical localization of malate dehydrogenase isoenzymes in watermelon cotyledons: a developmental study of glyoxysomes and mitochondria. Plant Physiol In press
23. WALK R-A, B HOCK 1977 Glyoxysomal and mitochondrial malate dehydrogenase of watermelon (*Citrullus vulgaris*) cotyledons. I. Molecular properties of the purified isoenzymes. Planta 136: 211–220
24. WALK R-A, B HOCK 1976 Mitochondrial malate dehydrogenase of watermelon cotyledons: time course and mode of activity changes during germination. Planta 129: 27–32
25. WALK R-A, B HOCK 1977 Glyoxysomal malate dehydrogenase of watermelon cotyledons: *de novo* synthesis on cytoplasmic ribosomes. Planta 134: 277–285
26. WALK R-A, B HOCK 1978 Cell-free synthesis of glyoxysomal malate dehydrogenase. Biochem Biophys Res Commun 81: 636–643
27. YANG N-S, JG SCANDALIOS 1975 Cytoplasmic synthesis of soluble and mitochondrial malate dehydrogenase isoenzymes in maize. Arch Biochem Biophys 171: 575–585
28. ZIMMERMANN R, W NEUPERT 1980 Biogenesis of glyoxysomes. Synthesis and intracellular transfer of isocitrate lyase. Eur J Biochem 112: 225–233