Electron transport in purified glyoxysomal membranes from castor-bean endosperm

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Abstract. Glyoxysomes isolated from castor-bean (Ricinus communis L.) endosperm were treated with water, 0.2 M KCl, 1 M KCl, or 0.1 M Na₂CO₃. Glyoxysomal sacs, i.e. membranes which retained some visible matrix, resulted from the treatments with water and KCl. Glyoxysomal ghosts, i.e. intact membranes free of matrix, were only obtained following treatment with carbonate. The ghosts were free of activities of matrix enzymes, particularly palmitoyl-CoA oxidation, isocitrate dehydrogenase (EC 1.1.1.42) and isocitrate lyase (EC 4.1.3.1), and contained only negligible amounts of malate synthase (EC 4.1.3.2), malate dehydrogenase (EC 1.1.1.37), β-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.98) and catalase (EC 1.11.1.6). Distribution and appearance of membrane-associated particles in the protoplasmic and ectoplasmic faces of freeze-fracture replicas of the glyoxysomal membrane were the same in intact tissue, isolated glyoxysomes, and ghosts. Membranes purified by treatment with 0.2 M KCl or 0.1 M carbonate catalyzed the reduction of cytochrome-c when NADH or NADPH was provided as the electron donor. β-Oxidation, localized in the matrix, could be linked to reduction of cytochrome-c or ferricyanide when purified membranes were combined with the matrix supernatant. Cytochrome-c could also be reduced by coupling enzyme activities in the matrix, NADP-isocitrate dehydrogenase or malate dehydrogenase, with those of the membrane. These results indicate that electrons from β-oxidation, malate oxidation or isocitrate oxidation can be transferred directly to the redox components of the glyoxysomal membrane. We, therefore, conclude that any NADH and NADPH formed by enzymes in the matrix can be recycled continuously within the organelle.

Key words: Glyoxysome - NADH-cytochrome-c reductase - NADH-ferricyanide reductase - β-Oxidation - Peroxisome - Ricinus

Introduction

In glyoxysomes of germinating oil seeds, NADH is generated by β-hydroxyacyl-CoA dehydrogenase and malate dehydrogenase, whereas NADPH is generated by isocitrate dehydrogenase (Cooper and Beevers 1969 a, b). β-Oxidation, the glyoxylate cycle, and isocitrate oxidation in glyoxysomes require a continuous supply of NAD and NADP. Latency studies indicate that NAD(H) may not be transported across the glyoxysomal membrane at rates equivalent to dehydrogenase activities (Donaldson et al. 1981; Luster and Donaldson 1987). It is possible that the reduced nucleotides are re-oxidized by linkage to enzymes in the membrane (Hicks and Donaldson 1982). The glyoxysomal membrane contains several redox components, flavin, NADH-ferricyanide reductase, NADH and NADPH-cytochrome-c reductases, cytochromes b₅ and P₄₅₀ (Hicks and Donaldson 1982). Since isolated glyoxysomal membranes catalyze the transport of electrons from exogenous NADH to ferricyanide, cytochrome-c, or endogenous cytochrome-b₅ (Hicks and Donaldson 1982), we considered the possibility that NADH and NADPH generated by dehydrogenases within glyoxysomes could donate electrons directly to reductases in the limiting membrane.

The study of electron-transport functions in the glyoxysomal membrane requires isolation of intact glyoxysomes free of other organelles and separation of the membrane from matrix components without altering the structural and functional integrity of the membrane. The methods used to isolate glyoxysomal membranes generally involve
osmotic shock in the presence of buffers, various concentrations of KCl, or detergents (Donaldson and Beevers 1978; Huang and Beevers 1973; Koller and Kindl 1977). However, preparations of this type contain visible remnants of the matrix (Donaldson and Beevers 1978). Recently a method has been developed for isolating the membranes of rat-liver endoplasmic reticulum (ER, microsomes), peroxisomes and mitochondria by means of treatment with 0.1 M Na2CO3 (Fujiki et al. 1982a, b). The peroxisomes and microsomes were converted to open membrane sheets devoid of matrix by this treatment. NADH-ferricyanide reductase and NADPH-cytochrome-c reductase were detected in the purified microsomal membranes (Fujiki et al. 1982b) but the peroxisomal membranes were not examined for these activities (Fujiki et al. 1982a).

Our objectives in this study were to obtain highly purified glyoxysomal membranes from Ricinus endosperm and to investigate the functional relationships between matrix dehydrogenases and membrane electron transport. We have compared the biochemical purity and morphological integrity of glyoxysomal membranes following treatment of isolated glyoxysomes with Na2CO3, KCl, or water. Thin sections and freeze-fracture replicas of the tissue, isolated organelles, and purified membranes were examined with transmission electron microscopy. When membrane preparations were combined with matrix fractions, it was possible to demonstrate coupling of fatty-acid oxidation, malate oxidation or isocitrate oxidation to membrane reductases. These results indicate that dehydrogenase activities of matrix enzymes can be coupled to electron-transport activities in the membrane. In addition, our findings indicate which glyoxysomal enzymes are soluble components of the matrix and which are integral to the membrane.

Material and methods

Isolation of glyoxysomes. Castor beans (Ricinus communis L., cv. Hale, purchased from Northrup King Co., Plainview, Tex., USA) were germinated in wet vermiculite at 30 °C in the dark for 4.5 d. The excised endosperm was homogenized and centrifuged, as described previously (Donaldson 1982). Nine milliters of the 270 g supernatant was slowly pipetted onto a linear sucrose gradient consisting of 2 ml 18% (w/w) sucrose and 2 ml 20% (w/w) sucrose, 0.05 M phosphate or Tricine buffer, pH 7.5. Gradients were centrifuged in a Dupont Sorvall (Wilmington, Del., USA) OTD-50 centrifuge with a TV 850 vertical centrifuge (Super 11). The carbonate supernatant was brought to pH 7.5 with the addition of 20% (w/w) sucrose, 0.05 M phosphate or Tricine buffer, pH 7.5, using a Potter-Elvehjem homogenizer. The final four membrane preparations studied were designated as (a) water, (b) 0.2 M KCl (glyoxysomal sars), (c) 1 M KCl, (d) carbonate-treated membranes (glyoxysomal ghosts).

Analytical methods. The following enzymes were assayed as referenced: isocitrate lyase and NADP-isocitrate dehydrogenase (Cooper and Beevers 1969a), catalase (Gerhardt and Beevers 1970), malate dehydrogenase (Huang and Beevers 1973), malate synthase (Miflin and Beevers 1974). Protein was determined by either of two methods (Bradford 1976; Lowry et al. 1951).

β-Oxidation activity was measured in an assay mixture consisting of 30 mM phosphate buffer (pH 7.5), 0.15 mg·ml−1 bovine serum albumin (BSA), 0.01% Triton X-100, 0.1 mM coenzyme A (CoA), 0.2 mM NAD, 2 mM KCl, 8-25 μg glyoxysomal protein and 10 μM palmitoyl-CoA in a final volume of 1 ml. The rate of NAD reduction was monitored at 340 nm.

Linkage of β-oxidation, isocitrate oxidation or malate oxidation to cytochrome-c reduction was determined by following the reduction of 0.02 mM cytochrome-c at 550 nm. The final concentration of reagents used for NADP-isocitrate oxidation linked to cytochrome-c reduction was 100 mM Tricine buffer (pH 7.5), 0.5 mM MnCl2, 1 mM KCN, 0.63 mM NADP, 1 mM isocitrate, 12-30 μg glyoxysomal protein and 0.02 mM cytochrome-c. β-Oxidation linked to ferricyanide reduction was determined by following reduction of ferricyanide at 420 nm upon addition of 1 mM potassium ferricyanide to the reaction medium. Malate oxidation linked to cytochrome-c reduction was assayed in a solution containing 100 mM potassium-phosphate buffer (pH 7.4), 0.14 mM NAD, 0.02 mM cytochrome-c, 6-25 μg glyoxysomal protein and 1 mM malate. Alternatively, malate could be generated independently by malate synthase when 0.1 mM acetyl-CoA and 1.5 mM glyoxylate were added to a reaction mixture containing 70 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) buffer (pH 8.0), 4 mM MgCl2.