Inorganic phosphate must be transported continuously into the mitochondrial matrix to maintain the steady-state oxidative phosphorylation of ADP. Specific uptake of this anion occurs via at least two distinct transport systems: an electroneutral inorganic phosphate-dicarboxylic acid exchange carrier and an inorganic phosphate-hydroxyl antiporter which catalyses the influx of about 90% of the mitochondrial phosphate.

An interesting property of these phosphate transporters, which has facilitated their identification, is that they are inhibited by a low concentration of sulphhydryl group reagents. The phosphate carrier protein is inhibited by N-ethylmaleimide, mersalyl and p-chloromercuribenzoic acid (Fonyo & Besman, 1968; Tyler, 1969; Coty & Pederson, 1974), whereas the phosphate-dicarboxylate exchange protein is blocked by mersalyl and p-chloromercuribenzoic acid but is insensitive to N-ethylmaleimide (Meyer & Tager, 1969; Meijer et al., 1970; Quagliariello & Palmiere, 1972; Coty & Pederson, 1974).

In rat liver mitochondria, the phosphate carrier protein has been purified by modifying the method of Kolbe et al. (1981). This involves selective extraction of the phosphate carrier protein in a Triton X-100 buffer (20 mM-LiCl, 20 mM-HIPO₄, 0.5 mM-dithiothreitol, 0.1 mM-EDTA and 8% (w/v) Triton X-100, pH 7.0), and hydroxylapatite chromatography before adsorption on Celite. Fig. 1 compares the Coomassie Blue staining pattern of the phosphate carrier purified from rat-liver and beef heart mitochondria, on a 14–20% sodium dodecyl sulphate/polyacrylamide gradient gel. The beef heart protein has been purified also as described by Kolbe et al. (1981). There is a major difference between the protein product(s) at the hydroxylapatite stage in the two preparations. In the rat liver sample, only one major band is observed, which migrates with a Mr of 34,000, whereas in the beef heart preparation there are two major bands, which migrate with Mr of 34000 and 31000. The 31000 Mr band is probably the adenine nucleotide translocase as shown by Wolrab (1980). The translocase protein can be removed by adsorption on Celite, as was shown by Kolbe et al. (1981), and is illustrated here in Fig. 1. In this laboratory, another method for separating the translocase from the phosphate carrier protein has also been utilized. This involves the differential elution of the proteins from a Procion Red A column with NaCl. The phosphate carrier protein can be eluted with 0.25 M-NaCl, whereas the translocase requires 3 M-NaCl for elution from the column.

The results discussed above, contrast markedly with the recent findings of Kolbe et al. (1982), who purified the phosphate carrier protein from bovine and pig heart mitochondria. The hydroxylapatite eluates, when resolved on 14–20% (w/v) sodium dodecyl sulphate/polyacrylamide gradient gels, demonstrated four to five Coomassie blue staining bands, which varied in Mr from 34,500 to 30,000. They explained their findings in terms of a model, which suggests that the phosphate carrier protein is proteolytically degraded during its isolation from mitochondria. Multiple banding was not observed in this laboratory. Densitometric scanning of the gel demonstrated that the protein was greater than 90% pure.

An important additional step in purifying the phosphate carrier proteins from rat liver, by the method of Kolbe et al. (1981), was the pre-extraction of the mitochondria with 0.5% (w/v) Triton X-100 buffer. Early preparations, although demonstrating only one major band, when overlayed with 3H-labeled concanavalin A showed the presence of numerous low and high Mr, glycoproteins. This initial extraction in 0.5% (w/v) Triton X-100 buffer solubilized about 80% of the mitochondrial protein and removed these contaminants.

The sulphhydril group sensitivity of the phosphate carrier protein was employed in the identification of the protein. Labelling studies with 3H-N-ethylmaleimide showed that only the 34000 Mr band was labelled in both the hydroxylapatite and Celite fractions, when purified from rat liver mitochondria. This result is consistent with the known sulphhydril group sensitivity of the phosphate carrier protein. Further confirmation is provided by preliminary experiments with an antiserum directed against the 34000 Mr protein. Partial inhibition of mitoplast swelling in isotonic ammonium phosphate buffer (Chappell & Crofts, 1966) was achieved when mitoplasts were incubated with the specific antiserum.
Organization and distribution of carbohydrate on mitochondrial membranes

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All eukaryotic membranes, including those of intracellular organelles, contain small amounts of covalently associated carbohydrate, mainly in the form of glycoprotein (Hughes, 1976). In mitochondria, it is clearly important to establish the presence, nature and topographical distribution of these glyco-conjugates, since resolution of these points will provide valuable information on the origins, mode of synthesis and assembly of outer and inner membranes.

Earlier reports on the presence of carbohydrate (De Bernard et al., 1971; Glew et al., 1973) and glycosyl transference activities in this organelle (Gateau et al., 1978) have not been developed to the stage where we can eliminate the possibility that these represent contaminants of non-mitochondrial origin. Moreover, no well-characterized glycoproteins have been isolated from the organelle, although a protein, M, 33000, involved in Ca2+ uptake, appears to contain associated carbohydrate (Panfili et al., 1976). There has also been controversy over possible glycosylation of the mitochondrial F1-ATPase (Andreu et al., 1978; Nalin et al., 1979).

In our laboratory, we can detect the presence of several (10-15) concanavalin A-reactive glycoproteins in highly purified mitochondria and derived subfractions. The broad specificity of this lectin enables over 70% of mitochondrially associated glycoprotein to be isolated by affinity chromatography on concanavalin A-Sepharose 4B.

Interestingly, using WGA, specific for sialic acid and GlcNAc, only two major glycosylated species with apparent M, of 92000 and 105000 are found to interact with this lectin in rat liver mitochondria. We have concluded that these components are located in mitochondrial membranes based on the following observations: (a) rat liver mitochondrial or mitoplasts (outer membrane removed) are readily agglutinated by WGA, a reaction which is inhibited by GlcNAc; (b) WGA can be employed to isolate sealed 'inside-out' vesicles containing no accessible carbohydrate from a mixed population of inner membrane fragments (D’Souza & Lindsay, 1981); and (c) fluorescein-WGA binds specifically to isolated mitochondria.

The external location of sialic acid on the 92000 M component has been confirmed by neuraminidase treatment of intact mitochondria or mitoplasts. This band exhibits a decreased mobility on SDS/polyacrylamide-gels on removal of sialic acid as revealed by fluorography after incubation with 125I-labelled WGA. In contrast, the higher M, species is unaffected by neuraminidase and is absent from mitoplasts, indicating a location in the outer membrane.

The unique nature of the major WGA-reactive glycoprotein has been confirmed by surface-specific modification of accessible galactosyl residues employing the galactose oxidase/tritiated borohydride procedure of Gahmberg (1976). Fig. 1 shows an SDS/polyacrylamide-gel profile of 3H-labelled glycoproteins in intact mitochondria as detected by fluorography. The most striking feature is the presence of two prominent bands with M, of 92000 and 105000 (track c). Prior incubation with neuraminidase, which exposes the presence of carbohydrate on mitochondrial membranes

![Fig. 1. External labelling of rat liver mitochondrial glycoproteins](image-url)

Succrose-density purified mitochondria are labelled with 1mCi Na3HBBH4 (5-20Ci/mmol) after pretreatment for 30 min at 4°C with neuraminidase and galactose oxidase (track b), galactose oxidase only (track c) or no pretreatment (track d) according to the procedure of Gahmberg (1976). For comparison, total mitochondrial sialoglycoprotein is oxidized with peridate (Blumenfeld et al., 1972) before titration with borohydride (track a). All samples are visualized by fluorography after resolution of SDS/polyacrylamide-gels. T.D., tracker dye front; k, kilodaltons.