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Dolichol kinase activity: a key factor in the control of *N*-glycosylation in inner mitochondrial membranes

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Inner mitochondrial membranes from liver contain a dolichol kinase which required CTP as a phosphoryl donor. Kinase activity was linear with protein concentration and unlike other reported kinases, activated almost equally well by Mg^{2+}, Mn^{2+} or Ca^{2+}. Thin-layer chromatography showed that the reaction product co-migrated with authentic dolichyl monophosphate. The phosphorylation of dolichol did not occur in presence of ATP, GTP or UTP but required exogenous dolichol for maximal activity. Newly synthesized [^{3}H]dolichyl monophosphate has been shown to be glycosylated in the presence of GDP[^{14}C]mannose or UDP[^{14}C]glucose. The double labeled lipids formed by the sugar nucleotide-dependent reactions were identified respectively as [^{14}C]mannosylphosphoryl[^{3}H]dolichol and [^{14}C]glucosylphosphoryl[^{3}H]dolichol. These results are discussed in terms of regulation of *N*-glycosylation processes in inner mitochondrial membranes from liver.

Introduction

Dolichol is a long chain polyisoprenoid, widely distributed in all tissues and organelles [1,2]. In their phosphorylated form, they are intimately involved in *N*-glycoprotein biosynthesis, serving as a lipid 'carrier' for oligosaccharide chains destined to be transferred to nascent polypeptides [3,4]. Numerous studies indicate that the concentration of dolichyl monophosphate may be a rate-limiting factor in the glycosylation processes. Dolichyl monophosphate concentration levels seem to be regulated by altering the relative activities of dolichol kinase and dolichyl monophosphate phosphatase. These enzymatic activities could influence the level of dolichyl monophosphate by a phosphorylation-dephosphorylation mechanism. The bulk of dolichols in many organs [2] is present in the non phosphorylated from. A number of reports have appeared regarding the occurrence and properties of dolichol kinase from several sources [5–7] using endogenous or exogenous dolichol as acceptor.

Inner mitochondrial membranes have been shown to catalyze the transfer of mannose from GDPmannose [8] and glucose from UDPglucose [9] into exogenous dolichyl monophosphate. In order to carry out an investigation of factors that control *N*-glycoprotein biosynthesis in mitochondria, we investigated the presence of dolichol kinase activity in inner mitochondrial membranes. Despite the low level of endogenous dolichol in these membranes [10], we present evidence here that inner mitochondrial membranes are able to catalyze the phosphorylation of dolichol. In another aspect of this study, experimental support has been obtained for the proposal that some dolichyl monophosphate molecules formed by dolichol kinase in inner mitochondrial membranes are available for the biosynthesis of dolichol-linked monosaccharide intermediates.

Experimental Procedures

Chemicals

[1-^{3}H]Dolichol was prepared from pig liver dolichol according to Keenan and Kruczek [11]. Standard dolichyl monophosphate, egg yolk phosphatidylcholine, guanosine 5′-triphosphate, uridine 5′-triphosphate, cytidine 5′-triphosphate and adenosine 5′-triphosphate were purchased from Sigma (St Louis, MO). DEAE-cellulose (DE 52, Whatman) was converted to the acetate form as described [12]. GDP[^{14}C]mannose (9.4 GBq/mmol) and UDP[^{14}C]glucose (10 GBq/mmol) were obtained from NEN Chemicals (U.S.A).

Animals

Mice, strain OF1, IFFA-Credo (France) with an average weight of 20 g were used. They were killed and

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underwent laparotomy. The livers were rapidly removed and placed in 250 mM sucrose, 10 mM Tris buffer, pH 7.4 at 4°C.

Preparation and purification of inner mitochondrial membranes

Mitochondria were isolated according to the procedure described by Weinbach [13] and modified by Bustamente et al. [14]. Mouse livers were homogenized and fractionated by differential centrifugations in 250 mM sucrose, 10 mM Tris (pH 7.4) as previously described [15]. Washed mitochondria were suspended in the same buffer and purified by mild ultrasonic treatment. Proteins were assayed according to the procedure of Gornall et al. [16]. Mitochondria were treated with digitonin solution (100 μg/mg of protein) for 15 min at 4°C. The action of digitonin was stopped with 250 mM sucrose buffer. A centrifugation at $10000 \times g$ allowed the association of inner membranes and matrix (mitoplasts) to sediment whereas outer membranes were eliminated in the supernatant. Inner membranes were prepared by swelling mitoplasts in 1 mM potassium phosphate buffer (pH 7.4) for 15 min at 4°C and purified on a discontinuous sucrose gradient [8]. Purified inner membranes (IM4 fractions) were washed in 10 mM Tris buffer, sedimented at $145000 \times g$ for 60 min and immediately frozen in liquid nitrogen.

Control of the quality of sub-fractions obtained

To assess the purity of inner membranes, cytochrome-*c* oxidase (EC 1.9.3.1) [17], monoamine oxidase (EC 1.4.3.4) [18] glucose-6-phosphatase (EC 3.1.3.9) [19], acid 5′-nucleotidase (EC 3.1.3.5) [20] and basic 5′-nucleotidase (EC 3.1.3.5) [19] were assayed as marker enzymes respectively of inner mitochondrial membranes, rough endoplasmic reticulum, lysosomes and plasma membranes.

Incorporation of exogenous dolichol into inner mitochondrial membranes

Phosphatidylcholine and [^{3}H]dolichol (4 Ci/mmol) were evaporated under nitrogen and resuspended in 20 mM Tris buffer (pH 7.4). The mixture was sonicated for 3 min at 4°C with a probe sonicator (Sonimass type 75T). These lipidic vesicles were incubated with purified inner membranes for 10 min at 37°C. The incubation medium was centrifuged at $10000 \times g$ in order to discard the unfused vesicles. [^{14}C]Phosphatidylcholine was sometimes included as tracer in order to evaluate phosphatidylcholine incorporation in inner mitochondrial membranes [21].

Dolichol kinase assay

Activity was monitored by measuring the formation of [^{3}H]dolichyl monophosphate from [^{3}H]dolichol. The reaction was carried out in a total volume of 1 ml containing 50 mM Tris buffer (pH 7.4), 10 mM NaF, 10 mM CTP, 25 mM $MgCl_2$, 20 mM UTP and 1 mg of inner mitochondrial membranes. The incubation was carried out for 15 min at 37°C. The reaction was terminated by the addition of a chloroform/methanol (2 : 1, v/v) mixture. The suspension was mixed vigorously, kept at room temperature for 10 min and centrifuged after addition of water. The lower phase was removed and washed with a chloroform/methanol/water (3 : 48 : 47, v/v) mixture.

Purification and characterization of the reaction product

The reaction product from the dolichol kinase assay was applied on a DEAE-cellulose column in order to separate [^{3}H]dolichyl monophosphate from unreacted [^{3}H]dolichol. Dolichol was eluted with chloroform/methanol (2 : 1, v/v) and dolichyl monophosphate with 0.25 M ammonium acetate in chloroform/methanol (2 : 1, v/v). The [^{3}H]dolichyl monophosphate fraction was desalted by washing with a chloroform/methanol/water (3 : 48 : 47, v/v) mixture and identified by thin-layer chromatography on precoated silica gel plates (60F-254, Merck). The plates were developed in chloroform/methanol/water/conc. ammonium hydroxyde (65 : 35 : 4 : 4, v/v) in presence of authentic dolichyl monophosphate. Products were visualized with anisaldehyde stain and the distribution of radioactivity on the plate was determined by counting small bands scraped from the plate. Radioactivity was assayed with a Packard Scintillation Spectrometer and counted in 299TM mixture.

Glycosylation assay of labeled dolichyl monophosphate

The reaction was initiated by addition of GDP[^{14}C]-mannose or UDP[^{14}C]glucose to the acellular system described above. The reaction was stopped by the addition of a chloroform/methanol (2 : 1, v/v) mixture. The suspension was mixed vigorously, kept at room temperature for 10 min and centrifuged after addition of water. Extraction of glycolipids and glycoproteins was performed as previously described [22]. Under these conditions, glycosylnucleotide pyrophosphatases have very low activities. Labeled products were identified by thin-layer chromatography on silica gel in the following solvent systems:

Solvent I: chloroform/methanol/water (65 : 25 : 4, v/v)

Solvent II: chloroform/methanol/water/conc. ammonia (65 : 35 : 4 : 4, v/v).

Results

Control of the quality of inner mitochondrial membranes

Purification of inner membranes on sucrose gradient leads to four fractions. Fraction IM4 represents purified inner mitochondrial membranes. Table I shows that this

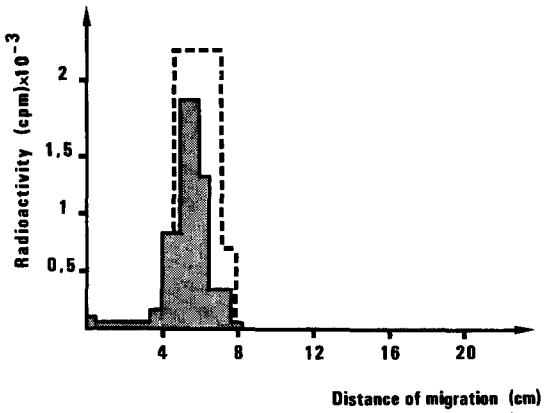

Fig. 1. Thin-layer chromatography in solvent II of the ^{3}H-labeled compound in the chloroform/methanol (2 : 1, v/v) fraction that bound to DEAE-cellulose. - - - - - -, authentic sample; ———, reaction product. Incubation and assay conditions were as described in Experimental Procedures.

fraction exhibits a great enrichment in cytochrome-*c* oxidase and can be considered as free of contaminants, especially endoplasmic reticulum and lysosomes.

Characterization of the reaction product

The reaction product was first fractionated on a DEAE-cellulose column equilibrated in chloroform/methanol (2 : 1, v/v) in order to separate neutral lipids from anionic lipids. Dolichyl monophosphate would be present in the portion of the chloroform/methanol (2 : 1, v/v) extract that bound to the column. The ^{3}H labelled product was retained on DEAE-cellulose and eluted with ammonium acetate. This anionic lipid was identified by thin-layer chromatography in solvent system II and comigrate with authentic dolichyl monophosphate (Fig. 1).

Requirement of exogenous dolichol for dolichol kinase activity

Since inner mitochondrial membranes contains relatively little dolichol [10], exogenous polyprenol must be added to the incubation mixture in order to obtain good rates of the reaction. Previous reports [23,24] suggested that this hydrophobic material must be suspended in Triton X-100 or some other detergent. Table II shows that dolichol kinase had low activity (minus 5 pmol per mg of protein) even in presence of exogenous [^{3}H]-dolichol dispersed with detergents. In contrast, the higher rates of reaction were obtained when phosphatidylcholine vesicles loaded with [^{3}H]dolichol were preincubated with inner mitochondrial membranes and at a lesser extent with phosphatidylethanolamine.

TABLE II

Effects of detergents and phospholipids on dolichol kinase activity

Aliquots of a scaled-up reaction mixture were taken after 15 min of incubation for the determination of dolichyl phosphate formation. [^{3}H]Dolichol was suspended in 0.5% (w/v) detergent solution or in presence of 0.7 mM phospholipid dispersion.

[^{3}H]Dolichol dispersion	Dolichyl phosphate formed (pmol per mg protein)
None	1
Triton X-100	5
Nonidet P-40	2
Phosphatidylcholine	42
Phosphatidylethanolamine	33

Effects of time and protein concentration on the dolichol phosphorylation

The time course of the reaction was linear for at least 10 min and the total amount of [^{3}H]dolichyl monophosphate was unchanged from 15 to 45 min (Fig. 2a). On the other hand, the product formation is directly proportional to the amount of membrane protein over the range of protein concentrations used in these studies (Fig. 2b) and proportional to the amount of exogenous [^{3}H]dolichol added (Fig. 2c) up to 35 μM. This finding confirmed that the amounts of dolichol available for phosphorylation in vivo are rate limiting in inner mitochondrial membranes.

Nucleoside triphosphate specificity of dolichol kinase

To investigate the nucleotide specificity of the mitochondrial enzyme, ATP, GTP and UTP were tested as

TABLE I

Distribution of specific activities in mitochondria fractions

Fraction	Cytochrome-*c* oxidase (nmol·min^{-1}·mg^{-1})	Monoamine oxidase (nmol·min^{-1}·mg^{-1})	Glucose-6-phosphatase [a]	Acid 5′-nucleotidase [a]	Basic 5′-nucleotidase [a]
Purified mitochondria	1250	18	3.4	9	7.5
Inner membrane	5820	4.2	1.7	3.4	1.3
Purified inner membrane					
IM1 fraction	142	13.3	0.08	0.09	0.05
IM2 fraction	0	6.6	0.07	0.05	0.04
IM3 fraction	3550	4	0.04	0.01	0.01
IM4 fraction	7110	0	0	0	0

[a] Expressed as percentage of total homogenate activity.

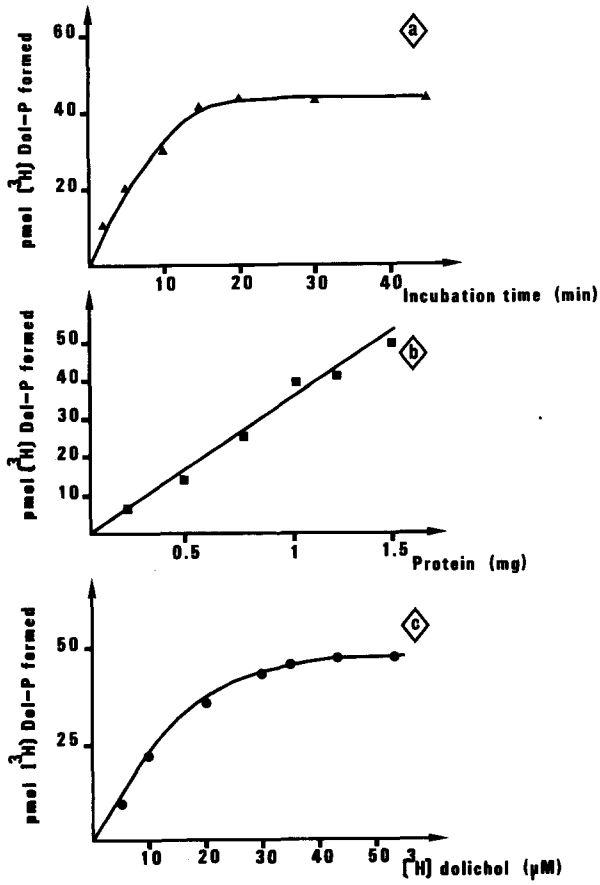

Fig. 2. Dolichol kinase activity as a function of time (a), protein concentration (b), and substrate (c). (a) The standard reaction mixture was used, except that the reaction was terminated at the times indicated. (b) The concentrations of inner membrane protein used in the assay are indicated. (c) Different substrate concentrations were used in the assay.

substrates and compared with CTP. Table III clearly demonstrates that CTP is several times more active than any of the other nucleotides examined. ATP, GTP and UTP were uneffective since the level of [^{3}H]dolichyl phosphate formed was inchanged compared to the control carried out in the absence nucleotide.

TABLE III

Effect of various nucleoside triphosphates as phosphoryl donors

The enzyme assay was carried out as described in Experimental procedures in the absence of excess of UTP. The reactions were terminated by the addition of a chloroform/methanol (2:1, v/v) mixture and assayed for product formation.

NTP added (10 mM)	[^{3}H]Dolichyl phosphate (dpm)
None	350
CTP	6350
CTP + 20 mM UTP	8520
ATP	710
GTP	370
UTP	362

TABLE IV

Effect of divalent cations and chelators on dolichol kinase activity

Dolichol kinase activity was performed with CTP as phosphoryl donor in presence of 20 mM UTP. The chloride salts of divalent cations and chelator were added as indicated and the reaction stopped after 15 min at 37 °C by the addition of a chloroform/methanol (2:1, v/v) mixture. [^{3}H]Dolichyl monophosphate synthesis was assayed as described under Experimental Procedures.

Divalent cations (mM)	Chelator (mM)	Dolichyl phosphate formed (pmol per mg protein)
None		1
Mg^{2+} (10)		28
Mg^{2+} (25)		43
Mn^{2+} (25)		39
Ca^{2+} (25)		40
Mg^{2+} (25)	EDTA (30)	0.5
Mn^{2+} (25)	EDTA (30)	0.3
Ca^{2+} (25)	EDTA (30)	0.2

However, if 20 mM UTP was included in the incubation mixture, the amount of dolichyl phosphate synthesized increased. The addition of UTP presumably inhibited the hydrolysis of CTP serving as a substrate for the phosphatase activity associated with inner mitochondrial membranes and does not proceed as an alternative phosphoryl donor.

Effect of divalent cations on the synthesis of [^{3}H]dolichyl phosphate

The basic properties of dolichol kinase have been studied in a variety of tissues [5,6,25] and it has been demonstrated that these enzymes all require a divalent cation for activation. The specific cation reported to activate each kinase most effectively differs among tissues. The effects of divalent cations on the activity of dolichol kinase in inner mitochondrial membranes are shown in Table IV. Magnesium, manganese and calcium ions were found to be about equally effective in promoting dolichol phosphorylation. Optimal activity was achieved at a divalent concentration of 25 mM. The biosynthesis of [^{3}H]dolichyl phosphate was completely abolished by the addition of EDTA, consistent with a divalent cation requirement.

Enzymatic glycosylation of newly synthesized labeled [^{3}H]dolichyl monophosphate

Considering the potential importance of acceptor lipid levels regulating the lipid intermediate pathway, enzymatic studies were conducted to see if the newly synthesized [^{3}H]dolichyl monophosphate, phosphorylated via CTP, could be utilized for the formation of mannosylphosphoryldolichol and glucosylphosphoryldolichol which are intermediates in the biosynthesis of asparagine-linked glycoproteins [3,26].

In these experiments, membranes were initially incubated with CTP in presence of exogenous [^{3}H]doli-

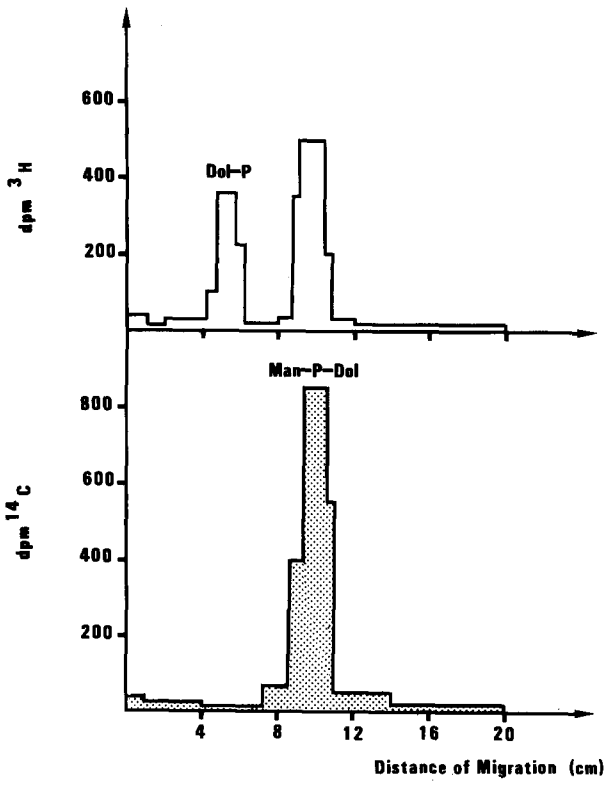

Fig. 3. Chromatographic behaviour of double labeled glycosyl-lipid in solvent II. Inner mitochondrial membranes were prelabeled with [^{3}H]dolichol and assayed for dolichol kinase activity as described in Experimental procedures. These membranes were reincubated in the presence of 5 mM $MgCl_2$ and 0.5 μM GDP[^{14}C]mannose as described before [8]. Radioactivity was measured along the chromatogram by scraping 0.5-cm segments from thin-layer plates and counting in the presence of liquid scintillation mixture.

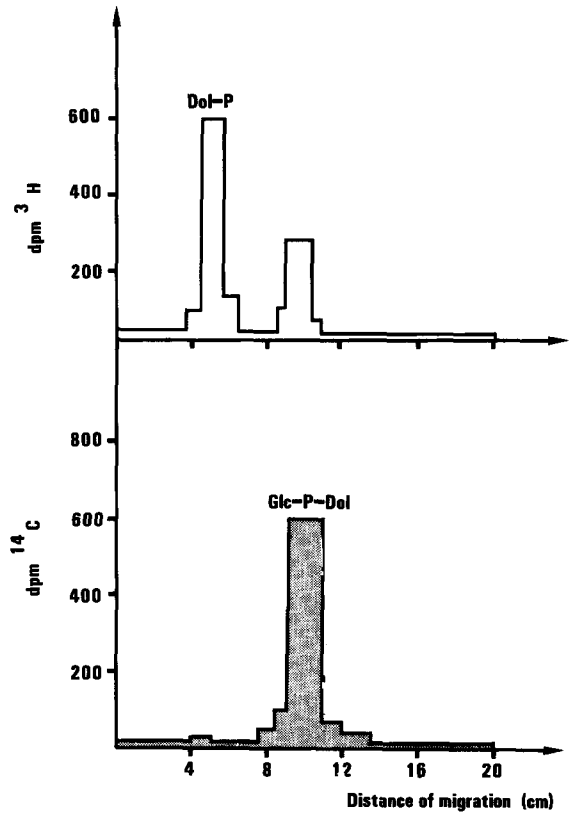

Fig. 4. Chromatographic behaviour of double-labeled glycosyl-lipid in solvent II. Inner mitochondrial membranes were prelabeled with [^{3}H]dolichol and assayed for dolichol kinase activity as described in Experimental Procedures. These membranes were reincubated in the presence of 5 mM $MgCl_2$ and 1.2 μM UDP[^{14}C]glucose as described before [9]. Radioactivity was measured along the chromatogram by scraping 0.5-cm segments from thin-layer plates and counting in the presence of liquid scintillation mixture.

chol. Then the washed membranes containing the prelabeled [^{3}H]dolichyl monophosphate were incubated in the presence of unlabeled or labeled sugar nucleotide. The labeled products in the lipid extracts were analyzed by thin-layer chromatography. When the ^{3}H-prelabeled membranes were incubated in the presence of GDP[^{14}C]mannose under conditions previously established for mannosylphosphoryldolichol biosynthesis in inner mitochondrial membranes [8], two labeled products were observed (Fig. 3). The second product had the chromatographic mobility of standard mannosylphosphoryldolichol.

When membranes containing endogenous prelabeled [^{3}H]dolichyl monophosphate were incubated in the presence of UDP[^{14}C]glucose, another major double labeled compound was seen in addition to [^{3}H]dolichyl monophosphate (Fig. 4). This double labeled lipid formed by the UDPglucose-dependent reaction had the same chromatographic mobility as glucosylphosphoryldolichol. These two products were subjected to mild acid treatment. In both cases, acid hydrolysis released [^{3}H]dolichyl monophosphate and ^{14}C-labeled sugars (data not shown).

The data presented in Table V show that 59% of the [^{3}H]Dol-*P* synthesized by the CTP-dependent kinase could be converted to [^{14}C]mannosylphosphoryl[^{3}H]dolichol. Under the same experimental conditions, only 21% of the [^{3}H]Dol-*P* could be converted to [^{14}C]glycosylphosphoryl[^{3}H]dolichol.

TABLE V

Accessibility of newly synthesized dolichyl monophosphate to lipid-mediated glycosyltransferases in inner mitochondrial membranes

Inner mitochondrial membranes containing prelabeled [^{3}H]dolichyl monophosphate were reincubated with 0.5 μM GDP[^{14}C]mannose or 1.2 μM UDP[^{14}C]glucose under conditions that are optimal for the synthesis of each phosphoglycolipid [8,9]. The percentage of [^{3}H]dolichol phosphoglycolipid was determined as described under Experimental Procedures.

Sugar nucleotide	Dolichol-linked saccharide formed	[^{3}H]Dol-*P* converted to [^{3}H]Dol phospho[^{14}C]glycolipid (%)
GDP[^{14}C]mannose	[^{14}C]Man-*P*-[^{3}H]Dol	59
UDP[^{14}C]glucose	[^{14}C]Glc-*P*-[^{3}H]Dol	21

These experiments clearly demonstrate that newly synthesized dolichyl monophosphate formed via CTP in inner mitochondrial membranes is accessible for the synthesis of mannosylphosphoryldolichol and glucosylphosphorydolichol.

Discussion

While numerous studies have examined the participation of dolichyl monophosphate as a glycosyl carrier lipid in the assembly of asparagine-linked oligosaccharides of glycoproteins in eukaryotic cells [3,4,26], the regulatory controls exerted on the lipid intermediate pathway remain unclear. Dolichol kinase has been previously shown to be located predominantly in heavy microsomes [27,28]. About 75–80% of the dolichol kinase in rat liver homogenates can be recovered in microsomes while low activities were still reported in nuclei and plasma membranes [29]. In brain, about 80% of the kinase was in heavy microsomes [28] with lesser amounts, equal to 9% and 8% in light microsomes and synaptic plasma membranes, respectively. In vitro studies with inner mitochondrial membranes indicate that the level of dolichyl monophosphate is rate limiting in the synthesis of mannosylphosphoryldolichol [8] and glucosylphosphoryldolichol [9]. This paper presents now firm evidence that the inner mitochondrial membranes have the enzymatic capacity to phosphorylate dolichol with CTP serving as the phosphoryl donor.

Preliminary studies to detect dolichol kinase activity have been to assess the purity of inner mitochondrial membranes. Estimation of enzymatic specific activities clearly demonstrate that inner mitochondrial membranes are free of contamination especially lysosomes in which high concentrations of dolichylphosphate phosphatase have been reported [30]. Inner mitochondrial membranes require exogenous dolichol for maximal dolichol kinase activity. This requirement can be satisfied in vitro by the addition of phospholipid vesicles loaded with dolichol to the membrane. Dolichol kinase activity was found to be dependent on the type of lipid used (Table II), phosphatidylcholine being the most effective. Dolichol is known to increase bilayer fluidity only in phosphatidylethanolamine systems [31] but a model is favored in which dolichol molecules are 'sandwiched' in between the two lipid monolayers, both in PC and PE systems. On the other hand, phosphatidylcholine has been reported to stimulate the phosphorylation of exogenous dolichol in yeast [32]. The phospholipid dependence of dolichol kinase activity in inner mitochondrial membranes is intringuing and at this stage of our investigation, we failed to determine if, according to their chemical composition, the phospholipids provide different modes of dolichol incorporation (conformation, orientation...) or if they provide different environments for the enzyme. Under conditions that allowed CTP-mediated phosphorylation of dolichol, ATP, GTP or UTP were not effective substrates for the mitochondrial kinase. All eukaryotic kinases reported thus far show the same particular nucleotide specificity, i.e, CTP as phosphoryl donor [25,33–35], with the exception of bacterial polyprenyl kinases which are primarily ATP-dependent [36].

To demonstrate appreciable dolichol kinase activity in mammalian systems it is necessary to control phosphatase activity. Results presented here indicate that inner mitochondrial membrane dolichylphosphate phosphatase was not very active since in the absence of 20 mM UTP, a competitive inhibitor, it can be calculated that only 25% of the dolichyl monophosphate formed during the reaction is lost due to phosphatase action. Inner mitochondrial membranes dolichol kinase was activated approximatively equally by Mg^{2+}, Mn^{2+} and Ca^{2+}. Our results according to Itami et al. [34] differ from other laboratories which found that dolichol kinases are dependent on either Mg^{2+} for rat liver [7], Mn^{2+} for hen oviduct [25] and Ca^{2+} for calf brain, sea Urchin and *Tetrahymena* [6,33,37].

Three properties of polyprenyl phosphate have been invoked to characterize the product of our reaction as dolichyl monophosphate:

(1) its anionic character on ion exchange chromatography;

(2) its R_f value and cochromatography with synthetic dolichyl monophosphate in several solvent systems;

(3) its ability to function as acceptor of sugars from nucleoside diphosphate sugars.

From this latter point, we felt that it was important to determine if the polyprenyl monophosphate was formed at a membrane site where glycolipid intermediates were synthesized. The data presented here demonstrate that newly formed dolichyl monophosphate is readily available to the enzymes that synthesize mannosylphosphoryldolichol and glucosylphosphoryldolichol. Thus this paper provides direct evidence that the dolichyl monophosphate, synthesized via CTP in inner mitochondrial membranes can participate in the lipid intermediates pathway for glycoprotein biosynthesis. Similar findings have been reported in central nervous tissues [6,28] where it was postulated that some Dol-*P* molecules formed by dolichol kinase are probably available for the synthesis of both glycolipid intermediates. However, the relatively small percentage of dolichyl monophosphate converted to glucosylphosphoryldolichol lead us to postulate, according to Hemming [38] that each glycosyltransferase may have a separate tightly bound pool of dolichyl monophosphate. Moreover, in addition of its amount, the structure of dolichyl monophosphate has been shown also to modulate the glycosyl transfer process [39]. Fully unsaturated polyprenylphosphates are much less effective as glycosyl

carriers than are the α-saturated forms [40] and recent data demonstrated that the optically active isomers of dolichol, the *S*- and *R*- forms, have different capacities as sugars acceptors [41,42]. Though, it is possible that individual sugars are translocated by defined forms of dolichyl monophosphate with specific chain length and structure.

In previous studies, we reported that outer mitochondrial membranes contain autonomous glycosyltransferase systems which transfer sugars into polyprenic endogenous acceptors [15,22,43,44]. More recently, we reported that each membrane (the outer membrane as well as the inner membrane) is able to synthesize *N*-glycoprotein products through their own dolichol intermediate pathway [45]. Up to now, very little information is available on the systems required for the regulation of the dolichol pool in mitochondria. Only few works reported that outer mitochondrial membranes effectively dephosphorylated both dolichol pyro- and monophosphates [46,47]. Considering the facts discussed above, an important aim for the study of the regulation of the *N*-glycosylation in the whole mitochondrion will be to learn more about the controls exerted on dolichol kinase as well as dolichyl monophosphate phosphatase and to evaluate the exact roles of these enzymes in both membranes for maintening the required levels of dolichyl monophosphate available for glycosylation processes.

On the other hand, inner mitochondrial membranes contains little dolichol compared to other membranes, especially outer mitochondrial membranes [10]. Uptake of dietary dolichol in the liver also indicates the involvement of outer mitochondial membranes in dolichol transport and metabolism [48]. Taking into the consideration these facts, it appears reasonable to assume that, in vivo, a part of the dolichol pool of the outer mitochondrial membranes redistribute to the inner membranes. At present, no system is available which allows time course studies and future work is required to clarify the intramitochondrial pathway of polyprenol movement.

Acknowledgments

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