SUBCELLULAR DISTRIBUTION OF DOLICHOL PHOSPHATE

Gustav DALLNER*, Nicolas H. BEHRENS, Armando J. PARODI
and Luis F. LELOIR
Instituto de Investigaciones Bioquímicas "Fundación Campomar"

and

Facultad de Ciencias Exactas y Naturales, Obligado 2490, Buenos Aires (28), Argentina

Received 12 June 1972

1. Introduction

A crude preparation of liver microsomes was shown to catalyze the transfer of glucose from UDP-Glc to Dol-P** to form Dol-P-Glc [1]. Subsequently it was observed that microsomes also transferred the glucose from Dol-P-Glc to an endogenous acceptor. This latter compound is probably another dolichol derivative containing about 20 monosaccharide units bound to dolichol through a phosphate or pyrophosphate bridge [2, 3]. The abbreviation Glc-acceptor will be used for this dolichol derivative until the details of its structure are known with certainty.

Liver microsomes also catalyze the transfer of mannose to Dol-P from GDP-mannose and of N-acetyl glucosamine from UDP-GlcNAc to form what seems to be dolichol monophosphate mannose and dolichol monophosphate *N*-acetyl glucosamine [4, 5].

It is possible that the dolichol derivatives are intermediates involved in the synthesis of the sugar portions of the various glycoproteins and glycolipids occurring in smooth microsomes, the Golgi system and other cellular membranes. It is therefore necessary to establish the subcellular localization of these derivatives.

The present work reports the subcellular distribution of Dol-P and of two enzymes related to its utilization.

2. Materials and methods

Radioactive UDP-Glc and Dol-P-Glc were prepared as earlier described [1]. Dol-P from whole liver and from the subcellular fractions was extracted and purified up to the DEAE-cellulose step as previously described [1]. The conditions for enzymatic assays are given in the text of each table.

The preparation of subcellular fractions from rat liver was done according to the following procedures described earlier: nuclear fraction [6], plasma membranes [7], Golgi system [8, 9], total as well as inner and outer mitochondrial membranes [10], and total microsomes and microsomal subfractions [11, 12].

Protein [13] and phospholipid [14] were determined as already described.

3. Results and discussion

The Dol-P content of the isolated subcellular fractions was determined enzymatically. It had been found that the amount of Dol-P-Glc formed in the reaction UDP-Glc + Dol-P → Dol-P-Glc + UDP is proportional to the concentration of Dol-P present [1]. Using labelled UDP-Glc very small amounts of Dol-P

* Permanent address: Department of Pathology II, Karolinska Institutet, Stockholm, Sweden.

** *Abbreviations:*
Dol-P, dolichol monophosphate. Dol-P-Glc, dolichol monophosphate glucose. Glc-acceptor, the glucosylated form of an endogenous acceptor.

Table 1
Dol-P content of subcellular fractions isolated from rat liver.

Fraction	Dol-P (in units per mg of phospholipid)
Nuclear	55.5
Plasma membranes	9.9
Golgi	42.1
Total mitochondria	12.3
Inner membranes	11.8
Outer membranes	10.6
Total microsomes	27.7
Rough	38.1
Smooth I	20.2
Smooth II	25.7

The Dol-P elicited increase in formation of Dol-P-Glc was measured with an incubation mixture which contained: 0.15 M Tris-malate, pH 7.7, 0.8% Triton X-100, 6 mM Mg-EDTA, 6 mM $MgCl_2$, 0.12 M 2-mercaptoethanol, 15 µl of a total microsomal fraction (0.5 mg protein), [^{14}C] UDPG (150,000 cpm, 309 Ci/mole) and increasing amounts of Dol-P, isolated from the subcellular fractions in a final volume of 165 µl. After 15 min incubation at 37°, the mixture was processed as previously described [3]. The increase of lower phase soluble radioactivity above endogenous activity by Dol-P isolated from 70 mg wet weight of whole liver homogenate, is defined as 10 arbitrary units. The endogenous activity obtained in the absence of externally added Dol-P was subtracted.

can be detected so that this reaction was used as an assay for Dol-P [3].

Results obtained show that Dol-P or a substance that mimics its action is found in all subcellular fractions with its highest concentration in nuclei, the Golgi fraction and rough microsomes and lowest in mitochondria and the plasma membrane (table 1). Morton et al. [15] studied the subcellular distribution of non-phosphorylated dolichol occurring as the free alcohol or esterified with fatty acids in pig liver. Expressed on a protein basis they also found a higher concentration of dolichol in the nuclear fraction than in either the mitochondrial or microsomal fractions.

Activities of the enzymes involved in the reaction sequence: UDP-Glc $\xrightarrow{1}$ Dol-P-Glc $\xrightarrow{2}$ Glc-acceptor, were measured. Table 2 shows data in which steps 1 and 2 were measured simultaneously, that is, using UDP-Glc as substrate, and also data in which direct transfer from Dol-P-Glc was assayed. The measurement of enzyme activity catalyzing the second step is based on the assumption that the acceptor for the glucose from Dol-P-Glc occurs in excess in each subfraction. However, this assumption cannot be verified until a method is found for separately assaying the acceptor.

The highest specific activity for transfer from UDP-Glc to Dol-P (step 1) is present in the outer mitochondrial membranes and smooth I microsomes while in the nuclear and Golgi fractions as well as in the rough and smooth II microsomes the activity is one half of the former. The transferase seems to be absent from plasma and inner mitochondria membranes. However negative results may reflect enzyme inactivation caused during the rather involved isolation of the membranes.

The distribution of the activity catalyzing step 2 depends on the way the reaction is measured. When starting from UDP-Glc the Golgi system and the smooth microsomes were the most active fractions. The nucleus also showed some activity. When measuring the direct transfer from Dol-P-Glc, a somewhat different distribution was found. Significant activity is detected only in the microsomes with some activity also in the plasma membranes. The remaining fractions gave only marginal activities. Parallel measurements of water soluble radioactivity in the Folch upper phase (see table 2), excluded the possibility that the absence of activity in some fractions could be due to a breakdown of the Glc-acceptor as observed previously [5]. The differences, depending on how step 2 was measured, were not unexpected since assay conditions were not the same.

However, the data are consistant in showing that mitochondria possess no transferase for step 2 and that some activity does exist in plasma membranes.

Summing up, it has been found that Dol-P and enzymes catalyzing the formation of Dol-P-Glc and the subsequent glucosylation of another dolichol intermediate, are widely but unevenly distributed in liver subcellular membranes. At this stage we can only venture to say that Dol-P plays different roles in the different liver membranes.

Table 2
Transfer of glucose from UDP-Glc and from Dol-P-Glc.

Substrate	UDP-Glc		Dol-P-Glc	
Product	Dol-P-Glc	Glc-acceptor	Glc-acceptor	Water soluble
	(pmoles of glucose transferred per mg of protein)			
Nuclear fraction	6.9	0.4	0	0
Plasma membranes	0.3	0.05	0.6	0
Golgi fraction	5.2	1.6	0.2	0
Total mitochondria	2.1	0.1	0.01	0
Inner membranes	0.6	0.03	0	0
Outer membranes	16.9	0.15	0.02	0
Total microsomes	9.4	0.9	3.3	1.0
Rough microsomes	8.3	0.9	1.6	0.8
Smooth I microsomes	17.6	1.1	1.3	0.3
Smooth II microsomes	9.3	0.5	0.1	0.1

For the measurement of Dol-P-Glc and Glc-acceptor formation from UDP-Glc, the incubation mixture contained: 0.33 M Tris-maleate pH 7.7, 0.6% Triton X-100, 30 mM Mg-EDTA, 30 mM $MgCl_2$, 0.1 M mercaptoethanol, 10 μl of the appropriate subfraction (0.5 mg of protein), [^{14}C] UDP-Glc (75 000 cpm, 309 Ci/mole) and 100 units (see table 1) of Dol-P from total liver, in a final volume of 31 μl.

Incubations were performed at 37° for 20 min and then 0.4 ml of methanol, 0.6 ml of chloroform and 0.2 ml of 4 mM $MgCl_2$ were added. After centrifugation the upper phase was removed and the phase decanted. If done carefully the precipitate from the interphase remains in the tube. Both the interphase and the lower phase were washed twice with 4 mM $MgCl_2$ in chloroform–methanol–water 3:48:47. The interphase was extracted three times with 0.5 ml of chloroform–methanol–water 1:1:0.3 [2]. This extract and the lower phase were transferred to aluminium planchets and counted in a gas flow counter.

The reaction mixture for the direct transfer of glucose from Dol-P-Glc to the acceptor containined: 70 mM Tris-maleate, pH 7.7, 0.18% deoxycholate, 3.5 mM Na-EDTA, 14 mM mercaptoethanol, [^{14}C] Dol-P-Glc (2,000 cpm) and an aliquot of the isolated fractions containing 0.5 mg of protein, in a final volume of 140 μl. After incubating for 15 min at 30°, 0.4 ml of methanol, 0.6 ml of chloroform and 0.2 ml of 4 mM $MgCl_2$ were added. After centrifugation both the upper and lower phases were discarded and the remaining precipitate was washed again with the same methanol–chloroform–$MgCl_2$ mixture. The Glc-acceptor was then extracted with three 0.5 ml portions of chloroform–methanol–water 1:1:0.3. The extracts were counted as outlined above. In both assays appropriate blanks were made and subtracted.

Acknowledgements

This work was supported by grants from the Consejo Nacional de Investigaciones Cientificas y Técnicas (Argentina). NHB and AJP are career investigators of this latter institution.

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