THE EFFECT OF DOLICHOL PHOSPHATE ON THE SYNTHESIS OF LIPID BOUND SUGARS IN EMBRYONIC CHICK BRAIN

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1. Introduction

It has been well established that polyisoprenol phosphate sugars are obligatory intermediates in the synthesis of oligosaccharide chains present in some bacterial cell walls [1]. Polyisoprenols are also present in mammalian tissues [2, 3], and their role as acceptors of hexoses from nucleotide sugars has been documented [4–10]. Leloir and his associates [5, 7, 8] have found that a liver system readily transfers hexoses from sugar nucleotides to dolichol monophosphate. On reincubation of one product, dolichol monophosphate glucose, the glucose is transferred to an oligosaccharide which is attached to dolichol pyrophosphate [9]. The fate of the oligosaccharide chain has not been established.

It has also been reported [11, 12] that brain homogenates readily transfer mannose from GDP mannose to chloroform soluble substances. The resulting compound has chemical properties very similar to undecaprenol phosphate mannose [17] and dolichol monophosphate glucose [5, 7, 8]. Furthermore, in rat brain it has been shown [13] that the transfer of glucose from UDP-glucose to chloroform soluble substances is stimulated 6-fold by the addition of dolichol phosphate. The concept that polyisoprenols may be important in the transfer of mannose to brain glycoproteins is supported by the present studies which show that the transfer of mannose from GDP-mannose to chloroform soluble lipids is highly stimulated by the addition of dolichol phosphate, while this reaction with other sugar nucleotides occurs to a much smaller extent.

2. Materials and methods

Dolichol was isolated from pig liver by the method of Burgos et al. [2]. Preparative thin-layer chromatography [3] was carried out developing first with chloroform and then eluting the dolichol band, which fluoresced when sprayed with Rhodamine G and gave a green-yellow colour when detected with anisaldehyde [3]. This material was chromatographed on a second plate with ethyl ester:petroleum ether (B.P. 40–60°) (20:80, v/v). This gave a product of >98% purity which on reversed phase chromatography [3] gave four major components corresponding to the C-90, C-95, C-100, C-105 dolichols, respectively. This material was chemically phosphorylated according to the method of Popjak [14]. For good yields the ditriethylamine phosphate had to be anhydrous. The crude phosphorylated product was treated with acid to destroy pyrophosphate [7] and, after a Folch partition with water, the chloroform soluble material was applied to a DEAE-cellulose column in chloroform–methanol (2:1, v/v). The column was first washed with chloroform–methanol (2:1, v/v) and subsequently with chloroform–methanol (2:1, v/v) saturated with 0.1 M ammonium acetate. This latter fraction was partitioned with 1/5 vol of water and the resulting chloroform layer was washed with chloroform–methanol–water (3:48:47, v/v/v). The chloroform layer was evaporated and the phosphorus content on an aliquot determined by the method of Rouser [15] to estimate the quantity of dolichol phosphate. Sugar nucleotides labelled with ^{14}C in the hexose moiety were obtained from New England

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Nuclear (Boston, Mass.) and Amersham/Searle (Don Mills, Ont.).

For the incubations the brain was removed from chick embryos of known age, weighed and homogenized in 0.32 M sucrose (10 ml/g tissue). The homogenate was centrifuged at 1000 *g* for 10 min, and the supernatant recentrifuged at 100,000 *g* for 60 min. The resulting pellet was resuspended in 0.32 M sucrose (2 mg/ml original wt). The conditions of the incubation are given in the legend to table 1. The dolichol phosphate was added to tubes initially, evaporated to dryness in a stream of nitrogen and suspended in the Triton X-100 buffer before the other additions. The reaction was terminated by the addition of 3 ml of chloroform–methanol (1:2, v/v). The protein was removed by centrifugation and washed with 1 ml chloroform–methanol (1:2, v/v). The solvent extracts for each tube were combined, chloroform (4 ml) and water (1 ml) were added and the phases allowed to separate. The upper was removed and the chloroform layer was washed twice with 1.5 ml of chloroform–methanol–water (3:48:47). Aliquots of the chloroform layer were evaporated to dryness, dissolved in 0.1 ml of methanol and the radioactivity determined in a standard toluene scintillation cocktail in a Packard TriCarb scintillation spectrometer. The efficiency of ^{14}C counting was 70%.

3. Results and discussion

Table 1 shows the transfer by chick brain of hexoses from sugar nucleotides into chloroform soluble lipids. In agreement with previous reports [11, 12], there was a significant transfer of mannose from GDP-mannose in the absence of dolichol phosphate. For other nucleotide sugars there was a much lower level of transfer of the hexoses to endogenous substrate. In 8-day embryo brain there was a 50-fold stimulation by dolichol phosphate in the transfer of mannose to a mannolipid. This stimulation fell to approx. 5-fold between 14 and 19 days. The stimulation by dolichol phosphate of the transfer of glucose and of fucose paralleled that of mannose. However, the total incorporation of these two sugars was low when compared to mannose. This may represent non-specific transfer by enzyme that uses mannose as the true substrate. The low incorporation and lack of stimulation by dolichol phosphate for galactose and

Table 1
Synthesis of lipid linked sugars in chick embryo brain.

Additions		Age of chick embryo			
Nucleotide sugar	Dolichol phosphate	8 days	12 days	14 days	19 days
		(pmoles hexose transferred/mg protein)			
GDPMan	–	2.1	4.9	5.4	5.4
	+	111.1	54.1	25.2	28.9
UDPGlu	–	0.4	1.3	1.0	1.4
	+	6.0	5.3	4.6	4.2
GDPFuc	–	0.6	0.5	0.4	0.4
	+	4.7	2.8	1.6	1.3
UDPGal	–	0.2	0.1	0.2	1.1
	+	0.6	0.2	0.4	0.8
UDPGluNAc	–	0.0	0.4	0.3	0.5
	+	0.0	0.5	0.4	0.3

The incubation mixture, pH 7.4, consisted of: Tris-HCl, 15 μmoles; $MnCl_2$, 5 μmoles; Triton X-100, 0.5 mg; radioactive nucleotide sugar, 1–2 nmoles (5×10^5 cpm); and brain postnuclear pellet (2.0–2.5 mg protein) in a volume of 0.5 ml. Dolichol monophosphate (0.116 μmoles) was added where indicated. Incubations lasted for 12 min and then were processed as indicated in the text. Zero time radioactivity, corresponding to 0.01–0.05 pmoles sugar has been subtracted from the above values. Each value is the result of one incubation.

glucosamine may indicate that the low level of radioactivity found represents endogenous synthesis of glycosphingolipids [16].

The radioactive mannolipid formed in the presence or absence of dolichol phosphate is: i) hydrolysed by 0.01 N HCl, 10 min 100° to yield radioactive water soluble products containing mannose, ii) stable to alkaline hydrolysis by 1 N NaOH, 15 min, 37°, and iii) behaves like a mannosyl monophosphoryl polyisoprenol in all the chromatographic systems developed by Scher et al. [17] in their studies on mannan synthesis by *Micrococcus lysodeikticus.*

In view of the fact that the stimulation of mannose incorporation with added dolichol phosphate decreases as the embryo develops while the incorporation in the absence of dolichol increases, it is quite possible that pig liver dolichol phosphates are not identical with the isoprenols present in chick brain. However, polyisoprenols with structures very similar to pig liver dolichols have been found in calf and rat brain (W.C. Breckenridge and L.S. Wolfe, unpublished observations). The nature of the synthesis of the mannolipid during brain development appears to correlate with other studies on the activities of glycosyl transferases in embryonic chick brain [16]. In general, these enzymes reach a peak of activity during the period of rapid brain development and increase in brain mass.

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References

[1] I.W.J. Lennarz and M.G. Scher, Biochim. Biophys. Acta 265 (1972) 417.
[2] J. Burgos, F.W. Hemming, J.F. Pennock and R.A. Morton, Biochem. J. 88 (1963) 470.
[3] P.J. Dunphy, J.D. Kerr, J.F. Pennock, K.J. Whittle and J. Feeny, Biochim. Biophys. Acta 136 (1967) 136.
[4] J.F. Caccam, J.J. Jackson and C.H. Eylar, Biochem. Biophys. Res. Commun. 35 (1969) 505.
[5] N.H. Behrens and L.F. Leloir, Proc. Natl. Acad. Sci. U.S. 66 (1970) 153.
[6] J. Molnar, H. Chao and Y. Ikehara, Biochim. Biophys. Acta 239 (1971) 401.
[7] N.H. Behrens, A.J. Parodi, L.F. Leloir and C.R. Krisman, Arch. Biochem. Biophys. 143 (1971) 375.
[8] N.H. Behrens, A.J. Parodi and L.F. Leloir, Proc. Natl. Acad. Sci. U.S. 68 (1971) 2857.
[9] A.J. Parodi, N.H. Behrens, L.F. Leloir and M. Dankert, Biochim. Biophys. Acta 270 (1972) 529.
[10] J.B. Richards, P.J. Evans and F.W. Hemming, Biochem. J. 124 (1971) 957.
[11] M. Zazt and S.H. Barondes, Biochem. Biophys. Res. Commun. 36 (1969) 511.
[12] L.S. Wolfe, J. Miller and J.T.R. Clarke, in: Transactions of the American Society for Neurochemistry, 3rd Annual Meeting, Seattle, March, 1972, p. 136.
[13] W. Jankowski and T. Chojnacki, Biochim. Biophys. Acta 260 (1972) 93.
[14] G. Popjack, J.W. Cornforth, R.H. Cornforth, R. Ryhage and D.S. Goodman, J. Biol. Chem. 237 (1962) 56.
[15] G. Rouser, A.N. Siakotos and S. Fleischer, Lipids 1 (1966) 85.
[16] B. Kaufman, S. Basu and S. Roseman, in: Inborn Disorders of Sphingolipid Metabolism, ed. S.M. Aronson and B.W. Volk (Pergamon Press, New york, 1967) p. 193.
[17] M. Scher, W.J. Lennarz and C.C. Sweeley, Proc. Natl. Acad. Sci. U.S. 59 (1968) 1313.