THE BIOSYNTHESIS OF A MANNOLIPID THAT CONTAINS A POLAR METABOLITE OF 15-^{14}C-RETINOL

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SUMMARY

A mannolipid synthesized by a membrane-rich fraction from rat liver in the presence of 15-^{14}C-retinol and GDP-mannose-^{14}C contained a polar metabolite of ^{14}C-retinol, as well as ^{14}C-mannose. Retinol stimulated the *in vitro* incorporation of ^{14}C-mannose from GDP-mannose-^{14}C into the mannolipid and glycoproteins.

Polyisoprenols form lipid-carbohydrate intermediates in the biosynthesis of bacterial polysaccharides and glycoproteins (1-4).

Caccam, Jackson, and Eylar (5), by using cell fractions from liver, have recently described the incorporation of ^{14}C-mannose from GDP-mannose-^{14}C into a mannolipid similar to the bacterial mannosyl-1-phosphoryl-isoprenols (3). Behrens and Leloir (6) reported the function of another polyprenol, dolichol, in the formation of dolichol monophosphate glucose.

Vitamin A (retinol), a tetraisoprenol derivative, is necessary for maintenance of differentiation of mucus-secreting epithelia and the biosynthesis of specific glycopeptides (7,8). We now report the incorporation of labeled retinol and mannose into a mannolipid by liver fractions.

A subcellular fraction from livers of mildly vitamin A-deficient rats, consisting of membrane-bound polyribosomes and smooth

endoplasmic reticulum, separated from free polyribosomes by discontinuous sucrose gradient centrifugation ("membrane-rich interface material") (9), incorporated ^{14}C-mannose from GDP-mannose-^{14}C into lipid and protein fractions (Fig. 1A). The addition of retinol at zero time or after 20 minutes greatly enhanced the incorporation (Figs. 1B and 1C). The reaction showed a requirement for ATP, since in its absence the incorporation of mannose dropped four-fold.

To test for incorporation of ^{14}C-retinol at the same time as GDP-mannose-^{14}C, an incubation was performed indentical to that shown in Figure 1B, except that 300 µg of 15-^{14}C-retinol[1] (10µCi) was used in place of the unlabeled retinol. The $CHCl:CH_3OH$ extract of the trichloroacetic acid (TCA) precipitate at the end of the incubation was placed on a DEAE-cellulose column (1 x 40 cm) (1), washed with 99% methanol and then treated with a linear gradient (0 to 0.1 M) of ammonium acetate in methanol. The ^{14}C-mannolipid was eluted from the column as a single peak of radioactivity with 0.0075 M ammonium acetate. To test its homogeneity, aliquots were chromatographed on silica gel G thin-layer plates in solvents (a) $CHCl_3:CH_3OH:CH_3COOH$:0.9% NaCl in H_2O (50:25:8:4); (b) $CHCl_3:CH_3OH:NH_4OH:H_2O$ (80:30:0.5:3); (c) $C_6H_6:CHCl_3:CH_3OH$ (50:12.5:12.5). In each solvent the mannolipid moved as one well-defined radioactive spot, whether prepared from incubations with GDP-mannose-^{14}C alone, ^{14}C-retinol alone, or from incubations containing both radioactive precursors together. When the incubation was stopped at zero time, after addition of either or both the precursors, no radioactivity was found in the mannolipid. The mannolipid had the same R_f in the above 3 solvent systems as retinoic acid; therefore, though ob-

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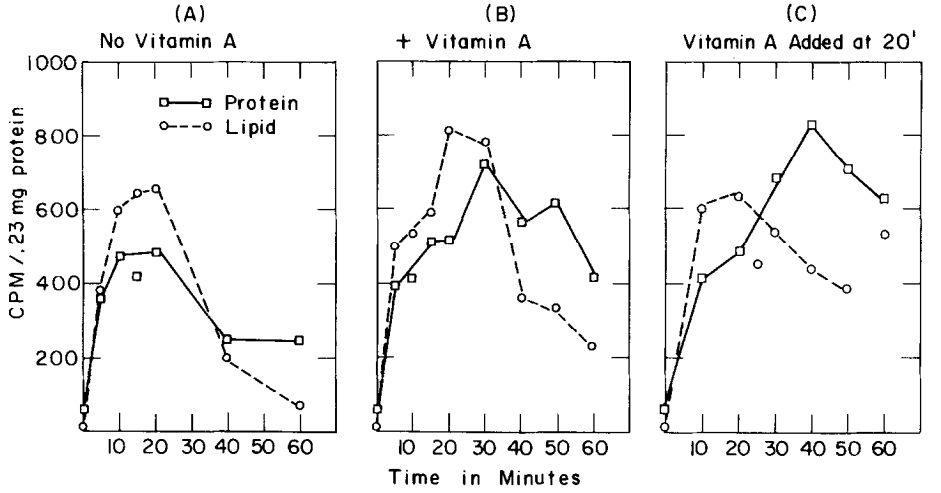

Figure 1. Time course of incorporation of ^{14}C-mannose into protein and lipid in vitamin A-deficient interface membranes.

A. The system contained 5 mg of membrane protein from vitamin A-deficient rat liver in 0.5 ml of medium A (14); 0.1 ml of 0.1 M $MnCl_2$; 0.1 ml of 0.025 M EDTA; 0.1 ml of 0.3 M Tris at pH 7; 0.1 ml of 1.5% Zonyl A (surface active agent; E.I. du Pont de Nemours & Co., Organic Chemicals Dept., Wilmington, Delaware); 0.1 ml of an aqueous solution of GDP-mannose-^{14}C (10 µCi contained in 40 µg) and 1 mg of ATP dissolved in 0.1 ml of H_2O. The incubation was carried out at 37° C and aliquots were taken at the indicated time intervals, precitated, and washed three times with 5% TCA, extracted with $CHCl_3$:CH_3OH (2:1) twice. The protein residue was dissolved in 0.2 N KOH and counted; the lipid extract, washed twice with 0.9% NaCl solution, was applied to paper, dried, and counted in 0.5% PPO in toluene. Tests showed that on chromatography on DEAE-cellulose, all the radioactivity in this lipid extract was recoverable in the mannolipid peak.

B. Same as A, with the addition of 1 mg of unlabeled retinol at zero time. Retinol was dissolved in 0.5 ml of ether. This was mixed with 0.1 ml of 1.5% Zonyl A and the ether removed under nitrogen. This retinol solution was added in place of the Zonyl A in the incubation A, above.

C. Same as B; however, the retinol solution was added at 20 minutes.

All the incubations and any subsequent operation were performed either in the dark or in red light.

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viously not identical with retinoic acid, its polarity must be similar. Thin-layer chromatography in C_6H_6:$CHCl_3$:CH_3OH:CH_3COOH (5:5:5:1) showed an R_f of 0.18, whereas retinoyl glucuronide (10) is reported in that solvent to have an R_f of 0.45.

Alkaline hydrolysis (1 N KOH for 45 min at 75° C) of the mannolipid labeled in both moieties and thin-layer chromatography of

Table I. Thin layer chromatography of the lipid moiety of the ^{14}C-labeled mannolipid compared to known standards after alkaline hydrolysis.

A. The mannolipid was hydrolyzed in 1 N KOH in ethanol at 75° C for 45 minutes. The hydrolysate was then neutralized with acetic acid and extracted with $CHCl_3$. The extract was placed on thin layers of silica gel G with standard retinol, retinal, and retinoic acid, which were visualized by UV absorption. Solvents were:

1. 6% acetone in hexane
2. Benzene:$CHCl_3$:CH_3OH (50:12.5:12.5)
3. 15% acetone in hexane
4. 5% Benzene in $CHCl_3$:CH_3OH:CH_3COOH (5:5:1)

	Solvent			
	1	2	3	4
Retinol	0.27*	0.78	0.29	
Retinal	0.39	0.95	0.61	
Retinoic Acid	0.00	0.78	0.05	1.0
Lipid Moiety	0.00	0.78	0.05	1.0

B. Standard retinoic acid and the lipid moiety of the mannolipid were esterified with CH_2N_2 in ether. They were then chromatographed in solvent 1.

Methyl-Retinoate	0.8*
CH_2N_2-Treated Lipid Moiety	0.0

* Numbers are R_fs.

the lipid extract of the hydrolysate in 5 solvents (Table I) gave one spot only, with an R_f of retinoic acid. However, after methylation with CH_2N_2, standard retinoic acid and the sample behaved differently. When the aqueous extract of the alkaline hydrolysate was chromatographed on Whatman No. 3MM paper in isobutyric acid: NH_4OH:H_2O (57:4:39), only one major radioactive peak, corresponding to mannose, was demonstrable.

In another experiment, 15-^{14}C-retinol was fed by stomach tube to vitamin A-deficient rats in the amounts of 2.96 μCi per day in two daily doses for five days. The rats were then killed, the interface material prepared from their livers and incubated with 10 μCi of GDP-mannose-^{14}C, and the constituents described under Figure 1A. The reaction was stopped by adding unlabeled GDP-mannose and 5% TCA. The mannolipid was then isolated by DEAE-cellulose chromatography. A peak of radioactivity was recovered from the CH_3OH wash of the column and identified as retinyl-palmitate. The ammonium acetate gradient eluted the mannolipid with 0.0075 M salt. The compound represented by this peak contained ^{14}C-mannose and a polar metabolite of retinol having the same R_fs as retinoic acid in the solvents described in Table I. When the reaction was stopped at zero time, the only radioactivity was found in the polar retinol metabolite.

Membrane-free polyribosomes collected as a pellet during the discontinuous gradient centrifugation were not able to incorporate ^{14}C-mannose from GDP-mannose-^{14}C into the mannolipid or the glycoproteins. This confirms the various reports (11-13) that secreted glycoproteins are synthesized on the membrane-bound polyribosomes.

The results presented here suggest that vitamin A, or one of its metabolites, may function in mucus-secreting tissues by acting as a lipid intermediate, carrying mannose from GDP-mannose for the biosynthesis of the secreted glycoprotein.

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