Synthesis of retinyl phosphate mannose and dolichyl phosphate mannose from endogenous and exogenous retinyl phosphate and dolichyl phosphate in microsomal fraction

Specific decrease in endogenous retinyl phosphate mannose synthesis in vitamin A deficiency

Luigi M. DE LUCA,* Michele R. BRUGH, Carol S. SILVERMAN-JONES and Yoshihiro SHIDOJI

Differentiation Control Section, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205, U.S.A.

(Received 9 March 1982/Accepted 9 July 1982)

Rat liver microsomal fraction synthesized Ret-P-Man (retinyl phosphate mannose) and Dol-P-Man (dolichyl phosphate mannose) from endogenous Ret-P (retinyl phosphate) and Dol-P (dolichyl phosphate). Ret-P-Man synthesis displayed an absolute requirement for a bivalent cation, and also Dol-P-Man synthesis was stimulated by bivalent metal ions. Mn$^{2+}$ and Co$^{2+}$ were the most active, with maximum synthesis of Ret-P-Man occurring at 5–10 mM; Mg$^{2+}$ was also active, but at higher concentrations. At 5 mM-Mn$^{2+}$ the amount of endogenous Ret-P mannosylated in incubation mixtures containing 5 µM-GDP-mannose in 15 min at 37°C was approx. 3 pmol/mg of protein. In the same assays about 7–10 pmol of endogenous Dol-P was mannosylated. Bivalent-cation requirement for Ret-P-Man synthesis from exogenous Ret-P showed maximum synthesis at 2.5 mM-Mn$^{2+}$ or -Co$^{2+}$. In addition to Ret-P-Man and Dol-P-Man, a mannosylipid co-chromatographing with undecaprenyl phosphate mannose was detected. Triton X-100 (0.5%) abolished Ret-P-Man synthesis from endogenous Ret-P and caused a 99% inhibition of Ret-P-Man synthesis from exogenous Ret-P. The presence of detergent (0.5%) also inhibited Dol-P-Man synthesis from endogenous Dol-P and altered the requirement for Mn$^{2+}$. Microsomal fraction from Syrian golden hamsters was also active in Ret-P-Man and Dol-P-Man synthesis from endogenous Ret-P and Dol-P. At 5 mM-Mn$^{2+}$ about 2.5 pmol of endogenous Ret-P and 3.7 pmol of endogenous Dol-P were mannosylated from GDP-mannose per mg of protein in 15 min at 37°C. On the other hand, microsomal fraction from vitamin A-deficient hamsters contained 1.2 pmol of Ret-P and 14.1 pmol of Dol-P available for mannosylation. Since GDP-mannose:Ret-P and GDP-mannose:Dol-P mannosyltransferase activities were not affected, depletion of vitamin A must affect Ret-P and Dol-P pools in opposite ways.

We have previously reported that bovine serum albumin, in the absence of detergent, stimulates the synthesis of Ret-P-Man in rat liver microsomal fraction (referred to below simply as microsomes) from GDP-mannose and exogenous Ret-P (Shidoji & De Luca, 1981).

It was therefore deemed important to investigate in detail the biosynthesis of Ret-P-Man from endogenous Ret-P present in the microsomal membrane, by using the assay in the presence of bovine serum albumin. In addition, it was decided to study how the condition of vitamin A deficiency affects the pool of endogenous Ret-P and also that of endogenous Dol-P, because vitamin A deficiency in vivo decreases mannosylipid and mannoprotein biosynthesis in hamster liver (De Luca et al., 1975) and allows the accumulation of Dol-P-GlcNAc$_2$-Man$_5$ in rat liver (Rosso et al., 1981).

Materials and methods

Materials

Bovine serum albumin (globulin-free, crystallized, freeze-dried), Dol-P (grade III) and GDP-mannose
were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Ret-P was synthesized as previously described (Bhat et al., 1980); Millipore filters were obtained from Millipore, Boston, MA, U.S.A. The following radioactive products were obtained from New England Nuclear Corp., Boston, MA, U.S.A.: GDP-[3H]mannose (sp. radioactivity 10 Ci/mmol) and GDP-[14C]mannose (0.2 Ci/mmoll). Precoated silica-gel plates (silica gel 60 layers from Merck, Darmstadt, West Germany) were obtained from Brinkman, Westbury, NY, U.S.A.

Preparation of liver microsomal vesicles

Normal male rats, Osborne–Mendel strain (weighing 150–200 g), starved overnight, were killed by bleeding under light diethyl ether anaesthesia. The livers were homogenized with 2 vol. of saline (0.9% NaCl) in a glass/Teflon homogenizer. After centrifugation of the homogenate at 2500 g for 20 min, the resulting supernatant was centrifuged at 105,000 g for 60 min. The pellet was resuspended in a small volume of saline and stored in liquid N2 until used. The incubation mixture contained: 0.4 μCi of GDP-[3H]mannose (or 0.1 or 0.2 μCi of GDP-[14C]mannose), 4 mg of bovine serum albumin/ml, GDP-d-mannose (24 μM final conc.), 30 mM-Tris/HCl buffer, pH 8, 5 mM-MnCl2 or as stated, 8 mM-NaF, 2 mM-ATP, 5 mM-AMP and about 1 mg of the microsomal protein in a final volume of 200 μl. After incubation for appropriate time intervals, the lipids were extracted by the following procedure, which yields two phases.

To the mixture was added 5 vol. (1 ml) of chloroform/methanol (2:1, v/v), the tube was stirred and two phases were allowed to separate by low-speed centrifugation. Under these extraction conditions approx. 98% of Dol-P-Man is recovered in the lower phase, whereas Ret-P-Man partitions about 40–50% in the lower phase and 60–50% in the upper phase.

Alternatively 15 vol. (3 ml) of chloroform/methanol (2:1, v/v) was added to yield a monophasic extract.

Extracts were dried under a stream of N2 and immediately dissolved in suitable volumes of chloroform/methanol (2:1, v/v) containing 10 μg of synthetic Ret-P ready for application on thin layers of silica gel. Chromatography was usually performed in chloroform/methanol/water (45:35:6, by vol.) (solvent A) on two identical plates. One plate was used for determination of radioactivity, the other one for fluorography. Sections (0.5 cm) of silica gel were collected into counting vials and radioactivity was determined after addition of 0.25 ml of methanol and 10 ml of Betafluor (National Diagnostics, Somerville, NJ, U.S.A.). The two bands obtained on occasion in the area of Ret-P-Man were both counted as Ret-P-Man, since variability in the relative proportions of the two compounds was noted. Such variability may be due to enzymic or non-enzymic isomerization. Fluorography was performed with Kodak film XR-5 after it had been sprayed with En3Hance (New England Nuclear), by following a procedure developed by Bonner & Laskey (1974).

Preparation of vitamin A-depleted Syrian golden hamsters

Vitamin A-depleted male Syrian golden hamsters were prepared essentially as previously described (De Luca et al., 1975), in accordance with the procedure of Rogers et al. (1974), by placing the mothers on a vitamin A-free diet at birth of the experimental animals. These were weaned on a vitamin A-deficient diet at about 21 days. At 40 days hamsters prepared in this way began to show signs of vitamin A deficiency, such as eye lesions and diminished growth rate. At about this stage of deficiency hamsters were anaesthetized with diethyl ether and killed by bleeding from the neck. Livers were removed and microsomes were prepared as described for rat liver microsomes. Microsomes from normal male Syrian golden hamsters were prepared in the same way.

Other procedures

Protein was determined by the method of Lowry et al. (1951), with bovine serum albumin as a standard. Ret-P was measured spectrophotometrically on the basis of A 1640 = 1440. Retinyl palmitate was measured by the high-pressure liquid-chromatography procedure of Bhat et al. (1980).

Results

Comparison of bovine serum albumin and Triton X-100 incubations for Ret-P-Man synthesis from exogenous Ret-P

Fig. 1 compares the rates of synthesis of Ret-P-Man from GDP-mannose and exogenous Ret-P in incubation mixtures containing 0.5% Triton X-100 or bovine serum albumin as the dispersal agent for Ret-P. A 200-fold increase in the incubations in the presence of bovine serum albumin compared with those in the presence of Triton X-100 is obtained. Therefore the biosynthesis of Ret-P-Man and Dol-P-Man from endogenous acceptor lipids was studied in the bovine serum albumin system.

Fig. 2(a) shows the time course of the synthesis of Ret-P-Man and Dol-P-Man from endogenous acceptors at 5 mM-MnCl2. Fig. 2(b) shows the fluorography of an identical thin-layer chromatograph showing a double spot in the area of Ret-P-Man, as previously reported for Ret-P-Man synthesized from exogenous Ret-P (Rosso et al., 1975). In addition to Dol-P-Man, a labelled mannolipid is also seen at a
slightly lower $R_f$. This compound co-chromatographs with standard undecaprenyl phosphate mannose, as shown in Fig. 2(c). Fig. 2(d) shows that Ret-P-Man partitions between the lower and the upper phase in the extraction procedure as used in these studies, where the reaction mixture (0.2 ml) is extracted with 1 ml of chloroform/methanol (2:1, v/v); nearly all the Dol-P-Man is found in the lower phase, as shown in Fig. 2(d). At 5 mM-MnCl₂ the ratio of Ret-P-Man to Dol-P-Man is about 1:4.2, and at 10 mM-MnCl₂ 1:3 (Expt. 1, Table 1). In Expt. 2 of Table 1 the upper phase was also examined, demonstrating that about 50% of Ret-P-Man partitions in the upper phase and that the actual ratio of Ret-P-Man to Dol-P-Man synthesized at 15 min of incubation is 1:3 at 5 mM-MnCl₂ and 1:2.3 at 10 mM-MnCl₂. The amounts of Ret-P-Man and Dol-P-Man and their ratios are similar to these values when calculated for monophasic extracts obtained by adding 15 vol. (3 ml) of chloroform/methanol (2:1, v/v) to the incubation mixture. The main products formed at 15 min are Ret-P-Man and Dol-P-Man with a ratio of 1:3 at 5 mM-MnCl₂ (results not shown).

Inclusion of 0.5% Triton X-100 in the bovine serum albumin incubation system maintains Dol-P-Man synthesis, albeit of about 50% of that in the incubation with bovine serum albumin alone, but completely inhibits Ret-P-Man synthesis from endogenous acceptor Ret-P (Figs. 3a and 3b). It should be noted that the detergent also inhibits the synthesis of the undecaprenyl phosphate mannose-like compound, though it greatly stimulates the synthesis of material A co-migrating with standard Dol-P-P-GlcNAc₂-Man (Fig. 3b).

It was decided to compare bivalent cations for their ability to stimulate Ret-P-Man and Dol-P-Man synthesis. Fig. 4(a) shows the Mn²⁺, Co²⁺-, and Mg²⁺-dependence of Ret-P-Man synthesis from endogenous Ret-P. A maximum between 5 and 10 mM with strong inhibition at 50 mM was found with MnCl₂ and CoCl₂; MgCl₂ also stimulated Ret-P-Man synthesis, but its optimal concentration was 10 mM and little inhibition was observed at 50 mM. These metal ions also stimulated Dol-P-Man synthesis by about 2-fold, but without a sharp maximum; however, optimal conditions were about 5 mM (Fig. 4b). The fluorographs for the t.l.c. profiles of the lower-phase extracts of incubation mixtures containing MnCl₂ (Fig. 4c), CoCl₂ (Fig. 4d) and MgCl₂ (Fig. 4e) are also shown (for only a few concentrations) and represent duplicate plates identical with those utilized for radioactivity measurement in Fig. 4(a). The metal ions also appeared to stimulate the liberation of mannose. Occasionally a doublet of bands is found opposite the Dol-P-P-GlcNAc₂-Man marker. Similar results were obtained when the analyses were conducted on monophasic extracts (Fig. 5a). Fe²⁺, Cu²⁺, Ca²⁺, Zn²⁺ were without activity (results not shown).

Triton X-100 at 0.5% completely inhibited Ret-P-Man synthesis from endogenous acceptors, as shown in Fig. 3. The same inhibitory effect is seen in the range of concentration of MnCl₂ 0-50 mM (Figs. 6a and 6b). However, Dol-P-Man synthesis in the presence of Triton X-100 shows an absolute requirement for MnCl₂ (2.5 mM maximum). Fig. 6(b) also shows the stimulatory effect of MnCl₂ on the biosynthesis of a compound migrating as Dol-P-P-GlcNAc₂-Man. The curve for the MnCl₂ requirement for the synthesis of Dol-P-Man from exogenous Dol-P is shown in Fig. 7, with maximum at 2.5 mM.

In the presence of exogenous Ret-P (0.15 mM) Ret-P-Man synthesis (2 min at 37°C) was highest at 2.5 mM-MnCl₂ or-CoCl₂ and at 10 mM-MgCl₂ (Fig. 8a). As for the studies on Ret-P-Man synthesis from
Fig. 2. Time course of the synthesis of Ret-P-Man (○) and Dol-P-Man (●) from endogenous Ret-P and Dol-P of rat liver microsomes

(a) GDP-[14C]mannose (0.2 μCi; 5 μM) was incubated with 1.0 mg of rat liver microsomes as described in the Materials and methods section at 37°C for the indicated times. Lower phases of the extracts were applied to 20 cm x 20 cm Merck t.l.c. plates, which were chromatographed in solvent A, and radioactivity was measured as described in the Materials and methods section. (b) A duplicate t.l.c. plate of (a) was utilized for fluorography. Exposure time was 14 days. A mixture (2000 c.p.m.) of Ret-P-Man and Dol-P-Man was applied in the column marked St. The positions of standard Dol-P-Man, undecaprenyl phosphate mannose (C55-P-Man), Ret-P-Man, mannoside, and GDP-mannose are indicated. Only the 5, 30 min and 90 min time points are shown. (c) Fluorography of t.l.c. plate of manno-olipids from incubations with and without exogenous undecaprenyl phosphate. Incubations were identical with those described for (a) except that the incubation mixture in lane II contained standard undecaprenyl phosphate (25 μg) solubilized in 0.5% Triton X-100 and incubation had proceeded for 15 min at 37°C. For the incubation mixture chromatographed in lane I incubation had proceeded for 30 min at 37°C. Similar amounts of radioactivity (10000 c.p.m.) were used for lanes I and II on a 5 cm x 20 cm plate developed as described in the text. Film was exposed for 3 days. (d) Fluorography of t.l.c. plates of lower-phase and upper-phase extracts. Incubation mixture and conditions were the same as in (a). The lower-phase extract was dried under N2, immediately dissolved in 60 μl of 99% (v/v) methanol containing 10 μg of standard Ret-P and applied to the plate. The upper-phase extract was dried under N2, resuspended in 60 μl of chloroform/methanol (2:1, v/v), and 20 μl of 99% methanol containing 10 μg of standard Ret-P was added. Samples were applied to a 5 cm x 20 cm t.l.c. plate of silica gel and developed as usual along with the extract of an incubation mixture containing exogenous Ret-P (marked St) to show the position of Ret-P-Man. The amount of radioactivity in the area of Ret-P-Man in the upper-phase extract was 440 c.p.m. and that in the lower-phase extract 350 c.p.m., whereas that of Dol-P-Man in the lower-phase extract was 2160 c.p.m., giving an approximate ratio of 1:3.6. The film was exposed for 5 days. The positions of the named compounds indicate the chromatographic mobilities of samples of these when used as chromatographic standards.
Table 1. Measurement of Ret-P-Man/Dol-P-Man ratios in rat liver microsomes as measured from the two-phase and the monophasic extraction procedures

For full experimental details see the text. Expt. 1. Rat liver microsomes were incubated in the usual incubation system to assay for endogenous Ret-P and Dol-P in quadruplicates at 5 mM- and 10 mM-MnCl₂. The procedure described for the ‘lower-phase extraction’ was utilized to measure Ret-P-Man and Dol-P-Man. Expt. 2. The same incubation systems as for Expt. 1 were used. Both upper and lower phases were dried and processed in the same way for Ret-P-Man and Dol-P-Man determination. Incubations were done in duplicate at 5 mM- and 10 mM-MnCl₂.

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<th>(c.p.m./15 min per mg of protein)</th>
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<th>(c.p.m./15 min per mg of protein)</th>
<th>Dol-P-Man synthesis</th>
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Fig. 3. Time course of the synthesis of Ret-P-Man (O) and Dol-P-Man (●) from endogenous acceptors by rat liver microsomes in the presence of Triton X-100

(a) The same conditions as described for Fig. 2(a) were used except that 0.5% Triton X-100 was included in the incubation mixture. (b) Fluorography of the t.l.c. plate of the experiment shown in (a). Purified standard Ret-P-Man was applied in the column marked St. Positions of standard Dol-P-Man, undecaprenyl phosphate mannose (C₁₅-P-Man), Ret-P-Man and Dol-P-P-GlcNAc₂-Man are shown. Film was exposed for 32 days. Only the 2 min, 10 min and 90 min time points are shown. The positions of the named compounds indicate the chromatographic mobilities of samples of these when used as chromatographic standards.

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endogenous Ret-P, optimal MgCl₂ concentration was at 10 mM, and Ret-P-Man synthesis was still very high (65% of maximum) at 50 mM-MgCl₂, whereas CoCl₂ and MnCl₂ completely inhibited at 50 mM. Fluorographs of t.l.c. profiles (representative points only are shown) of the lower-phase extract of incubation mixture containing MnCl₂ (Fig. 8b), CoCl₂ (Fig. 8c), and MgCl₂ (Fig. 8d) are shown for comparison. Here again, as for Figs. 4 and 9(d), a multiplicity of bands is observed with RF close to that of Dol-P-P-GlcNAc₂-Man.

Studies on hamster liver in normal and vitamin A-depleted animals

A remarkable similarity between the rat and the hamster liver microsomes was observed for Mn²⁺-dependence of Ret-P-Man synthesis from endogenous Ret-P (Fig. 9a). As for the rat, MnCl₂ constituted an absolute requirement for Ret-P-Man synthesis, but not for Dol-P-Man synthesis, by hamster liver microsomes in the bovine serum albumin incubation system. However, the ratio of
Ret-P-Man to Dol-P-Man in the lower-phase extract was greater (0.3:1) with the hamster preparation than that (0.23:1) with the rat preparation. Vitamin A-depleted and normal hamster liver microsomes were compared for their ability to synthesize Ret-P-Man and Dol-P-Man from endogenous lipids. Fig. 9(b) shows a maximum (15 min at 37°C) for

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**Fig. 5. Dependence on Mn\(^{2+}\) and Co\(^{2+}\) of Ret-P-Man synthesis by rat liver microsomes as measured in monophasic extracts**

Incubation mixtures were the same as described for Fig. 2(a) except that either MnCl\(_2\) (●) or CoCl\(_2\) (△) was used. The monophasic extraction procedure was used for the assay. The positions of the named compounds indicate the chromatographic mobilities of samples of these when used as chromatographic standards.

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**Fig. 6. Effect of Triton X-100 on Mn\(^{2+}\)-dependence of Ret-P-Man (●) and Dol-P-Man (○) synthesis from endogenous acceptors by rat liver microsomes**

The incubation system to test 'endogenous lipid acceptors' (see the Materials and methods section) was used except that 0.5% Triton X-100 was included. Duplicate t.l.c. plates were used: one (a) for scraping and counting of radioactivity, the other (b) for fluorography; only concentrations of 0 mM, 5 mM, and 50 mM MnCl\(_2\) are shown. The film was exposed for 2 weeks. The positions of the named compounds indicate the chromatographic mobilities of samples of these when used as chromatographic standards.

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Ret-P-Man synthesis at 2.5 mM-MnCl₂ for vitamin A-deficient microsomes; however, the actual amount of Ret-P-Man synthesized is greatly decreased compared with microsomes from normal hamsters (Fig. 9a), and the ratio of Ret-P-Man to Dol-P-Man is greatly diminished, this being also due to a concomitant accumulation of Dol-P-Man in vitamin A-deficient microsomes, as shown in Table 2. This is readily realized from the fluorographs shown in Fig. 9(c). Lower-phase extracts of incubation mixtures containing 2.5 mM-, 5 mM- and 10 mM-MnCl₂ are compared in microsomal systems from normal and vitamin A-deficient hamsters. A decrease in radioactivity associated with Ret-P-Man is observed. Here, as before, the doublet of bands opposite the Ret-P-Man marker was considered as Ret-P-Man.
because of the variability in the relative proportion of the two bands, possibly due to isomerization. It does seem, however, that a specific depletion of the lower band takes place in the vitamin A-deficient membrane. At the same time Dol-P-Man increases, and multiple bands migrating in the area of standard undecaprenyl phosphate mannose are also seen (materials B and C). Material B is chromatographically similar to undecaprenyl phosphate mannose, and material C is slightly less hydrophobic.
Table 2. Effect of vitamin A deficiency on the Ret-P-Man/Dol-P-Man ratio from endogenous lipid acceptor at different concentrations of MnCl₂.

For explanation and full experimental details see the text.

<table>
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<tr>
<th>[MnCl₂] (mM)</th>
<th>Ret-P-Man synthesis</th>
<th>Dol-P-Man synthesis</th>
<th>Ret-P-Man/Dol-P-Man ratio</th>
<th>[MnCl₂] (mM)</th>
<th>Ret-P-Man synthesis</th>
<th>Dol-P-Man synthesis</th>
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shown by the arrow. Moreover, an accumulation of a compound with chromatographic characteristics of Dol-P-P-GlcNAc₂-Man is also found (Fig. 9c). This is confirmed in Fig. 9(d), where a study of mannolipid synthesis in the 0–50 mM range of MnCl₂ concentrations is shown for the vitamin A-deficient microsomes. The results of an experiment designed to compare the synthesis of Ret-P-Man and Dol-P-Man and their ratios in normal and severely vitamin A-deficient hamster liver microsomes are shown in Table 2.

Discussion

The incubation in the presence of bovine serum albumin permits the detection of the following mannolipids from endogenous lipids: (1) Ret-P-Man; (2) a slightly more hydrophobic compound at times also found in incubations containing endogenous Ret-P and possibly representing an isomeric form of Ret-P-Man (Rosso et al., 1975); (3) a mannolipid with the same chromatographic characteristics as undecaprenyl phosphate mannoside; (4) Dol-P-Man. Radominska-Pyrek et al. (1979) have characterized a fully unsaturated decaprenol from bovine pituitary gland, and the putative undecaprenyl phosphate mannoside may be related to that structure.

The amount of Ret-P available for mannosylation is about 1 ng/mg of microsomal rat liver protein for the rat (Shidoji & De Luca, 1981) and 0.71 ng/mg for the hamster, as assayed at 5 mM-MnCl₂ for 15 min at 37°C. These concentrations compare with Dol-P values of 20 ng/mg of microsomal rat protein and 6 ng/mg in the hamster. The finding that about 50–60% of Ret-P-Man partitions in the upper phase of the extraction procedure is not surprising, if one considers the relatively short polyisoprenoid chain (C₂₀) of retinol. A more rapid assay for both Ret-P-Man and Dol-P-Man synthesis from endogenous lipid acceptors involves extraction with 15 vol. of chloroform/methanol (2:1, v/v) to yield a monophasic extract. However, determinations of Ret-P-Man are rendered difficult by large amounts of mannose present in monophasic extracts, and for this reason the procedure involving the use of the biphasic extract is preferable. Whatever the extraction procedure, it is crucial to dry down the extract, immediately redissolve it in chloroform/methanol (2:1, v/v) containing carrier Ret-P and perform the t.l.c. procedure within the same day, if one wants to avoid selective breakdown of Ret-P-Man within 24 h, even in the refrigerator at 4°C. Storage at −20°C does not protect Ret-P-Man from breakdown in the presence of salts. It should also be emphasized that membrane preparations used in this work were always utilized within 1–2 weeks. Prolonged storage of the membrane preparation in liquid N₂ (2–3 months) preferentially destroyed Ret-P-Man synthesis from endogenous Ret-P compared with Dol-P-Man synthesis from endogenous Dol-P.

By using these procedures, distinctive characteristics between Ret-P-Man and Dol-P-Man synthesis are found. Thus a bivalent cation (Mn²⁺, Co²⁺ or Mg²⁺) is essential for Ret-P-Man synthesis, but not for Dol-P-Man synthesis, in the assay in the presence of bovine serum albumin. The optimal concentration for Ret-P-Man synthesis from endogenous Ret-P is higher for Mg²⁺ (10 mM) than for Co²⁺ and Mn²⁺ (5–10 mM). Triton X-100 (0.5%) completely inhibits Ret-P-Man synthesis; it also causes partial inhibition of endogenous Dol-P-Man synthesis as assayed in the bovine serum albumin system. A similar inhibitory effect of Triton was
observed at 0.4% for Dol-P-Man synthesis in the rough endoplasmic reticulum but not the smooth endoplasmic reticulum of rat liver by Nilsson et al. (1978). Similarly to Kerr & Hemming (1978), the metal-ion requirement becomes absolute in the presence of the detergent in Dol-P-Man synthesis from both endogenous and exogenous Dol-P. The optimal concentration for this synthesis shifts from 5 mM to 2.5 mM in the presence of the detergent. Similarly, optimal Ret-P-Man synthesis from exogenous Ret-P in rat liver microsomes has been consistently observed to require 2.5 mM-MnCl₂ instead of 5–10 mM, the observed maximum range for the endogenous system.

In the present paper we have shown for the first time that vitamin A deficiency specifically lowers the amount of Ret-P by 50% in the liver microsomes, and it increases the amount of Dol-P. The amount of enzyme activities responsible for Ret-P-Man and Dol-P-Man synthesis were the same as in normal liver microsomes (results not shown). It should be said that the hamsters displayed typical symptoms of vitamin A deficiency, including weight loss, closed eye lids and an un-co-ordinated walking pattern, and that their vitamin A liver stores had fallen to undetectable values. (Retinyl palmitate concentrations in matched normal hamsters were about 30 µg/g wet wt. of liver tissue.) Ret-P-Man behaved as either one or two chromatographic bands, without a predictable pattern. Therefore the total amount was always considered in the calculations of Ret-P-Man synthesis. The two bands may represent isomeric forms of Ret-P-Man, and their possible biological significance remains to be established.

The increase in the amount of Dol-P available for mannosylation appears to be at variance with our own previous findings obtained in vitro, in which a decrease in the incorporation of [14C]mannose into both Ret-P-Man and Dol-P-Man was observed in livers from severely vitamin A-deficient hamsters (De Luca et al., 1975). Apparently this discrepancy cannot be resolved on the basis of differences in precursor pool sizes in vivo, inasmuch as the relatively small difference in mannose pools (699 nmol/g wet wt. of liver in normal and 560 nmol/g in deficient tissue) could not explain the large decrease (90%) in Dol-P-Man labelling in vivo (De Luca et al., 1975). However, the relatively large accumulation of radioactive precursor (from 1.6 × 10⁵ d.p.m./g in normal to 4.9 × 10⁵ d.p.m./g of deficient liver after 30 min from intraperitoneal injection) suggests lack of utilization of the mannose as deficiency becomes more severe.

Taken together, the results obtained in vitro and in vivo suggest that the decrease in Ret-P-Man synthesis is the primary result of vitamin A deficiency, and that, in the highly organized structure of the intact cell, Ret-P depletion causes accumulation of Dol-P, possibly because less Dol-P-Man is being utilized for protein mannosylation, consistent with a converging pathway for these lipid intermediates. Such accumulation is rendered evident in the microsomal system, where membrane continuity and control is at least partially broken down. These findings, though preliminary, are consistent with a regulatory role of Ret-P pools in glycoprotein synthesis, particularly if one considers recent findings that Dol-P-P-GlcNAc₂-Me, also appears to accumulate in vitamin A-deficient rat liver (Rosso et al., 1981) and that our results suggest an increased pool of Dol-P-P-GlcNAc₂ available for mannosylation in the microsomes from vitamin A-deficient hamsters.

In addition to Dol-P, less hydrophobic mannosyl acceptors appear to accumulate in the vitamin A-deficient hamster liver. The function, if any, and the structure of these compounds (B and C) is unknown, but their accumulation becomes of relative interest if one considers that ubiquinone, a substituted benzquinone with a C₄₅ polyisoprenoid side chain, also accumulates in vitamin A-deficient rat liver (Lowe et al., 1953).

In conclusion, the present work has demonstrated unequivocally that mammalian liver contains Ret-P and that the condition of vitamin A deficiency specifically decreases the pool of Ret-P. It has also shown that a mannolipid of chromatographic characteristics similar to undecaprenyl phosphate mannose is synthesized in the endogenous system. Therefore the conclusion that at least three mannolipids are synthesized by rat liver microsomes seems warranted by these findings. The reason for the apparent accumulation of Dol-P and Dol-P-P-GlcNAc₂ in vitamin A deficiency is not clear, but it is consistent with a regulatory role of Ret-P in mannosylation of dolichyl pyrophosphate oligosaccharides.

References