FORMATION OF RETINOL [α,β-^{32}P]PYROPHOSPHATE WITH [γ-^{32}P]ATP CATALYSED BY WHOLE HOMOGENATES OF RAT THYROID

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Summary. When retinol was incubated in the presence of ATP, UDP-galactose, magnesium ions and Triton X-100, with a whole homogenate of rat thyroid as an enzyme source the formation of a retinol containing phosphate compound was observed besides retinol galactoside described before. This phosphate compound is soluble in chloroform-methanol (3:2, v/v) and can be separated by chromatography on a column prepared with butanol-washed cellulose powder. The isolated compound was retinol pyrophosphate since it contained retinol and phosphate in a molar ratio of 1:2 shown by double isotopic labelling techniques and was found to be free of galactose.

Introduction. With the exception of the activity of vitamin A in the visual process, there is little known of its general metabolic action at the molecular level (1,2). Only recently, complex saccharides containing retinol have been described (3-6). Apparently, retinol monophosphate sugars are used by various tissue cells in the biosynthesis of glycoproteins (4,6). Retinol glycosides (5) may play a metabolic role in transglucosidation reactions, although this has still to be shown. In the present communication the separation and identification of retinol pyrophosphate as revealed by double labelling techniques is reported. The physiological function of this pyrophosphate will be discussed briefly.

Materials and Methods. UDP-galactose, trans-retinol and Triton X-100 were purchased from Sigma Chemical Company, St. Louis, Mo., USA.; UDP-[^{14}C] galactose (specific activity 254 μCi/μmole) and adenosine 5'-[γ-^{32}P]triphosphate (specific activity 21.7 Ci/mmole at the date of delivery) were obtained from New England Nuclear, Boston, Mass., USA. n-Butanol was from J.T. Baker Chemical Co., Phillipsburg, N.J., USA; Whatman cellulose powder,

standard grade, from W. and R. Balston, Ltd., Maidstone, Kent, England. [6,7-^{14}C]Retinol (specific activity 47 μCi/mg), and [11,12-^{3}H]retinol acetate (specific activity 253 μCi/mg) were a kind gift of Prof. O. Wiss of Hoffman La Roche and Co., Basel, Switzerland.

The homogenate preparation from rat thyroid and the incubation method used have been described previously (5). Using double labelling techniques two different experiments have been performed during the present study:

a) [6,7-^{14}C]Retinol (0.5 μmole, 1.6 x 10^5 c.p.m.) was incubated with cold UDP-galactose. Mg^{++} ions (5 mM) and Triton X-100 (1.2% v/v) were added to the incubation mixture of a final volume of one ml. A Tris-buffered (pH 7.5) thyroidal whole homogenate was used as an enzyme source. Contrary to earlier experiments (5) [γ-^{32}P]ATP (0.5 μmole, 1.1 x 10^5 c.p.m.) has been added.

b) [11,12-^{3}H]Retinol acetate (0.5 μmole, 1.0 x 10^5 c.p.m.) was incubated with UDP-[^{14}C (U)]galactose (0.5 μmole, 3.8 x 10^5 c.p.m.) and ordinary ATP (0.5 μmoles). Enzyme source and the concentration of Mg^{++} ions and the detergent were the same as given under a).

The reaction was stopped after one hour of incubation at 37°C by mixing the incubation medium with chloroform-methanol (3:2, v/v) which served also as an extraction solvent. The separation of the reaction products was performed on a column with butanol washed Whatman cellulose powder. The first 100 fractions were eluted with aqueous butanol, as reported in detail earlier (5), the following ones with water.

Radioactivity of the ^{14}C, ^{3}H and ^{32}P-labelled compounds was determined in a Packard Tri-Carb liquid scintillation spectrometer using current techniques. When the resulting compound was double labelled, the counts have been corrected for spillover.

Results and Discussion. It has been observed earlier (3,5) that the rat

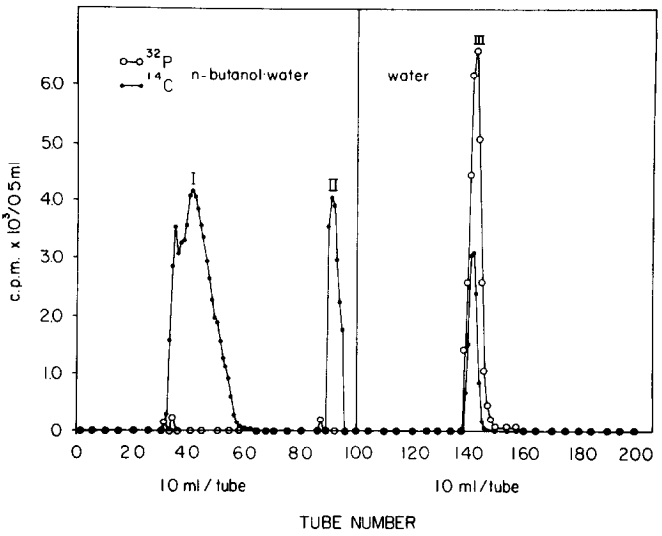
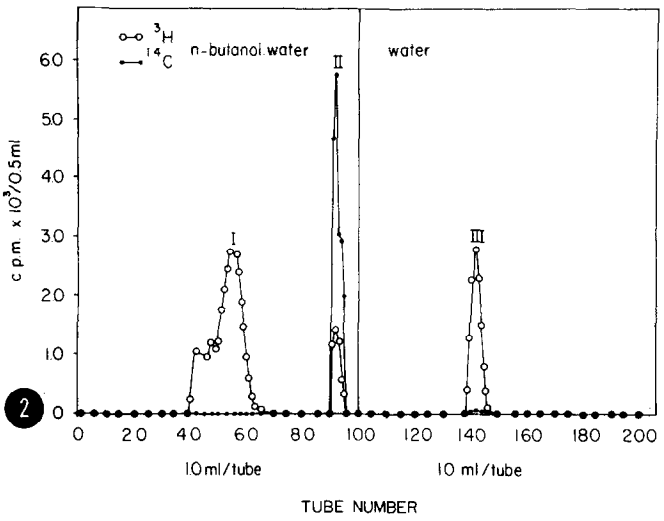


Fig. 1. Separation of [^{14}C]retinol [α,β-^{32}P]pyrophosphate (III) from [^{14}C]retinol galactoside (II) and free [^{14}C]retinol (I) by column chromatography. [^{14}C]Retinol and [γ-^{32}P]ATP were incubated with ordinary UDP-galactose in the presence of Mg^{++}, Triton X-100 and a whole homogenate of rat thyroid. Resulting reaction products were extracted with chloroform-methanol and submitted to chromatography on a cellulose powder column as described in the text.

thyroid catalyses the synthesis of retinol glycosides when homogenates of this tissue are incubated with retinol and nucleotide sugars in the presence of Mg^{++} ions, and a relatively high concentration of Triton X-100. Without addition of ATP glycosides were the only retinol containing compounds to be detected besides free, unchanged retinol (5). In the presence of ATP, unexpectedly, not retinol phosphate sugars, but the simple prenol pyrophosphate is formed as shown in Fig. 1 and 2.

The elution pattern on column chromatography shown in Fig. 1 is the result of an incubation experiment using [6,7-^{14}C]retinol and [γ-^{32}P]ATP besides cold UDP-galactose. As seen in Fig. 1 during the elution with aqueous butanol there appear two peaks of ^{14}C-radioactivity formed by free retinol (peak I)



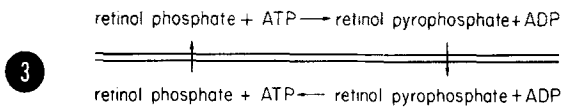


Fig. 2. Separation of [^{3}H]retinol pyrophosphate (III) from unreacted [^{3}H]retinol (I) and [^{3}H]retinol [^{14}C]galactoside (II) by chromatography after incubating [^{3}H]retinol acetate and UDP-[^{14}C]galactose in the presence of ordinary ATP. Other additions to the incubation mixture and separation the same as in figure 1.

Fig. 3. Hypothetical scheme for the function of retinol pyrophosphate as a lipid-soluble phosphate carrier.

and retinol galactoside (peak II). Continued elution of the column after the 100th fraction with water produces a third peak, not observed when ATP is absent during the incubation (5). This third peak corresponds to a compound which contains retinol and phosphate as concluded from its content of ^{14}C and ^{32}P (Fig. 1). From the isotopic content the molar concentration of the retinol moiety has been calculated to be 0.09 µmoles and that of phosphate, corrected for decay during the experiment, as 0.176 µmoles. Therefore, within the experimental error, the molar ratio of retinol and phosphate is well in the range of 1:2.

However, these results are not conclusive whether the newly formed compound contains galactose or not. Therefore, another experiment was performed using [11,12-^{3}H]retinol acetate, UDP-[^{14}C (U)]galactose and cold ATP. It was assumed that the retinol acetate would be hydrolyzed by endogenous tissue esterases. As shown in figure 2 the hydrolysis took place. Only a small fraction of unchanged retinol acetate appears in front of the free retinol (peak I) in the chromatogram. Furthermore, the results of this experiment (Fig. 2) indicate unambiguously the absence of galactose in the compound of peak III. For this reason it must be concluded that the novel compound is retinol pyrophosphate.

It seems surprising that no retinol monophosphate could be detected since it is the first product from the reaction of [γ-^{32}P]ATP with retinol (equ. 1).

$$\text{Ado-P-P-}^{32}\text{P} + \text{retinol} = \text{retinol-}^{32}\text{P} + \text{ADP} \qquad (1)$$

$$\text{Ado-P-P-}^{32}\text{P} + \text{retinol-}^{32}\text{P} = \text{retinol-}^{32}\text{P-}^{32}\text{P} + \text{ADP} \qquad (2)$$

As shown in the equations ^{32}P can have been transferred from [γ-^{32}P]ADP to the prenol only in two consecutive reactions forming in that way retinol [α,β-^{32}P]pyrophosphate (equ. 2).

The proof of the enzymatic production of retinol pyrophosphate by a mammalian tissue preparation raises the question about its metabolic use. There are in the opinion of the authors various possible metabolic functions for this compound. One function may be that of a precursor in the synthesis of retinol pyrophosphate saccharides, although this kind of complex saccharides so far has been found only in bacteria (7,8). Another possibility may consist in a co-factor function of the lipid-soluble retinol pyrophosphate in the permeation of energy-rich phosphate through lipid bilayer membranes according to the following scheme (Fig. 3). However, this hypothetical function as a lipid-soluble phosphate carrier has to be established in future investigations.

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