# URIDINE DIPHOSPHATE RHAMNOSE FROM THE GOLDEN BROWN ALGA OCHROMONAS

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In bacteria in most cases thymidine diphosphate rhamnose (TDPRh) serves as a donor for L-rhamnosyl groups (Kornfeld and Glaser 1961, Okazaki et al. 1962, Pazur et al. 1962) and the occurrence of uridine diphosphate rhamnose (UDPRh, Smith et al. 1959) seems to be the exception. In green plants L-rhamnose is a common constituent of glycosides, gums and structural polysaccharides, but little is known about the biosynthesis of rhamnosyl groups in plants. Barber (1963) found that enzyme preparations from tobacco leaves synthesize UDPRh only and that UDPRh is a better precursor than TDPRh in rutin synthesis by mung bean enzymes. This suggests that in plants rhamnosylation may proceed with UDPRh, although the nucleotide as such has not yet been found in green plants.

The present communication deals with the isolation and identification of UDP-L-Rhamnose from the chrysomonade algae Ochromonas malhamensis and Ochromonas danica after photosynthesis in $C^{14}O_2$.

Ochromonas was grown under sterile conditions in 1 liter bottles placed in a thermostat with fluorescent lamps as a light source. The complex nutrient medium from Hutner et al. (1953) was used and 1 % $CO_2$ in air bubbled through the flasks. A 4 day culture was centrifuged, washed with sterile water and transferred for additional 3 days to the same medium lacking glucose. This procedure strongly increases the photosynthetic capacity. The algae were then suspended in half concentrated nutrient solution and the density standardized

so that a 1:50 dilution at 510 mμ (1 cm cuvette) had an optical density of 0.6. The fixation experiments were performed in a small "lollipop" or with longer fixation times in a small stoppered flask. At a light intensity of 40 000 lux 0.3 - 1.5 ml of a M/100 solution of $KHC^{14}O_3$ (25 mcurie/mmole) was added to 0.5 ml suspension of Ochromonas. The algae were killed in 10 parts of hot ethanol, washed twice with 20 % ethanol and the whole extract evaporated and analyzed by two-dimensional chromatography on Whatman paper 1 with the solvents 1 and 2. The different solvent systems used for chromatography are: 1.) 88 % phenol (w:w):HOH:1M $Na_2EDTA$:acetic acid = 840:160:1:10. 2.) n-butanol:propionic acid:HOH = 750:352:498. 3.) n-butanol:pyridine:HOH:acetic acid = 60:40:30:3. 4.) ethylacetate:pyridine:HOH = 140:70:30. 5.) isobutyric acid:conc. $NH_4OH$: HOH = 57:4:39. 6.) 99 % ethanol:0.5 M ammoniumacetate pH 7.5 = 5:2. 7.) 99 % ethanol:0.5 M ammoniumacetate pH 3.8 = 5:2. 8.) isopropanol:1 % $(NH_4)_2SO_4$ = 2:1.

The phosphate ester area from a chromatogram is shown in fig. 1. In addition to the common fixation products such as PGA, sugar monophosphates and sugar diphosphates there is a large spot of polysaccharides, containing mainly chrysolaminarin, the reserve ß-1-3-glucan of the chrysophyta (Kauss and Kandler 1963). The spot marked UDPG etc, apparently is a mixture of UDPG and nucleotide-activated galactose, mannose and xylose which was shown by hydrolysis in N/100 HCl for 10 min. From the two additional unknown weak spots one was identified as uridine diphosphate N-acetylglucosamine (UDPAG) by two-dimensional cochromatography of the hydrolysis products in the solvents 1/2 and 3/4 and by cochromatography of the whole spot with authentic material (Sigma Chem. Comp.) in the solvents 5/6 and 7/8.

The other unknown substance was identified as UDPRh in the following manner. Hydrolysis with N/100 HCl for 10 min. at 100° C released all

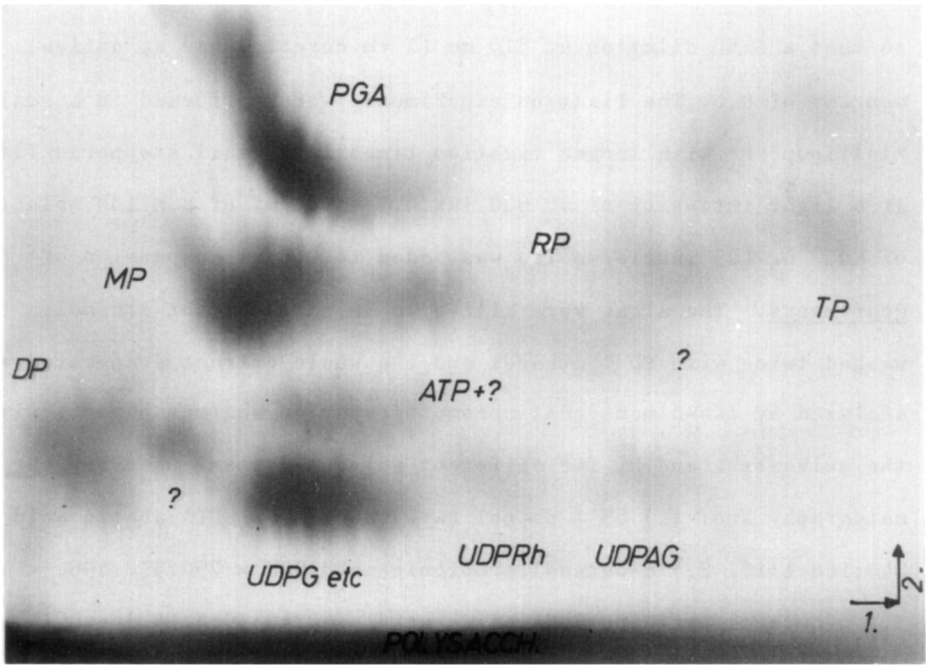


Fig. 1: Radioautogram from the phosphate-ester-area of a chromatogram in the solvents 1 (48 h) and 2 (24 h). Extract of Ochromonas malhamensis after 5 minutes photosynthesis in radioactive carbon dioxyde. DP = sugar diphosphates, MP = sugar monophosphates, PGA = phosphoglyceric acid, RMP = ribose monophosphate, TP = triose monophosphate, ATP = adenosine triphosphate, UDPG etc = uridine diphosphate glucose+ other activated sugars containing galactose and mannose, UDPAG = uridine diphosphate N-acetylglucosamine, UDPRh = uridine diphosphate-L-rhamnose, St = start.

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the rhamnose, which was identified by cochromatography in the solvents 1/3 and 4/2. This indicates the presence of a bond which is as easily hydrolyzable as a sugar pyrophosphate bond. When the unknown spot ($R_{glucose}$ = 0.35) was rechromatographed one-dimensionally in the solvent 1 a new fast-running rhamnose phosphate with $R_g$ = 0.95 appeared besides free rhamnose ($R_g$ = 1.58). From the possible rhamnose phosphates the $\alpha$-L-Rh-1-$PO_4$ (kindly supplied by G.A.Barber) has a $R_g$ value of 0.45. This suggests, that the fast running substance can only be the rhamnose-1-2-cyclic-phosphate. An analogous behaviour was observed with ADPG or UDPG rechromatographed in the same solvent system (Kauss and Kandler 1962). The formation of a 1-2-cyclic-mono-

phosphate is possible only if a nucleoside-$PO_4$-$PO_4$-sugar configuration is present in the molecule.

The L-configuration of the rhamnose in the sugar nucleotide was indicated when its reactivity to L-rhamnose isomerase from Escherichia coli B was compared with that of authentic L-rhamnose according to the procedure of Barber (1963). Under the conditions used, 23 % of the rhamnose from UDPRh and 25 % of the authentic L-rhamnose were isomerized to rhamnulose.

To identify the nucleoside, part of the activated rhamnose material was hydrolyzed in N/100 HCl for 3 hours and incubated at pH 8.0 with bull semen plasma 5'-nucleotidase (Heppel and Hillmoe 1951, simplified method). After photosynthesis experiments of 5 minutes and longer the resulting nucleoside was radioactive and ran exactly with added uridine in the solvents 1/3 and 4/2.

The UDP-L-rhamnose present in Ochromonas seems to be related to the biosynthesis of a heteropolysaccharide which is released into the nutrient medium by this alga (paper in preparation).

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