Novel Dolichyl Derivatives in Rat Spleen

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Novel dolichyl derivatives were found in rat spleen. The compounds were eluted from reverse phase HPLC after eluting dolichyl fatty acid ester. The elution profiles of the unsaponified forms of the unknown compounds were coincident with that of dolichol from spleen on reverse phase HPLC. The compounds were not dolichol dolicholate, which are present in bovine thyroid. The compounds were not found in young rats (4 months of age) but were found in old rats (above 12 months of age), and they were not detected in other tissues under our conditions.

Key words: dolichol; polisoprenoid; aging; spleen; lipids

The accumulation of dolichols (free dolichol and dolichyl fatty acid ester) has been observed in many animal tissues with aging.\textsuperscript{1-5} In particular, there are huge amounts of dolichols in several human glands.\textsuperscript{6} The latter have dolichol contents on the mg order as compared with the μg order in other tissues, although their function is not clear. On the other hand, it is well known that dolichol phosphate is associated with the biosynthesis of glycoprotein. In addition to these three types of dolichol, dolichyl dolicholate has been found in bovine thyroid.\textsuperscript{7,8} However, it has not been detected in other tissues or other animals. Recently, Sagami et al. found that yeast enzymatically synthesizes dolichol.\textsuperscript{9} In human colon carcinoma cells, it was suggested that there are not prenylated but dolichylated proteins.\textsuperscript{10} These results indicate the metabolism of dolichol, as suggested by Dallner and Chojnicki.\textsuperscript{6}

We showed previously that diets containing casein greatly increased dolichol in rat spleen, compared with diets containing soy protein.\textsuperscript{5} Rip and Carroll also showed that rat spleen had a high dolichol content.\textsuperscript{11} Thus, we have searched for novel dolichyl derivatives in rat spleen.

Four-week-old male Wistar rats were maintained on a diet containing 15% (w/w) fat and 20% (w/w) casein between 1 and 18 months of age.\textsuperscript{4,5} Rat spleens were homogenized in a cold chloroform/methanol (1:2) solution containing 0.01% butylhydroxytoluene with a Bio- mixer. Lipids were extracted as described in our previous paper.\textsuperscript{5} The crude lipids (corresponding to 0.4 g of spleen) were passed through a Sep Pak silica, and then the neutral lipid fraction was eluted with 30 mL of chloroform. Reverse phase HPLC on a Cosmosil 5C18 column (4.6 × 250 mm, Nacalai Tesque) and saponification of lipid fractions was done by the methods previously described.\textsuperscript{4,5,12} Dolichol was analyzed fluorometrically using a Cosmosil 5C18 column (4.6 × 150 mm, Nacalai Tesque) after derivatization with anthracene-9-carboxylic acid by the method of Yamada et al.\textsuperscript{13} Elution was done with ethyl acetate/acetonitrile/water (68:32:2) at the flow rate of 1.5 mL/min at 25°C. Dolichyl dolicholate was isolated by modification of Steen's method.\textsuperscript{7} Briefly, the neutral lipid fraction, which was obtained by silica column chromatography of crude lipids from bovine thyroid, was put on a Cosmosil 5C18 column (4.6 × 250 mm) kept at 55°C. Stepwise solvent programming was used such that isopropanol accounted for 60, 80, and 95% of the eluate volume at 0, 10, and 30 min, respectively.

There were great increases in dolichols in the rat tissues, especially spleens of rats fed the casein diets containing lard, with aging.\textsuperscript{4,5} We expected that there were novel dolichyl derivatives in the spleen. Thus, we spent more than two hours for the HPLC analysis of the neutral lipid fraction, though the running time of HPLC analysis for dolichol was about one hour usually. Figure 1 shows a chromatogram of the reverse phase HPLC of the neutral lipid fraction from the spleen of a 18-month-old rat. Three peaks were eluted after that of dolichyl fatty acid ester. We did not detect these peaks for 6-month-old rat spleen, or for liver, kidney, heart, or brain under the same conditions. These three unknown compounds (UK1, UK2, and UK3) were saponified. Each unsaponified compound gave almost the same elution profile as free dolichol of rat spleen on reverse phase HPLC (Fig. 2). On the other hand, retention times of these compounds roughly corresponded to these from prenol-34 to -36, respectively (Fig. 1). Steen et al. has reported that dolichyl dolicholate was present in bovine thyroid.\textsuperscript{7} Under the conditions of Fig. 1, the dolichyl dolicholate did not elute until 140 minutes (data not shown). As shown in Fig. 3, when HPLC analysis had been done on a reverse phase column at 55°C, the unknown compounds were not dolichyl dolicholate. These results indicated that the unknown compounds consisting of dolichol are novel dolichyl derivatives. The contents of dolichol in the unknown compounds (UK1 + UK2 + UK3) were estimated to be 50–80 μg/g in 18-month-old rat spleen.

Though there is the possibility that these compounds are dolichyl fatty acid esters, because an esterified docosahexaenoic acid exists in the dolichyl fatty acid ester fraction of the liver (Fig. 1), longer esterified fatty acids might not exist. Furthermore, since the new...
Fig. 1. Chromatogram of the Neutral Lipid Fractions of 18-month-old Rat Spleen and Liver. HPLC analysis on a Cosmosil 5C18 column (4.6 × 250 mm) was done as described in our previous paper. DOL, DOL-FA, and UK represent free dolichol, dolichyl fatty ester, and an unknown compound, respectively. Figure in a square is part of the elution profile of prenol 30–40.

Fig. 2. Chromatogram of Fluorescent Derivatives of Dolichol. (A); Free dolichol fraction obtained in Fig. 1 (spleen) (B); UK1 fraction obtained in Fig. 1 (spleen). The fluorescent derivatives of dolichol were analyzed by the method of Yamada et al. The numbers denote dolichol with 16–21 isoprene units.

Fig. 3. Chromatogram of Dolichyl Dolicholate and the Unknown Compounds. (A); the unknown compounds, (B); dolichyl dolicholate from bovine thyroid. HPLC analysis were done by reverse phase column maintained at 55°C as described in the text.

dolichol derivatives were not dolichyl dolicholate (Fig. 3), it is possible that dolichol is associated with glycerolipids in an esterified form. A study on this is now in progress.

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References