Short Communication

The Terminal Reactions in Polyisoprenoid Biosynthesis*

Johan Ericsson,[a] Tomas Ekström,[a] Tadeusz Chojnacki[a] and Gustav Dallner[a,b]

[a]Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm and [b]Department of Pathology, Karolinska Institutet, Huddinge Hospital, S-141 86 Huddinge, Sweden

Ericsson, J., Ekström, T., Chojnacki, T. and Dallner, G., 1988. The Terminal Reactions in Polyisoprenoid Biosynthesis. – Acta Chem. Scand., Ser. B 42: 202–205.

Polyisoprenoid compounds are broadly distributed in animal tissues and are, in fact, present in all cells and membranes.[1] The function of phosphorylated dolichol has been studied in detail, since this lipid is an obligatory intermediate in the synthesis of oligosaccharide chains bound in *N*-glycoside linkage to proteins.[2] It has been proposed that under certain circumstances the level of dolichyl-P may be rate-limiting in protein-glycosylating reactions and, thereby, an important factor during embryonic development.[3] On the other hand, the biological role of free dolichol is much less well established. In model membranes, polyisoprenols influence phospholipid fluidity, membrane stability and permeability.[4] The level of dolichol in different tissues has been found to be greatly dependent on age, and on various experimental and human pathological conditions.[5,6]

The mevalonate pathway operative in cytoplasm has been extensively studied; it results in the formation of farnesyl-PP, which is the common precursor of cholesterol, ubiquinone and polyisoprenoid compounds. The terminal part of the synthesis of the latter lipids occurs on the endoplasmic reticulum and begins with the *cis* addition of isopentenyl-PP to *trans,trans*-farnesyl-PP. Through a number of condensation reactions a polyprenyl-PP of appropriate length is obtained, but the nature of subsequent reactions is less well known. It is assumed that α-saturation followed by dephosphorylation gives rise to dolichyl-P, whereas further dephosphorylation produces dolichol. Previous studies could not, however, demonstrate a precursor–product relationship between dolichyl-P and dolichol. Consequently, the possibility was raised that the terminal reactions in the biosynthesis of these compounds are different.[7,8]

The investigations described here employed an *in vitro* system consisting of microsomes, supernatant, divalent cations, phosphate buffer, NADH and an effective ATP-generating system. A rapid rate of labeling of both the total polyisoprenol and polyisoprenyl-P fractions was obtained using [^{3}H]mevalonate as substrate (Table 1). The degree of labeling of the phosphorylated compounds was five times greater than that of the free alcohol. When incubations were performed in the presence of NADH and NADPH, the labeling in the polyprenol fraction decreased by 50 %, whereas labeling of the phosphorylated form was doubled. In the absence of reduced nucleotide no α-saturation of the free alcohol was detected, while in the presence of NADH 25 % of the total radioactivity was associated with the saturated fraction. NADPH was less effective as cofactor for this saturation process. In contrast, neither NADH nor NADPH resulted in α-saturation of the phosphorylated counterpart. These results agree with previous suggestions that synthesis and saturation of dolichol involve a concerted mechanism. The presence of reduced nucleotide increases the rate of synthesis of polyprenyl-P, which is independent of the completion of formation of the final product, since there is no

*Communication at the Meeting of the Swedish Biochemical Society in Uppsala, August 21–22, 1987.

Table 1. In vitro α-saturation of polyisoprenols using 10000 *g* supernatant and NADH and NADPH. The values are the means of 5 experiments ± SEM.

Addition	Incorporation (/dpm) of [^{3}H]mevalonate into:		α-Saturation/% of total product	
	Polyprenol	Polyprenyl-P	Polyprenol	Polyprenyl-P
None	12.539±985	24572±2390	0	0
NADH	5762±381	38866±2960	25	4
NADPH	6164±605	42445±4410	15	3

Table 2. Effect of isopentenol on incorporation of [^{3}H]mevalonate into polyprenols by the 10000 *g* supernatant fraction. The values are the means of 5 experiments ± SEM.

Addition	Radioactivity (/dpm) as a function of number of isoprenes		
	18	19	20
10 μM [^{3}H]mevalonate	2810±346	5882±616	2807±338
10 μM [^{3}H]mevalonate + 2 μM isopentenol	2375±286	5042±418	2727±291
10 μM mevalonate + 10 μM [^{3}H]isopentenol	163±18	326±37	195±22

concomitant α-saturation. In the case of polyprenol, the rate of appearance of final product is limited by the rate of α-saturation, and for this reason biosynthetic labeling of the alcohol is also limited.

The terminal condensation reaction results in chain completion and the signal for this termination has not yet been identified. We tested the possibility that isopentenol, rather than isopentenyl-PP, is added in the final step and that the lack

Table 3. Influence of various factors on the synthesis and α-saturation of polyprenols *in vitro*. The values are the means of 6 experiments ± SEM.

Additions	Incorporation of [^{3}H]mevalonate/dpm	α-Saturation/% of total
Detergents		
None	6700±494	26
Triton X-100, 0.05 %	3540±341	11
Chaps, 0.5 %	1972±226	0
β-octylglycoside, 0.5 %	1230±120	0
Deoxycholate, 0.05 %	3599±262	4
Taurodeoxycholate, 0.1 %	4044±438	0
Divalent cations		
Mg^{2+}, 5 mM	5440±522	24
Mn^{2+}, 5 mM	3122±371	22
Zn^{2+}, 5 mM	4192±402	23
Ca^{2+}, 5 mM	6290±556	22
Fatty acid		
None	4821±521	24
Palmitoyl-CoA, 50 μM	7986±882	11

of a pyrophosphate leaving group, rather than final dephosphorylation, results in termination. When *in vitro* biosynthesis starting with [^{3}H]mevalonate was performed in the presence of unlabeled isopentenol, the levels of radioactivity in the major polyprenols 18 and 19 decreased by about 10 % (Table 2). This result may be explained by dilution of the radioactivity in the final isoprene residue by the unlabeled isopentenol. The lack of decrease of radioactivity in polyprenol-20 is probably due to two factors: Firstly, this compound is present in lower amounts than are the shorter polyprenols. Secondly, during the short incubation time (20 min) two independent processes take place, i.e., complete *de novo* synthesis and completion of shorter or longer precursor polyprenyl-PP. These two processes label a variable number of isoprene residues, but the relative importance of these processes is not yet known. The above-described experiment was also performed using unlabeled mevalonate and [^{3}H]isopentenol. The appearance of limited amounts of radioactivity in the various individual polyisoprenols in this case lends further support to the hypothesis that the biosynthetic process is terminated by the addition of an isopentenol unit.

The two parallel processes involved in the termination of polyprenol synthesis differ considerably in several respects (Table 3). Incorporation of [^{3}H]mevalonate decreases moderately in the presence of Triton X-100, deoxycholate and taurodeoxycholate, and more extensively in the presence of chaps and β-octylglycoside. In contrast to the ongoing synthesis in the presence of these detergents, the saturation process is eliminated completely or almost completely. Divalent cations influenced the rate of incorporation to various extents, but did not affect α-saturation. In the presence of palmitoyl-CoA, α-saturation was decreased and this partial elimination of the parallel reaction resulted in increased total labeling of lipids with [^{3}H]mevalonate. The difference between the two terminal processes is further emphasized by the different optimal pH values for synthesis and saturation.

The properties of the α-saturase were also investigated after isolation of this enzyme from the high-speed supernatant fraction of rat liver using affinity chromatography. Successful isolation, however, required stabilization of the protein in several ways. Chromatographic removal of all supernatant lipids or addition of bovine serum albumin proved to be essential for maintenance of the α-saturase activity. In addition, the presence of sulfhydryl reducing agents was necessary. Saturase activity is preserved during the incubation assay by the ATP-generating system.

The results described support our suggested, complex mechanism for polyisoprenoid biosynthesis in the liver. *De novo* synthesis of dolichol does not appear to occur to any great extent by the dephosphorylation of dolichyl-P, but rather via a terminal condensation involving isopentenol. Condensation and saturation are strictly concerted processes which regulate the process of polyisoprenoid completion. However, these two processes are quite different in many respects. The product of the cytoplasmic α-saturase is only dolichol and not dolichyl-P. The enzyme is extremely labile, requiring stabilization for successful isolation by chromatography. Further investigation of polyisoprenoid biosynthesis using the purified α-saturase promises to be very useful in the elucidation of the biosynthetic mechanism involved.

Experimental

Livers from male Sprague-Dawley rats were homogenized in 0.1 M potassium phosphate buffer, pH 7.5, in 0.25 M sucrose containing 2 mM dithiothreitol and 2 mM EDTA. The 10 000 *g* supernatant was adjusted to a protein concentration of 60 mg ml^{-1} and stored at −20 °C. α-Saturated and α-unsaturated polyprenols were isolated by reverse- and straight-phase HPLC, as described previously.[7] Radioactivity was determined by scintillation counting.

Acknowledgement. This work was supported by the Swedish Medical Research Council.

References

1. Hemming, F. W. In: Porter, J. W. and Spurgeon, S. L., Eds., *Biosynthesis of Isoprenoid Compounds,* Wiley, New York 1983, p. 305.
2. Struck, D. K. and Lennarz, W. J. In: Lennarz, W. J., Ed., *The Biochemistry of Glycoproteins and Proteoglycans,* Plenum Press, New York 1980, pp. 35–73.

3. Rossignol, D. P., Lennarz, W. J. and Waechter, C. J. *J. Biol. Chem. 256* (1981) 10538.
4. Valtersson, C., van Duyn, G., Verkleij, A. J., Chojnacki, T., de Kruijff, B. and Dallner, G. *J. Biol. Chem. 260* (1985) 2742.
5. Pullarkat, R. K. and Reha, H. *J. Biol. Chem. 257* (1982) 5991.
6. Wolfe, L. S., Ng Ying Kin, N. M. K., Palo, J. and Haltia, M. *Lancet ii* 99 (1982).
7. Ekström, T. J., Chojnacki, T. and Dallner, G. *J. Biol. Chem. 259* (1984) 10460.
8. Ekström, T. J., Chojnacki, T. and Dallner, G. *J. Biol. Chem. 262* (1987) 4090.

Received September 15, 1987.