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The Esterification of Dolichol by Rat Liver Microsomes†

Roy W. Keenan* and Michael E. Kruczek

ABSTRACT: The incubation of 1-[^{3}H]dolichols with cell-free preparations from various rat tissues resulted in the formation of a labeled material which possessed the characteristics of synthetic dolichol palmitate. Rat liver microsomes were found to be a good source of the acyltransferase activity, and the properties of the reaction were investigated using microsomal preparations. The reaction did not require ATP, CoA, or Mg^{2+} and was stimulated by the addition of phosphatidylcholine. The esterification of dolichol appears to be similar to the esterification of retinol. The fact that the esterification of dolichol is not depressed even in the presence of a several-fold excess of retinol is evidence that the two reactions are catalyzed by different enzymes.

Dolichols are polyisoprenoid alcohols containing 16 to 22 isoprene units and possessing a saturated α residue. These compounds function in eucaryotes as intermediates in transglycosylation reactions (see review by Hemming, 1974). The richest practical source of dolichol yet discovered is pig liver which contains approximately 100 μg/g of wet weight. More than half of the dolichol in pig liver is present as the fatty acid ester, which occurs in greatest concentration in the nuclear fraction of the cell (Butterworth and Hemming, 1968). Whether the esters represent a storage form of dolichol or serve some other function is not known at this time. In the course of cell culture studies in which [^{3}H]dolichol metabolism was being investigated, we observed the production of what we assumed to be dolichol esters. Since no data are available on the manner in which dolichol esters are formed, we undertook to study this reaction.

The present paper deals with the partial characterization of the reaction product and an investigation of the properties of the enzymatic system. The results indicate that dolichol is esterified by rat liver microsomes in a reaction in which the acyl moiety may be derived from phosphatidylcholine.

Experimental Section

Materials. Pig liver dolichols, a mixture of isoprenologues containing polyprenols with 16 to 22 isoprene units, was subjected to oxidation with the chromium trioxide–pyridine complex followed by reduction with [^{3}H]$NaBH_4$ as recently described (Keenan and Kruczek, 1975) to yield 1-[^{3}H]dolichol. Reversed-phase chromatography and autoradiography revealed a series of labeled polyisoprenoids as the only significantly labeled products. The specific activity of the dolichol used for these experiments (132 μCi/μmol) was calculated by using the molecular weight of the 95-carbon polyprenol, the predominant compound in the mixture as an average molecular weight. The palmityl ester of dolichol was synthesized from [^{3}H]dolichol and palmityl chloride by the procedure of Pinter et al. (1964) and purified by alumina chromatography. Retinol, retinol esters, phytol, and sphingomyelin were obtained from Sigma Chemical Company. Phosphatidylcholine was isolated from egg yolk lipids by chromatography on alumina as described by Luthra and Shetawy (1972). Lysophosphatidylcholine was prepared by the enzymatic hydrolysis of phosphatidylcholine and was also isolated by column chromatography on alumina. Phosphatidylethanolamine was prepared by alumina chromatography of rat liver lipids. Aqueous dispersions of the phospholipids produced by sonication were added to the incubation mixtures.

Enzyme Preparations. Livers obtained from 160–180-g female Sprague-Dawley rats were homogenized with a Potter-Elvehjem type homogenizer in 3 volumes of isotonic sucrose containing 0.001 M EDTA. Nuclei and cell debris were removed by centrifuging at 600*g* for 10 min, and the supernatant was centrifuged 20 min at 22 000*g* to obtain the mitochondrial fraction. The supernatant from this run was spun at 100 000*g* for 1 h to yield a microsomal pellet. Mitochondrial and microsomal pellets were generally suspended in sufficient sucrose solution to give a concentration of 50–60 mg of protein per ml.

For the experiments in which the effect of phosphatidylcholine was demonstrated, a post-mitochondrial supernatant was fractionated on a discontinuous sucrose density gradient as described by Palamarczyk and Hemming (1975). We obtained two fractions, a pellet (fraction I), and

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fraction of density less than 1.30 M sucrose (fraction II).

Analytical and Chromatographic Methods. Phosphorus was estimated by the procedure of Bartlett (1959). Protein was determined by the biuret method using bovine serum albumin as a standard. Radioactivity measurements were made in a Beckman LS 230 liquid scintillation counter using procedures previously described (Keenan et al., 1975). Dolichol, dolichol palmitate, and the biosynthetic product were all purified by column chromatography on Merck neutral alumina adjusted to Brockmann grade III by the addition of water. The palmitate ester and biosynthesized product were both eluted with 2% ether in petroleum ether (30–60 °C), while free dolichol was eluted in 20% ether in petroleum ether.

Silica gel G plates were obtained from Analtech. Kieselguhr plates were prepared with Kieselguhr G obtained from EM Laboratories Inc. and impregnated with 2.5% paraffin oil in light petroleum ether. Solvents A, B, C, and D were chloroform–methanol–water (10:20:1), acetone–water (95:5), acetone saturated with paraffin oil, and light petroleum ether–ether–acetic acid (90:10:1). Ratios expressed are v/v ratios.

Determination of [^{3}H]Dolichol Incorporation. A typical incubation mixture consisted of 85 μmol of acetate buffer, pH 5.6, 0.092 μmol of [^{3}H]dolichol (specific activity 132 μCi/μmol) suspended in Triton X-100, and approximately 2 mg of microsomal protein in a final volume of 0.185 ml. After 1 h incubation at 38 °C, the reaction was terminated by the addition of 0.411 ml of chloroform–methanol 1:1 containing approximately 1 mg/ml of phytol. After mixing and centrifugation, aliquots of the lower layer were streaked on silica gel G and the plates were developed with solvent D. The solvent was allowed to evaporate from the plates which were then exposed to iodine vapor to reveal the location of the phytol band. Dolichol ran slightly ahead of phytol in this system with an R_f of 0.3 to 0.4, while dolichol palmitate and the biosynthetic product ran near the solvent front. The iodine was driven off by warming the plates and the areas of the plate corresponding to dolichol and its ester were scraped into scintillation vials and counted in 5.0 ml of liquifluor. The level of radioactivity found in the dolichol fractions was observed to be variable, but the radioactivity near the solvent front could be determined reproducibly and with good efficiency, probably because the reaction product is eluted from the silica gel into the scintillation solvent, while the free dolichol is at least partially bound to the adsorbant. For this reason the extent of dolichol incorporation was determined on the basis of the radioactivity appearing near the solvent front following incubation after deducting the values obtained in the appropriate zero time or boiled controls. The amount of radioactivity found in the dolichol ester band in samples from these controls was small (usually less than 5% of those in the incubated samples) and consistent. Approximately 76 000 cpm of [^{3}H]dolichol incorporation is equivalent to 1 nmol of product under these conditions.

Results

Nature of the Reaction Product. Chick embryo cell cultures grown for 48 h in the presence of [^{3}H]dolichol were found to contain only one principal radioactive component in addition to dolichol. This material which was judged to be less polar than dolichol was also synthesized from dolichol by cell-free preparations from liver and other tissues. The fact that the dolichol was labeled on carbon one limits

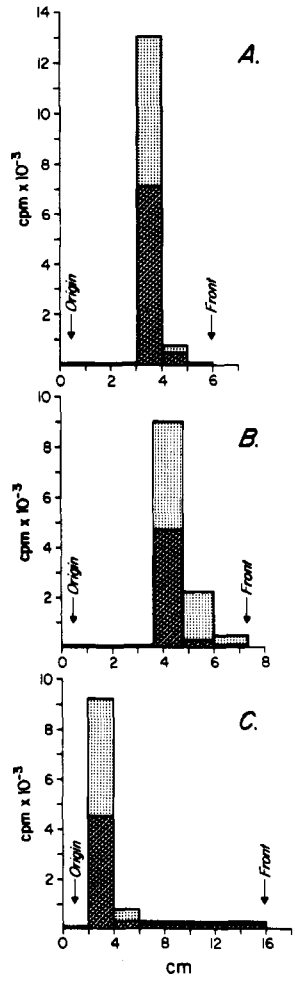

FIGURE 1: Radioactive profile of thin-layer chromatograms of [^{3}H]dolichol palmitate and the ^{3}H-labeled reaction product. [^{3}H]Dolichol palmitate and the pooled samples of ^{3}H-labeled reaction product were purified by column chromatography on alumina as described in the Experimental Section. Equal quantities of radioactivity from each sample were mixed and chromatographed in the systems shown. The shaded areas represent superimposed radioactive profiles of plates run with [^{3}H]dolichol palmitate alone under identical conditions. (A) Silica gel G plates developed with solvent A; (B) silica gel G plates developed with solvent B; (C) Kieselguhr impregnated with 2.5% paraffin oil and developed with solvent C.

the number of possible radioactive compounds which could be produced. The biosynthetic product has properties completely analogous to those of synthetic dolichol palmitate. These materials exhibit identical chromatographic properties on alumina columns and, in most of the thin-layer systems tested, the radioactive compounds both moved at or near the solvent front. Figure 1 shows that the synthetic dolichol palmitate and the reaction product co-chromatographed in three different systems. The radioactive product could be completely converted to dolichol by treatment with alkali under mild conditions but was stable to 0.1 N acid at 50 °C for 2 h. A comparison of the rates of alkaline hydrolysis of dolichol palmitate and the reaction product is given in Figure 2. Although we have not been able to obtain sufficient material to permit chemical characterization, the results discussed above constitute good evidence that this material is probably dolichol esterified to long-chain fatty acids.

Tissue and Subcellular Distribution of Acyltransferase Activity. A survey was made to determine a practical source of the enzyme system by comparing homogenates

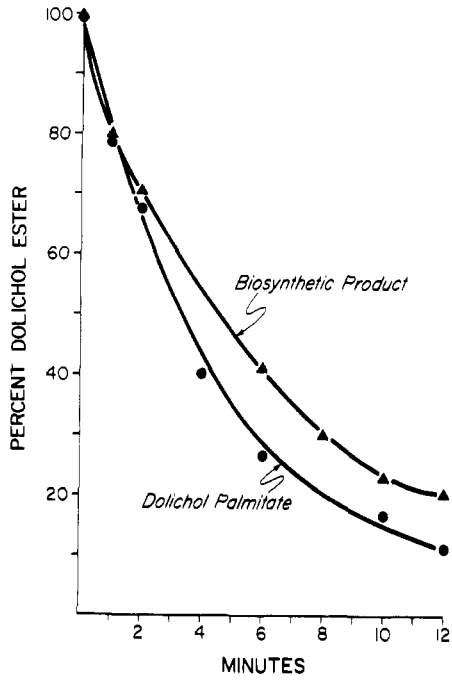

FIGURE 2: The alkaline hydrolysis of dolichol palmitate and the enzymatic product. Samples of [^{3}H]dolichol palmitate and the ^{3}H-labeled reaction product, purified as described under Figure 1, which each contained about 100 000 cpm were dissolved in 1.0 ml of tetrahydrofuran and 200 μl of 0.66 N methanolic KOH. The samples were incubated at 38 °C and aliquots withdrawn at the indicated times and chromatographed on silica gel G TLC plates in solvent D. The radioactivity in the area corresponding to the dolichol ester was determined as described in the Experimental Section.

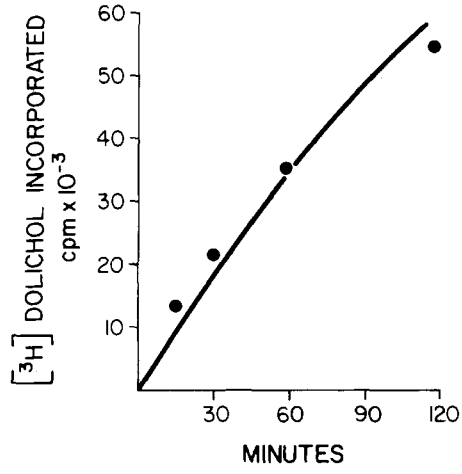

FIGURE 3: The effect of incubation time on the incorporation of [^{3}H]dolichol. [^{3}H]Dolichol was incubated with 10 mg/ml of microsomal protein for the indicated times under the conditions employed in Table II. The assay was performed as described in the Experimental Section.

from various tissues of the rat. The results of this experiment which are summarized in Table I show that liver was the most active of the tissues tested, although all the other tissues appeared to possess some activity.

Liver was fractionated into various subcellular fractions and the activities of these fractions were compared. The data presented in Table II show that the microsomal fraction possessed the highest total and specific activity. The addition of post-microsomal supernatant to the microsomal fraction caused a significant increase in activity although the post-microsomal supernatant is a relatively poor source

Table I: The Distribution of Dolichol Esterifying Activity in Various Rat Tissues.[a]

Tissue	Relative Incorporation of [^{3}H]Dolichol
Liver	1.0
Spleen	0.62
Kidney	0.56
Pancreas	0.41
Brain	0.33
Intestinal mucosa	0.28

[a] Ten percent homogenates of the tissues shown were prepared in 0.25 M sucrose containing 1 mM EDTA. One to 4 mg of protein from each homogenate was incubated 1 h with 0.024 μmol of Triton-suspended [^{3}H]dolichol in acetate buffer at pH 5.6. The assay procedure and other conditions are as described under Experimental Section. The relative values given are based on the same quantity of protein.

Table II: Subcellular Localization of Activity.[a]

Cell Fraction	[^{3}H]Dolichol Incorporated (nmol/mg of Protein)
Whole homogenate	0.31
Nuclei	0.26
Mitochondria	0.29
Microsomes	0.51
Post-microsomal supernatant	0.14
Microsomal + post-microsomal supernatant[b]	0.83

[a] Approximately 2 to 5 mg of protein from each subcellular fraction was incubated for 1 h with 0.092 μmol of Triton-suspended [^{3}H]dolichol and 85 μmol of acetate buffer, pH 5.6, in a final volume of 185 μl. Other conditions and the assay technique were as described under Experimental Section. [b] Per milligram of microsomal protein corrected for activity in supernatant fraction.

of enzymatic activity. This indicates that the post-microsomal supernatant contains substrate or cofactors which are limiting in the microsomal fraction, an observation which will be discussed later. Unless otherwise indicated, rat liver microsomes were employed as the enzyme source in all subsequent experiments.

Properties of the Microsomal Enzyme Preparations. Dolichol esterifying activity was rapidly lost at room temperature or above, and when the pH was 6 or less. If enzyme preparations were kept at neutral or slightly alkaline pH in the refrigerator or freezer, the loss of activity was gradual and the microsomes could be used up to 1 month after preparation. The reaction rate was stimulated several fold by the addition of Triton X-100 up to 1% final concentration, but about 3% Triton was inhibitory. The detergent concentration which was employed in the experiments described in this paper was 0.6%.

The effect of time on the rate of dolichol esterification was studied under the usual conditions, but the time of incubation was varied. The results in Figure 3 show the extent of dolichol incorporation is directly proportional to time for approximately 2 h. Figure 4 shows that the esterification of dolichol is also directly proportional to the concentration of microsomes over a fairly broad concentration range.

The effect of pH was determined using both acetate and Tris-maleate buffers. The results given in Figure 5 show that the enzyme has only one pH optimum in the pH range from 4 to 8.5. This is clearly different from the retinol esterifying activity in microsomes described by Futterman and Andrews (1964) who reported two distinct optima, one at 4.5 and one at 8.2. In the experiment shown in Figure 5, the

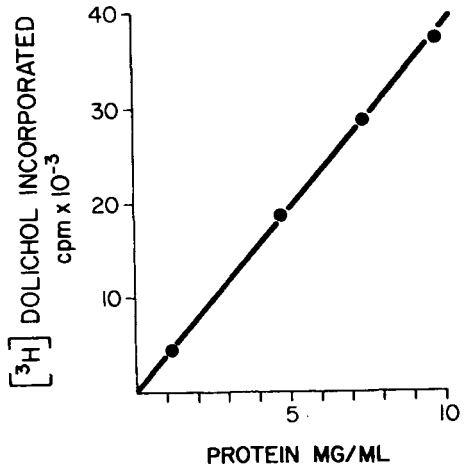

FIGURE 4: The incorporation of [^{3}H]dolichol as a function of protein concentration. The indicated amounts of microsomal protein were employed in 1-h incubations with [^{3}H]dolichol under the conditions and using the assay procedure described in the legend to Figure 3.

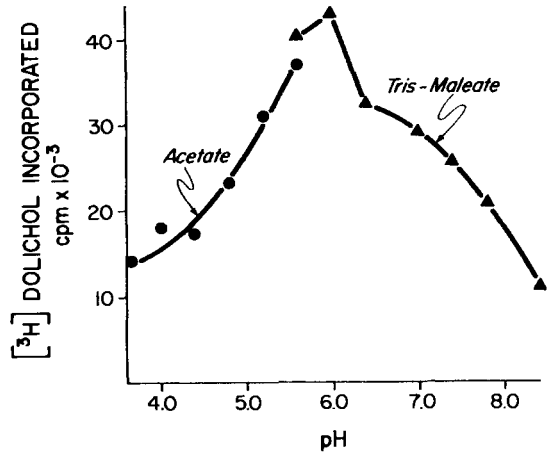

FIGURE 5: The effect of pH on the incorporation of [^{3}H]dolichol. The conditions described in the legend to Figure 3 were employed in this reaction except that the incubation was for 1 h and each tube contained 230 μmol/ml of the indicated buffer. The assay was performed as described in the Experimental Section.

highest reaction rate was obtained in Tris-maleate, pH 6, but most of our experiments were conducted using acetate buffer, pH 5.6.

The effects of ATP, coenzyme A, and Mg^{2+} on the rate of formation of dolichol ester by dialyzed liver microsomes are given in Table III. The lack of effect of these cofactors seen in this experiment is typical of the results of several other experiments. It is also apparent that the enzyme is inactivated to only a small extent by dialysis for 18 h. Palmityl coenzyme A also failed to effect the rate of dolichol esterification, providing further support for the idea that the source of the acyl group was not the coenzyme A thioester.

The influence of phosphatidylcholine on dolichol ester formation is shown in Table IV. A rat liver post-mitochondrial fraction was separated into two subfractions by density gradient centrifugation, as described in the Experimental Section. Fraction I or the pellet which consisted of material with a density greater than 1.30 M sucrose contained most of the enzymatic activity, but phosphatidylcholine had no effect on the rate of dolichol esterification. The lower density fraction (fraction II) had relatively little activity but was markedly stimulated by phosphatidylcholine as shown in Table IV. Phosphatidylethanolamine, lysophosphatidylcholine, and sphingomyelin were without effect on the reaction when tested at several concentrations in the range used with phosphatidylcholine. These results and those in Table II suggest that phosphatidylcholine may serve as an acyl donor in this system. We attempted to test this possibility with uniformly labeled phosphatidylcholine, but incorporation was too low to give significant results.

Table III: The Effect of ATP, CoA, and Mg^{2+} on [^{3}H]Dolichol Incorporation.[a]

Cofactor Added	[^{3}H]Dolichol Incorporated (nmol/mg of Protein)
None[b]	0.32
ATP	0.31
CoA	0.33
Mg^{2+}	0.28
ATP, CoA, Mg^{2+}	0.19

[a] The microsomal fraction used in the experiment described in Table II was dialyzed 18 h against 0.05 M Tris-malate buffer, pH 7.0, and incubated in the presence and absence of the cofactors shown. The concentrations used were ATP, 1 mM; CoA, 1 mM; and $MgCl_2$, 4 mM. All other conditions were as described in Table II. [b] Undialyzed enzyme incorporated 0.4 nM under these conditions.

Table IV: The Effect of Phosphatidylcholine on Dolichol Esterification by Fraction II.[a]

Phosphatidylcholine Added (μmol/ml)	[^{3}H]Dolichol Incorporated (nmol/mg of Protein)
0	0.004
1.1	0.011
2.2	0.018
4.5	0.026
7.3	0.030

[a] Each tube contained 1.2 mg of fraction II prepared as described in the Experimental Section and the quantity of phosphatidylcholine shown. All other conditions were as described in Table II.

The effect of dolichol concentration on the extent of dolichol incorporation is shown in Figure 6. The maximum rates of dolichol incorporation were obtained with dolichol concentrations of between 0.4 and 0.7 μmol/ml of incubation medium. The K_m determined for dolichol was 3×10^{-4} M. The concentration of endogenous dolichol which could be present in the microsomal enzyme preparations was calculated from the data of Gough and Hemming (1970) to be about three orders of magnitude lower than the amount of dolichol added in most of the experiments reported here and, thus, might be expected to have negligible effects on the results.

The effect of retinol on the rate of dolichol esterification was investigated because of the similar nature of the two compounds and the fact that both are apparently stored as esters in the liver. Retinol esterification has also been shown to occur in the absence of coenzyme A, presumably with a phospholipid as the acyl donor (Futterman and Andrews, 1964). The data given in Figure 7 clearly demonstrate that, even at relatively high levels, the retinol had no effect whatever on the incorporation of dolichol.

Discussion

Incubation of radioactive dolichol with cell-free preparations from various tissues resulted in the formation of ra-

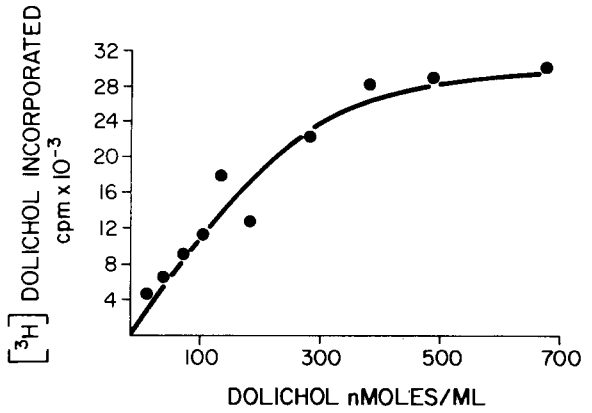

FIGURE 6: The incorporation of dolichol into dolichol ester as a function of dolichol concentration. Aliquots of an acetone solution of [^{3}H]dolichol were dried in microcentrifuge tubes under nitrogen and suspended by mixing with 60 μl of 2% Triton X-100. Acetate buffer, pH 5.6, was added, and the reaction was initiated with the addition of 1.9 mg of microsomal protein. The final volumes were 185 μl and incubation was for 1 h. All other conditions and the assay were as described in Table II.

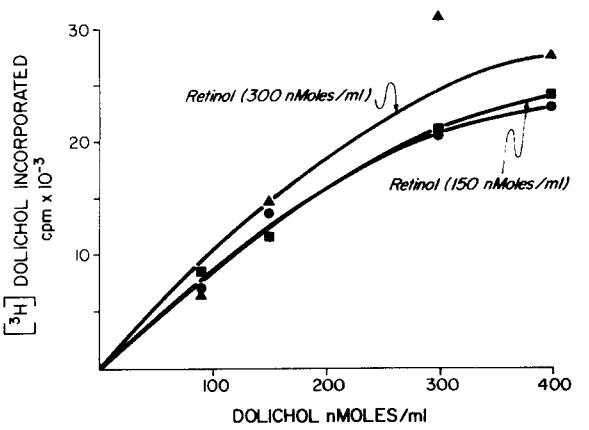

FIGURE 7: The incorporation of [^{3}H]dolichol in the presence of retinol. Aliquots of an acetone solution of [^{3}H]dolichol were dried in microcentrifuge tubes under nitrogen as described in the legend to Figure 6. In the indicated samples, an acetone solution of retinol containing either 150 or 300 μmol/ml of incubation mixture was also taken to dryness with the dolichol. All other conditions and the assay were as described in the legend to Figure 6.

dioactive products with chromatographic properties which differed from those of dolichol. Despite the fact that only microgram quantities of material have been obtained, the physical and chemical properties which have been investigated are identical with those of synthetic palmityldolichol and provide strong evidence that this material is an acyl ester of dolichol. The nature of the acyl group has not as yet been established; to the author's knowledge the only reference to the acyl groups of dolichol in the literature (Butterworth and Hemming, 1968) states that they are predominantly saturated. The fatty acids from a related polyprenol ester mixture (hexahydropolyprenyl esters) from the mold *Aspergillus fumigatus* have been shown to consist primarily of saturated fatty acids (Stone and Hemming, 1968). We are currently engaged in the determination of the acyl groups in rat liver dolichols, but the difficulties involved in the complete elimination of lipid contaminants have prevented us from obtaining unequivocal data on their fatty acid composition.

A survey of various tissues for acyl transferase activity revealed that this reaction is widespread in rat tissue, the liver being most active tissue tested. The microsomal fraction which had the highest specific activity of any subcellular fraction was used as an enzyme source to study the properties of the reaction. The microsomal esterification reaction was only moderately diminished by dialysis and appeared not to require CoA, ATP, or Mg^{2+}. Fractionation of the microsomal preparation enabled us to demonstrate that the reaction was markedly stimulated by phosphatidylcholine, but not by other phospholipids. We attempted to show the incorporation of labeled fatty acid from the uniformly labeled phosphatidylcholine, but the activity of the preparations was too low. The enzyme system appears to function in a manner analogous to that of the plasma lecithin:cholesterol transferase (Glomset, 1968) or the retinol esterifying system of liver microsomes (Futterman and Andrews, 1964).

The incorporation of dolichol into the ester by microsomes takes place at a relatively slow rate (<nmol per h per mg of protein) and could not have been detected without the use of labeled dolichol of high specific activity. Although the microsomal enzyme activity can be partially solubilized with Triton X-100, preliminary attempts to purify the enzyme have not been successful. For this reason, we cannot be sure that the enzyme is specific for dolichol. It is unlikely that the esterification of dolichol is catalyzed by the same enzyme as that responsible for cholesterol esterification since the cholesterol acyltransferase from liver requires ATP, CoA, and Mg^{2+} (Goodman et al., 1964). Although the mechanism of dolichol and retinol acylation (Futterman and Andrews, 1964) appears to be similar, there are large differences in pH optima, and our competition experiments demonstrated that retinol does not affect the acylation of dolichol even when present in excess. The microsomal fractions did acylate betulaprenol at a rate equivalent to that observed with dolichol. Betulaprenol is a mixture of isoprenologues containing 6 to 9 isoprene units with an allylic hydroxyl. Whether this represents a lack of specificity in the acyltransferase or the presence of still another polyprenol acylating system is not known.

The investigations of Butterworth and Hemming (1968) and Gough and Hemming (1970) indicate that the dolichols of rat and pig liver are the same except for some differences in the ratios of the individual isoprenologues. The only detailed information available on dolichol content and distribution has been obtained with pig liver, but probably applies to rat liver as well. Approximately 60% of pig liver dolichol is esterified and its intracellular distribution is quite different from that of retinol or cholesterol esters. The greatest portion of the dolichol esters was found to be associated with the nuclear fraction of the cell. Precisely what the biological role of these esters is, is not yet clear. They may represent a storage form of dolichol or serve some as yet undescribed purpose. The role of the enzymatic activity described in this communication is apparently to synthesize the dolichol esters.

Acknowledgments

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Preparation of Fv Fragment from the Mouse Myeloma XRPC-25 Immunoglobulin Possessing Anti-Dinitrophenyl Activity†

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ABSTRACT: The myeloma IgA protein, produced by plasmacytoma XRPC-25, was isolated by affinity chromatography on dinitrophenyllysine-Sepharose. The affinity constant of the intact protein or its Fab′ toward 2,4-dinitrophenyl-L-lysine (Dnp) was found to be 2.6×10^5 M^{-1}. In order to prepare an Fv fragment (Hochman, J., Inbar, D., and Givol, D. (1973), *Biochemistry 12,* 1130) from this protein, the heavy and light chains were separated and the light chain was digested with trypsin at pH 8.2 to yield half a light chain. This digest was reassociated with the heavy chain and the recombinant was digested with papain at pH 5.7. Fractionation of this digest on a Sephadex G-75 column and Dnp-lysine-Sepharose resulted in the isolation of an Fv fragment which possesses one binding site for Dnp-lysine ($K_a = 2.0 \times 10^5$ M^{-1}). The active Fv fragment has a molecular weight of 23 400 and is composed of two peptide chains, each having a molecular weight of approximately 12 000. The N-terminal residues of these chains are aspartic and glutamic acids, which are also N-terminal in the heavy and light chains, indicating that the Fv is composed of V_L and V_H.

The heavy (H)[1] and light (L) chains of immunoglobulins are comprised of a linear array of compact domains resulting from the folding of homology regions of approximately 110 residues each. The L and H chains have two and four such domains, respectively (Edelman, 1971; Poljak et al., 1972). It has been demonstrated that the antibody combining site is contained entirely in the Fv region which is composed of the N-terminal V_L and V_H domains (see Givol, 1975, for a review). An Fv fragment was indeed isolated from the mouse myeloma protein 315 and was shown to possess an intact combining site (Inbar et al., 1972; Hochman et al., 1973) and all the idiotypic determinants of protein 315 (Wells et al., 1973). Hence, Fv is the elementary unit of recognition and specificity in the immune system and it is desirable to develop methods for its preparation from other immunoglobulins.

The preparation of Fv from human myeloma IgM, using pepsin digestion, was reported by Kakimoto and Onou (1974). Various attempts in our laboratory to prepare Fv fragments by digesting the Fab′ fragment from mouse myeloma proteins other than protein 315 were unsuccessful. Similarly, digestion with papain, pepsin, or trypsin of the intact Ig, reduced and alkylated Ig, or molecules recombined from separated H and L chains, did not yield Fv fragment. A reasonable explanation for this may be that protein 315 has a unique type of L chain (λ2) which is not present in other mouse immunoglobulins (Schulenburg et al., 1971). This λ2 differs significantly from other L chains in its constant (C_L) portion and therefore its interaction with C_H1 may be less tight, thus rendering these domains susceptible to peptic digestion, leaving an intact Fv of protein 315.

We therefore used another approach for the preparation of Fv, based on the following considerations. Human L chain can be split into two halves (Solomon and McLaughlin, 1969; Karlsson et al., 1969; Poulsen et al., 1972) and conditions for the preferential recovery of either V_L or C_L were described (Seon et al., 1972). The preparation of V_H domain from rabbit heavy chain was also reported recently (Huston et al., 1972; Mole et al., 1975). It is conceivable that either V_L or V_H can recombine with the other chain to yield an active recombinant in which the Fv region will be protected from enzymic digestion.

We have tried this approach on the mouse myeloma IgA produced by plasmacytoma XRPC-25 which possesses anti-Dnp[2] activity. The L chain was split by trypsin and the digest was recombined with the H chain. Papain digestion of this recombinant yielded an active Fv fragment of this protein.

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[1] Nomenclature and abbreviations for immunoglobulins correspond to those recommended by the World Health Organization (1964). The proteins and their fragment are referred to as protein 25, Fv 25, or Fv 315, corresponding to the plasmacytomas that secrete these proteins.