short communication

Identification and quantification of dolichol and dolichoic acid in neuromelanin from substantia nigra of the human brain[S]

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Abstract **Neuromelanin (NM) isolated from the substantia nigra of the human brain is found to contain a series of dolichoic acids (dol-CA) containing 14–20 isoprene units. This is the first observation of dol-CA in a natural system. Using internally spiked nor-dolichol and nor-dolichoic acid standards, the concentrations of dolichol (dol) and dol-CA present in NM were determined. Remarkably, dol was only four times as abundant as dol-CA in NM. The distribution of dol-CA chains lengths in NM also differed from that of dol, suggesting that the enzyme(s) responsible for the conversion of dol to dol-CA prefer a dolichol substrate containing 19 isoprene units.**—Ward, W. C., Z. Guan, F. A. Zucca, R. G. Fariello, R. Kordestani, L. Zecca, C. R. H. Raetz, and J. D. Simon. **Identification and quantification of dolichol and dolichoic acid in neuromelanin from substantia nigra of the human brain.** ***J. Lipid Res.*** **2007.** 48: **1457–1462.**

Supplementary key words dolichyl • isoprenoid • Parkinson's disease • melanin

The potential interplay between neuromelanin (NM) and the loss of pigmented neurons in Parkinson's disease has prompted a significant effort to understand its structure and interactions. Although NM is commonly referred to as a "lysosome-related organelle", it is a complex entity with melanic components bound to metals, peptides, and lipids, the structures of which are still unknown (1–7). While there have been many studies examining the properties of the melanic pigment, less is known about the lipids and proteins present.

Dolichol (dol) reportedly accounts for 14% of the dry weight of NM pigment (2). Yet to date, the quantification of the distribution of dol present in NM has not been reported, and it is not known whether the lipid distribution in NM is the same as that in the surrounding midbrain tissue. Determining such information could be important to developing a better understanding of potential link between NM, brain aging, and Parkinson's disease.

Dolichols are polyisoprenoid α-saturated alcohols occurring in species-specific series, typically 14–22 residues in human cells. Dol are present in all cellular membranes and can accumulate in some tissues. Accumulation of dol in the brain is characteristic of ageing (8). Increased dol levels are also observed in hepatocarcinogenesis and ceroid lipofuscinosis (9). On the other hand, dol concentrations in brains of Alzheimer's disease patients are drastically decreased (8).

Roles for membrane-associated dol have been postulated; an increase in the membrane dol may alter membrane structure, fluidity, and functionality (10). The significance of the separate dol pool located in the lysomal luminal compartment remains to be determined (11). Luminal dol is believed to be a possible source of increased dol in ageing (12) and may be the source of dolichol seen in NM. A correlation between the amount of lipofuscin and the concentration of dol indicates the two may be related (13, 14).

Herein, we report the characterization by mass spectrometry of dol and its oxidized derivative, dolichoic acid (dol-CA), in NM granules from human substantia nigra (SN). The concentrations of specific dol and dol-CA molecular species in NM were determined to be within about a factor of four of each other. Only very small amounts of dol-CA were detected in total pig brain lipids, requiring that the dol-CA be separated from the more abundant dol

Abbreviations: dol, dolichol; dol-CA, dolichoic acid; LC/MS, liquid chromatography-mass spectrometry; NM, neuromelanin; SN, substantia nigra.

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by ion exchange chromatography prior to mass spectrometry. This is the first report of dol-CA as a natural product in mammalian tissues.

EXPERIMENTAL PROCEDURES

Isolation of neuromelanin and lipid extraction

This study was approved by the Institutional Review Board and performed in accordance with the ethics committee of Duke University Medical Center. NM was isolated from the SN pars compacta region of the human midbrain of neurologically normal adult individuals within 48 h after death and immediately frozen at −80°C as described previously (15, 16). The dissection of the SN from the frozen human midbrain was performed on a cold plate at −10°C, homogenized in distilled water (0.03 g/ml) in a glass-Teflon homogenizer, followed by centrifugation at 12,000 *g* for 10 min. The pellets were washed twice with 30 ml of phosphate buffer (0.05 M, pH 7.4)/g of tissue. The SN pellets were next incubated at 37°C for 3 h with 20 ml Tris buffer (0.050 M, pH 7.5) containing SDS (5 mg/ml)/g of tissue, as standard protocol for the isolation of NM granules. The suspension was centrifuged at 18,000 *g* for 20 min at 20°C. The supernatant was removed, and 20 ml/g of tissue of the previously described incubating solution containing 4 mg of proteinase K/g tissue was added. The sample was incubated for 3 h at 37°C. The NM pigment was separated by centrifugation at 18,000 *g*, and washed/centrifuged (12,000 *g*) twice with 5 ml of NaCl (9 mg/ml). The NM was then washed/centrifuged (12,000 *g*) with 5 ml of water.

After dialysis against water (to remove low molecular weight compounds and remaining salts), the NM was suspended in 1 ml of methanol, sonicated for 5 min, and centrifuged (12,000 *g*, 30 min, 20°C). The supernatant containing the lipid component was aspirated. The precipitate was resuspended in 1 ml of hexane and centrifuged identically to the methanol fraction. The solvent extracts were combined and evaporated under nitrogen.

Next, the 1.2 mg dried lipid fraction was redissolved in 200 μl chloroform. Then 25 μl was transferred and diluted to 100 μl in chloroform:dimethyl sulfoxide (DMSO) (1:1, v/v). A nor-dolichol internal standard synthesized by Avanti Polar Lipids (Alabaster, AL) with n = 13–21 (see supplementary Scheme I) was added to give a final concentration of 100 ng/μl, and 5 μl of the final mixture was injected onto a reversed-phase column for liquid chromatography-mass spectrometry (LC/MS) analysis as described below. The levels of dol species in NM granules were estimated by comparing the monoisotopic ion abundance of the naturally occurring dol with those of the nor-dol standard. A more rigorous quantitation was not pursued due to sample limitations and the lack of a stable isotope-labeled dol standard. Because there is no standard for dol-CA commercially available, the concentration of dol-CA relative to dol in NM was estimated by measuring the signal response ratio of the nor-dol-CA (Avanti Polar Lipids) (see supplementary Scheme II) to the nor-dol standards, When analyzed by LC/MS, equal weight quantities (50 ng) (about equal molar) of nor-dol and nor-dol-CA yielded $[M+Ac]^-$ to $[M-H]^-$ monoisotopic ion abundance ratio of 1.7 (see supplementary Figs. I and II for ^{1}H-NMR of nor-dol and nor-dol-CA).

Preparation of dol-CA

A 2.5 mg dol mixture (Avanti Polar Lipids) was dissolved into 1 ml acetone. Jones reagent was prepared using 6.7 ml water, 3.35 g CrO_3, and 2.9 ml H_2SO_4 (18 M). Water was added until no precipitate remained (approximately 2 ml). Next, 8 μl of Jones reagent were added drop-wise to the dol mixture. The liquid portion, including the lipids, was aspirated; the salt precipitate was rinsed twice with 100 μl acetone to ensure complete extraction of lipids, and the rinses were added to the original supernatant. The solution was neutralized by adding $NaHCO_3$ until reaching pH ~7, filtered, and centrifuged. Three ml of aqueous NaCl (saturated) were added to the solution. The lipids were then extracted three times with 3 ml portions of ethyl acetate. The organic layers were combined and dried down. Half of the final product was redissolved in 100 μl of isopropyl alcohol for LC/MS analysis.

HPLC/MS experimental conditions

LC/MS analysis was performed using a Shimadzu LC system (comprising a solvent degasser, two LC-10A pumps and a SCL-10A system controller) coupled to a QSTAR XL high-resolution quadrupole time-of-flight mass spectrometer (Applied Biosystems, Foster City, CA). The negative ion TOF mode of Q-Star XL mass spectrometer was calibrated using PPG3000 (Applied Biosystems). LC was operated at a flow rate of 200 μl/min with a linear gradient as follows: 100% A was held isocratically for 2 min and then linearly increased to 100% B over 14 min and held at 100% B for 4 min. Mobile phase A consisted of methanol/acetonitrile/aqueous 1 mM ammonium acetate (60/20/20, v/v/v). Mobile phase B consisted of 100% ethanol containing 1 mM ammonium acetate. Five μl of the lipid sample dissolved in 50/50 chloroform/DMSO were injected onto a Zorbax SB-C8 reversed-phase column (5 μm, 2.1 × 50 mm) obtained from Agilent (Palo Alto, CA). The postcolumn split diverted ~10% of the LC flow to the ESI source of the mass spectrometer.

Anion-exchange fractionation of porcine brain lipids

The anion-exchange fractionation of porcine brain lipids (Avanti Polar Lipids, Part number: 131101) was performed using an Agilent 1200 HPLC system. The DEAE column (DEAE-5PW, 10 μm, 7.5 mm × 7.5 cm) was from Sigma. The LC flow rate was 2 ml/min. Solvent A consisted of chloroform/methanol/water (2/3/1, v/v/v). Solvent B consisted of chloroform/methanol/480 mM ammonium acetate (2/3/1, v/v/v). Lipid fractionation was carried out by step-elution: after loading 100 mg porcine brain total lipids in 1.5 ml of chloroform/methanol/water (2/3/1, v/v/v) onto the DEAE column, the lipids were eluted with 16-ml steps of chloroform/methanol/aqueous ammonium acetate (2:3:1, v/v), with ammonium acetate concentrations of 0, 30, 60, 120, 240, and 480 mM successively in the aqueous component. Each fraction was then converted to a two-phase Bligh-Dyer system consisting of chloroform/methanol/water (2/2/1.8, v/v/v) by adding appropriate volumes of chloroform and water. The lower phases were dried under a stream of nitrogen. For LC/MS analysis, the dried lipids were dissolved in 100 μl chloroform, followed by adding 100 μl DMSO. Typically, 10 μl of the sample solution was injected onto a C8 reversed-phase column for LC/MS analysis as described above.

RESULTS

Figure 1A shows the total ion chromatogram of the LC-MS of the lipid extract of NM isolated from the human SN. The major lipid species, as identified by MS/MS, include phospholipids, sulfatides, sphingolipids, as well as prenol lipids, which are eluted off the reversed-phase (C8) column in later part the gradient. Figure 1B is the averaged mass spectrum showing the NM lipid

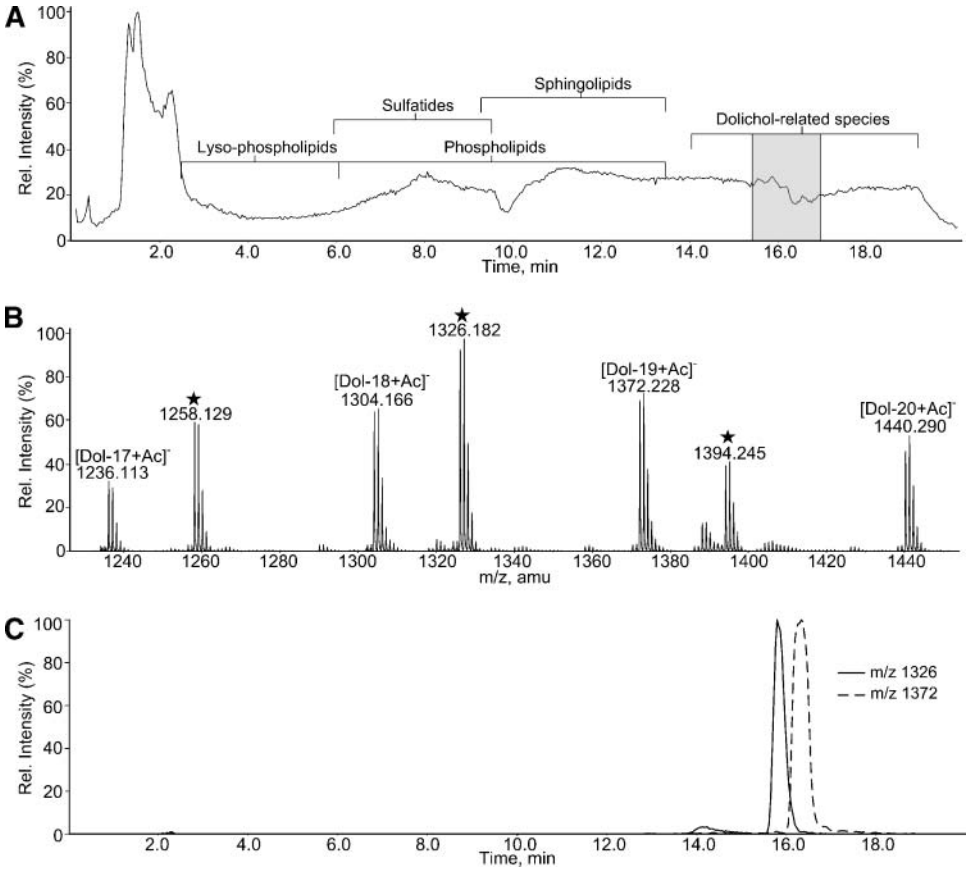


Fig. 1. LC/MS analysis of prenol lipids from human NM granules. A: The total ion chromatogram of LC/MS (negative ion mode) of the lipid extract of NM isolated from the human SN. B: Averaged mass spectrum (*m/z* 1180–1440) of the NM lipid species eluting from 15.4–16.8 min [shaded region in (A)]. The series of singly charged ions near *m/z* 1236, 1304, 1372, and 1440 correspond the acetate adduct $[M+Ac]^-$ ions of dol species with chain lengths of n = 17, 18, 19, and 20 respectively. The starred peaks near *m/z* 1258, 1326, and 1394 are unknown species, later identified as the $[M-H]^-$ ions of dol-CA species with n = 18, 19, and 20, respectively. C: Extracted ion chromatograms of peaks near *m/z* 1326 and 1372.

species with *m/z* 1180–1440 that elute from 15.4–16.8 min. The series of peaks near *m/z* 1236, 1304, 1372, and 1440 are interpreted as the acetate adducts $[M+Ac]^-$ of dol species with chain lengths of n = 17, 18, 19, and 20, respectively (n denotes the number of isoprene units). The peaks near *m/z* 1258, 1326 and 1394 represent an unknown series of polyisoprenoid derivatives, not reported in previous studies (17–19). To show their relative ion abundance, extracted ion chromatograms near *m/z* 1372 and 1326 corresponding to $[Dol\text{-}19+Ac]^-$ and the major starred peak are given in Fig. 1C. Note that the ion intensities shown in the averaged mass spectrum of Fig. 1B do not reflect the relative abundance of various dol and unknown series of polyisoprenoid derivatives.

To identify these unknown species, MS/MS was performed. **Fig. 2A** is the MS/MS spectrum of the ion near *m/z* 1258.1 from the human NM sample. Based on data from MS/MS and accurate mass measurement ($[M-H]^-$ at *m/z* 1258.129, Fig. 1B), the structure of n = 18 dol-CA was proposed for the *m/z* 1258 species (Scheme 1, calculated exact mass of the $[M-H]^-$ ion is 1258.125). To verify the proposed dol-CA structure, we synthesized dol-CA from dol as described in the Experimental Procedures. Figure 2B is the MS/MS spectrum of $[M-H]^-$ of the n = 18 dol-CA standard. Although the mass accuracies of most fragment ions are within 20 ppm, the unusually large mass error of the *m/z* 183 ion (measured at 183.093, calculated at 183.138) is intriguing and remains to be explained. Interestingly, substantiating the structural assignment of the *m/z* 183 ion is the MS/MS of the nor-dol-CA standard (Avanti Polar Lipids, refer to supplemental data) which exhibited similar error for the corresponding ion at *m/z* 169 (measured at 169.082, calculated at 169.123; see supplementary Fig. III).

The MS/MS fragmentation pattern for the naturally occurring molecule and the synthetic standard are virtually identical, confirming that the peak near *m/z* 1258 is dol-CA. Thus, the starred peaks in Fig. 1B near *m/z* 1258, 1326, and 1394 are assigned to $[M-H]^-$ of a series of dol-CA species with n = 18, 19, and 20, respectively.

Free dol and dol-CA account for approximately 12.6 and 3.1% of the dry weight of NM, respectively (data shown in **Table 1**). Table 1 also lists the absolute amount of dol and dol-CA, with n = 14–22 isoprene units, present per mg

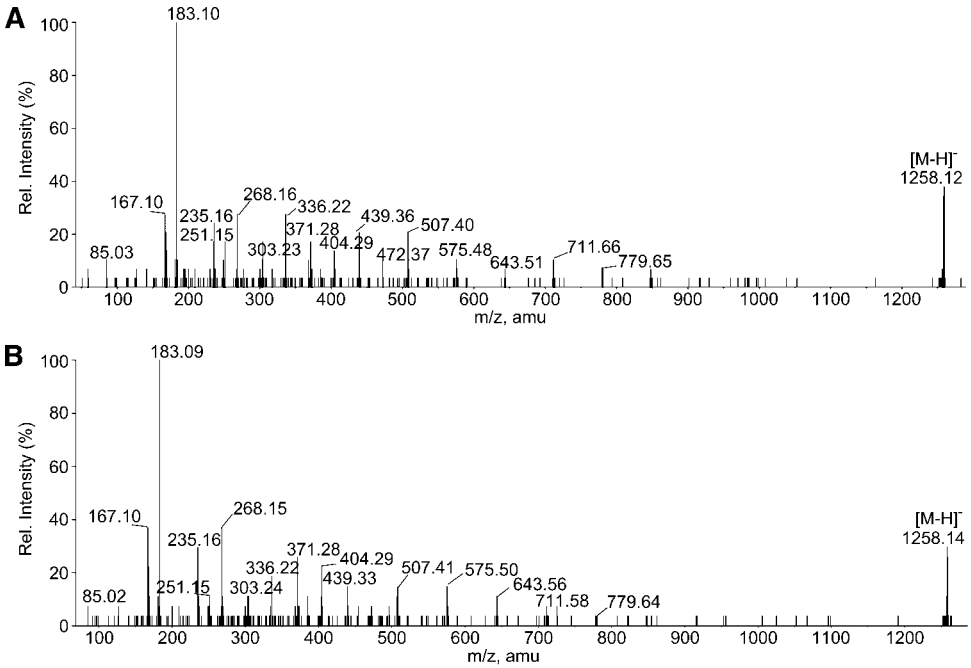


Fig. 2. MS/MS comparison of the *m/z* 1258 species from the human NM (A) with the synthetic dol-CA standard (B). The ion near *m/z* 1258 ion corresponds to $[M-H]^-$ of the n = 18 dol-CA . The proposed bond cleavages are illustrated in Scheme 1. Similarly, the *m/z* 1326 and 1394 ions in the NM sample (Figure 1) are assigned to the $[M-H]^-$ of dol-CA with n = 19 and 20, respectively.

of NM, and the percentage of each chain length present. The most prevalent dol species is n = 19.

LC/MS detection of dol-CA in porcine brain lipids

No dol-CA ion signals were observed during total lipid profiling of mouse brains by mass spectrometry (data not shown) or pig brain (**Fig. 3A**). However, it remained possible that dol-CA species might be present at very low levels in the total brain lipid extract and detection of them might require prefractionation. We therefore performed anion-exchange fractionation on 1 g of porcine brain lipids (Avanti Polar Lipids) prior to LC/MS analysis. As shown in Fig. 3C, dol-CA species are indeed detected in the lipid fraction eluted from DEAE cellulose with 30 mM ammonium acetate as the aqueous component, while free dol emerge in the DEAE cellulose run-through fractions (Fig. 3B). The presence of dol-CA molecules in the 30 mM fraction is consistent with the net negative charge (−1) associated with these species. Without DEAE cellulose prefractionation, only free dol species (n = 18–20) are detected as negative acetate adduct ions by LC/MS (Fig. 3A).

TABLE 1. Quantification of dol and dol-CA in NM from the SN of the human midbrain using nor-dol and nor-dol-CA as internal standards

Dol chain length	% dol-gray matter[a]	μg dol per mg NM	% total dol	μg dol-CA per mg NM	% total dol-CA
14	0.5	0.6	0.5	0.5	1.5
15	1.4	2	1.6	0.5	1.6
16	1.9	3.3	2.6	0.6	2
17	2.4	9.1	7.2	1.7	5.5
18	14.1	22.6	18.0	7.7	25
19	43.8	34.5	27.4	13.6	44.3
20	29.4	34.1	27.1	5.4	17.6
21	5.5	15.8	12.6	0.7	2.4
22	1	3.8	3.0	0	0
Total	100	125.8	100	30.7	100

[a] Molecular species distribution of dol from gray matter of human brain, determined by integrating HPLC-UV peak area at 215 nm (14).

DISCUSSION

Mammalian dol is a mixture of molecular species, usually consisting of 16–22 isoprene units (19). Species with 18–20 units are the most abundant. All of double the bonds, except for those derived from the farnesyl diphosphate (or in some systems the geranygeranyl diphosphate) primer, have the *cis* configuration (**Scheme 1**) (20). The prenyl transferase responsible for the elongation of farnesyl diphosphate to dol, which sequentially incorporates 13–19 isoprene units using isopentenyl diphosphate, orients its substrates differently than does the farnesyl diphosphate synthase (20). Although free dol is the predominant prenol lipid present in animal cells, dol is initially biosynthesized as the diphosphate derivative (21). The latter is then cleaved to dol-phosphate and free dol (21). However, kinases exist that can recycle dol back to dol-phosphate (21). In our experience, dol-phosphate is much less abundant in animal cells than is free dol (data not shown). Furthermore, dol-phosphate is converted to several important dol-phosphate sugar derivatives. These include dol-phosphate mannose and the dol-diphosphate oligosaccharide ($GlcNAc_2Man_9Glc_3$) used for protein *N*-glycosylation (21, 22). Like dol-phosphate itself, these

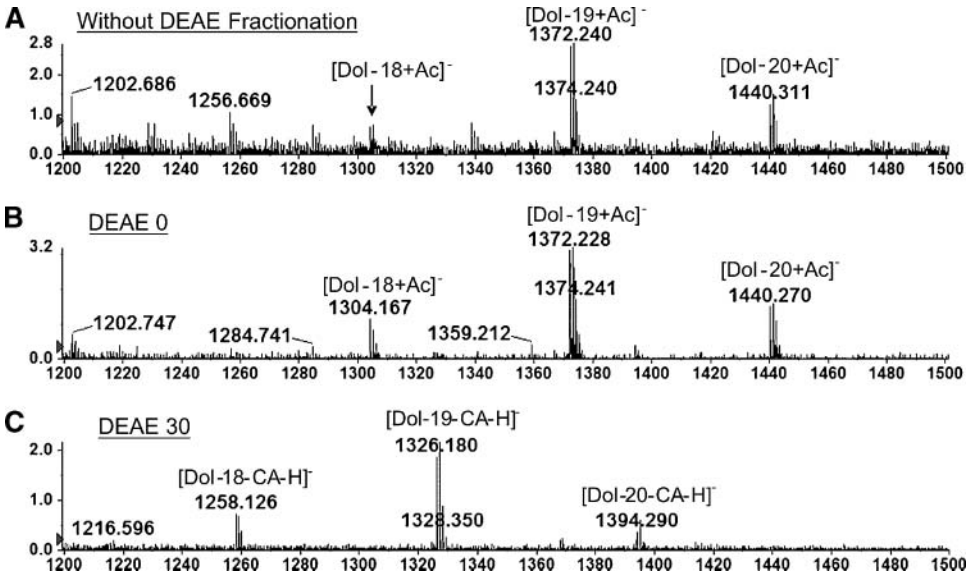
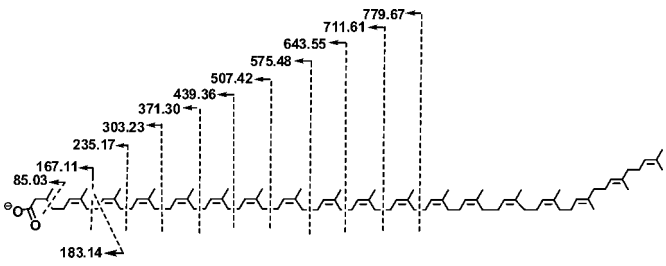


Fig. 3. Negative ion LC/MS detection of dol-CA in total porcine brain lipids after prefractionation on DEAE cellulose. A: Total lipids without DEAE cellulose prefractionation. B: Run-through fraction (0 mM NH_4Ac). C: Lipids eluting with 30 mM NH_4Ac as the aqueous component.

dol-phosphate sugar derivatives are much less abundant than free dol.

While ours is the first isolation and unequivocal analysis by mass spectrometry of dol-CA as a natural product from any tissue, this class of molecules has been partially characterized previously, using an in vitro system (23). In particular, NAD-dependent enzymatic conversion of dol to dol-CA in bovine thyroid extracts was demonstrated (23), but no evidence was presented showing the occurrence of this substance in vivo.

Based on knowledge of the catabolism of the plant isoprenoid, phytol, it may be speculated that the first step in dol catabolism is the oxidation of dol to dol-CA. The hypothesized biological degradation of dol-CA could involve the peroxisomal system for β-oxidation, ultimately yielding propionyl-CoA (24). However, incubation studies of MDCK cells (canine distal tubulus cells) with synthetic dol-CA indicated that the acid is degraded via α-oxidation, producing formic acid, which is further oxidized to CO_2 (24). Whatever the enzymatic pathway, the latter study demonstrates that there is a mechanism for the metabolism of dol-CA if it were to be produced naturally within tissues.



Scheme 1. Proposed fragmentation scheme for dol-CA (n = 18).

The distribution of free dol in NM is of great interest because it is atypical of the dol distribution in gray and white matter in the human brain (10, 14). In NM from the SN, dol 18, 19, and 20 show comparable relative abundances, but in gray matter (14) dol-19 exceeds dol-20 and dol-18 by factors of 1.5 and ~3, respectively (Table 1). In all reports of human tissues, dol-19 is the predominant species (25); thus the lipid distribution in NM seems to be shifted in favor of slightly longer lengths and a broader distribution (Table 1). Interestingly, the distribution of dol-CA species is more narrowly centered around 19 isoprene units (Table 1), suggesting that the putative dehydrogenase(s) that synthesize dol-CA from dol prefer dol-19 over the other available molecular species.

In previous studies of the gray matter of human and mouse brain, dol-CA was not observed in detectable quantities, but high-resolution mass spectrometry was not used to analyze the lipids. This and our analysis of total pig brain lipids (Fig. 3) suggests that the NM granules are greatly enriched in dol-CA and may therefore contain the enzyme(s) responsible for its biosynthesis. As shown in Fig. 3, we were able to detect dol-CA in commercial porcine brain lipids only if they were subjected to extensive prefractionation on an anion exchange resin to remove the major lipids, which probably cause signal suppression of minor components like dol-CA during mass spectrometry.

Future work remains in determining the biosynthetic pathway for dol-CA in NM and elucidating both its turnover and function within the organelle. The development of a quantitative in vitro assay for following the enzymatic conversion of dol to dol-CA in various brain subcellular fractions will be a necessary first step. After that, expression cloning of the gene(s) encoding the dol dehydrogenase(s) should enable incisive genetic and functional studies.

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REFERENCES

1. Zecca, L., P. Costi, C. Mecacci, S. Ito, M. Terreni, and S. Sonnino. 2000. Interaction of human substantia nigra neuromelanin with lipids and peptides. *J. Neurochem.* **74:** 1758–1765.
2. Fedorow, H., R. Pickford, J. M. Hook, K. L. Double, G. M. Halliday, M. Gerlach, P. Riederer, and B. Garner. 2005. Dolichol is the major lipid component of human substantia nigra neuromelanin. *J. Neurochem.* **92:** 990–995.
3. Aime, S., M. Fasano, B. Bergamasco, L. Lopiano, and G. Valente. 1994. Evidence for a glycidic-lipidic matrix in human neuromelanin, potentially responsible for the enhanced iron sequestering ability of substantia-nigra. *J. Neurochem.* **62:** 369–371.
4. Zecca, L., and H. M. Swartz. 1993. Total and paramagnetic metals in human substantia-nigra and its neuromelanin. *J. Neural Transm. Park. Dis. Dement. Sect.* **5:** 203–213.
5. Zecca, L., C. Mecacci, R. Seraglia, and E. Parati. 1992. The chemical characterization of melanin contained in substantia-nigra of human brain. *Biochim. Biophys. Acta.* **1138:** 6–10.
6. Zecca, L., T. Shima, A. Stroppolo, C. Goj, G. A. Battiston, R. Gerbasi, T. Sarna, and H. M. Swartz. 1996. Interaction of neuromelanin and iron in substantia nigra and other areas of human brain. *Neuroscience.* **73:** 407–415.
7. Tribl, F., M. Gerlach, K. Marcus, E. Asan, T. Tatschner, T. Arzberger, H. E. Meyer, G. Bringmann, and P. Riederer. 2005. "Subcellular proteomics" of neuromelanin granules isolated from the human brain. *Mol. Cell. Proteomics.* **4:** 945–957.
8. Edlund, C., M. Soderberg, and K. Kristensson. 1994. Isoprenoids in aging and neurodegeneration. *Neurochem. Int.* **25:** 35–38.
9. Ericsson, J., E. L. Appelkvist, M. Runquist, and G. Dallner. 1993. Biosynthesis of dolichol and cholesterol in rat-liver peroxisomes. *Biochimie.* **75:** 167–173.
10. Andersson, M., E. L. Appelkvist, K. Kristensson, and G. Dallner. 1987. Distribution of dolichol and dolichyl phosphate in human-brain. *J. Neurochem.* **49:** 685–691.
11. Low, P., E. Peterson, C. Edlund, U. Brunk, and E. L. Appelkvist. 1992. Nonmembrane associated dolichol in rat-liver. *Lipids.* **27:** 1–9.
12. Edlund, C., M. Soderberg, K. Kristensson, and G. Dallner. 1992. Ubiquinone, dolichol, and cholesterol metabolism in aging and Alzheimer's Disease. *Biochem. Cell Biol.* **70:** 422–428.
13. Kril, J. J., R. K. Pullarkat, P. S. Pullarkat, and C. G. Harper. 1990. Brain and liver dolichol in chronic-alcoholism - a necropsy study. *Alcohol. Clin. Exp. Res.* **14:** 528–530.
14. Pullarkat, R. K., and H. Reha. 1982. Accumulation of dolichols in brains of elderly. *J. Biol. Chem.* **257:** 5991–5993.
15. Double, K. L., D. Ben-Shachar, M. B. H. Youdim, L. Zecca, P. Riederer, and M. Gerlach. 2002. Influence of neuromelanin on oxidative pathways within the human substantia nigra. *Neurotoxicol. Teratol.* **24:** 621–628.
16. Zecca, L., R. Fariello, P. Riederer, D. Sulzer, A. Gatti, and D. Tampellini. 2002. The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease. *FEBS Lett.* **510:** 216–220.
17. Pennock, J. F., F. W. Hemming, and R. A. Morton. 1960. Dolichol: A naturally occurring isoprenoid alcohol. *Nature.* **186:** 470–472.
18. Behrens, N. H., and L. F. Leloir. 1970. Dolichol monophosphate glucose: An intermediate in glucose transfer in liver. *Proc. Natl. Acad. Sci. USA.* **66:** 153–159.
19. Chojnacki, T., and G. Dallner. 1988. The biological role of dolichol. *Biochem. J.* **251:** 1–9.
20. Poulter, C. D. 2006. Farnesyl diphosphate synthase. A paradigm for understanding structure and function relationships in E-polyprenyl diphosphate synthases. *Phytochemistry Rev.* **5:** 17–26.
21. Schenk, B., F. Fernandez, and C. J. Waechter. 2001. The ins(ide) and outs(ide) of dolichyl phosphate biosynthesis and recycling in the endoplasmic reticulum. *Glycobiology.* **11:** 61R–70R.
22. Lehle, L., S. Strahl, and W. Tanner. 2006. Protein glycosylation, conserved from yeast to man: A model organism helps elucidate congenital human diseases. *Angew. Chem. Int. Ed. Engl.* **45:** 6802–6818.
23. Vandessel, G., A. Lagrou, H. J. Hilderson, and W. Dierick. 1993. Characterization of the *in vitro* conversion of dolichol to dolichoate in bovine thyroid. *Biochim. Biophys. Acta.* **1167:** 307–315.
24. VanHoute, H. A., P. P. VanVeldhoven, G. P. Mannaerts, M. I. Baes, and P. E. Declercq. 1997. Metabolism of dolichol, dolichoic acid and nor-dolichoic acid in cultured cells. *Biochim. Biophys. Acta.* **1347:** 93–100.
25. Swiezewska, E., and W. Danikiewicz. 2005. Polyisoprenoids: Structure, biosynthesis and function. *Prog. Lipid Res.* **44:** 235–258.