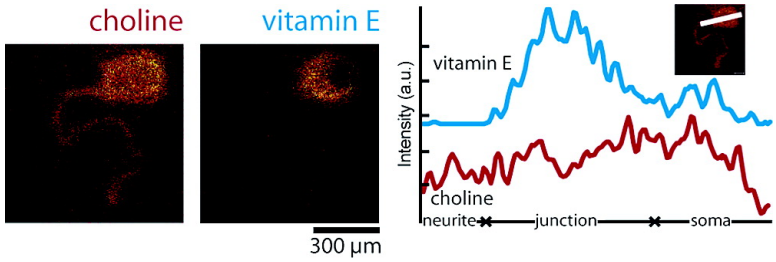
Communication

Vitamin E Imaging and Localization in the Neuronal Membrane

Eric B. Monroe, John C. Jurchen, Jinju Lee, Stanislav S. Rubakhin, and Jonathan V. Sweedler

J. Am. Chem. Soc., **2005**, 127 (35), 12152-12153• DOI: 10.1021/ja051223y • Publication Date (Web): 12 August 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 7 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 08/12/2005

Vitamin E Imaging and Localization in the Neuronal Membrane

Eric B. Monroe,[†] John C. Jurchen,[†] Jinju Lee,[‡] Stanislav S. Rubakhin,[†] and Jonathan V. Sweedler*,[†]

Department of Chemistry and the Beckman Institute, University of Illinois, 600 South Mathews Avenue, Urbana, Illinois 61801, and Center for Microanalysis of Materials, Frederick Seitz Materials Research Laboratory, 104 South Goodwin Avenue, Urbana, Illinois 61801

Received February 25, 2005; E-mail: sweedler@scs.uiuc.edu

What does vitamin E[1] do in the brain? Since its discovery in 1922, functional studies of vitamin E have focused primarily on its antioxidant properties, although more recent research has pointed to other nonantioxidant functions.[2] We utilize single cell time-of-flight secondary ion mass spectrometry[3] (ToF-SIMS) to show that vitamin E has a unique localization in neuronal membranes, suggesting important neurological functions.

Extended periods of deprivation reduce vitamin E levels below detectable levels in tissues outside the nervous system where it remains at ~5% of original levels although reduced axonal transport, slowed signal conduction, axonal dystrophy, and swelling are observed.[4] In contrast, recent evidence suggests that high doses of vitamin E increase mortality, while the biochemical reason for such effects are not known.[5] In addition, lipid oxidation, which is likely modulated by vitamin E, has been linked to several neurodegenerative disorders.[6] The addition of vitamin E to the diet of Alzheimer's patients has been shown to slow the progression of the disease.[7] Vitamin E may affect the activity of membrane-dependent enzymes by modifying the properties of the lipid membrane.[8] Despite these varied roles of vitamin E, no technique has been available to observe the subcellular localization of vitamin E in a single cell by immunohistochemical or other imaging techniques.

We use ToF-SIMS to study the subcellular localization of vitamin E in the membranes of single, isolated neurons from a widely studied neurobiological model, *Aplysia californica*. In recent years, imaging mass spectrometry has been developed as a means of understanding the localization of specific molecules.[9] ToF-SIMS has traditionally been limited to the analysis of atomic ions due to extensive fragmentation of organic molecules although gold and cluster ion sources have increased the mass range available for analysis above 2000 Da and have made the investigation of biological materials attractive. ToF-SIMS has been applied to elemental imaging of single cells[10,11] as well as molecular imaging in drug delivery systems,[12] kidney[13] and nervous tissue,[14] rat pheochromocytoma (PC12) cells,[15] leukocytes,[16] paramecia,[17] *Tetrahymena*,[18] and liposomes.[19]

Ion images corresponding to the relative distributions of specific molecules are presented in Figure 1. The ion images for choline (*m*/*z* 86) (Figure 1a) and acyl chain fragments (*m*/*z* 69) (Figure 1b) represent cellular lipids and show a uniform distribution across the cell. The ion image corresponding to vitamin E (*m*/*z* 430) exhibits localization at the junction of the cell soma and neurite when compared to other subcompartments (Figure 1c), suggesting a physiological significance of the corresponding molecule. Line scans also illustrate the increase of vitamin E at the soma−neurite junction (Figure 1d). Although slight fluctuations are observed, which are expected with molecular measurements on the micrometer scale, the choline signal remains relatively constant across the cell, while the vitamin E signal increases in the junction region.

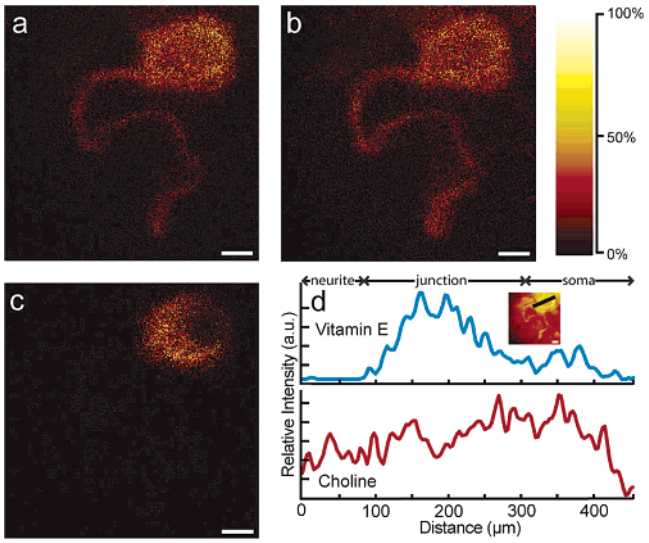


Figure 1. Ion-specific images of an isolated single neuron using a relative thermal scale, in which light areas represent a higher ion yield. (a) Cellular lipids are represented by the choline fragment (*m*/*z* 86) from sphingosine and the phosphatidylcholine lipid headgroup (0−11 counts). (b) Acyl chain fragment (*m*/*z* 69) is also indicative of cellular membranes and serves as an internal standard (0−10 counts). (c) Vitamin E (*m*/*z* 430) is localized at the soma−neurite junction as shown by the molecular ion (0−7 counts). (d) Line scans for normalized vitamin E (top) and choline (bottom) begin in the neurite and continue across the cell soma. The line scans have been normalized with the acyl chain fragment signal. Images have a pixel size of 3 μm. Scale bars are 100 μm.

The identity of vitamin E is verified by a comparison of mass spectra from cellular samples to spectra of vitamin E standard (Figure 2). The co-localization of the signal corresponding to *m*/*z* 165 with that of the molecular ion of vitamin E is observed. The peak at *m*/*z* 205 also appears to be a fragment of vitamin E. Similar ion signals have been observed but not identified as vitamin E in previous applications of SIMS to biological tissues.[14,20−22] A recent report by Piersma et al.[22] identified the molecule from which the *m*/*z* 430 signal originated in *Lymnaea* neurons as the neuropeptide, APGWamide. In the *Aplysia* neurons we present, this reported identification does not correspond to our findings based on the observed (Figure 2a) and previously reported[23] fragmentation products of this peptide.

ToF-SIMS may generate semiquantitative information when the intensity of a signal of interest is normalized to a ubiquitous ion. This ion serves as an internal standard to account for fluctuations in ionization efficiency across a sample caused by, for example, topographical effects. In this case, the lipid acyl chain fragment ($C_5H_9^+$, *m*/*z* 69), which produces a relatively constant signal across the cell membrane,[18] is used. Briefly, spectra are obtained from selected regions of interest (junction region or entire cell), the vitamin E and phosphatidylcholine signal intensities are collected

[†] University of Illinois, Urbana, Illinois 61801.
[‡] Frederick Seitz Materials Research Laboratory, Urbana, Illinois 61801.

10.1021/ja051223y CCC: $30.25

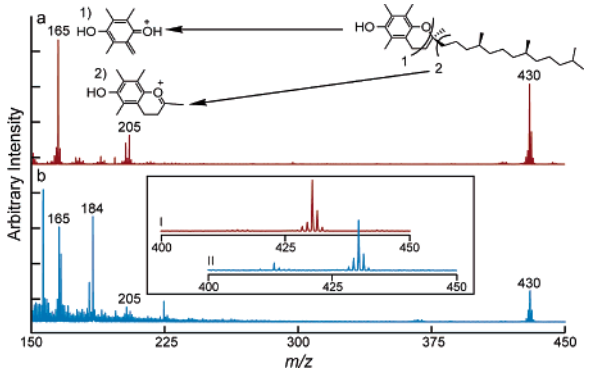


Figure 2. Comparison of mass spectra obtained from: (a) a vitamin E standard and (b) a single, isolated neuron. The spectra show similar peaks at *m*/*z* 430, 205, and 165. The proposed structures correspond to fragmentation along the corresponding line in the vitamin E molecule. The phosphatidylcholine lipid headgroup (*m*/*z* 184) is observed in the neuron sample (b). (Inset) Region of the molecular ion from (I) the vitamin E standard and (II) the neuron.

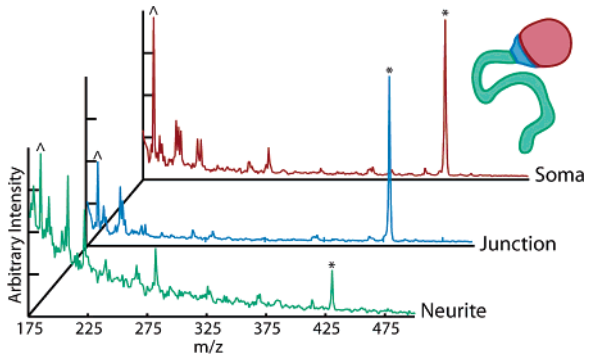


Figure 3. Vitamin E levels across neuronal membrane. Vitamin E is detected in each cellular region as expected but in varying amounts. This is apparent when the vitamin E signal (*) (*m*/*z* 430) is compared to that of phosphatidylcholine (∧) (*m*/*z* 184). Vitamin E is most abundant in the junction region and is at particularly low levels in the neurite.

and normalized by the acyl chain signal prior to comparison. The normalized vitamin E signal is significantly increased at the soma−neurite junction, while the normalized phosphatidylcholine signal remains constant. Specifically, the vitamin E:acyl fragment ratio in the soma−neurite junction is 165 ± 11% of the ratio found across the cell, a statistically significant increase ($p = 0.004$, $n = 12$). This localization is observed in all freshly isolated cells analyzed and includes neurons from several different animals and ganglia.

Surprisingly, the amount of vitamin E observed in membranes is greatly reduced in the neurite as opposed to the average for the entire cell as shown in Figure 3. A similar trend is observed in other cells, although the irregular shape and size of the neurites as well as the low levels of vitamin E detected in the neurite make a semiquantitative comparison difficult. Vitamin E is, however, readily detectable in all regions of the neuron as could be expected given its lipophilic antioxidant properties.

Vitamin E localization is not observed when samples are analyzed several days following freeze-drying, during which time a redistribution of molecules in the lipid membrane via diffusion could be expected. Similarily, vitamin E localization is not observed when cells are incubated in a vitamin E saturated physiological solution, resulting in nonspecific integration into the cellular membrane. Vitamin E begins to relocalize within hours following the replacement of a saturated culture solution with fresh physiological solution (data not shown).

The concentration of vitamin E in the junction region between the cell soma and the low levels in the neurite itself is a new finding that is consistent with several reported roles of the molecule. Perhaps most intriguing is that the cellular localization supports work that shows reduced axonal transport in cells lacking vitamin E, supporting an active functional role in transport mechanisms and/or cellular signaling of neurons.[24] Recently, vitamin E has also been shown to bind specifically to several proteins.[25] As the formation and maintenance of neuronal connections is a central aspect of memory, the role of vitamin E in this process merits further study.

Acknowledgment. We thank Dr. Donald M. Cannon for discussions. The support of P30DA018310 to the UIUC Center on Neuroproteomics on Cell to Cell Signaling and an NIAAA NRSA training grant to J.J. are gratefully acknowledged. Portions of this work were carried out in the Center for Microanalysis of Materials, UIUC, which is partially supported by the U.S. Department of Energy under Grant DEFG02-91-ER45439.

Supporting Information Available: Cell culture parameters and TRIFT III instrument parameters are available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

References

(1) The term vitamin E describes an entire family of lipid-soluble tocopherols and tocotrienols, of which α-tocopherol is the most biologically relevant and constitutes the vast majority of vitamin E found in tissues. References to vitamin E used here correspond to α-tocopherol.
(2) Brigelius-Flohé, R.; Kelly, F. J.; Salonen, J. T.; Neuzil, J.; Zingg, J.; Azzi, A. *Am. J. Clin. Nutr.* **2002**, *76*, 703−716.
(3) Belu, A. M.; Graham, D. J.; Castner, D. G. *Biomaterials* **2003**, *24*, 3635−3653.
(4) Muller, D. P. R.; Gosssampson, M. A. *Ann. N. Y. Acad. Sci.* **1989**, *570*, 146−155.
(5) Miller, E. R., III; Pastor-Barriuso, R.; Dalal, D.; Riemersma, R. A.; Appel, L. J.; Guallar, E. *Ann. Intern. Med.* **2005**, *142*, 37−46.
(6) Butterfield, D. A.; Castegna, A.; Drake, J.; Scapagnini, G.; Calabrese, V. *Nutr. Neurosci.* **2002**, *5*, 229−239.
(7) Sano, M.; Ernesto, C.; Thomas, R. G.; Klauber, M. R.; Schafer, K.; Grundman, M.; Woodbury, P.; Growdon, J.; Cotman, C. W.; Pfeiffer, E.; Schneider, L. S.; Thal, L. J. *N. Engl. J. Med.* **1997**, *336*, 1216−1222.
(8) Bradford, A.; Atkinson, J.; Fuller, N.; Rand, R. P. *J. Lipid Res.* **2003**, *44*, 1940−1945.
(9) Todd, P. J.; Schaaff, T. G.; Chaurand, P.; Caprioli, R. M. *J. Mass Spectrom.* **2001**, *36*, 355−369.
(10) Clerc, J.; Fourre, C.; Fragu, P. *Cell Biol. Int.* **1997**, *21*, 619−633.
(11) Chandra, S.; Smith, D. R.; Morrison, G. H. *Anal. Chem.* **2000**, *71*, 104A−114A.
(12) Belu, A. M.; Davies, M. C.; Newton, J. M.; Patel, N. *Anal. Chem.* **2000**, *72*, 5625−5638.
(13) Nygren, H.; Malmberg, P.; Kriegeskotte, C.; Arlinghaus, H. F. *FEBS Lett.* **2004**, *566*, 291−293.
(14) Sjovall, P.; Lausmaa, J.; Johansson, B. *Anal. Chem.* **2004**, *76*, 4271−4278.
(15) Roddy, T. P.; Cannon, D. M.; Meserole, C. A.; Winograd, N.; Ewing, A. G. *Anal. Chem.* **2002**, *74*, 4011−4019.
(16) Sjovall, P.; Lausmaa, J.; Nygren, H.; Carlsson, L.; Malmberg, P. *Anal. Chem.* **2003**, *75*, 3429−3434.
(17) Colliver, T. L.; Brummel, C. L.; Pacholski, M. L.; Swanek, F. D.; Ewing, A. G.; Winograd, N. *Anal. Chem.* **1997**, *69*, 2225−2231.
(18) Ostrowski, S. G.; Van Bell, C. T.; Winograd, N.; Ewing, A. G. *Science* **2004**, *305*, 71−73.
(19) Cannon, D. M.; Pacholski, M. L.; Winograd, N.; Ewing, A. G. *J. Am. Chem. Soc.* **2000**, *122*, 603−610.
(20) Nygrenm, H.; Malmberg, P.; Kriegeskotte, C.; Arlinghaus, H. F. *FEBS Lett.* **2004**, *566*, 291−293.
(21) Touboul, D.; Halgand, F.; Brunelle, A.; Kersting, R.; Tallarek, E.; Hagenhoff, B.; Laprevote, O. *Anal. Chem.* **2004**, *76*, 1550−1559.
(22) Altelaar, A. F. M.; van Minnen, J.; Jiménez, C. R.; Heeren, R. M. A.; Piersma, S. R. *Anal. Chem* **2005**, *77*, 735−741.
(23) Li, K. W.; Kingston, R.; Dreisewerd, K.; Jiménez, C. R.; van der Schors, R. C.; Bateman, R. H.; Geraerts, W. P. M. *Anal. Chem.* **1997**, *69*, 563−565.
(24) Rimbach, G.; Minihane, A. M.; Majewicz, J.; Fischer, A.; Pallauf, J.; Virgli, F.; Weinberg, P. D. *Proc. Nutr. Soc.* **2002**, *61*, 415−425.
(25) Kaempf-Rotzoll, D. E.; Traber, M. G.; Arai, H. *Curr. Opin. Lipidol.* **2003**, *14*, 249−254.

JA051223Y