The biological role of Vitamin E

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Vitamin E was discovered in 1922 as a necessary dietary component for healthy reproduction in rats. It is now known to consist of two families of four compounds each, known as the tocopherols and tocotrienols. All the tocopherols are well known to be very useful, fat-soluble, anti-oxidants, and together with water soluble anti-oxidants like ascorbic acid, are very common additives in the food and cosmetic industries. Despite receiving all eight types in our diets, only α-tocopherol is retained in our bodies. The unanswered question for the last 80 years has been whether α-tocopherol serves as an antioxidant in living organisms as well.

Traber and Atkinson write that ". . . all of the observations concerning the in vivo mechanism of action of α-tocopherol result from its role as a potent lipid-soluble antioxidant" [1]. On the other hand, Dr. Azzi expresses another opinion when he writes that ". . . α-tocopherol is not able, at physiological concentrations, to protect against oxidantinduced damage or prevent disease allegedly caused by oxidative damage" [2]. We are looking more closely into this diverging of opinions though a series of biophysical experiments on Vitamin E, including neutron diffraction.

We use neutron diffraction data to determine whether α-tocopherol could in fact serve as an anti-oxidant or is merely a sensor of oxidative stress. We are fortunate to have tocopherol with specific deuterium labels (Fig. 1); first, in the three hydrogen attached to the methyl group of the 5' carbon on the phenol ring in the headgroup, and second, another synthesis with deuterium on the 9 carbon of the acyl chain. We begin with simple phosphocholine lipids with mono-unsaturated acyl chains; 16:0-18:1 PC (POPC) and 18:1-18:1 PC (DOPC). By determining the location of tocopherol in a model bilayer, we might be able to answer whether it's in a position to sacrifice itself to stop lipid peroxidation events, or just simply respond to the presence of free radicals where they shouldn't be.

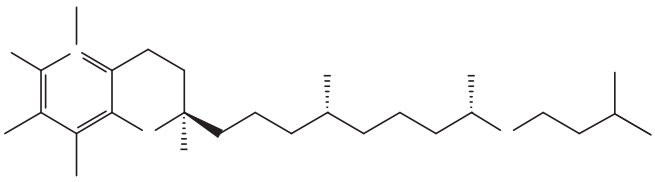

Fig 1. alpha-tocopherol, deuterium-labelled.

Neutron diffraction data was collected at the CNBC D3 beamline, using 2.37 Å wavelength neutrons from a pyrolytic graphite (PG) monochromator and PG filter. Using a sample cell specially constructed at Brock University for the purpose, samples were kept at 30°C, and hydrated at fixed humidity using saturated salt solutions of KNO_3 (90.79 ± 0.83% relative humidity (RH)), KCl (82.95 ± 0.25% RH), $NaNO_3$ (72 ± 0.32% RH), and K_2SO_4 (96.71 ± 0.38% RH), with 70, 16, 8 and 0 mol % D_2O. Note that at 8 mol % D_2O, the contribution to the total scattering intensity from the inter-bilayer water is null, which means the only contribution to the scattering arises from the lipid bilayer and tocopherol. Care was taken to prepare all samples in an oxygen-free environment.

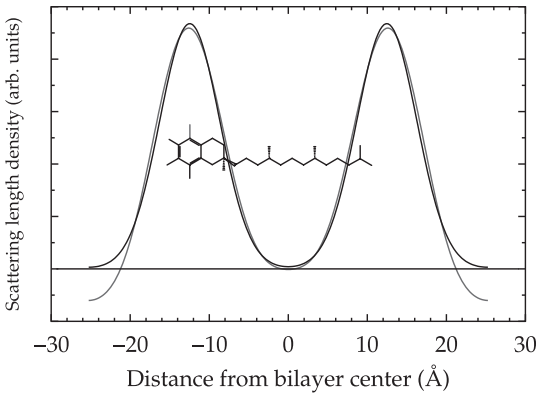

Fig 2. Mass distribution of C5 headgroup deuterium label in DOPC bilayers.

Fourier reconstruction of the scattering length density (SLD) bilayer profile followed the method outlined in Harroun et al. [3], including corrections for neutron absorption, geometry of beam and sample widths, and the Lorentz factor. The SLD profile ρ(*z*) is calculated from the Fourier transform of the structure factors, and placing the data on a "SLD per mole" basis. The difference between deuterium labeled (L) and protonated, or unlabelled (U) samples can also be calculated using the difference in the structure factors $F_h = F_h^L - F_h^U$, as long as the structure factors for the labeled and unlabelled experiments can be placed on the same relative scale. If they can, then the difference SLD profile is simply the center of mass of the isotopic/isomorphic substitution label, with all other molecular components subtracted away.

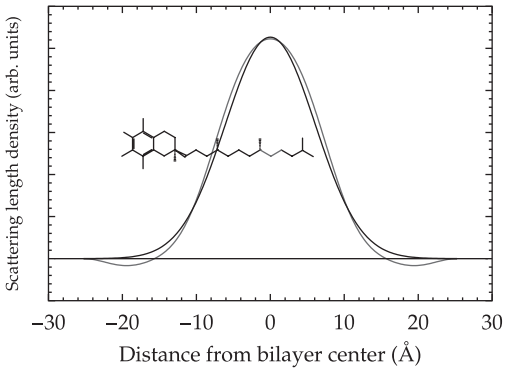

Fig 3. Mass distribution of C9 acyl chain deuterium label in DOPC bilayers. POPC bilayers are similar.

Figure 2 shows the subtracted SLD profiles of the center of mass of the deuterium label at the headgroup of tocopherol, in a DOPC bilayer. It's located ~ 16 Å from the center of the bi-

layer, and the cartoon of tocopherol in Fig. 2 shows how it sit upright in the bilayer. This orientation is confirmed in Figure 3, where the 9 carbon of the acyl chain is located in a broad region around the center of the bilayer. From the width of this distribution, the chain must enjoy a high degree of mobility and freedom, whereas the headgroup adopts a more localized position at the hydrophobic/hydrophilic interface.

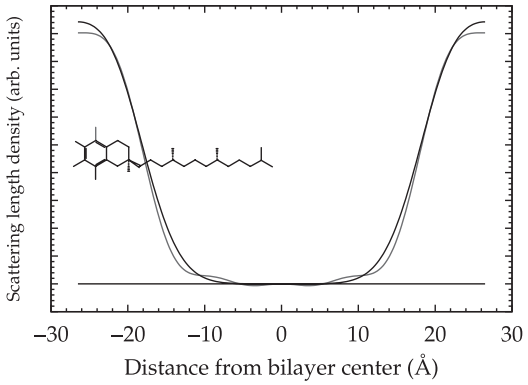

Fig 4. *Highly unexpected mass distribution of C5 headgroup deuterium label in POPC bilayers.*

Strangely, in POPC bilayers shown in Figure 4, the phenol ring of α-tocopherol sits well above the bilayer, although the acyl chain is still oriented into the lipid hydrocarbons, as determined by the C9 data (data not shown). This highly unusual position the hydroxyl group is solvated by water, and raises more questions than answers. Is this location because tocopherol should be more accessible to free radicals, rather then lipid radicals? Is this an artifact of the data analysis, especially the scaling and phasing procedures?

Scaling can be done by: 1. Equating $\rho^{L}(x)$ and $\rho^{U}(x)$ at one point, resulting in a set of simultaneous equations in the Bragg peak with scaling factors and label parameters as unknowns. 2. Equating $\rho^{L}(x)$ and $\rho^{U}(x)$ at two points, which yields an analytical result for scaling factors. 3. Scale inter-bilayer water profiles to be the same. In each case, the result shown in Fig. 4 remains the same. Clearly, more data on additional lipids is required to fully answer these questions.

References

[1] Vitamin E, Antioxidant and Nothing More. Free Radical Biology & Medicine 43 4-15 (2007).

[2] Molecular mechanism of α-tocopherol action. Free Radical Biology & Medicine 43 16-21 (2007).

[3] Biochemistry 47, 7090-7096 (2008).