ELSEVIER

Journal of Nutritional Biochemistry 14 (2003) 421–422

THE JOURNAL OF Nutritional Biochemistry

Letter to the editor

Is tocopherol associated protein a misnomer?

Dear editor:

In a recent Current Topics review in your journal [1], Dr. Porter argued that a single protein (SPF/TAP) is both a key regulator of vitamin E (tocopherol) activity, and of cholesterol biosynthesis. Understanding different biochemical activities demonstrated by the same enzyme is of high impact, and thus addressing this issue in a mini-review is indeed important. We wish, however, to alert readers to several claims and conclusions made in this review that we believe to be erroneous, and that cannot be reconciled with available information.

The history of SPF (supernatant protein factor) is, as Dr. Porter has pointed out, a long and convoluted one. Its initial discovery as a factor required for squalene monooxygenase activity [2,3] has remained incompletely understood for some years, and has been made more puzzling by the apparent inability of SPF to bind neither the substrates nor the products of the reactions it facilitates (squalene, oxido-squalene or lanosterol; [4]). The rediscovery of SPF based on its ability to bind ^{3}H-α-tocopherol (when it was re-named as tocopherol associated protein, or TAP; [5,6]) has added yet another enigmatic property to this protein, leading to the claim that it is a key regulator of vitamin E (α-tocopherol) activity in cells.

As the author noted, SPF is a member of the CRAL_TRIO protein family, members of which include the yeast PI/PC transfer protein sec14, the retinal 11-cis retinaldehyde binding protein CRALBP, and the hepatic tocopherol transfer protein, αTTP. Members of this family share a homologous substrate-binding pocket, commonly referred to as the "Sec14 domain". Upon close inspection of the literature, one finds that members of this family also share a broad substrate profile. For example, while Sec14 is believed to function mainly in transferring phosphatidylinositol, it exhibits measurable affinity toward phosphatydilcholine and β-octyl-glucoside. Similarly, CRALBP binds both the aldehyde and alcohol forms of 11-*cis* retinal. Since ligands of CRAL_TRIO proteins are all small lipids, it is not surprising that their ligand binding pockets are somewhat similar, typified by a cavity lined with hydrophobic residues. This presents a critical experimental issue: in typical binding assays, the ligand is diluted into an aqueous buffer containing the binding protein. Given the pronounced insolubility of CRAL_TRIO ligands in water, it is only expected that most **small lipids will exhibit some** affinity toward any binding pocket that can accommodate them. Thus, the fact that a certain ligand exhibits a measurable affinity toward a binding protein is, on its own, insufficient grounds for declaring it as the protein's "true" physiological ligand. We believe that this issue is at the root of some of the confusion surrounding the assignment of a cellular function(s) to SPF.

Motivated by these concerns, we recently undertook a systematic study of substrate specificity among all CRAL_TRIO proteins (TTP, SPF, Sec14 and CRALBP, [7]). If we must point at a single conclusion from these studies, it is that ligand promiscuity is a common and significant feature of this family. Thus, while all four proteins exhibited some measurable α-tocopherol binding activity, only αTTP had high-affinity toward this ligand (25 nM; Kd values for the other proteins range from 350 to 615 nM). Of further importance is that α-TTP is *specific* to α-tocopherol, binding other forms of vitamin E with 5 to 23 folds weaker affinity.

SPF, on the other hand, binds α-tocopherol much weaker (actually, SPF is weakest α-tocopherol binding protein in this family). We also find that SPF binds γ-tocopherol, with ca. two-fold **higher** affinity than α-tocopherol (268 nM vs. 615 nM, respectively). Hence, this protein shows non-selective, weak affinity toward tocopherols. In fact, SPF's affinity for γ-tocopherol is essentially identical to its affinity for phosphatidylinositol!

What, then, is the "true" ligand of SPF? Clearly, more experiments are required before the physiological ligand(s) of SPF is/are identified, and critically important will be analyses of metabolite pools in SPF "knock-out" animals. Based on presently available data, we see no basis for assigning SPF a central role in tocopherol biology, as proposed in Dr. Porter's review.

If there is a lesson in this example from this small corner of lipid biochemistry, it is that the conjecture of cause and effect in complex systems can easily be misleading. While vague and lacking in panache, the name "supernatant protein factor" remains brutally honest.

Sincerely,

D. Manor,[a]
J. Atkinson[b]
[a]*Division of Nutritional Sciences*
Cornell University
Ithaca, NY 14853, USA
[b]*Dept. of Chemistry*
Brock University
St. Catharines
Ontario, Canada L2S 3A1

doi:10.1016/S0955-2863(03)00073-1

References

[1] Porter TD. Supernatant protein factor and tocopherol-associated protein: an unexpected link between cholesterol synthesis and vitamin E (review). J Nutr Biochem 2003;14:3–6.

[2] Yamamoto S, Bloch K. Studies on squalene epoxidase of rat liver. J Biol Chem 1970;245:1670–4.

[3] Tai HH, Bloch K. Squalene epoxidase of rat liver. J Biol Chem 1972;247:3767–73.

[4] Ferguson JB, Bloch K. Purification and properties of a soluble protein activator of rat liver squalene epoxidase. J Biol Chem 1977;252:5381–5.

[5] Stocker A, Zimmer S, Spycher SE, Azzi A. Identification of a novel cytosolic tocopherol-binding protein: structure, specificity, and tissue distribution. IUBMB Life 1999;48:49–55.

[6] Zimmer S, et al. A novel human tocopherol-associated protein: cloning, in vitro expression, and characterization. J Biol Chem 2000;275:25672–80.

[7] Panagabko, C, et al. Ligand specificity in the CRAL-TRIO protein family. Biochemistry 2003;42:6467–74.