Supplementary Material

3-Hydroxyacyl-CoA and Alcohol Dehydrogenase Activities of Mitochondrial Type 10 17β-Hydroxysteroid Dehydrogenase in Neurodegeneration Study

Supplementary Material 1. Abstract of SCHAD report [*Biochem. J.* **345**, 139-143, 2000]

The alcohol dehydrogenase (ADH) activity of human short-chain l-3-hydroxyacyl-CoA dehydrogenase (SCHAD) has been characterized kinetically. The k(cat) of the purified enzyme was estimated to be 2. 2 min(-1), with apparent K(m) values of 280 mM and 22 microM for 2-propanol and NAD(+), respectively. The k(cat) of the ADH activity was three orders of magnitude less than the l-3-hydroxyacyl-CoA dehydrogenase activity but was comparable with that of the enzyme's hydroxysteroid dehydrogenase (HSD) activity for oxidizing 17beta-estradiol [He, Merz, Mehta, Schulz and Yang (1999) *J. Biol. Chem*. 274, 15014-15019]. However, the k(cat) values of intrinsic ADH and HSD activities of human SCHAD were found to be two orders of magnitude less than those reported for endoplasmic-reticulum-associated amyloid beta-peptide-binding protein (ERAB) [Yan, Shi, Zhu, Fu, Zhu, Zhu, Gibson, Stern, Collison, Al-Mohanna *et al*. (1999) *J. Biol. Chem*. 274, 2145-2156]. Since human SCHAD and ERAB apparently possess identical amino acid sequences, their catalytic properties should be identical. The recombinant SCHAD has been confirmed to be the right gene product and not a mutant variant. Steady-state kinetic measurements and quantitative analyses reveal that assay conditions such as pH and concentrations of coenzyme and substrate do not account for the kinetic differences reported for ERAB and SCHAD. Rather problematic experimental procedures appear to be responsible for the unrealistically high catalytic rate constants of ERAB. Eliminating the confusion surrounding the catalytic properties of this important multifunctional enzyme paves the way for exploring its role(s) in the pathogenesis of Alzheimer's disease.

Supplementary Material 2. Communications with the *JBC* Editorial Board.

The *JBC* editorial board ignored any criticism of the magical ABAD/ERAB story and has refused to make corrigenda by unbelievable reasons. For example, the published data of the oxidation of (-)-2-octanol catalyzed by ERAB/ABAD were demonstrated here to be unreliable. However, some members of the *JBC* editorial board had orally claimed that such experiments were performed in their labs frequently. A formal *JBC* reply in writing was also found to be based upon incredible reasons. For example, it claimed that 'the k_{cat}/K_m derived from the *initial slope of Fig. 2B* is probably reasonable enough not to challenge the overall conclusions. As a matter of the fact, the referred 'Fig. 2B' of the *JBC* article [34] was, indeed, the v versus [S] curve of the nonreproducible experiments (see Fig. 4). Even if someone could reproduce those reported experiments in the unknown future, who knows what is meant by 'the initial slope' of a v versus [S] curve? Furthermore, the *one-site* competitive inhibition (see Fig. 6) equation '*or some derivative*' shown in that *JBC* official letter is also meaningless. The left side, v/V_{max}, was found not equal to the right side. Apparently, it cannot illustrate competitive, noncompetitive, and/or uncompetitive enzyme inhibition.

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F. PETER GUENGERICH
Deputy Editor

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Dear Dr. Yang:

Dr. Gierasch and I received your message of 21 February, following up my response of 17 February.

We do not plan to take any action on this paper, in that we are unable to define any manipulation of data, which is what Dr. Sakabe and I are charged to evaluate in submitted papers. As I indicated, we now have a "Letter to the Editor" mechanism for criticisms of recently published articles but this has a time limit of six months after publication.

With regard to the experimental design and interpretation of results, you have already made your point in the J. Steroid Biochem. Mol. Biol. review you published.

I gave you some information about solubility of hydrophobic substances in my response, but I do not believe that I need to try to repeat the Yan et al. experiments myself. I could elaborate on work we have done in other systems but I doubt if you would consider it relevant anyway. In reading the paper, I would conclude that the k_{cat}/K_m derived from the initial slope of Fig. 2B is probably reasonable enough not to challenge the overall conclusions. I am not sure how much of a factor this is in the overall conclusions.

In Segel's book in the context of the paper, I would only conclude that the standard expression for competitive inhibition has been applied:

$$(v/V_{max}) = S/[K_s (1 + (I/K_i)) = S]$$ or some derivative.

As I indicated before and earlier in this message, we review the experimental design and interpretation of results in our initial review of papers. With regard to issues of manipulation of published data, we inspect some submitted papers and reserve the right to review and correct/retract any published paper. This one does not fall into that category.

We appreciate your interest in The Journal of Biological Chemistry, although you may not be satisfied with our decision not to pursue this 18-year-old paper. However, I have tried to explain our policy and I hope that you will understand it. We will not take any action. The file is closed.

Sincerely yours,

F. Peter Guengerich, Ph. D.
Deputy Editor

cc: Dr. K. Sakabe
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