Expression of concern

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Expression of concern

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We would like to highlight the following concerning issues in the paper by Gu et al.:

1. Conformation of the ferulate ligand
The conformation of ferulate as modelled by the authors is not realistic. In fact, considering as a reference the crystal structure of ferulate retrieved from the Cambridge Structural Database (code GASVOL; Nethaji & Pattabhi, 1988), we find unacceptable features around the insaturation within the alkyl chain (bond angle C1-C7-C8: 101.9º [mean CSD value:127º ± 2º]; bond angle C7-C8-C9: 141.9º [mean CSD value:123º ± 3º]; torsion C8-C7-C1-C6 54º [CSD: distributed around 0º or 180º]), and also within the methoxy moiety (bond angle C10-O3-C3: 128.3º [mean CSD value:118º ± 2º]).

2. Ligand electron density
The unbiased electron density (calculated by omitting the ligand) of the FAD:ferulic acid complex (3NX2) deposited in the PDB does not convincingly fit the ferulate ligand. In particular, the difference electron density does not support the ligand fitted by the authors. Rather, a tetrahedral structure is clearly visible at the end pointing towards Tyr27. Since HEPES has been present in the reported crystallisation conditions, we have fitted a HEPES molecule into the ligand density with the buffer molecule's sulfonyle group positioned next to Tyr27. We conclude that the ligand should be HEPES rather than ferulate, because:
   (i) Visually, HEPES fits the density better than ferulate
   (ii) Preliminary refinement of the complex structure FAD:HEPES yields similar R/Rfree values as for the reported FAD:ferulic acid complex
   (iii) Atoms C9, O1 and O2 of the ligand in the FAD:ferulate structure show significantly lower B-factors than the other ligand atoms; additionally, positive difference density is observed in this area. This suggests more/heavier atoms in the C9/O1/O2 region. In contrast, the B-factors of the ligand in the refined FAD:HEPES structure show no significant outliers and the sulfonyle group of HEPES does not give rise to significant difference electron density.

3. Proposed mechanism
In the absence of ferulate in the ligand binding site, there is no proof of the mechanism proposed by the authors in Figure 6. The suggested mechanism is entirely speculative. Moreover, the enzymatic activity observed for the FAD mutant E134A (around 40% of the wild type), which involves the removal of the proposed catalytically essential E134 amino acid side chain, is inconsistent with the proposed mechanism, unless of course an ancillary mechanism is present.

4. There are concerns regarding the mechanism reported in Figure 6
The statement of the authors claiming that “However, the precise catalytic mechanism of FADase remains largely unknown...” should be revised to a great extent considering that the mechanism proposed in Figure 6 of the current study shows identical chemical steps to the one previously proposed by Hashidoko and Takara (1998), which is not included in the references of the paper, and also by Rodriguez et al. (2010) (Figure 8). In this regard, it is a cause of concern that a print error present in the latter figure (a missing a single bond between carbon atoms C7 and C8 of the p-quinone methide intermediate) also occurs in Figure 6 of the paper by Gu et al.
References


