LETTER

Ambiguous evidence for assigning DddQ as a dimethylsulfoniopropionate lyase and oceanic dimethylsulfide producer

Li et al. (1) describe a structure and a mechanism for DddQ, an enzyme identified in marine microbial genomes, and putatively annotated as a dimethylsulfoniopropionate (DMSP) lyase (2). We surmise, however, that the presented data are insufficient to support its identification as a marine microbial DMSP lyase, let alone the claim for novel insights regarding the bacterial cleavage of oceanic DMSP.

Key data missing in Li et al. (1) are kinetic parameters. The initial identification of DddQ was based on activity in crude *Escherichia coli* lysates in which the explored DddQ variants was overexpressed (2). Even a modest estimate gives a catalytic efficiency that falls much shorter than expected for an enzyme acting on its natural substrate ($k_{cat}/K_M \sim 20\ M^{-1} \cdot s^{-1}$) (Table 1). The average k_{cat}/K_M from all published parameters in Brenda is $10^5\ M^{-1} \cdot s^{-1}$ (www.brenda-enzymes.org). Enzymatic DMSP conversion to DMS, being a simple concerted elimination, is expected to be well within this range (3). The only kinetic data provided by Li et al. (1) regards relative activities. The assay conditions suggest very low activity, $k_{cat}/K_M < 200\ M^{-1} \cdot s^{-1}$ (Table 1): this while using a crystallographic-grade protein preparation and optimal conditions, as far as this work identified.

Catalytic promiscuity is widely recognized; nearly every enzyme exhibits latent, weak promiscuous activities. These activities may be completely coincidental or may indicate the native activity of related family members (other DddQs may be parologs, rather than orthologs, given the limited sequence identity). Accordingly, annotations of enzyme families by what eventually turned out to be merely a promiscuous activity are common (4). A Dali search for structural homology identified cysteine dioxygenase (PDB code: 3EQE) as structural homolog of DddQ. Indeed, as indicated by Li et al, the metal ligands in DddQ are highly similar to non-heme oxygenases (reference 26 in ref. 1). Furthermore, active-site tyrosine similar to Y131 in DddQ interacts with the catalytic iron of cysteine dioxygenase, although its location in the DddQ cupin-fold differs. Despite these obvious similarities, iron was not included in the metals tested with DddQ (figure 1*D* in ref. 1). Thus, there is the possibility that the observed DMSP lyase activity of DddQ is merely promiscuous, and the native substrate and activity remain unknown. Similar reservations explicitly expressed by the original discoverers of DddQ (2), and by others (5), cannot be ruled out at this stage.

Another major concern regards the structural models in Li et al. (1). The assignment of a 2-(*N*-morpholino) ethanesulfonic acid (Mes) is questionable. Foremost, the sulfonate moiety should be tetrahedral, and as such would not fit the observed density (Fig. 1). The resolution of the Y131A mutant in which DMSP was assigned is low, 2.7 Å. In fact, both structures may carry the same ligand in their active sites that, in our view, may be neither Mes nor DMSP.

Finally, Li et al. (1) provide no data supporting the claim that *Ruegeria lacuscaerulensis* ITI_1157 DddQ mediates oceanic DMS release, or even that DddQ mediates DMS production in its original species, *Roseovarius nubinhibens* ISM or *Ruegeria pomeroyi* DSS-3 (2). The latter is of importance given the limited sequence identity (44.5% and 34.5%, respectively) with the originally identified DddQ.

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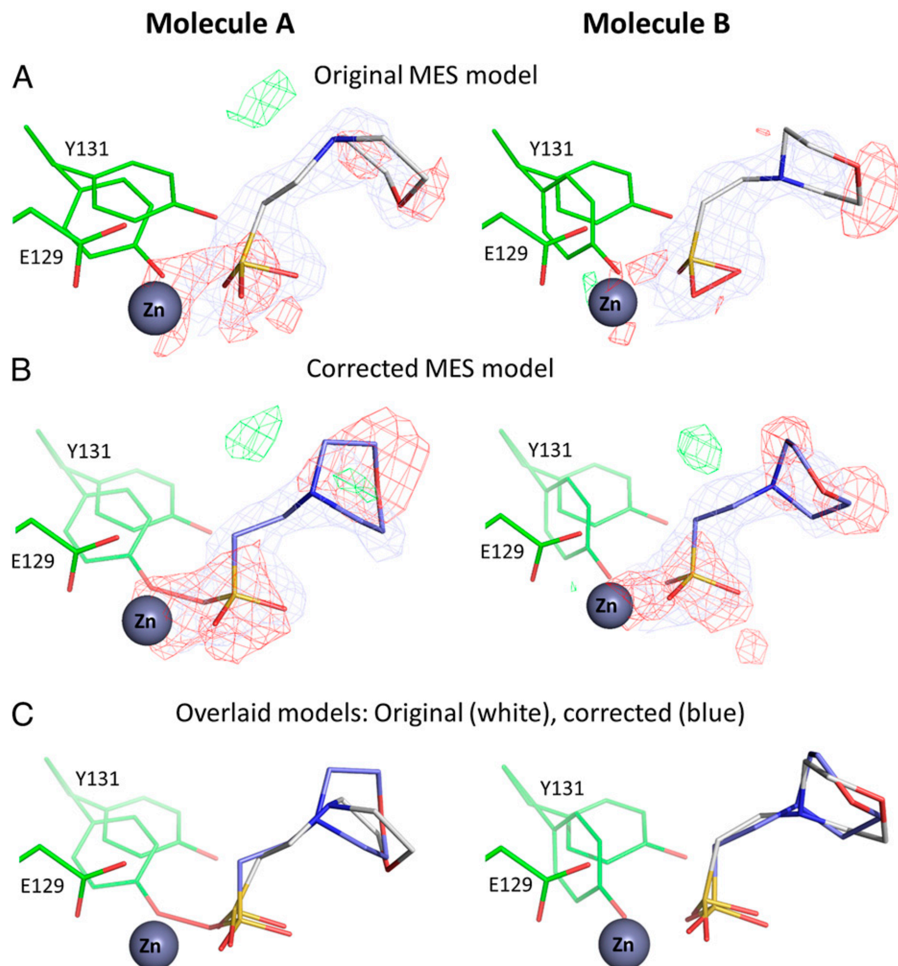

Fig. 1. The assignment of 2-(*N*-morpholino) ethanesulfonic acid (Mes) in DddQ's active site, in both monomers A and B (PDB code: 4LA2). (*A*) Conformation and electron density fit of the Mes molecule in the PDB file 4LA2. The Mes model used by Li et al. (1) relates a noncanonical configuration. (In molecule B the bridging bond between the two sulfonate oxygens was annotated as such by PyMol because the distance assigned by Li et al.'s model corresponds to a covalent bond). (*B*) Electron density fit of a Mes molecule whose geometry has been regularized into a canonical configuration. The observed density does not fit this corrected model. Note that the fit to the originally proposed Mes model is also marginal, particularly in "molecule A" of the asymmetric unit. (*C*) Superposition of original and regularized Mes modeling. The figure denotes electron density maps that were calculated using the deposited structure factors files corresponding to 4LA2. The $2F_o–F_c$ is contoured at 1 σ (blue mesh), and the Fourier difference map ($F_o–F_c$) is contoured at 3 σ (positive density in green, negative density in red mesh).

Table 1. Estimation of catalytic parameters

DMSP lyase (origin)	DMSP concentration	Observed V_{max}	Enzyme source	Estimated k_{cat}/K_M ($M^{-1}·s^{-1}$)	Ref.
DddQ (*Roseovarius nubinhibens* ISM/ *Ruegeria pomeroyi* DSS-3)	5 mM	2–5 $nmol·min^{-1}·mg^{-1}$	*E. coli* crude lysate, over expression	<20*	2
DddQ (*Ruegeria lacuscaerulensis* ITI_1157)	75 nM	Only relative activity was provided	Purified protein, crystallographic grade	<200†	1

*Assuming the overexpressed enzyme comprises 5% of total protein (a conservative estimate, as overexpression of small, well-folded proteins usually exceeds 20%), the observed rate, 5 $nmol·min^{-1}·mg^{-1}$ protein in crude lysate, corresponds to 0.1 $\mu mol·min^{-1}·mg^{-1}$ enzyme. Assuming a molecular weight of 25 KDa, 1 mg of DddQ equals 4×10^{-8} mole. Because $k_{cat} = V_{max}/[E]_0$, and assuming that applied DMSP concentration equals to the K_M (i.e., K_M = 5 mM; the two published parameters for DMSP lyases indicate an average of ∼5 mM, a value that is in fact even lower relative to DMSP concentrations in marine organisms), we obtained, $k_{cat} = 5\ min^{-1} = 0.083\ s^{-1}$, and $k_{cat}/K_M = 0.083/5 \times 10^{-3} = 16.6\ M^{-1}·s^{-1}$.

†Li et al. (1) provide only the assay conditions: the use of 3 μM enzyme, $[S]_o$ = 75 nM DMSP, and reactions were followed for 30 min. These assay conditions are unusual, as $[E]_o > [S]_o$, and do not indicate catalytic turnover. Notwithstanding, assuming initial, linear rate (else, the comparison of relative activities is invalid), the maximal possible rate is: $V_o = 75 \times 10^{-9}$ M/1800 s = 4.2×10^{-11} M/s. Assuming $[S]_o << K_M$ (the latter is taken as 5 mM), $V_o = [E]_o [S]_o (k_{cat}/K_M)$. Hence, the estimated k_{cat}/K_M is: $4.2 \times 10^{-11}\ Ms^{-1}/[(3 \times 10^{-6}\ M) \times (75 \times 10^{-9}\ M)] \sim 190\ M^{-1}·s^{-1}$.