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Fold prediction and comparative modeling of Bdm1: a probable α/β hydrolase associated with hot water epilepsy

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Abstract Hot water epilepsy (HWE) is a benign and rare form of reflex epilepsy that occurs most commonly in humans. Bdm1 is one of the proteins whose mRNA transcript is overexpressed during HWE in a rat model. We show, by sequence analysis and fold recognition methods, that Bdm1 has strong structural similarities to α/β hydrolases like the thioesterases. A three-dimensional model derived by comparative modeling methods allowed the search for catalytic residues using a flexible functional template characteristic of these enzymes. We predict that Bdm1 might be regulated by homocysteine levels by means of direct participation in degradation pathways.

Keywords α/β hydrolases · Kindling · Function prediction · Homology modeling · Distant relationships · Homocysteine

List of abbreviations *Bdm1*: Brain-derived development molecule-1 · *HWE*: Hot water epilepsy · *RT-PCR*: Reverse-transcript polymerase chain reaction · *HTL*: Homocysteine thiolactone · *NDR*: N-myc downstream regulated proteins · *BPHD*: 2-Hydroxyl-6-oxo-6-phenylhexa-2,4-dienoic acid hydrolase

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Introduction

Hot water epilepsy (HWE) is a type of reflex epilepsy, exhibited in the form of psychomotor seizures, that occurs in humans. Seizure is precipitated when the patient is subjected to hot water (above 37 °C) over the head. [1] Ictal EEG, SPECT and CT scan studies on a human patient show focal epileptic discharges in the temporo-occipetal region associated with local hypometabolism but without any structural lesions. [2] In about one-fourth of the cases, a kindling-like progression in the pattern of seizures has been observed. [1] HWE has remained elusive due to the absence of molecular markers.

Most Wistar rats manifest seizures on stimulation by application of hot water to the head; such seizures intensify on repetition of stimulation, as expected for kindling. [3] Animal models have been used previously to study febrile convulsions, in which hyperthermic seizures are precipitated by subjecting the animals to higher ambient temperature [4, 5] or by repeated exposure to hot water. [3] Pathological findings in some HWE patients [6, 7] and also the rat model have revealed varying degrees of anoxic changes in the brain, including neurocytologic changes in the hippocampus. [8] Kindling reflects progressive activity-dependent changes occurring in the brain on repeated stimulation. These may arise from loss of groups of neurons or rearrangement of synaptic connections, which in turn could be attributed to changes in the expression patterns, or level of expression of different genes in individual neurons. [9] Differential-display RT-PCR and Northern blot experiments have shown that HWE is associated with the overexpression of an mRNA transcript (L. Krishnaswamy, M.M. Panicker and G.M. Ullal, unpublished results), corresponding to Bdm1 [10] in the cerebral cortex of kindled animals. Bdm1 is a close homologue of the N-myc downstream developmentally regulated proteins (NDR). In this paper, we report the structural compatibility of Bdm1 to α/β hydrolases, particularly the thioesterases and enol-lactone hydrolysing enzymes involved in the degradation of toxic biphenyl compounds.

Materials and methods

Sequence searches were performed using methods like PSI-BLAST, [11] WU-BLAST2, [12] PFSCAN (Phillip Bucher, unpublished results) and IMPALA. [13] Three fold prediction methods were used to predict the fold of the sequence of Bdm1: GenThreader, [14] 3D-PSSM [15] and the UCLA hybrid fold prediction. [16] The three-dimensional structure of the protein was built using MODELLER. [17] Validation of the structure was carried out using VERIFY3D. [18] Polar residue clusters around conserved His residues and glycine-rich loops were examined for their similarity to the catalytic triad in α/β hydrolases, using flexible distance restraints (R. Sowdhamini, unpublished results). The distance restraints were obtained by an analysis of three-dimensional structures of representative members of that superfamily.

Results and discussion

Homologous sequences

Closely related sequences to Bdm1 include N-myc downstream regulated proteins (NDR) (average sequence identity of 60%) and pollen-specific proteins (SF21 from *Arabidopsis thaliana* and *Oryza sativa*) (average sequence identity of 25%). Figure 1 shows the alignment of Bdm1 with representative NDR and SF21 sequences. In addition, Bdm1 has homology with the misexpression suppressor of KSR in *Drosophila melanogaster* and several proteins annotated as "hypothetical" but belonging to either NDR or SF21 families. An advanced WU-BLAST2 search showed partial similarities with DNA-binding homeobox proteins: hox-10 of zebrafish, indicating that Bdm1 might interact with DNA.

Search for similar sequences and assignment of function

An iterative PSI-BLAST search of Bdm1 against the NR sequence database identifies homologous NDR1 and NDR3 sequences, followed by 3-oxoadipoic enol-lactone hydrolase with higher significance (E-value: 3e–47) than NDR2 proteins. Other proteins identified with significant E-values, before pollen-specific proteins (SF21) and some NDRs, include epoxyhydrolases, BPHD (E-value at the tenth iteration is 2e–37 to 5e–37), hydroxymuconic semialdehyde hydrolase esterase, 2-hydroxy-6-oxo-7-methylocta-2,4-dienoate hydrolase and EtBD1 from *Rhodococcus sp.*, a polychlorinated biphenyl degrader and an aromatic hydrolase, all belonging to the α/β hydrolase superfamily (E-values 5e–38 to 5e–34). 3-oxoadipoic enol-lactone hydrolase (pcaD) appears as an intermediate relative to Bdm1 in PSI-BLAST searches [11] (E-value: 4e–53). Purified from benzoate-grown cells of *Rhodococcus opacus* (erythropolis), 1CP (pcaD) belongs to a protocatechuate catabolic gene cluster. Higher similarity between Bdm1 and pcaD than the other NDR sequences suggests that Bdm1 has evolved divergently from the enol-lactone hydrolases (like pcaD and HPCD) and the diene-lactone hydrolases (BPHD).

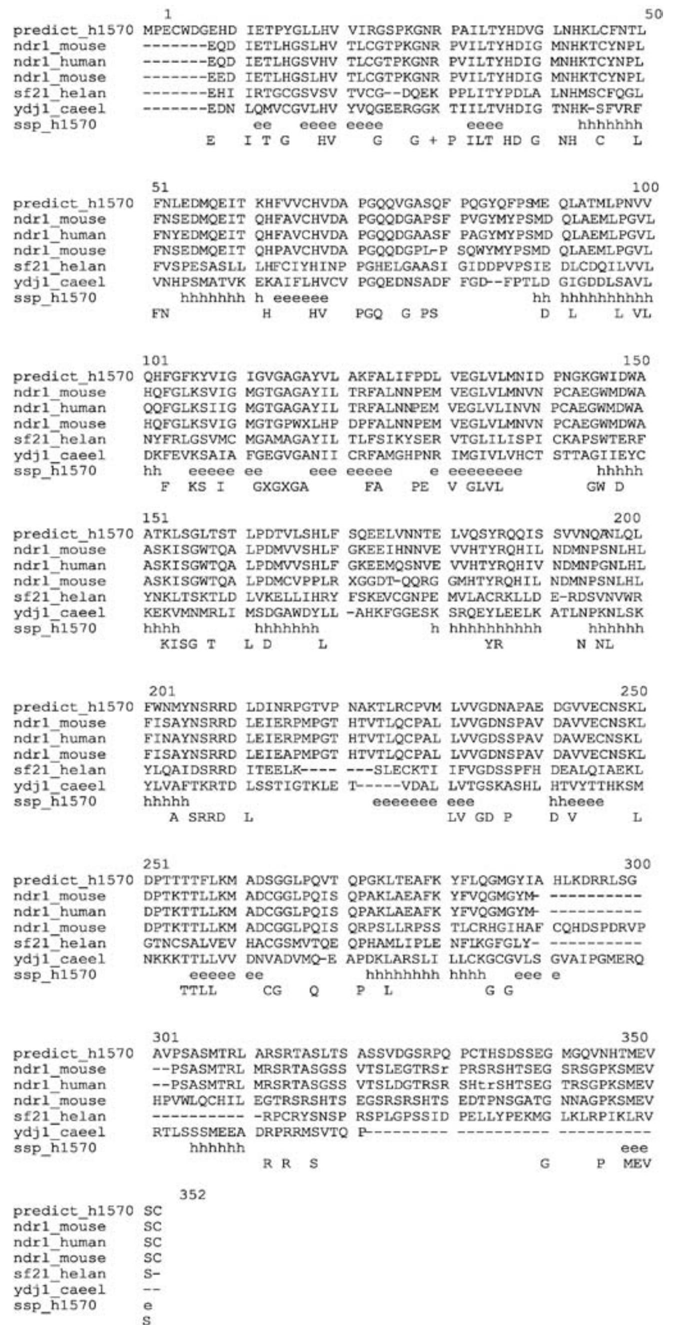


Fig. 1 Alignment of Bdm1 with homologous sequences. N-myc downstream regulated proteins (NDR) from various sources and pollen-specific proteins (SF21) are aligned. Predicted secondary structures (PHD [35]) for Bdm1 and conserved residues are marked along the alignment. Several polar and charged residues are conserved at the end of predicted β strands

Fold prediction of BDM1

WU-BLAST2, PSI-BLAST and several fold prediction techniques were employed to identify distantly related proteins and to relate to proteins of known three-dimensional structure and function (Tables 1, 2 and 3). Predominant hits were with thioesterases, haloalkane

Table 1 Proteins predicted to share a common fold with Bdm1 by different fold recognition methods. All hits correspond to the fold of α/β hydrolases. Results from the hybrid fold prediction method [16]

Fold prediction method	Protein	PDB code	Function
Hybrid fold recognition method	Hpda hydrolase	1C4XA	C–C bond cleavage
	Haloalkane dehalogenase	1CV2A	C–Cl bond cleavage
	Epoxide hydrolase	1EHYA	C–O bond cleavage
	Haloalkane dehalogenase	1EDE	C–Cl bond cleavage
	Haloalkane dehalogenase	1CQW	C–Cl bond cleavage

Table 2 Proteins predicted to share a common fold with Bdm1 by different fold recognition methods. All hits correspond to the fold of α/β hydrolases. Results from the 3D-PSSM fold prediction method [15]

Fold prediction method	Protein	PDB code	Function
3D-PSSM fold prediction method	Lipase	1HLG	C–O bond cleavage
	Hpda hydrolase	1C4XA	C–C bond cleavage
	Haloalkane dehalogenase	1BN7A	C–Cl bond cleavage
	Proline iminopeptidase	1QTRA	C–N bond cleavage
	Lipase	1CVL	C–O bond cleavage
	Haloalkane dehalogenase	1B6G	C–O bond cleavage
	Epoxide hydrolase	1A8S	C–O bond cleavage
	Haloalkane dehalogenase	1CV2	C–Cl bond cleavage
	Haloperoxidase	1A88	C–Br bond formation
	Haloperoxidase	1BRT	C–Cl bond formation
	Proline iminopeptidase	1A8Q	C–N bond cleavage

Table 3 Proteins predicted to share a common fold with Bdm1 by different fold recognition methods. All hits correspond to the fold of α/β hydrolases. Results from the GenThreader [14] fold prediction method

Fold prediction method	Proteins	PDB Code	Function
GenThreader fold prediction method	Haloalkane dehalogenase	1BN7A	C–Cl bond cleavage
	Epoxide hydrolase	1CR6A	C–O bond cleavage
	Epoxide hydrolase	1CR6A2	C–O bond cleavage
	Hpda hydrolase	1C4XA	C–C bond cleavage
	Epoxide hydrolase	1EHYA	C–O bond cleavage
	Haloperoxidase	1BRT	C–Br bond formation
	Haloperoxidase	1A8Q	C–Cl bond formation
	Proline iminopeptidase	1QTRA	C–N bond cleavage
	Epoxide hydrolase	1QO7A	C–O bond cleavage
	Haloalkane dehalogenase	1B6G	C–Cl bond cleavage

dehalogenases, epoxyhydrolases and BPHD that are ⁄α/β hydrolases. Search for proteins related to Bdm1 using PFSCAN against Hidden-Markov model profiles provided a single match with the thioesterase family. These results were supported by IMPALA, where the sole hit was thioesterase of the α/β hydrolase superfamily.

Structural compatibility to α/β hydrolases

Proteins belonging to the α/β hydrolase superfamily include a variety of protein hydrolases (detoxification enzymes, lipases, haloalkane dehalogenases) and catalyze a specific bond cleavage (C–C, C–X, C–S etc.) reaction. They are characterized by a common catalytic domain whose topology is largely similar: a central eight-stranded, largely parallel β-sheet surrounded by helices on either side with 12435678 as the strand order; the first three β-strands of this basic fold are continuous hairpin structures, whereas the rest of the fold is alternating α/β interrupted by a helical sub-domain after β5. There are at least 15 families under this broad superfamily: the sequence identity between proteins belonging to two such family members is in the order of 7–18%. Due to poor sequence identity, structural deviations exist as insertions (exhibited as extra peripheral secondary structures), differences in the helical sub-domain, positional displacements of core secondary structures and the lengths of loop regions.

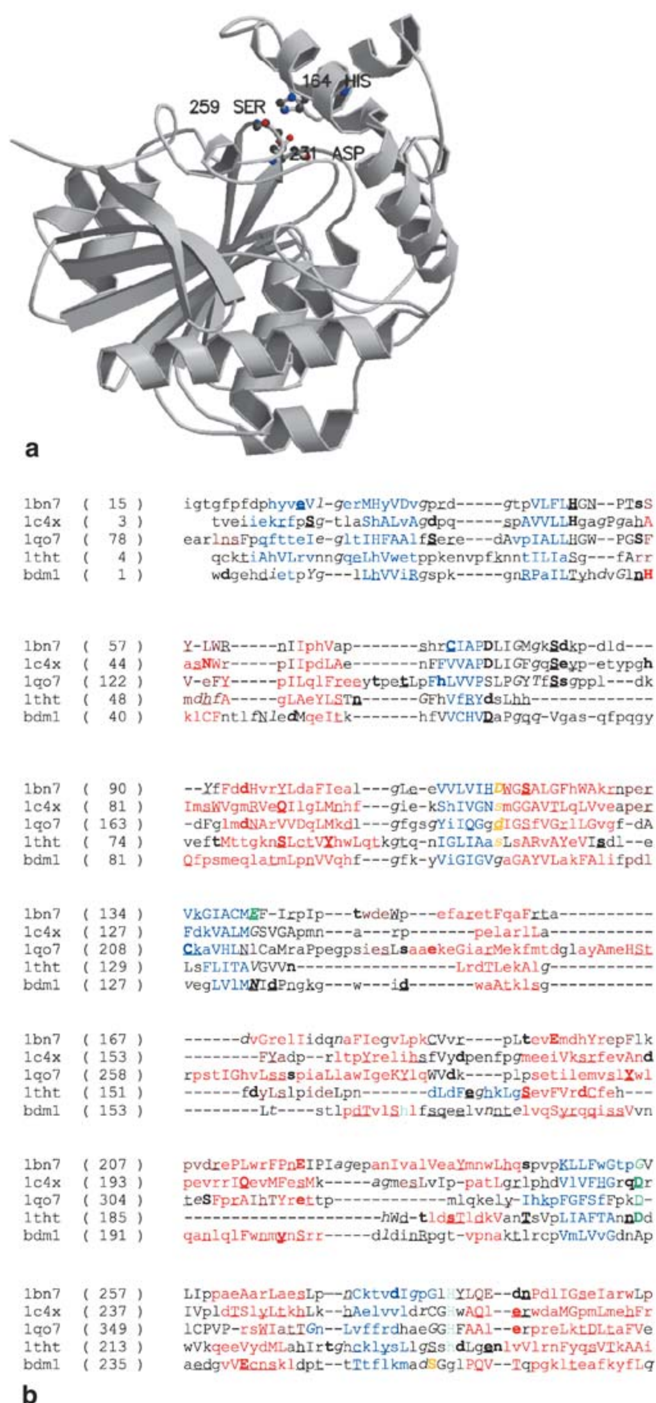
Three-dimensional modeling

The three-dimensional structure of Bdm1 was generated (Fig. 2a) using BPHD (2-hydroxyl-6-oxo-6-phenylhexa-2,4-dienoic acid hydrolase, whose crystal structure has been solved to 2.4 Å resolution (Protein Data Bank code 1c4x [19]), as template and the model validated. Threading the sequence of Bdm1 on the fold of thioesterase, haloalkane dehalogenase and validating the structures showed that the best model is obtained for Bdm1 with BPHD as the template (data not shown). Structural similarity of Bdm1 with this hydrolase has been suggested by iterative PSI-BLAST, advanced-WU-BLAST2 searches and UCLA fold prediction server.

Functionally important residues

All the proteins in this superfamily require three residues that form a catalytic triad: Asp/Ser (a nucleophile at the end of β5), Asp (at the end of β7) and the invariant His (proton donor at the end of β8) for the catalysis and the mechanism of action has been worked out in detail for various families. [20]

The alignment of protein representative sequences from thioesterase, haloalkane dehalogenases, epoxyhydrolase families, BPHD along with Bdm1 (Fig. 2b) shows that the functional aspartate is aligned but the catalytic nucleophile is replaced by a glycine-rich loop in Bdm1. We searched, starting from the Bdm1 model, around all the conserved His residues within a sphere of radius 14 Å for other conserved (probably functionally important) residues. The method is similar to that employed for α/β hydrolase superfamily in the *E. coli* genome database. [21] Table 4 shows several spatially proximate triads, resembling the catalytic triad observed in α/β hydrolases, which have been identified in the Bdm1 model by the flexible distance restraint approach. However, it is possible that Bdm1 adopts a three-dimensional structure similar to α/β hydrolases but lacks hydrolytic activity.



Conclusions

Sequence searches and fold prediction suggest that Bdm1 belongs to the α/β hydrolase superfamily like

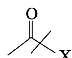
Table 4 Spatially interacting triads detected in Bdm1 model

S. No	Invariant His	Nucleophilic residue	Catalytically important acidic residue
1	His (164)	Ser (259)	Asp (231)
2	His (39)	Asp (65)	Asp (34)
3	His (39)	Asp (65)	Asp (51)
4	His (164)	Ser (167)	Asp (34)
5	His (164)	Ser (180)	Asp (34)
6	His (33)	Ser (84)	Asp (34)
7	His (33)	Ser (151)	Asp (34)
8	His (69)	Cys (62)	Asp (65)

Fig. 2 **a** Three-dimensional model of Bdm1 obtained by threading the Bdm1 sequence onto the fold of BPHD (PDB code 1c4x) using MODELLER. The side-chains of three residues His164, Ser259 and Asp231, one of the hits for the presence of a catalytic triad identified by the functional template approach and defining the putative α/β hydrolase active site, are shown. This figure was generated using MOLSCRIPT. [36] **b** Alignment of Bdm1 with α/β hydrolase sequences. Crystal structures are available for all the hydrolases considered (see Tables 1, 2 and 3 for name of protein against PDB code). Residues observed as solvent inaccessible and accessible are denoted in capital and small letters, respectively. Active site residues, nucleophile (usually a Ser), acidic residue (Asp/Glu) and proton acceptor–donor (invariant His) are highlighted by colour-shaded boxes with arrows to indicate alignment positions. Structural features of Bdm1 model (1BDM) are also marked. The active sites are not in equivalent positions in 1bn7 as well as in Bdm1

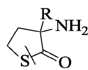
Solvent inaccessible	*upper case*
Solvent accessible	*lower case*
Alpha helix	*red*
Beta strand	*blue*
3–10 helix	*maroon*
Hydrogen bond to main-chain amide	*bold*
Hydrogen bond to main-chain carbonyl	*underlined*
Disulphide bond	*cedilla*
Positive phi	*italic*
Nucleophile (S/D)	*green*
Acidic residue (D/E)	*yellow*
Basic residue (H)	*light turquoise*

Plausible cleaving bond of α/β hydrolase

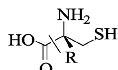


Plausible cleaving bond for BDM1

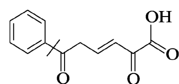
1) Thiolactone



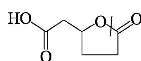
2) Homocysteine



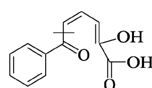
Substrate of the homologs brought up by PSI BLAST



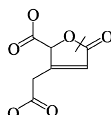
1) 2,6-dioxo-6-phenyl-hex-3-enoate



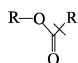
2) 3-oxo adipaete enol lactone



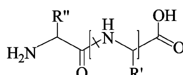
3) 2-hydroxy-6-oxo-6-phenyl hex-2,4 δ dienoate



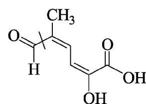
4) 4-carboxymucano lactone



5) Esters



6) Peptide



7) 2-hydroxy-4-methyl-6-oxo-hex2,4 dieonic acid

Fig. 3 Substrates for different members of α/β hydrolase family. The site of proteolytic cleavage is marked in each substrate. Thiolactone is a possible substrate for Bdm1

thioesterases and diene-lactone hydrolases. Both homocysteine thiolactone (HTL) and homocysteine have been implicated to play a role in epilepsy. Under HWE conditions, Bdm1 might catalyze the degradation of more stable HTL to homocysteine for further catabolism, explaining the distant similarity between Bdm1 and enol-lactone hydrolysing enzymes (Fig. 3).

We show, by comparative modeling approaches, that the hydrolase fold is entirely compatible with the Bdm1 sequence. Although the catalytic triad characteristics of α/β hydrolases are not absolutely conserved, the identification of active site residues in non-equivalent positions amongst superfamily members is not uncommon. [22] It will be interesting to perform site-directed mutagenesis experiments on the other residue clusters. However, it is conceivable that Bdm1, whose mRNA expression during HWE is high, accumulates in high levels as a non-functional α/β hydrolase. A similar example has been observed in the genetic disease, infantile neuronal ceroid lipofuscinosis, where a Arg122-to-Trp mutation near the active site of a palmitoyl thioesterase, leads to an intracellular accumulation of the polypeptide and undetectable enzyme activity. [23]

The mRNA expression of several proteins, including NDR1_HUMAN – a close homologue of Bdm1, is up-regulated during high homocysteine levels. [24, 25] Increases in levels of homocysteine also cause seizures [26] and increased levels of homocysteic acid have been observed during high-frequency stimulation of hippocampal slices. [27] Homocysteine is generated as a metabolic intermediate when the normal methionine to cysteine conversion is affected by reduced levels of folate-dependent enzymes. Increased levels of homocysteine have been associated with a variety of physical disorders, starting from cardiovascular diseases such as artherosclerosis to birth complications such as pre-eclampsia and Alzheimer's disease and others including homocystinuria, osteoporosis and presbyopia. It is believed that, like cholesterol, homocysteine can clog the blood vessels and thereby increase the risk of heart diseases. [28, 29, 30] Homocysteine can confer secondarily generalized convulsive status epilepticus in actively epileptogenic cobalt-leisoned animals and diazepam arrests such seizures [31]. Interestingly, in human patients it has been observed that prior treatment with diazepam prevents HWE. While the main pathway of homocysteine formation from homoserine involves enzymes such as acyltransferase and sulfhydrolase, a subsequent pathway suggests the conversion of homocysteine to HTL by methionyl tRNA synthetase. [32] This alternate mechanism is shown to reduce the levels of homocysteine; however, HTL has recently been shown to damage proteins by modifying lysine residues. [33] Other enzymes such as homocysteine thiollactonase belonging to the detoxifying enzyme family of paraxonases are recruited in humans for the degradation of HTL. [34] The results derived here propose structure and function for one of the HWE-induced proteins and form useful inputs in our general understanding of events during such epileptic seizures and in brain development.

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