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Mini-review

Diverse catalytic activities in the αβ-hydrolase family of enzymes: activation of H_2O, HCN, H_2O_2, and O_2

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Abstract

The article describes the observation of novel catalytic activities in the αβ-hydrolase superfamily apparently unrelated to ester hydrolysis and unexpected biochemical observations relating to the structure and function of the serine catalytic triad in these enzymes. One common feature of these novel activities is the activation of a small diatomic molecule, but via diverse chemistry. Possible mechanisms of catalysis are discussed.

Keywords: Enzyme mechanism; αβ-hydrolase; C–C hydrolase; Haloperoxidase; Hydroxynitrile lyase; Dioxygenase

1. Introduction

The existence of "superfamilies" of proteins and enzymes sharing a similar three-dimensional protein fold is an important principle of modern structural biology [1]. In most cases the members of an enzyme superfamily catalyse similar types of reaction, however, in some cases a superfamily contains enzyme activities of apparently diverse function. For example, the enolase superfamily contains enzymes that catalyse racemisation reactions (mandelate racemase), lactonisation reactions (muconate

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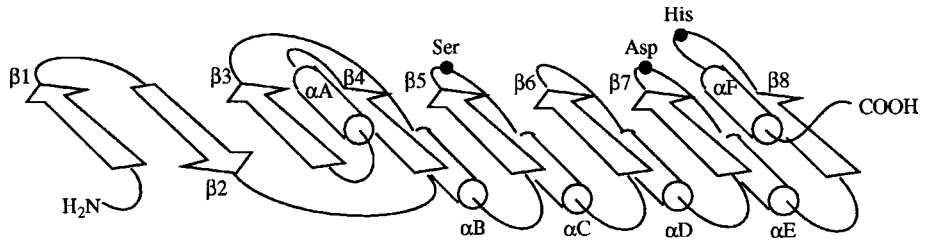

Fig. 1. Topology diagram for canonical α/β hydrolase fold, showing position of catalytic triad.

lactonising enzyme), and hydration reactions (enolase) [1]. In these cases, it seems reasonable to suppose that there is some common mechanistic feature of the reactions catalysed (in the case of the enolase superfamily, the formation of a carbanion adjacent to a carbonyl group), in order to account for the evolution of new function.

The αβ-hydrolase enzyme superfamily was first identified in 1992: five hydrolytic enzymes (dienelactone hydrolase, haloalkane dehalogenase, wheat serine carboxypeptidase II, acetylcholinesterase, and *Geotrichum* lipase) were found to share a very similar structural fold, even though they shared no significant sequence similarity [2]. This canonical α/β hydrolase fold consists of an eight-stranded β-sheet, all parallel β-strands apart from the second antiparallel β-strand, with α-helices or an additional small domain between each pair of parallel β-strands (see Fig. 1). The catalytic machinery of these enzymes consists of a serine-histidine-aspartic acid triad, similar to the catalytic triad of the serine proteases. In haloalkane dehalogenase and epoxide hydrolase, the active site serine is replaced by an aspartic acid, which functions as a nucleophile during catalysis. Structurally, the serine nucleophile is positioned on a sharp turn after strand β5; the aspartic acid residue is positioned immediately after strand β7, and the histidine base is located near the C-terminus, after strand β8 (see Fig. 1) [2–4]. The purpose of this article is describe recent observations in this enzyme family of: (1) novel catalytic activities apparently unrelated to ester hydrolysis; (2) unexpected biochemical data relating to the structure and function of the serine catalytic triad in these enzymes.

Most members of the α/β hydrolase family are esterase and lipase enzymes that catalyse ester hydrolysis reactions [3,4]. The enzymes haloalkane dehalogenase [5] and epoxide hydrolase [6], containing an aspartic acid nucleophile in place of serine, carry out the nucleophilic attack on haloalkane or epoxide substrates, respectively, followed by hydrolysis of a covalent ester intermediate. There are also enzymes that catalyse peptide hydrolysis reactions, such as prolyl oligopeptidase [7], using the serine triad in an analogous fashion to the serine proteases.

1.1. C–C hydrolase enzymes in the αβ-hydrolase family

Enzymes that catalyse the hydrolytic cleavage of C–C bonds adjacent to carbonyl groups, termed C–C hydrolases, are found on bacterial *meta*-cleavage pathways responsible for the degradation of aromatic compounds in soil. Amino acid sequence alignments revealed that *Pseudomonas putida* XylF, on the catechol

meta-cleavage pathway, shared sequence similarity with the αβ-hydrolase family [8]. The catalytic triad was identified as Ser-107, Asp-228, and His-256, and each of these residues was demonstrated using site-directed mutagenesis to be essential for catalytic activity [8]. It was therefore logical to suppose that the role of the catalytic serine residue was to act as a nucleophile during the catalytic mechanism (see Fig. 2A).

Studies on *Escherichia coli* MhpC, a C–C hydrolase on the phenylpropionic acid catabolic pathway, had shown that the reaction mechanism involved two half-reactions: an initial keto–enol tautomerisation to form a discrete keto-intermediate, followed by a stereospecific C–C fragmentation reaction [9,10]. However, attempts to identify a covalent acyl enzyme intermediate via radiochemical trapping experiments gave $<1\%$ of protein-bound ^{14}C label, under conditions, where stopped flow kinetic measurements had indicated that hydrolysis of the acyl enzyme intermediate should be rate limiting [11]. The alternative catalytic mechanism, involving nucleophilic attack of water on the keto-intermediate (see Fig. 2B), was investigated using ^{18}O

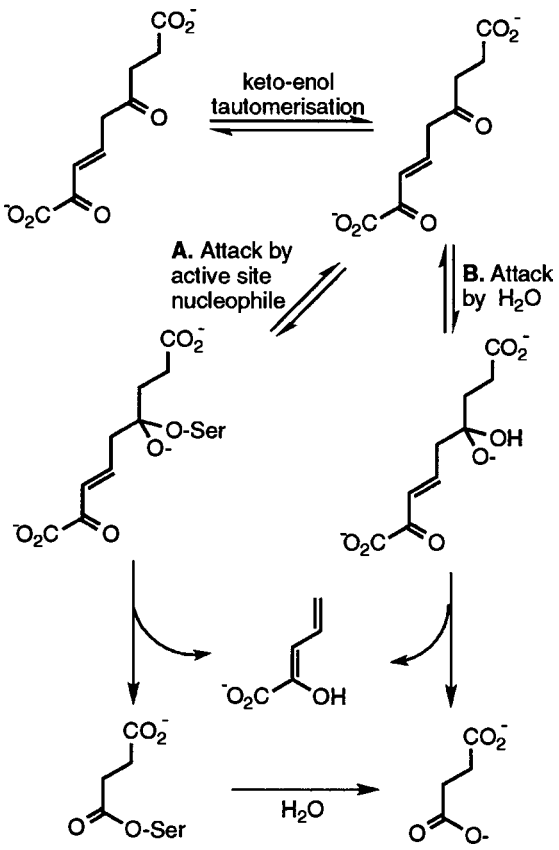

Fig. 2. Catalytic mechanisms of C–C hydrolases involving (A) nucleophilic attack of a catalytic serine residue, (B) nucleophilic attack of water upon a keto-intermediate.

labelling studies. Enzymatic processing of the natural substrate in $H_2{}^{18}O$ was accompanied by 4–6% incorporation of a second atom of ^{18}O, consistent with the reversible formation of a *gem*-diol intermediate. The enzyme was also found to catalyse exchange of ^{18}O from $H_2{}^{18}O$ into the carbonyl group of a non-cleavable ketone analogue, consistent with enzyme-catalysed attack of water, rather than nucleophilic attack of an active site serine residue [11].

The crystal structure of *Rhodococcus* sp. BphD, a C–C hydrolase on the biphenyl degradation pathway, was published in 2001 [12]. The structure contains the canonical αβ-hydrolase fold, together with an additional four-α-helix 'lid' domain after the β6 strand. Two unusual features of the catalytic triad were observed: the hydroxyl sidechain of Ser-110 is not hydrogen-bonded to His-263, and furthermore a 'blob' of extra electron density was observed immediately adjacent to the sidechain of Ser-110, which was interpreted as a covalent modification of Ser-110 [12]. A similar arrangement has been observed in the crystal structure of *E. coli* MhpC, and a similar 'blob' of electron density adjacent to the catalytic serine, although it is known in this case that no covalent modification has occurred, since the expected M^+ is observed by electrospray mass spectrometry [13]. It is therefore interesting that unusual structural features are observed in the serine catalytic triad, when the catalytic role of the active site serine is also in question.

1.2. Enzymatic activation of HCN in the αβ-hydrolase family

The enzyme hydroxynitrile lyase from *Hevea brasiliensis* catalyses the reversible addition of hydrogen cyanide to aldehydes and ketones to give cyanohydrins [14]. Structure determination of this enzyme in 1996 revealed that it belongs to the αβ-hydrolase family, containing a catalytic triad consisting of Ser-80, His-235, and Asp-207 [14]. A high resolution 1.1 Å structure published in 1999 revealed that two conformations of Ser-80 existed: one in which Ser-80 was hydrogen-bonded to His-235, and a second conformation in which they were not hydrogen-bonded, but Ser-80 was instead hydrogen-bonded to the substrate carbonyl [15]. Although the structure of an enzyme complex with trichloroacetaldehyde showed a covalent linkage to Ser-80, complexes with other substrates and inhibitors showed no covalent attachment [16]. Consequently, Kratky and co-workers [16] have proposed a catalytic mechanism involving deprotonation of HCN by His-235, and attack of CN^- upon the substrate carbonyl, with protonation of the carbonyl group by Ser-80, which is in turn protonated by His-235.

1.3. Enzymatic activation of H_2O_2 in the αβ-hydrolase family

A novel class of haloperoxidase enzymes, catalysing the reaction of hydrogen peroxide with an organic acid to form a peracid which in turn reacts with a halide ion, have also been found to be members of the αβ-hydrolase family [17], with a very similar structure to that of the C–C hydrolase BphD. The structures of cofactor-independent haloperoxidases from *Streptomyces aureofaciens*, *Streptomyces lividans*, and *Pseudomonas fluorescence* each contain a catalytic triad composed of

Ser-98, His-257, and Asp-228 [18]. In each case there was additional electron density adjacent to the sidechain of Ser-98, which was interpreted as a covalent adduct [17,18]. Also, it was observed that there were two conformations of Ser-98: one in which it was hydrogen-bonded to His-257, and one in which it was swung away to a distance of 3.2 Å [18]. By analogy with the serine protease reaction mechanism, a catalytic mechanism involving nucleophilic attack of Ser-98 upon the organic acid was proposed [18].

Recently, three bifunctional esterase-haloperoxidase enzymes have been discovered, which are αβ-hydrolases. A Co^{2+}-activated bromoperoxidase-esterase has been found in *P. putida* IF-3 [19]. The enzyme catalyses ester hydrolyses at specific activity 439 U/mg, bromination at 11.7 U/mg, and also catalyses a side reaction of peracid hydrolysis to hydrogen peroxide at 21 U/mg. Curiously, the enzyme is inert to the serine-directed reagents DFP and PMSF, unlike most serine hydrolases [19]. 3,4-Dihydrocoumarin hydrolase from *Acinetobacter calcoaceticus* catalyses coumarin hydrolysis at 4.0 U/mg, bromination of monochlorodimedone at 0.44 U/mg, and peracetic acid hydrolysis at 27 U/mg [20]. Given that the downstream gene encodes a catalase enzyme, it seems that the physiological role of this enzyme is in oxidative stress defence against peracids [20]. The third bifunctional enzyme is a thiocarbamate-inducible enzyme ThcF from *Rhodococcus erythropolis*, which shows v_{max} 2.58 nmol/min for ester hydrolysis, and 0.45 nmol/min for bromination [21]. Studies of the pH activity of this enzyme showed quite different pH/rate profiles for the two activities: ester hydrolysis activity was highest at pH 8.0–8.5, whereas bromination activity was highest at pH 5.5–6.0 [21]. These data imply different catalytic mechanisms for ester hydrolysis and haloperoxidase activity: in particular, it is likely that the catalytic histidine will be protonated at pH 5.5, and therefore unable to act as a base. It is known that certain lipases are able to catalyse the conversion of organic acids into peracids in organic solvents [22], thus there is clearly some connection between ester hydrolysis activity and haloperoxidase activity.

1.4. Enzymatic activation of O_2 in the αβ-hydrolase family

Remarkably, two cofactor-independent dioxygenase enzymes have been discovered that contain the αβ-hydrolase fold. 1H-3-hydroxy-4-oxoquinoline 2,4-dioxygenase (Qdo) from *P. putida* 33/1 and 1H-3-hydroxy-4-oxoquinaldine 2,4-dioxygenase (Hod) from *Arthrobacter ilicis* Rü61a catalyse the oxidative cleavage of heterocyclic substrates, yielding a ring-opened product, and carbon monoxide [23]. Amino acid sequence alignments revealed that these enzymes were members of the αβ-hydrolase family, containing a serine catalytic triad, with low, but significant sequence similarity to esterase and C–C hydrolase enzymes [23]. Site-directed mutagenesis of the catalytic serine, Ser-95, gave a S95A mutant enzyme with 8% k_{cat} compared to the native enzyme, implying that the active site serine is not absolutely required for catalytic activity [23,24]. The catalytic mechanism is likely to involve the chelotropic ring opening of a cyclic endoperoxide, liberating carbon monoxide [25].

2. Discussion

The observation of esterase, C–C hydrolase, haloperoxidase, cyanohydrin lyase, and dioxygenase activities (see Fig. 3) within the same structural family of enzymes is intriguing, and begs the question of whether there are any mechanistic similarities between these reactions. At the same time, there are several observations that imply differences in the structure and function of the serine catalytic triad in these enzymes from the "classical" serine hydrolase paradigm. From independent lines of study, indications have emerged of a non-nucleophilic role for the active site serine residue: a non-nucleophilic mechanism has been proposed on the basis of mechanistic studies for C–C hydrolase MhpC [11]; and a non-nucleophilic mechanism has been proposed for hydroxynitrile lyase on the basis of detailed crystallographic studies [16]. Furthermore, crystallographic studies have, in several cases, revealed two conformations of the active site serine, one of which is not hydrogen-bonded to the active site histidine—an observation that has also been made for acetylxylan esterase from *Penicillium purpurogenum* [26]. It is therefore prudent to consider possible alternatives to the "classical" nucleophilic mechanism.

Comparing the active site structures of the enzymes described in this article (see Fig. 4), one can see that, compared with the "classical" serine catalytic triad of trypsin, the catalytic triad of C–C hydrolase BphD is somewhat twisted, with the serine hydroxyl group swung away from the histidine base, as described above. Unlike the trypsin active site, where there is a very clear "oxyanion hole" formed by the backbone N–Hs of Gly-193 and Ser-195, there is no clear oxyanion hole in C–C hydrolase BphD. The active site of *P. fluorescens* chloroperoxidase is virtually

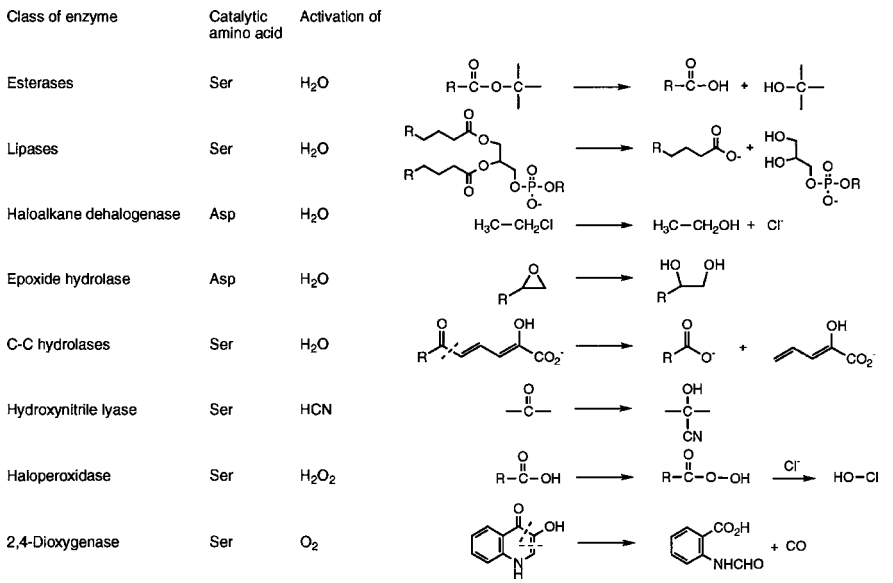

Fig. 3. Classes of enzymes within the αβ-hydrolase superfamily.

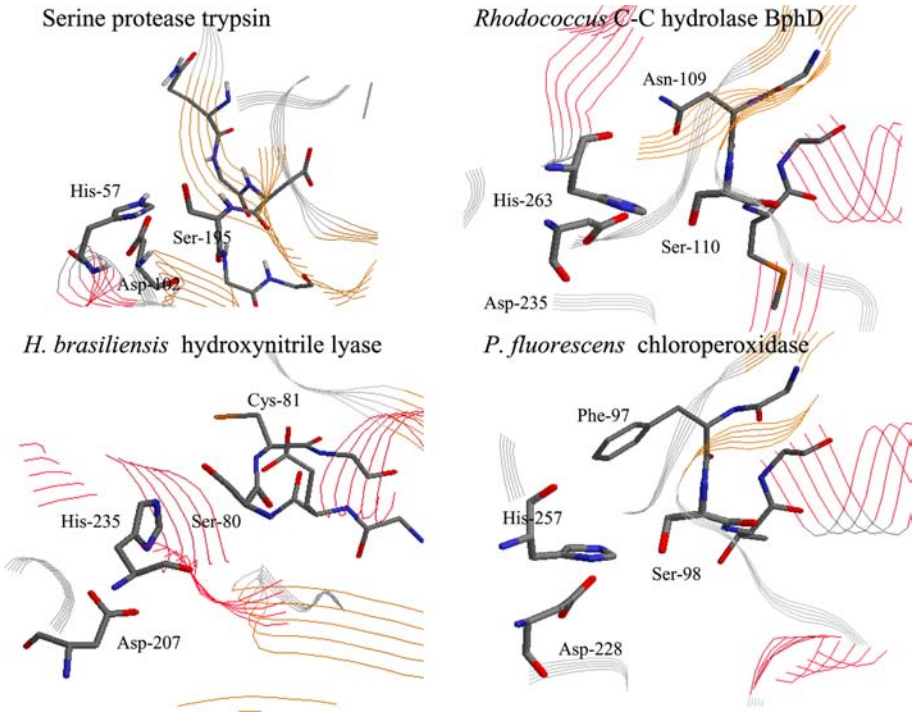

Fig. 4. Active sites of trypsin, C–C hydrolase BphD, *P. fluorescence* chloroperoxidase, and hydroxynitrile lyase from *H. brasiliensis.*

superimposable upon the active site of C–C hydrolase BphD, with the exception of certain specific amino acid alterations, such as the residue adjacent to the active site serine, which is Met in BphD, but Phe in the chloroperoxidase structure.

What might then be the connection between the diverse catalytic activities described in this article? One possible connection is that each activity involves activation of a small, diatomic molecule. While the majority of αβ-hydrolases activate water for the hydrolysis of esters (and in some cases peptides), these enzymes are capable of activation of hydrogen cyanide (HCN), hydrogen peroxide (H_2O_2), or dioxygen (O_2). Although the chemistry involved is apparently, quite different, the study of structural biology has shown that protein structure is often conserved more than function in Nature [1], implying that through a few point mutations in the course of evolution, it is possible to evolve new catalytic function in a structural family.

One can speculate that the greater separation of the active site serine and histidine residues might allow the binding of diatomic molecules, rather than water, and would allow a change of mechanism, whereby the histidine residue could act as a base. In Fig. 5 possible non-nucleophilic mechanisms are illustrated, that might account for the observations described in this article. In the C–C hydrolases, as in hydroxynitrile lyase, the active site histidine could act as a base to deprotonate water (or HCN), with the active site serine acting as a hydrogen bond donor. In the cofactor-independent

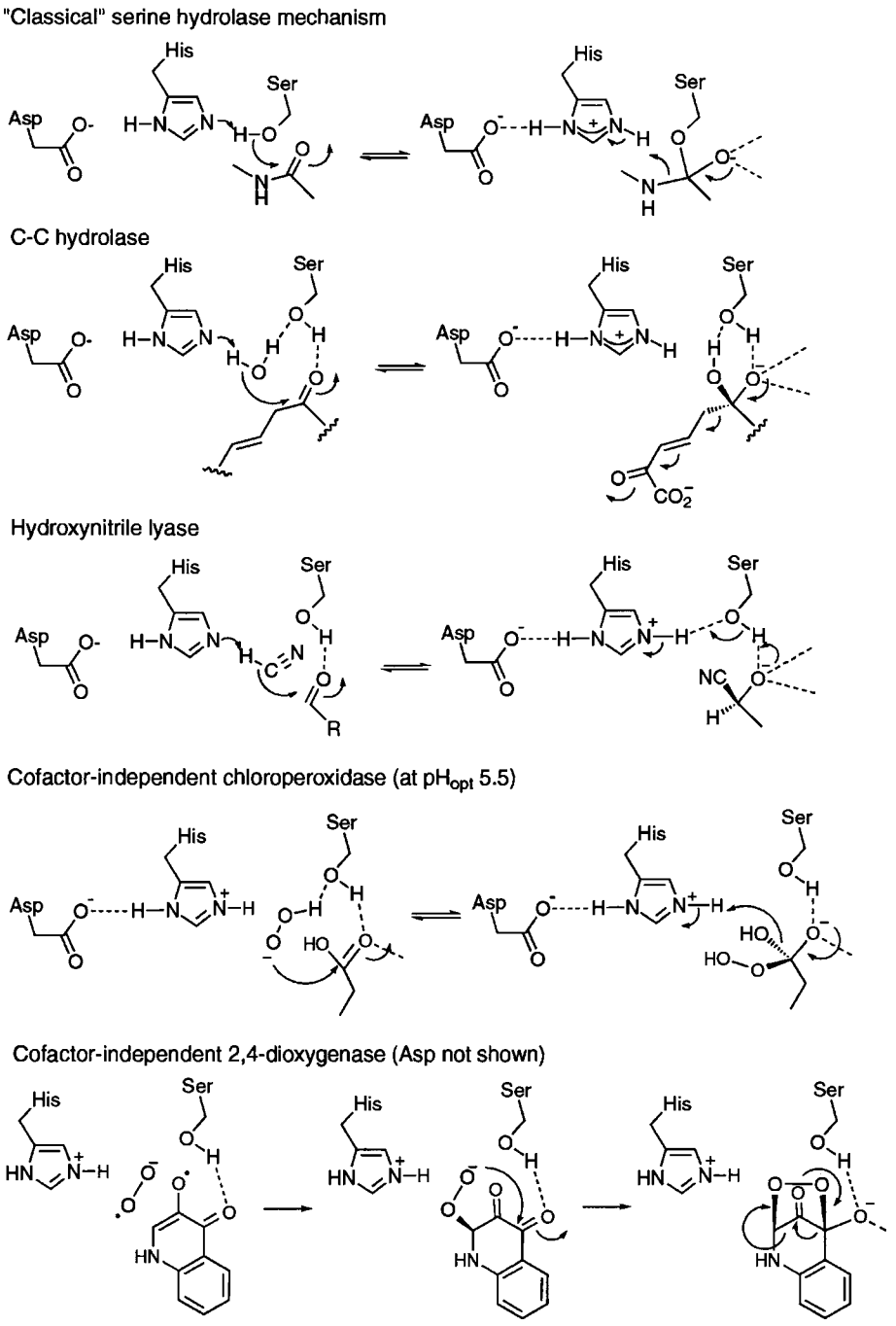

Fig. 5. Possible mechanistic rationalisation of the diverse catalytic activities described in the article.

haloperoxidases, the more nucleophilic peroxide ion may require no activation, instead it is likely that at pH 5.5 the active site histidine residue acts as an acid to protonate the departing hydroxide ion. The role of the catalytic triad in the cofactor-independent dioxygenases is not at all obvious: the existence of a protein radical is one possibility [25]; alternatively the active site may form a convenient diatomic binding site and assist oxygen activation through hydrogen bonding. More definitive explanations await detailed structural studies on these enzymes.

The αβ-hydrolase superfamily continues to expand rapidly, with the identification of enzymes of unknown biological function from a breast carcinoma cDNA library [27,28], and from an emphysemal lung cDNA library [29]. Annotation of genome sequencing data will provide many new members of the family, whose precise function is now perhaps less certain, in view of the diversity of catalytic function. It seems likely that Nature will have further surprises in store in this remarkable family of enzymes.

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