It can be a complicated life being an enzyme

Department of Biochemistry, Trinity College, Dublin 2, Ireland

Abstract

It is becoming increasingly apparent that many well-known enzymes have alternative non-enzymic functions. Similarly, several proteins that were identified as having non-catalytic functions were subsequently found to have enzyme activities. Some examples are considered to illustrate the diversity of alternative functions. The semicarbazide-sensitive amine oxidase (EC 1.4.3.6) is considered in more depth as an example. It was originally believed to be a detoxifying enzyme, but the reaction products may have important signalling functions. Furthermore, this enzyme, from some sources, also behaves as a vascular-adhesion protein. Finally, the challenges posed by such multiplicity of functions for the interpretation of genetic deletion, \textit{in vivo} inhibition and the development of functional protein databases are briefly considered.

Introduction

Most of us working in what used to be called academia are now well used to having to do several different tasks, often simultaneously. Some of these tasks have little to do with the research and teaching that we were appointed to do. It is a relief to know that others are in the same position. In particular, it is becoming increasingly apparent that many of the proteins that we know as enzymes have radically different alternative functions. Perhaps we should not be surprised about this, since we have known for very many years that every time we looked at something it was through the courtesy of glycolytic enzymes forming lens crystallin. However, the number of enzymes known to have spare-time jobs has increased dramatically in recent years.

Table 1 shows a few such enzymes (see [1–13]), chosen to illustrate the diversity of alternative functions. Much more space would be required for a comprehensive list. The list also excludes those enzymes that appear to catalyse widely different reactions, such as the multicatalytic Ku protein (e.g. see [14]), maleylacetoacetate isomerase (EC 5.2.1.2), which is also a glutathione-S-transferase (EC 2.5.1.18) [15], and the Haemophilus influenzae NadR protein, which contains both nicotinamide-nucleotide adenyllytransferase (E.C 2.7.7.1) and ribosylnicotinamide kinase (EC 2.7.1.22) activities [16]. In some cases, it was an enzyme that was found to have an alter ego, whereas in others a protein whose function was believed to be well established was found to be moonlighting as an enzyme. This multitasking poses a number of challenges, which will be discussed later, but first we will discuss a specific example of an overworked enzyme, which initially seemed to be particularly uninteresting, the so-called semicarbazide-sensitive amine oxidase (SSAO).

What is SSAO and where is it?

‘Semicarbazide-sensitive amine oxidase’ was a name coined to distinguish a group of enzymes catalysing the oxidative deamination of some aliphatic and aromatic primary amines from the mitochondrial monoamine oxidases [MAOs; with the systematic name of amine:oxygen oxidoreductase (deaminating) (flavin-containing); EC 1.4.3.4]. Both classes of enzyme catalyse the same overall reaction:

\[
\text{RCH}_2\text{NH}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{RCHO} + \text{NH}_3 + \text{H}_2\text{O}_2
\]

The substrate specificities of these two enzymes overlap, although the MAOs are also able to oxidize some secondary and tertiary amines, whereas SSAO is not.

The experimental distinction between the two enzymes is often made by their inhibitor sensitivities. The MAOs, which are flavoproteins, are inhibited by some propargylamine derivatives, including clorgyline, deprenyl and pargyline, which are mechanism-based inhibitors that react covalently with the enzyme-bound FAD (see [17]). In contrast, SSAO is insensitive to inhibition by such compounds. Indeed, the even less helpful name of clorgyline-resistant amine oxidase (‘CRAO’) can be found in some of the earlier literature. Since
The MAOs are not inhibited by semicarbazide and some other carbonyl-group reagents, it seemed like a good idea at the time to coin the name SSAO in order to distinguish it from MAO. Earlier work on SSAO has been reviewed extensively [18–20].

The systematic name for SSAO is amine:oxygen oxidoreductase (deaminating) (copper-containing); EC 1.4.3.6, a group which also contains some other, quite distinct enzymes. Unfortunately, several other amine oxidases are also inhibited by semicarbazide. These include diamine oxidase, which is also classified in EC 1.4.3.6, but differs from SSAO in having a high activity towards histamine. Lysyl oxidase (protein-lysine 6-oxidase; EC 1.4.3.13) [21,22], and the flavoprotein polyamine oxidase [N1-acetylspermidine:oxygen oxidoreductase (deaminating), EC 1.5.3.11] [23] are also inhibited by semicarbazide. This adds to the excitement of often not knowing which enzyme is being referred to as SSAO. However, by convention, SSAO is generally used only for the members of the enzymes within EC 1.4.3.6 that are not good at catalysing histamine oxidation.

SSAO is present in plants, animals and microbes. Most mammalian species contain a soluble form of SSAO in blood plasma plus a membrane-bound form of the enzyme that has a relatively high activity in cardiovascular tissue, the lung and adipocytes. It is mainly located on the plasma membrane, and it is believed that the plasma enzyme arises from proteolytic cleavage of the membrane-bound SSAO. The cardiovascular location of the enzyme might suggest that it is involved, together with plasma SSAO, in the removal of potentially toxic amines from the circulation.

**Why is SSAO at all interesting?**

For many years, SSAO appeared to be a particularly boring enzyme. Its best substrate was found to be benzylamine, which is also a substrate for MAO, and the name ‘benzylamine oxidase’ was sometimes used. Clearly, to name an enzyme after a substrate that is not known to occur in mammalian tissues is some indication of a lack of faith in its physiological relevance.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Some enzymes with part-time jobs</th>
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<tbody>
<tr>
<td><strong>Enzyme</strong></td>
<td><strong>EC number</strong></td>
</tr>
<tr>
<td>Aconitase (cytoplasmic)</td>
<td>4.2.1.3</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>4.2.1.1</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>1.2.1.13</td>
</tr>
<tr>
<td>Some glycolytic enzymes – Lens crystallin</td>
<td>–</td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>2.5.1.18</td>
</tr>
<tr>
<td>Glucose-6-phosphate isomerase</td>
<td>5.3.1.9</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase &amp; transketolase</td>
<td>1.2.1.3 and 2.2.1.1</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>3.1.1.7</td>
</tr>
<tr>
<td>Dipeptidyl peptidase IV</td>
<td>3.4.14.5</td>
</tr>
<tr>
<td>5′-Nucleosidase</td>
<td>3.1.3.5</td>
</tr>
<tr>
<td>Peptidyl glycine mono-oxygenase</td>
<td>1.14.17.3</td>
</tr>
<tr>
<td>SSAO</td>
<td>1.4.3.6</td>
</tr>
<tr>
<td>Ketol-acid reductoisomerase (Yeast I1v5p)</td>
<td>1.1.1.86</td>
</tr>
</tbody>
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<thead>
<tr>
<th>Table 2</th>
<th>Altered levels of SSAO in some disease states</th>
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</thead>
<tbody>
<tr>
<td><strong>Condition</strong></td>
<td><strong>Plasma SSAO</strong></td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>↑ (cerebral blood vessels)</td>
</tr>
<tr>
<td>Burns</td>
<td>↓</td>
</tr>
<tr>
<td>Cancer (solid tumour)</td>
<td>↓</td>
</tr>
<tr>
<td>Cancer (breast)</td>
<td>↓ (rat, chemically induced)</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>↑</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>↑ (increases with severity)</td>
</tr>
<tr>
<td>Diabetes (Types 1 and 2)</td>
<td>↑ (human, rat and sheep)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>↑ (rat plasma and kidney)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Unchanged (rat aorta, lung and pancreas)</td>
</tr>
<tr>
<td>Diabetic retinopathy</td>
<td>↑↑</td>
</tr>
<tr>
<td>Diabetic atherosclerosis</td>
<td>↑↑</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>Unchanged (placenta)</td>
</tr>
<tr>
<td>Stroke</td>
<td>No change</td>
</tr>
</tbody>
</table>

There was a flurry of interest when the carbonyl-containing cofactor that was originally thought to be pyridoxal phosphate was reported to be pyroquinoline quinone (‘PQQ’), and this was subsequently shown to be TOPA (2,4,5-trihydroxyphenylalanine; also called 6-hydroxydopa) quinone (see [24]). The enzyme contains 1 mol of copper per active site, which appears to be essential for the conversion of the tyrosine residue in the polypeptide chain of SSAO into TOPA cofactor [25]. The copper does not appear to have any redox role in amine oxidation, and may simply act as a suitably placed positive charge in the active site [26]. In view of what we know of the effectiveness of most copper oxidases, this might appear to be a strange design fault. The observation that the activity of SSAO appeared to be altered in a number of disease states, as summarized in
SSAO, in common with MAO, catalyses a reaction in which each of the products is potentially nasty. Hydrogen peroxide can act as a source of reactive-oxygen-containing radicals, ammonia is known to be toxic in a number of systems, and the aldehyde products formed may also be toxic. The products common to the oxidation of all amines (ammonia and hydrogen peroxide) are, of course, also important metabolic intermediates in a number of different reactions and, before wildly speculating about the toxicity of the SSAO-catalysed reaction products, it would be necessary to show the enzyme to be capable of producing, or significantly contributing to, toxic levels of these compounds under some conditions. They also have other metabolic and, perhaps, signalling functions. However, since there are many metabolic sources of ammonia and hydrogen peroxide, it necessary to know whether there is something special about the localization or regulation of SSAO that makes its metabolites likely to be particularly important in such responses. Unfortunately, except in the case of glucose transport (discussed below), there is little evidence for this.

It has been suggested that one of the roles of SSAO is the formation of $H_2O_2$ for the regulation of the function of the glucose transporters GLUT1 and GLUT4 [39,40]. The hydrogen peroxide formed stimulates the transport of the glucose transporters to the cell surface, and thus promotes glucose transport. This is not a specific property of SSAO, since the externally added $H_2O_2$ and that formed by the MAO-catalysed reaction act similarly. However, a proportion of SSAO in adipocytes has been shown to be associated with the intracellular vesicles containing the GLUT4 glucose transporter, which may suggest a more specific function for this enzyme.

The potential toxicity of some aldehyde products is well documented. The cardiotoxicity of the xenobiotic allylamine has been shown to result from its oxidation to acrolein by SSAO [41]. Formaldehyde, which is the product of methylamine oxidation, can produce irreversible adducts with proteins and single-stranded DNA, among other harmful cross-linkage reactions (see [42]). It might be expected to be oxidized rapidly to formate by the glutathione-dependent formaldehyde dehydrogenase (EC 1.2.1.1). However, that enzyme is not present in the blood plasma, so any formaldehyde produced there would have to be transported into cells, such as the erythrocytes, for metabolism. This may be significant with regard to formaldehyde-induced toxicity in blood vessels [43]. Indeed, the toxicity in vivo of methylamine in the rat was shown to be decreased by the inhibition of SSAO [43].

The consumption of relatively large amounts of creatine as a nutritional supplement, in attempts to enhance sports performance, would result in increased methylamine formation, and it has been suggested that the formation of formaldehyde and $H_2O_2$ from its oxidation might underlie some of the deleterious effects of long-term creatine consumption [44].

Aminoacetone oxidation by SSAO produces methylglyoxal, which is highly cytotoxic. Since the levels of methylglyoxal, as well as those of SSAO activity, are raised in diabetes, this had led to the suggestion that this might contribute to some of the diabetic complications, such as advanced-glycation end product (‘AGE’) formation [38].
may function in this way [45]. MAO is also an amiloride-binding protein, but the plasma and endothelial-surface location of SSAO may make it more important. The enzyme from different sources differ considerably in their binding abilities, and SSAO from ox lung has, for example, been shown to have a low affinity [37]. The possible significance of this binding, which inhibits SSAO activity, for those taking such drugs remains unclear.

Recently, an adhesion protein, vascular-adhesion protein 1 (VAP-1), was found to have sequence identity with SSAO at the cDNA level [46], and was subsequently found to possess SSAO activity. VAP-1 is an endothelial glycoprotein that supports the adhesion of lymphocytes to endothelial cells and mediates lymphocyte recirculation in an L-selectin-dependent manner (see [47] for a review). VAP-1 has been shown to support sialic-acid-dependent adhesion under shear stress, and to mediate tethering of T-cells to the tumour endothelium in human hepatocellular carcinoma [48]. Its cell-surface expression is induced under inflammatory conditions, and this may provide an explanation for the observations that SSAO activity increases under such conditions (see Table 2) [49]. This might appear to be a totally unrelated function of SSAO, although our studies on the interaction of SSAO with amino sugars suggest the H₂O₂ generated in the enzyme-catalysed reaction to be necessary for adhesion [50]. VAP-1 type adhesion is, perhaps not surprisingly, found only in SSAO from some tissues. Since, with the exception of retinal SSAO [51], there appears to be only a single gene for SSAO (see [52]), this suggests some further complexities in the processing of SSAO/VAP-1.

As a final complexity, SSAO appears to be necessary for adipocyte maturation [53,54] and in extracellular matrix development and maintenance, and specifically in the development of normal elastin in vascular smooth muscle [55]. In the case of adipocyte maturation, H₂O₂ formed as a result of substrate oxidation by SSAO appeared to be necessary but, intriguingly, that resulting from MAO-catalysed substrate oxidation was ineffective [53].

Some implications of multitasking

The complexities in the functions of what, at first, seems to be a protein of only minor interest provide an indication of the challenges in determining its quantitative roles in the different processes and under different physiological conditions. The suggestion that SSAO has been put there by God so that you can have complications if you are diabetic appears to underlay some of the literature on this enzyme. Indeed, similar ideas have been commonly expressed for other proteins. Clearly, the effects of inhibition may be far more diverse than might be concluded from a narrow focus on its substrates.

Effects of enzyme modulation

Knock-out experiments are already more difficult to interpret than was thought to be the case as judged from several earlier experiments that did not appreciate the possibilities of redundancy, or that an enzyme may have different functions during development than it does in the adult. An example of this is the A isoenzyme of MAO (EC 1.4.3.4; MAO-A). The human phenotype resulting from the loss of the gene for this enzyme is characterized by compulsive aggression [56], whereas inhibition of the enzyme in the adult is anti-depressive, but without the extreme symptoms seen with the ‘knock-outs’. Similarly MAO-A knock-out mice behave aggressively [57], whereas essentially complete inhibition of the enzyme in the adult has much less marked effects. These differences may be attributable to 5-HT, a substrate for this enzyme, having a key role in brain development. Interestingly, maintenance of 5-HT levels is also important for the development of the whisker barrel in the rat [58], but apparently not in its maintenance in the adult.

If these developmental aspects are viewed in the light of multiple protein and, in the case of enzymes, substrate/product functions, knock-out experiments might be expected to result in a more complex, and interesting, phenotype than the simple loss of a single function. As mentioned above, there are also implications for the development and use of enzyme inhibitors for therapeutic purposes.

Classification

Attempts to provide functional classifications for proteins have been fewer than those determined by sequence, superfamily or parent DNA sequences. The well-established IUBMB enzyme nomenclature and classification list [59] classifies enzymes according to the reaction catalysed. It is parsimonious in that, if a number of quite different proteins catalyse the same reaction, such as yeast and mammalian alcohol dehydrogenase, they have the same classification (EC) number. Similarly, isoenzymes are treated as a single entity. Only if there is a significant difference in the reactions catalysed are different EC numbers used (see [60]). This procedure made sense when it was introduced, because nothing was known about the structures of the proteins involved. Despite its limitations, it still forms the basis for enzyme identification in a large number of databases. In the rather few cases where an enzyme was known to have another, unrelated, function, this might be referred to in a comment. For example, the entry for NADPH:quione reductase (EC 1.6.5.5) has the comment, ‘In some mammals the enzyme is abundant in the lens of the eye, where it is identified with the protein ζ-crystallin’.

This will be hardly sufficient in the future. Linkage of each enzyme, perhaps by way of a sequence database, to one of the many separate databases that contains some classification based on other functions might be a way forward. However, this will not be entirely straightforward. In the case of SSAO, for example, not all SSAOs within an organism are VAPs. Whereas the enzyme associated with the vascular endothelium functions in this way, that associated with adipocytes does not. So far, there is no evidence for primary structural differences between these two proteins. It is possible that the adipocyte enzyme is associated with
adhesion to something else, but there are no data to support such an hypothesis. So, at the present state of the art, the binding protein that can be classified as VAP-1 is only a part of the enzyme group known as SSAO.

We are grateful to the Health Research Board for financial support. Thanks are also owed to many people, most of whom are cited in the reference list, for stimulating and convivial discussions.

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Received 17 March 2003

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