N-TERMINAL EXTENSION AND STRUCTURAL-FUNCTIONAL CONSIDERATIONS

With the exception of CysI, the N-terminal sequences of plant AOXs are not well conserved, and this region has been considered independently from the structurally defined four-helix bundle. For instance, although the distance between CysI and CysII in AOXs from 13 different plant species is 50 amino acids, the distance between the predicted N-terminal sequence and CysI varies. However, functional regulation might occur via phosphorylation of the N-terminal extension of AOX. The Musite tool, which has been developed for global prediction of general and kinase-specific phosphorylation sites (46), predicted at least three potential phosphorylation sites in SgAOX [Ser-68, Thr-82, and Tyr-102 (SgAOX numbering)]. Because some of these amino acid residues are well conserved in both thermogenic and nonthermogenic plant AOXs, protein phosphorylation might play a role in controlling the activity through charge-induced conformational changes and/or an interaction with other mitochondrial proteins.

RETROGRADE REGULATION OF AOX

Response to mitochondrial perturbations of the nuclear gene for AOX is the most well studied mitochondria-to-nucleus signaling pathway—referred to as the retrograde regulation system—in plants (31, 32, 52, 79, 101, 110, 129, 130). The transcription factor ABSCISIC ACID INSENSITIVE 4 (ABI4), a regulator of plastid retrograde signaling (79, 70), was first identified as a repressor of AOX1a (49). Although ABI4 seems to be one of the downstream components of a mitochondrial retrograde signaling pathway, the entire signaling cascade is still unknown.

Another regulator that may play a role in the retrograde signaling of *AOX* gene expression is INCREASED SIZE EXCLUSION LIMIT 2 (ISE2) (24). *ISE2* was first discovered as a gene encoding a putative DEVH-box RNA helicase involved in plasmodesmata function during *Arabidopsis* embryogenesis (69). Of particular interest is that in the *ise2* mutant, genes for AOX1a and several external rotenone-insensitive alternative mitochondrial NAD(P)H dehydrogenases (NDBs) were coinduced (24). In this case, the NDBs oxidize NADH to reduce ubiquinone to ubiquinol, which is in turn oxidized by AOX1a. Kakizaki et al. (65) showed that in *A. maculatum*, not only *AOX* gene expression but also the *NDB* transcript and protein levels are significantly higher in thermogenic appendices. Such coordinated gene expression for AOX1a and NDBs also contributes to an energy-dissipating system in thermogenic plants, and *ISE2* should be one of the future target genes for a deeper understanding of retrograde regulation of AOX in plants.

ISE2: INCREASED SIZE EXCLUSION LIMIT 2

DEVH box: a motif found in RNA helicase

NDB: external rotenone-insensitive NAD(P)H dehydrogenase

SUMMARY POINTS

1. The cyanide- and antimycin-A-resistant AOX is a diiron carboxylate protein that is located on the substrate side of the mitochondrial respiratory chain, is non-protonmotive, and results in the net oxidation of ubiquinol and the reduction of oxygen to water.
2. Crystallographic studies have revealed that AOX is a homodimer, with each monomer comprising six long and four short α-helices arranged in an antiparallel fashion, thereby forming a four-helix bundle that acts as a scaffold to bind the two iron atoms.
3. In AOX's oxidized state, the two iron atoms within each active site are linked by a hydroxo bridge and ligated by four highly conserved glutamate residues.

4. A highly conserved tyrosine residue plays a key role in the catalytic cycle, and we propose a catalytic cycle to account for the four-electron reduction of oxygen to water, which involves several short-lived intermediates and radical species.
5. In addition to the classical conserved CysI residues, plant AOX sequences contain an E/DNV or QDC motif that appears to play a role in the posttranslational regulation of activity, the presence of which could be related to the phenotype of thermogenic plants.
6. Mitochondrial retrograde regulation involves coordinated gene expression of AOX and several external alternative mitochondrial NDBs.

FUTURE ISSUES

1. Crystal structures of other AOX proteins are needed to determine whether a homodimeric structure is universal. Information on the N-terminal region is of particular importance because the current structure indicates that the N-terminal region of each monomer is intimately associated with its neighbor and probably plays a key regulatory role in altering the conformation of the four-helix bundle.
2. It is important to obtain structures of both wild-type and mutant forms of AOX in a reduced state to determine whether the reduction results in carboxylate shifts, thereby changing the conformation and primary ligation sphere of the diiron center.
3. Further spectroscopic studies are needed to confirm the role of tyrosine in the catalytic cycle. Such studies should reveal whether short-lived radical species are generated during the turnover of the cycle. The use of mutants, which have altered turnover rates, may prove important in trapping such intermediates.
4. More information is needed on the native structure of AOX, in particular its interaction with other mitochondrial components and the extent to which these interactions are governed by various physiological conditions.
5. Exploring the mechanisms of AOX turnover should reveal the mitochondrial proteases that digest AOX and the possible involvement of the retrograde signaling pathway(s) specifically induced by degradation products.

DISCLOSURE STATEMENT

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The alternative oxidases: simple oxidoreductase proteins with complex functions

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Abstract

The alternative oxidases are membrane-bound monotopic terminal electron transport proteins found in all plants and in some agrochemically important fungi and parasites including *Trypansoma brucei*, which is the causative agent of trypanosomiasis. They are integral membrane proteins and reduce oxygen to water in a four electron process. The recent elucidation of the crystal structure of the trypanosomal alternative oxidase at 2.85 Å (1 Å = 0.1 nm) has revealed salient structural features necessary for its function. In the present review we compare the primary and secondary ligation spheres of the alternative oxidases with other di-iron carboxylate proteins and propose a mechanism for the reduction of oxygen to water.

Introduction

It is generally recognized that the distribution of the AOX (alternative oxidase) is substantially wider than previously thought [1]. In addition to being ubiquitous among the plant kingdom, it is also common among some agrochemically important fungi [such as *Chalara fraxinea* (U.K. die-back Ash disease) and *Magnaporthe grisea* (rice blast fungus)] and protists [1,2]. The AOX is also widespread among human parasites such as *Trypanosoma brucei* (the causative agent of African sleeping sickness) [3,4], intestinal parasites such as *Cryptosporidium parvum* and *Blastocystis hominis* [5,6] and opportunistic human pathogens such as *Candida albicans* [7].

Prior to the publication of the structure of any AOX, sequence analysis, extensive site-directed mutagenesis, EPR and spectroscopic studies [8–18] predicted that it was an integral (~32 kDa) interfacial membrane protein that interacted with a single leaflet of the lipid bilayer, which contained a non-haem di-iron carboxylate active site [10] comparable with that observed in other di-iron proteins such as RNR R2 (ribonucleotide reductase R2 subunit) and MMO (methane mono-oxygenase). Interestingly, the recombinant protein self-assembles when expressed in *Schizosaccharomyces pombe* or *Escherichia coli* without the requirement of any specific co-factors (apart from iron) and attaches itself into the membrane to become a fully viable member of the electron transport chain [19].

Non-haem di-iron-containing enzymes are a ubiquitous and diverse superfamily of metalloenzymes [20]. They can be divided into different subfamilies with a wide range of distinct catalytic functions, such as peroxidation, oxidation, hydroxylation, desaturation, NO reduction and even aging-related disorders and utilize a wide variety of substrates (see [21]). Despite their diverse range of activities, however, the majority of the enzymes in this di-iron family share very common structural elements. These include a common fold involving a four-helix bundle, a bridging carboxylate group across the di-iron centre, the presence of common ligands, such as the widespread motif comprising two histidine residues and four carboxylate groups and importantly the possession of a common catalytic function, namely the activation of molecular oxygen [20,21]. Given these common structural characteristics and yet diverse chemical reactivities, the question arises as to whether changes in chemical reactivity are a reflection of the type or position of the amino-acid residues in the primary and secondary ligation spheres.

The AOX is one of the newest members of the di-iron group of proteins whose structure has recently been elucidated [1,22] and in the present review we compare the structure of this protein with that of other di-iron proteins. We believe that both a structural and mechanistic understanding of this protein is important not only to direct and inform future rational phytopathogenic or anti-parasitic drug design, but also because the structural simplicity and stability of this family of proteins make them an ideal choice for structure–function correlation studies. Furthermore, we also believe that since the AOX is a naturally occurring oxidoreductase containing a minimal four-helical scaffold structure which lacks spectral features that complicate other such respiratory chain proteins, such studies should reveal salient structural features that not only are necessary for its oxidoreductase function, but also will inform how minor variations in the geometry or ligation sphere of the active site of such proteins result in such a diverse range of functions.

Key words: di-iron carboxylate protein, electron transfer, protein engineering, respiration, ubiquinol oxidase.

Abbreviations used: ACP, acyl carrier protein; AOX, alternative oxidase; MMO; methane mono-oxygenase; PCET, proton-coupled electron transfer; RNR R2, ribonucleotide reductase R2 subunit; TAO, trypanosomal AOX.

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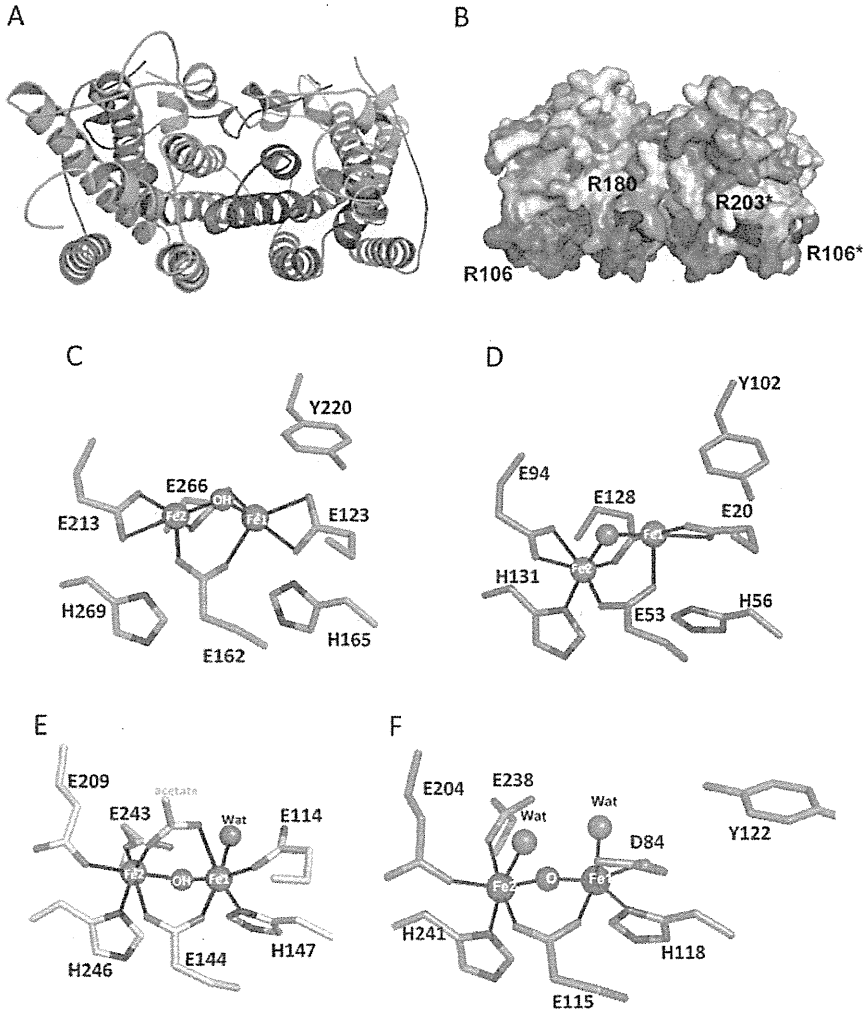
Figure 1 | Structure of TAO and comparison of its active site with other di-iron carboxylate proteins

(**A**) Cartoon representation of the dimeric structure of TAO in which helices are visualized as cylinders roughly parallel to the membrane. (**B**) Surface representation of the TAO dimer showing the hydrophobic and hydrophilic surfaces. Colours are according to the following hydrophobicity scale: red, high hydrophobicity; white, low hydrophobicity (http://www.pymolwiki.org/index.php/Color_h). Conserved basic residues located on the interface between TAO and the membrane (Arg^{106}, Arg^{180}, Arg^{203*} and Arg^{106*}) are coloured blue. (**C**) Di-iron active sites of TAO (green), (**D**) rubrerythrin (cyan), (**E**) α-subunit of MMO (yellow) and (**F**) ribonucleotide reductase R2 subunit (orange). Oxygen and nitrogen atoms are coloured red and blue respectively. Di-iron, hydroxo and μ-oxo atoms are shown as spheres. Co-ordinate bonds to di-iron centres are shown by continuous lines. The non-protein ligand acetate in (**E**) is shown as dark yellow sticks. Wat stands for water molecule. This Figure was generated using PDB code 3VV9.



AOX crystal structure

We were able to recently solve the structure of the AOX in the absence and presence of AOX-specific inhibitors at 2.6 and 2.3 Å (1 Å = 0.1 nm) respectively [PDB codes 3VV9 for TAO (trypanosomal AOX), 3VVA for TAO-AF2779OH complex and 3W54 for TAO-colletochlorin] [22]. This structure represents a major research milestone, for it is not only the first structure of any AOX irrespective of species to be crystallized, but also the first membrane-bound di-iron protein and the last of the respiratory chain oxidoreductases to be solved. Crystal structures indicate that the AOX is a homodimer with each monomer comprising six long α-helices and four short α-helices [22] (Figure 1A). The four long α-helices (2, 3, 5 and 6) are arranged in an antiparallel fashion and form a four-helix bundle which acts as a scaffold to bind the two iron atoms. Within the homodimer, the two monomers are related by a two-fold axis perpendicular to the membrane surface and the dimer interface is built

around α-helices 2, 3 and 4 of one monomer and α-helices 2*, 3* and 4* of its neighbour (Figure 1B). A large hydrophobic face is visible on one side of the dimer surface which is formed by α-helices 1 and 4 plus the C-terminal region of α-helix 2 and the N-terminal region of α-helix 5 (Figure 1B). Similar to other monotopic proteins such as the yeast NADH dehydrogenase (Ndi1) [23] and prostaglandin H_2 synthase [24], this hydrophobic region undoubtedly anchors the protein to the inner surface of the inner mitochondrial membrane via a series of conserved arginine residues which probably interact with the phospholipid headgroups (Figure 1B).

Nature of the active site

The active site is buried deep within the AOX molecule in a hydrophobic environment. Under oxidized conditions the iron atoms within the active site of the AOX are ligated by a single hydroxo-bridge and four glutamate residues, but, unusually for di-iron proteins, the two highly conserved histidine residues are too far away (>4 Å) from the di-iron centre to act as ligands. Evidence in favour of a hydroxo-bridge (rather than an oxo-bridge) include the lack of absorbance above 340 nm, the result that a mixed-valence EPR signal is seen only at very low temperatures and the small value of the exchange coupling constant ($-J$) [16,17]. In addition to the hydroxo-bridge, the iron atoms are bridged by Glu^{162} and Glu^{266}, whereas Glu^{123} and Glu^{213} act as bidentate ligands to Fe1 and Fe2 respectively. The Fe1–Fe2 distances are 3.3 Å, which are compatible with a diferric state rather than a diferrous state in which the distances are ~4.2 Å [21,25]. Such a primary ligation sphere gives rise to a five-co-ordinated di-iron centre with a distorted square pyramidal geometry (Figure 1C) similar to that observed in the reduced form of the castor acyl-ACP (acyl carrier protein) desaturase [25]. The redox-active tyrosine residue (Tyr^{220}), which is highly conserved across all AOXs and is critical for activity, is within 4 Å of the active site (Figure 1C). Figures 1(C)–1(F) compare the primary ligation spheres of the AOX with other di-iron centres under oxidized conditions and it is obvious from such a comparison that the AOXs are unusual inasmuch that all of the other centres are ligated by at least one (such as rubrerythrin; Figure 1D), if not two (such as MMO and RNR R2; Figures 1E and 1F) histidine residues. AOX does share some features common to other di-iron proteins such as the hydroxo-bridge being on the same side of the di-iron axis (compare with MMO)

Although His^{165} and His^{269} appear to be too distant from Fe1 and Fe2 under oxidized conditions to act as ligands, Figure 2 shows that they are within hydrogen-bond distances of Glu^{123}, Gln^{161}, Glu^{162}, Glu^{213} and Asp^{265} (Figure 2A). Gln^{161} and Asp^{265}, which are highly conserved across all AOX sequences, are in the centre of this secondary ligation sphere and extend the hydrogen network to also include Trp^{65}, Tyr^{246} and Trp^{247}. Apart from Trp^{65}, both Tyr^{246} and Trp^{247} are also very highly conserved and Trp^{247}, in addition to playing a key role in the stabilization of the active site, may also be critical for electron and proton transfer, since mutagenesis of this residue results in 100% inhibition (M.S. Albury and A.L. Moore, unpublished work). In RNR R2, Trp^{48} plays a key electron transfer role in the catalytic cycle of this important di-iron protein [26] (Figure 2B) and it is remarkable how similar in terms of ligand position and components of the PCET (proton-coupled electron transfer) network the two functionally dissimilar proteins are (Figure 2C). Such a finding adds further credence to the notion that in the AOXs Trp^{247} may equally have a role in electron transfer, since the PCET network is so highly conserved. Although both AOX and RNR R2 possess a redox-active tyrosine residue, their positions within the ligation sphere differ. For instance, Tyr^{220} (AOX) is located on α-helix 5 (equivalent to helix 3 in RNR R2) in a position where it could be hydrogen-bonded to Glu^{123} or Fe1 and spatially is in a position similar to that of Tyr^{177} of RNR R2 with respect to its distance from Fe1. Tyr^{177}, however, is located on helix 2 (equivalent to helix 3 in AOX), but in both cases the tyrosine residues are within 5 Å of the di-iron centre [1,10].

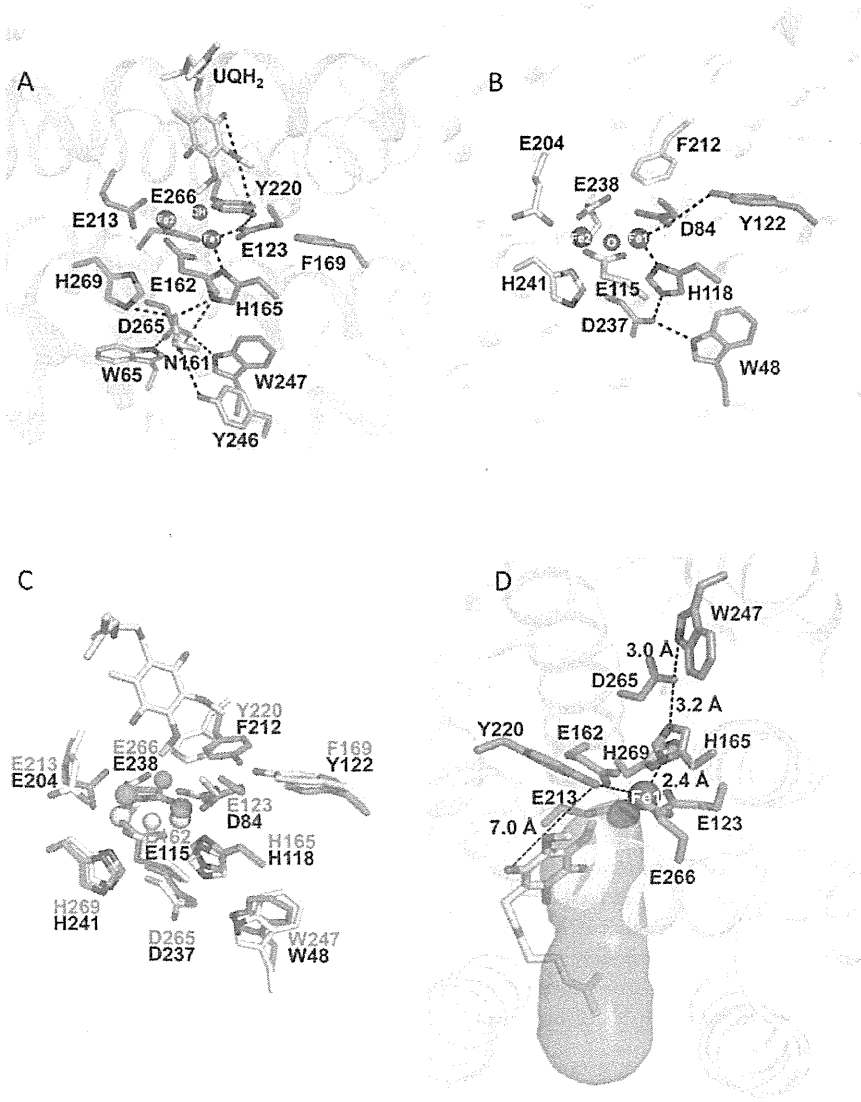
As indicated above, a key research objective within our groups is to determine whether modification of the ligation spheres affects catalytic activity. Indeed, there is evidence in the literature to suggest that minor modifications of the active site can elicit profound changes in enzyme reactivity. For instance, Guy et al. [27] reported that a single amino acid substitution in the active site within the castor bean desaturase enzyme (from threonine to aspartate) switched reactivity from desaturation to oxidation. More recently DeGrado and co-workers [28] described the rational reprogramming of a designed *de novo* di-iron protein that exhibited a dramatic switch of function through a single active-site mutation. With respect to whether it is possible to modify AOX activity through modification of the active site, we mutated Thr^{124}, positioned adjacent to the ligand Glu^{123}, the effect of which resulted in a considerable change in both oxygen affinity and catalytic activity [29]. We have suggested previously that this change in affinity was unlikely to have a direct effect upon AOX, but was more likely to be due to subtle secondary-structure rearrangements that affect iron-ligating residues such as Glu^{123}. In this respect, it is worth noting that mutation of the equivalent carboxylate ligand in RNR R2 causes accumulation of a peroxodi-iron intermediate [30]. Such a species is part of the catalytic cycle of several other di-iron proteins and has indeed been proposed as a possible AOX reaction intermediate [10,31] and such mutants should prove helpful in the elucidation of the catalytic cycle.

Catalytic cycle for the reduction of oxygen to water

It is well established that many di-iron proteins, including stearoyl-ACP Δ^9-desaturase [32], MMO [33] and rubrerythrin [34], are capable of fully reducing oxygen to water and not peroxide as a side reaction to their main respective catalytic activities, and the AOX is no exception to performing such a function. Oxygen activation is a key step in this reductive process [10,31], but, before AOX can react with oxygen,

Figure 2 | Route of proton-coupled electron transfer pathway through the AOX and RNR R2

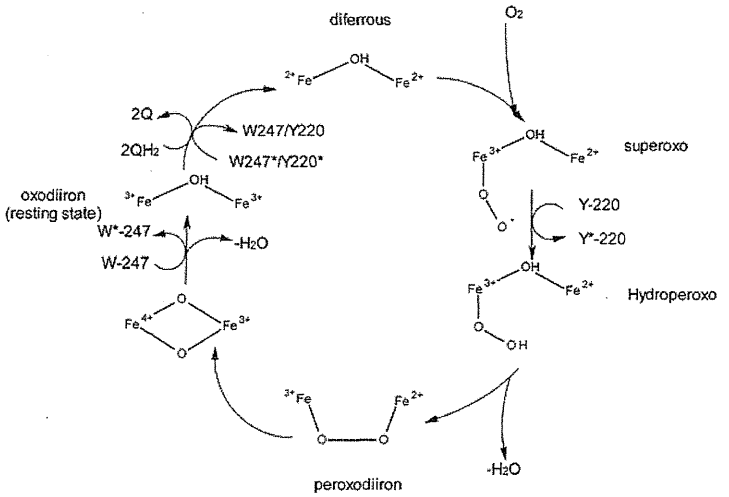
(**A**) In TAO, Asn^{161} is located in the centre of the PCET pathway and forms hydrogen bonds (broken lines) with Tyr^{246}, Asp^{265} and His^{165}, which in turn is within hydrogen-bonding distance of Fe1. Asp^{265} forms a hydrogen-bond network with Trp^{65} and Trp^{247}. Docking modelling suggests that ubiquinol forms a PCET network with Tyr^{220}, Glu^{123} and Fe1. (**B**) PCET pathway in RNR R2 in which Asp^{237} forms hydrogen bonds with His^{118} and Fe1, whereas Tyr^{122} is hydrogen-bonded through Asp^{84} to Fe1. (**C**) Overlay of the proposed PCET pathway of TAO (green residues) with RNR R2 (grey residues). (**D**) Location of the primary (green residues) and secondary (orange residues) ligation sphere of TAO, indicating hydrogen-bond distances between Trp^{247}, Asp^{265} and Fe1. Location of a hydrophobic cavity predicted by CAVER protein analysis software [36] is indicated in green, whereas the predicted position of ubiquinol and its hydrogen-bond distance to Tyr^{220} is indicated in yellow. The hydrogen-bond network is depicted by broken lines. This Figure was generated using PDB code 3VV9.



the resting oxidized state must be reduced by ubiquinol to generate the diferrous centre. FTIR (Fourier-transform infrared) spectroscopy studies [18] suggest that the iron-ligating carboxylates are protonated during the generation of this state. Upon binding of oxygen to the diferrous centre (Figure 3), we have previously suggested that two short-lived intermediates are generated prior to the formation of the peroxodi-iron species [1], similar to that observed in other systems [35]. The first electron, which is transferred from the di-iron core, forms a superoxo species (Fe-O-O•) which is immediately reduced to a hydroperoxo intermediate following the transfer of a proton and electron from a nearby

Figure 3 | Potential intermediates in the oxygen reduction cycle of the AOX

The mechanism is based on that previously proposed for the AOX [1], but differs by proposing that two rather than one transient protein-derived radicals are formed. For details refer to text.



source that can form a stable radical (probably Tyr^{220} or a bound ubiquinol). Following these transfers, the di-iron core rearranges to form a peroxodi-iron species, losing water in the process. A second rearrangement follows with the bridging peroxo migrating between the irons, allowing for homolytic cleavage of the O-O bond and the formation of the diamond core. Transfer of protons from the carboxylates and reformation of the bidentate arrangement around the irons allows for the formation of the second molecule of water and regeneration of the resting state occurs following the transfer of a proton and electron from a second amino acid which is in close vicinity of the active site. The oxidation of two ubiquinol molecules completes the cycle by reducing the amino acid radicals and the iron atoms and re-protonating the carboxylates as indicated above and in Figure 3.

Evidence in favour of such a catalytic cycle include: (i) the generation of a mixed-valence Fe(II)/Fe(III) EPR signal observed following the introduction of molecular oxygen to a fully reduced sample [16]; (ii) the occurrence of a carboxylate shift following reduction of the oxodi-iron species to form the diferrous state [18]; and (iii) the location of Tyr^{220} in a catalytically active position (within 4 Å of the bimetallic active site) [22] and the fact that this residue is universally conserved across all AOX species and is essential for catalytic activity [1].

In the above scheme we suggest that, in addition to ubiquinol and tyrosine, the catalytic cycle may also include a second protein-derived radical (Figure 3) as is the case with RNR R2 [26]. We have previously suggested that, similar to RNR R2, a highly conserved tryptophan residue (Trp^{150}) may play such a role since site-directed mutagenesis studies suggested that this residue was critical for activity [29,31]. The crystal structure reveals, however, that this residue is too far away from the di-iron centre to play a role in electron transport and is more than likely to be involved in π–π interactions with Phe^{276} [22]. As indicated in Figure 2, however, Trp^{247} is within the secondary ligation sphere and hence could act as an electron donor in a manner analogous to that described for RNR R2. What is not immediately obvious, however, is the route of the electron transfer pathway for the re-reduction of the Trp^{247} radical. It is conceivable that oxidation of Trp^{247} is merely part of a back-up mechanism to prevent the build-up of a highly reactive Fe(IV), thereby allowing a more stable Fe(III) species to exist when there is no bound ubiquinol. Alternatively, it is also possible that loosely bound ubiquinol serves as the re-reductant for both protein-based radicals as depicted in the catalytic cycle.

Future directions

Although recent major advances in our understanding of the structure of the AOX has allowed us to definitively characterize AOXs as monotopic membrane-bound ubiquinol oxidoreductases that possess a di-iron carboxylate active-centre, many important problems remain to be solved. Further high-resolution structures of the TAO protein under reducing conditions are required to understand at an atomic level whether carboxylate shifts occur as a result of conformational changes due to substrate binding. For instance, the extent to which two protein-based radicals are utilized and whether high-valence iron species are generated in the diamond core during the oxidation/reduction cycle. Furthermore, species-specific differences in the N-terminal tails and the presence or absence of regulatory motifs within the main sequence suggest that a more complete model of how activity affects function will have to await high-resolution structures of the plant and fungal enzymes. An interesting question from attempts to crystallize the plant

and fungal enzymes to date is whether difficulty in obtaining such structures derives from the dynamic properties of the N-terminal tail interaction. Indeed N-terminal truncated versions of the plant AOX lack activity, but they do form dimers (M.S. Albury and A.L. Moore, unpublished work), suggesting that the N-terminal tail is important for activity, but not dimerization.

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Review

Recent advances in the diagnosis and control of *Schistosoma japonicum* infection in animals

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ABSTRACT

Other than human, more than 40 mammals have been known to host the blood fluke *Schistosoma japonicum*. These animals served as "silent" sources of this parasitic disease in the transmission ecosystem of the parasite. However, diagnosis and control for animal schistosomiasis are not so well-developed and are almost lacking in endemic countries such as the Philippines. Although massive efforts have been done to control schistosomiasis in humans, the lack of preventive measures directed to the animal reservoir hosts might be an important loophole in the existing control mechanics for this parasitic disease. This paper will tackle the updates on disease surveillance and control programs currently directed to important animal reservoirs like water buffaloes, cattles and dogs. These advances should be utilized effectively towards the convergence of the public health and veterinary sectors for the possible elimination of the zoonotic schistosomiasis.

Keywords : *Schistosoma japonicum*, schistosomiasis, reservoir hosts, zoonoses, disease surveillance, disease control.

1. INTRODUCTION

Zoonotic schistosomiasis is mainly caused by the Asian human schistosomes *Schistosoma japonicum* and *Schistosoma mekongi*. *S. japonicum* is different from other human schistosomes because of its wide range of domestic and wild mammalian reservoir hosts. It has been identified in over 40 different species of wild and domestic animals [11]. These zoonotic infections considerably complicate the control activities implemented in endemic countries. In China and the Philippines, reservoir hosts play a role in maintaining the parasite, especially in areas where dense populations of humans co-exist side by side with dense snail population. The close proximity of animals such as the dogs and water buffaloes to the human hosts (Fig. 1) is an important factor in maintaining this parasite in endemic localities. On the other hand, *S. mekongi* was also found among dogs [20] and pigs [31]. However, the transmission mechanism between animals and human has only been indicated so far with *S. japonicum* [25] but not yet in *S. mekongi*.

2. Role of Animals in Schistosomiasis

Animals have been proven to be possible sources of the schistosome parasite in schistosomiasis endemic

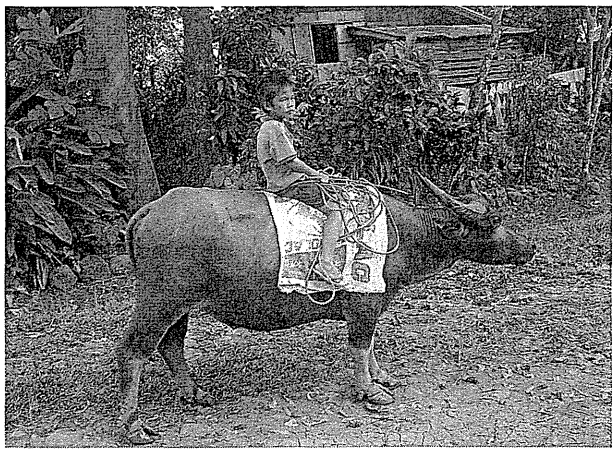

Fig. 1. An active transmission of *Schistosoma japonicum* infection between human and animals including the water buffalo was seen in endemic areas such as the Calatrava, Negros Occidental, the Philippines.

countries. In China, the most important reservoir hosts for *S. japonicum* are bovines which include cattle and water buffalo [9, 27]. It has been known that in schistosomiasis-endemic areas in China, a large proportion of the environmental contamination is due to bovine defecation [39]. In Indonesia, a small portion of domestic animals such as water buffaloes and wild animals were found to be infected with schistosomes according to one study done [12]. A wide range of animals in the Philippines such as rats, cats, dogs, pigs, cattle and water buffaloes was found to be potential hosts for schistosomiasis using various parasitological and immunological assays [4, 8, 19]. A population genetic study done among these animal reservoir hosts in one endemic province in the Philippines suggests that there is a very high level of transmission between humans and dogs [26]. On the other hand, half of the water buffaloes examined in two separate studies were found to be infected with *S. japonicum* [1, 34]. These results somehow suggest that dogs and water buffaloes are the important reservoir hosts of schistosomiasis in the Philippines.

3. Zoonotic Surveillance

Detection of schistosome eggs remains to be the gold standard for schistosomiasis diagnosis in both humans and animals but due to the issues in the sensitivity of this coprological method, crude egg antigen-based immunodiagnostic techniques like COPT and ELISA are usually used instead. However, crude antigens can cause cross-reactions affecting its specificity to schistosomiasis diagnosis and massive production of crude egg antigen for large scale diagnostic purposes such as epidemiological surveys is difficult and tedious. Recombinant antigens which are easier to produce have been characterized and studied for their diagnostic potentials to possibly replace crude antigens. And so far, only a few defined antigens have been identified for serological diagnosis of animal schistosomiasis as seen in Table 1. These antigens were tested using animal samples and yield high sensitivity and specificity ranging from 78 to 100%. These recombinant antigens therefore can be used to improve schistosomiasis surveillance in endemic areas. However, sensitivity and specificity alone will not ensure the success of zoonotic surveillance. Another criterion to consider is the universality of the antigen for schistosomiasis diagnosis. Majority of these antigens were examined in either cattle or water buffalo. A tandem repeat protein Sj1TR was also tested for both humans [2] and water buffaloes [1] but was only found good for the latter. Only thioredoxin peroxidase-1 (SjTPx-1) stood out among these antigens as it was tested and proven to have good diagnostic potentials in human [2], cattle [13] and water buffalo [1] serum samples. Examining the potential of SjTPx-1 on other animal reservoir hosts like dogs and rats to develop a more universal diagnostic test for both human and animals is now underway. A multi-species diagnostic test will surely strengthen the disease surveillance for schistosomiasis.

As for the coprological techniques, a new procedure known as the formalin-ethyl acetate sedimentation-digestion technique has been developed for improved

Table 1. List of recombinant antigens characterized and tested for the diagnosis of animal schistosomiasis with good immunodiagnostic potentials

Antigen	Property	Samples Tested
Sjc26GST	26 kDa Glutathione-S-transferase (GST)	Water buffalo [6]
FBPA	Fructose-1,6-biphosphate aldolase	Water buffalo [24]
rSjGCP-Sj23-Sj28	Mixture of gynecophoral canal protein (GCP), tetraspanin fragment (Sj23) and 28 kDa GST	Cattle [13]
SjTPx1	Thioredoxin peroxidase-1	Cattle [13], human [2], water buffalo [1]
rSjEF1	Elongation factor 1-alpha	Cattle [13]
LHD-Sj23	Large hydrophilic domain of Sj23	Cattle [15]
Sj1TR	Dentin sialophosphoprotein (tandem repeats)	Human [2], water buffalo [1]

visualization of *S. japonicum* eggs in bovine feces [37]. This technique which involves filtration, sedimentation, potassium hydroxide digestion and centrifugation steps prior to microscopy discriminates nearly 70% of debris from the fecal samples and renders the remaining debris translucent. This improvement will be useful in poor endemic localities as it is not costly and does not require expensive equipments.

However in the case of epidemiological studies and surveillance of animal infection in areas that have reached elimination level, a more sensitive and specific diagnostic test is highly required. To answer this need, molecular techniques like polymerase chain reaction (PCR) have also been developed for detecting animal infection. One study has compared microscopy with real-time PCR detecting *S. japonicum* mitochondrial DNA in water buffalo stool samples [26]. Their results showed a very big difference in prevalence between the two techniques and that microscopic-based techniques dramatically underestimate the prevalence of *S. japonicum* infection among the water buffaloes. This has proved that there is really a need for better and improved diagnostic techniques to see the real picture of animal transmission for schistosomiasis.

4. Animal Schistosomiasis Control

Control measures for schistosomiasis include community-based praziquantel chemotherapy, health education, environmental modification and snail control. Despite these available control mechanisms, these parasites are so thoroughly integrated into the ecosystems in which they occur making the schistosomiasis control at the community level very difficult. However, this was not the case in Japan. Largely based on ecological approaches, *S. japonicum* was eliminated in Japan in 1977 just before the drug praziquantel became available [23]. In contrast, most underdeveloped countries cannot do so as they neither have the political will, infrastructure, nor enough funding for the control measures.

In China, schistosomiasis remains to be a major public health concern despite over 50 years of concerted efforts for its control [41]. Until the mid-80's, transmission interruption had reached 60% and transmission control at 14.5% of all the schistosome-endemic areas in China. However, ecological factors like flooding led to snail diffusion and re-emergence of schistosomiasis in the Yangtze River in the recent years [40]. In the Philippines, schistosomiasis has dropped significantly in the prevalence and morbidity of the disease in many areas by chemotherapy [14]. But due to the mobility of the people and other ecological factors, emergence of two new endemic foci has been reported for the past few years [3]. New strategies in the schistosomiasis control therefore should be studied and applied in order to eradicate this disease of antiquity.

A much-needed strategy is the inclusion of zoonotic interventions in the national control programs done in these endemic countries. Previous researches have shown that animal interventions can successfully reduce the prevalence of *S. japonicum* infection among humans [9, 10]. In a five-year praziquantel-based intervention study done around the Poyang Lake in Jiangxi Province, China, simultaneous treatment of water buffaloes has proven to be effective in the decline of human schistosomiasis cases.

The search for a protective vaccine both for humans and animals has been a hot topic of researches in the past decades. Development of transmission-blocking veterinary vaccines in livestock animals is a complementary approach in schistosomiasis control. UV-attenuated cercarial vaccines have been tested successfully in water buffaloes [29] and pigs [30] under field conditions in China. However, there were many inherent problems found such as difficulty on producing quality-controlled, reproducible batches of these vaccines. Thus, vaccine researches resorted to the use of either the protein or DNA vaccines against schistosomiasis [21, 36]. Table 2 shows the list of the *S. japonicum* protein and DNA vaccines tested in animal reservoir hosts. The use of protein vaccines resulted to a worm burden reduction ranging from 17 to 60% in these animals. On the other hand, DNA vaccines lead to reduction of worm burden (16-65%) and liver egg burden (19-72%). Field trials as to measure the effect of this vaccine in the prevalence among humans are still under study in China.

Table 2. List of *Schistosoma japonicum* protein and DNA vaccines tested in reservoir hosts.

Antigen	Code	Property	Reservoir Hosts Tested
Protein Vaccines			
Paramyosin	Sj97	Contractile protein	Native: sheep [33]
			Recombinant: water buffalo [22], pig [5]
Integral membrane protein	Sj23	Membrane protein	Native: water buffalo [28]
			Recombinant: Water buffalo [28], cattle [27], sheep [33]
26-kDA GST	Sj26GST	Enzyme	Native: sheep [38]
			Recombinant: water buffalo [17], cattle [35], pig [18], sheep [33]
FABP	Sj14	Fatty acid binding protein	Recombinant: rat [16], sheep [16]
DNA Vaccines			
Triose-phosphate isomerase	SjTPI	Enzyme	Pig [43], water buffalo [7]
Integral membrane protein	Sj23	Membrane protein	Pig [42], sheep, [28] water buffalo [7, 28], cattle [21]
28-kDa GST	Sj28GST	Enzyme	Water buffalo [21], cattle [21]
31/32-kDa protein	Sj31/32	Enzyme	Goat [32]

Schistosomiasis has been a public health problem for centuries now. Recent advances in the *Schistosoma* research have opened many pathways for designing new strategies in the elimination of this parasitic disease. The real challenge therefore in schistosomiasis control lies in the integration of the public health and veterinary sectors in the battle to schistosomiasis.

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