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REVIEW

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Azoreductase: a key player of xenobiotic metabolism

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Abstract

Azoreductases are diverse flavoenzymes widely present among microorganisms and higher eukaryotes. They are mainly involved in the biotransformation and detoxification of azo dyes, nitro-aromatic, and azoic drugs. Reduction of azo bond and reductive activation of pro-drugs at initial level is a crucial stage in degradation and detoxification mechanisms. Using azoreductase-based microbial enzyme systems that are biologically accepted and ecofriendly demonstrated complete degradation of azo dyes. Azoreductases are flavin-containing or flavin-free group of enzymes, utilizing the nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate as a reducing equivalent. Azoreductases from anaerobic microorganisms are highly oxygen sensitive, while azoreductases from aerobic microorganisms are usually oxygen insensitive. They have variable pH, temperature stability, and wide substrate specificity. Azo dyes, nitro-aromatic compounds, and quinones are the known substrates of azoreductase. The present review gives an overview of recent developments in the known azoreductase enzymes from different microorganisms, its novel classification scheme, significant characteristics, and their plausible degradation mechanisms.

Keywords: Azo dye, Azoreductase, Bioremediation, Biotransformation, Detoxification, Xenobiotics

Introduction

Azo dyes and nitro-aromatic compounds are considered as potential xenobiotics. They are extensively used worldwide in textile, paint, printing, cosmetics, and pharmaceutical industries. A high discharge of untreated wastewater from these industries is the major source of azo dyes to enter into the ecosystem (McMullan et al. 2001; Stolz 2001). Azo dyes containing nitro and amine moieties are toxic and mutagenic to biological systems. Synthetic nitro-aromatic compounds are also potential mutagenic and carcinogenic to biological system (Chung and Cerniglia 1992; Rafii and Cerniglia 1995).

A typical azo dye contains one or more characteristic azo bonds (–N=N–) in their complex aromatic structure. This semicovalent azo linkage makes azo dyes more recalcitrant to microbial degradation, and it is not readily broken down under the environmental conditions. Varieties of physical, chemical, and biological treatment procedures are employed to degrade and detoxify the chemical content and to remove color from dye-containing industrial wastewater. The complex aromatic structures of azo dyes and nitro-aromatic compounds are not being efficiently degraded by conventional treatment methods. Biological treatment methods include microbial biodegradation in aerobic, anaerobic, anoxic, or combined anaerobic/aerobic conditions (McMullan et al. 2001; Robinson et al. 2001). However, all of these methods have limitations and some drawbacks. It has been proven that the use of one individual process may often be not sufficient to achieve complete decolorization or mineralization of dye. Therefore, the dye degradation strategies should consist of a combination of different physical, chemical, as well as biological techniques. Recently, some investigators demonstrated that the biological dye degradation techniques by pure and mixed cultures of bacteria, fungi, and algae are more useful, and technically and economically feasible. Microbial enzyme-based technologies would also be highly efficient methods for removing xenobiotics from environment (Stolz 2001; Robinson

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et al. 2001; Bürger and Stolz 2010; Oturkar et al. 2013). Utilization of different microbial oxidases and reductases demonstrated the complete dye degradation at experimental conditions (Lang et al. 2013; Oturkar et al. 2013).

In higher organisms, degradation of azo compounds is considered to be a detoxification metabolic pathway. Most of the azo compounds are considered as potent pre-carcinogens. The metabolites generated after the breakdowns of azo bonds are supposed to be non-carcinogenic, but in some cases, it may lead to be more carcinogenic (Stolz 2001). Once the azo compounds enter into the human body through ingestion, inhalation, or skin contact, they are initially metabolized via azoreductases to aromatic amines in the skin and the gastrointestinal tract, and further metabolized through the liver (Rafii and Cerniglia 1995). The human gastrointestinal tract contains a complex micro flora comprising at least 400–500 bacterial species. Some of them are isolated and characterized with high activity of azo nitroreductase in the presence of flavins and NAD(P)H (Rafii and Cerniglia 1995; Koppel et al. 2017). However, the precise role of azoreductases in drug metabolism is not yet known. It is well known that azoic drugs are used in the treatment of inflammatory bowel disease. They are activated in the gut by NAD(P)H quinone oxidoreductase. NAD(P)H quinone oxidoreductase is the human ortholog of azoreductase (Ryan et al. 2010a, b). Moreover, the colon-specific drug delivery could also be possible by the azoreductase sensitive system (Rao and Khan 2013). However, the precise biochemical mechanism is still unclear. Recently, Ryan et al. (2011) studied the mechanism of azoreduction and the ability of azoreductase to reduce nitro-aromatic and quinone compounds. The nitro-aromatic compounds are commonly used in nimesulide, nitrofurazone, and tolcapone, and it may lead to hepatotoxicity upon reduction of a nitro group or azo bond by azoreductase or nitroreductase (Rafii and Cerniglia 1995; Ryan et al. 2010a). Therefore, it is very important to explore the drug metabolism pathways in detail. The bacterial azoreductases are known to be involved in drug metabolism via flavin or nicotinamide cofactors. Few metabolic pathways have been suggested for azo dye degradation and detoxification in aerobic and anaerobic conditions. An aerobic pathway involves the azoreductase as the major player and an anaerobic pathway in which the azo compound reduction is mediated by reduced quinone compounds resulting from quinone reductase activities (Kudlich et al. 1997; Liu et al. 2008; Gonçalves et al. 2013). Liu et al. (2009) suggested that the azoreductases might be involved in the detoxification of quinones. There are several therapeutic compounds and drugs, which are quinone based, and it is known that pro-drugs are activated upon reduction by azoreductases in the human body (Wang et al. 2010; Ryan et al. 2010a). In all the suggested pathways, azoreductases are involved in the reductive cleavage of the azo bond as the initial stage in degradation of azo dyes. This has been the topic of interest in the recent years as these enzymes are also involved in carcinogenic compound metabolism and their cleanup from environment. Indeed, in all these processes, the precise biochemical role and the mechanism of azoreductase are still not well explored.

This review presents an overview and evaluation of the properties of the known azoreductase enzymes from different microorganisms, and sheds a light on its significant characteristics. Moreover, it can also provide new insights into its new classification scheme and demonstrate the versatile nature in biodegradation, biotransformation, and detoxification of xenobiotic procedures.

Azoreductase classification

Azoreductases are the diverse group of enzymes widely present in a variety of microorganisms with variations in their structures and functions. Although there is diversity in structure and function, they have a common potential for reduction of azo dyes (Fig. 1). Several azoreductases have been isolated, purified, and biochemically characterized from aerobic and anaerobic microorganisms, and their encoding genes were identified (Zimmermann et al. 1982; Bryant and DeLuca 1991; Punj and John 2009; Matsumoto et al. 2010; Bürger and Stolz 2010; Misal et al. 2011; Morrison et al. 2012). Azoreductases from different sources are diverse in their catalytic activity, cofactor requirement, and biophysical characteristics. Therefore, the interests have been evinced in categorizing the known enzymes showing azoreductase activity and discovering their common evolutionary origin.

Abraham and John (2007) initially classified azoreductases on the basis of secondary and tertiary structures of azoreductase predicted from their amino acid sequences. Due to the low level of sequence homology, azoreductases were further classified on the basis of oxygen tolerance or cofactor requirement, predominantly their flavin and NADH-dependence (Bafana and Chakrabarti 2008). The diversity in gene sequence and structures of aerobic and anaerobic azoreductase appeared to be two distinct azoreductases (Rafii and Coleman 1999; Suzuki et al. 2001; Chen et al. 2004). Based on the oxygen tolerance, azoreductases were classified in two major groups, oxygen-sensitive and oxygen-insensitive azoreductases (Chen et al. 2005; Nakanishi et al. 2001). Bürger and Stolz (2010) characterized the flavin-free oxygen-tolerant azoreductase from *Xenophilus azovorans* KF46F and proposed a cofactor-based three-group classification system but that does not cover every azoreductase enzyme.

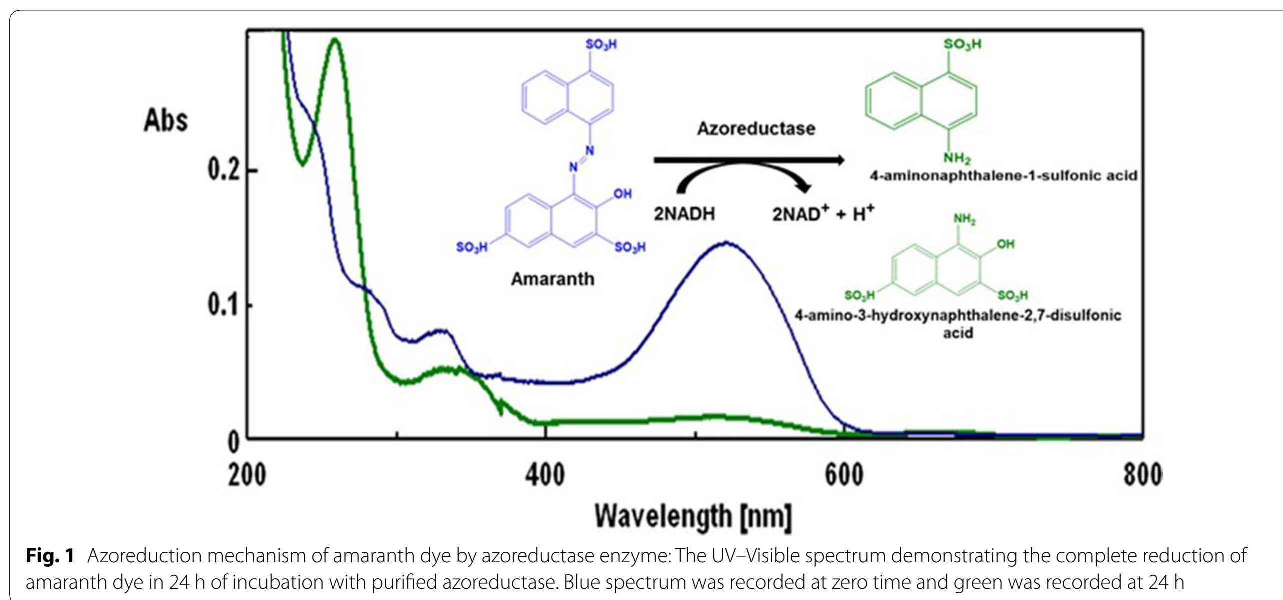

Fig. 1 Azoreduction mechanism of amaranth dye by azoreductase enzyme: The UV–Visible spectrum demonstrating the complete reduction of amaranth dye in 24 h of incubation with purified azoreductase. Blue spectrum was recorded at zero time and green was recorded at 24 h

Flavin-free and NAD(P)H-dependent azoreductases were previously isolated and characterized from alkaliphilic and neutrophilic bacterial strains that are different from flavin-containing azoreductases (Misal et al. 2011, 2013, 2014, 2015). In addition, flavin-containing, NADH and NADPH-dependent azoreductases were isolated from *Enterococcus faecalis* (AzoA) (Chen et al. 2004; Punj and John 2009) and *S. aureus* (Azo1) (Chen et al. 2005). The gene sequence alignment of flavin nucleotide binding domain indicates that this is a highly conserved domain across many azoreductases. The flavin-free azoreductases might have lost the flavin-binding domain due to the mutation. Furthermore, the residues involved in NAD(P)H binding were also found to be conserved in bacterial species (Bafana and Chakrabarti 2008).

It has been observed that there is a significant difference in substrate specificities of all types of azoreductases. Flavin-containing NADH-dependent azoreductase from *Geobacillus stearothermophilus* showed higher activity toward Acid red 88 than Orange II, while NAD(P)H-dependent azoreductase from *Pseudomonas* KF46 showed the highest activities with Orange I and Orange II (Matsumoto et al. 2010; Zimmermann et al. 1982, pp 84). Methyl red is found to be the best substrate of flavin-containing NADPH azoreductases (Chen et al. 2005; Wang et al. 2007). It was also shown that Amaranth, Orange I, and Orange II azo dyes were the efficient substrates of flavin-free NADH azoreductase (Blumel et al. 2002; Chen et al. 2010; Misal et al. 2014). Nitro compounds, 2-nitrophenol, 4-nitrobenzoic acid, 2-nitro-benzaldehyde, and 3-nitrophenol, were also efficiently reduced by flavin-free azoreductases (Misal et al. 2014, 2015). Previously, it was demonstrated that menadione (quinone) is a better substrate for flavin-containing NAD(P)H-dependent azoreductase compared with azo and nitro compounds (Liu et al. 2008). Therefore, azo, nitro, and quinone reductions could also be another criterion to be considered in classification of azoreductases. Some investigators demonstrated a major role of azoreductase in the detoxification of quinones. The quinone reduction activities of azoreductase from different microorganisms and their dependence on NAD(P)H cofactor need to be classified as NAD(P)H quinone oxidoreductases (NQOs) (Liu et al. 2008, 2009; Ryan et al. 2010a, b; Hervas et al. 2012). As reviewed by Ryan (2017) and Koppel et al. (2017), there are very few NQOs characterized from human gut bacterial strains. Recently, Ryan et al. (2014) identified the NAD(P)H quinone oxidoreductase activity in azoreductase from *P. aeruginosa* and demonstrated that the azoreductases and the NAD(P)H quinone oxidoreductases belong to the same FMN-dependent azoreductase superfamily.

Based on flavin cofactor content and nicotinamide dependence or preference, azoreductase superfamily can be classified into five major groups—(1) flavin-containing NADH-dependent azoreductase (Matsumoto et al. 2010; Liu et al. 2007), (2) flavin-containing NADPH-dependent azoreductase (Chen et al. 2005; Wang et al. 2007), (3) flavin-containing NAD(P)H-dependent azoreductase (Qi et al. 2016, 2017a, b; Oturkar et al. 2013), (4) flavin-free NAD(P)H-dependent azoreductase (Blumel et al. 2002; Chen et al. 2010; Misal et al. 2011, 2014, 2015), and (5) flavin-containing NAD(P)H-dependent quinone

oxidoreductases from homo sapiens and human gut bacteria (Liu et al. 2008; Ryan et al. 2014).

Significant characteristics of azoreductase

Aerobic and anaerobic enzyme activity

An azoreductase was initially identified by its ability to break the azo linkage during the dye metabolism under aerobic and anaerobic conditions (Zimmermann et al. 1984; Rafii et al. 1990). Under both conditions, the aromatic amines were generated upon reduction, which are eventually degraded under aerobic conditions by microbial enzymes like mono, dioxygenases, and hydrolases (Idaka et al. 1987; Russ et al. 2000; Hu 2001). The ability of the intestinal microorganism to reduce the azo dyes and nitro groups of various xenobiotic compounds has been known since many years (Rafii and Cerniglia 1995). This nitro-reduction activity is the additional important property of the azoreductase enzyme utilized to reduce toxic nitro-aromatic compounds (Ryan et al. 2011; Misal et al. 2014). Predominant anaerobic bacteria, with azoreductase and nitroreductase activities found in the human intestinal tract, include *Clostridium leptum*, *Eubacterium* sp., *C. clostridiiforme*, *C. paraputrificum*, *Clostridium* sp., and *C. perfringens* (Rafii et al. 1990; Morrison et al. 2012; Morrison and John 2015). Although the azoreductased from aerobic and anaerobic strains have differential properties including molecular weight, optimal pH, temperature, and thermal stability, most of them, however, have structural homology (Bryant and DeLuca 1991; Morrison et al. 2012). More members of anaerobic intestinal bacteria including *Butyrivibrio* sp., *Sphingomonas* sp., *Eubacteria* sp., and *Clostridia* sp. have been reported to possess an azoreduction activity (Rafii and Coleman 1999). However, very few of the anaerobic azoreductases have been isolated and characterized systematically. Morrison et al. (2012) isolated and characterized the novel azoreductase (AzoC) from *Clostridium perfringens* and demonstrated the best enzyme activity in an anaerobic environment at alkaline pH and at room temperature, as summarized in Table 1. Moreover, some researchers demonstrated that flavin reductases are indeed anaerobic azoreductases having the highest activity in the presence of flavins (Kudlich et al. 1997; Russ et al. 2000). Azoreductases having the activities in both aerobic and anaerobic conditions are extremely useful in developing the detoxification technology.

Optimal pH, temperature, and thermal stability

Many of the known azoreductases are stable at pH within a range of 5–9 including some alkaliphilic azoreductases, but most of them show optimal activity at physiological pH and the thermal stability is within a temperature range of 25–85 °C (Table 1). Azoreductases from *Bacillus* sp. (AzrA, AzrB, and AzrC) are stable up to 55, 50, and 70 °C, respectively (Ooi et al. 2007, 2009). The majority of azoreductases shows optimal activity at 35–40 °C. There are some azoreductases (AzrG) stable at higher temperature reported previously from thermophilic *Geobacillus stearothermophilus,* showing optimal activity at 85 °C. Another azoreductase from alkaliphilic *B. badius* has an optimal activity at 60 °C and is stable up to 85 °C (Matsumoto et al. 2010; Misal et al. 2011, 2013). The temperature stability, flavin/nicotinamide dependence, and pH with aerobic/anaerobic natures of azoreductases from various microorganisms are compared and summarized in Table 1. Previously, Misal et al. (2014) demonstrated the highly stable flavin-free NADH-dependent azoreductase from neutrophilic bacterial strain having optimal activity at 70 °C and at neutral pH. The stabilities of azoreductases at alkaline pH and higher temperatures could make them more proficient for industrial as well as the pharmaceutical purposes. Such highly stable and catalytically efficient azoreductases are expected to be investigated in the coming years to combat with xenobiotic contamination.

Kinetic properties and substrate specificities of azoreductases

Azoreductases from a variety of microorganisms demonstrated the diverse kinetic properties and substrate specificities. The kinetic analysis shows that the azoreduction follows a Ping–Pong bi–bi mechanism in all flavin-containing azoreductases. This has been supported with double-reciprocal plots of initial velocity versus concentration of substrate (Nakanishi et al. 2001; Bin et al. 2004; Ito et al. 2008; Misal et al. 2011). Bürger and Stolz (2010) suggested differently ordered bi-reactant reaction mechanism for the flavin-free azoreductases. Zimmermann et al. (1982, 1984) reported the apparent kinetic constants for Orange II and carboxy-Orange II azoreductase, and they demonstrated that the affinity of the enzyme is higher for NADPH compared with NADH. Similar observations were reported from different microorganisms (Table 1). Although these azoreductases are from different sources, they utilize the NADH or NADPH as a source of electron in the presence or the absence of flavin.

In previous reports, the substrate recognitions of respective azoreductases including azo dyes, nitro-aromatic compounds, and quinones have been determined (Zimmermann et al. 1982; Liu et al. 2008; Ryan et al. 2010b). A broad substrate specificities of azoreductases were reported from alkaliphilic *Bacillus badius*, *Aquiflexum* sp., and neutrophilic *Lysinibacillus sphaericus* against a range of azo dyes and nitro compounds (Misal et al. 2011, 2013, 2014, 2015). More interestingly, flavin-containing and flavin-free monomeric azoreductases

Table 1 Summary of known azoreductases from different bacteria

Sr. no	Organism	Aerobic/anaerobic	Flavin/NAD(P)H dependence	Thermal stability in °C (time)	Molecular mass (kDa)	References
1	*Pseudomonas* KF46	Aerobic	NAD(P)H	30	32	Zimmermann et al. (1982)
2	*Escherichia coli*	Aerobic	FMN/NADH	NA	23	Nakanishi et al. (2001)
3	*Bacillus* sp. OY1-2	Aerobic	NAD(P)H	70	20	Suzuki et al. (2001)
4	*Xenophilus azovorans* KF46F	Aerobic	NAD(P)H (flavin free)	NA	30	Blumel et al. (2002)
5	*Pigmentiphaga kullae* K24	Aerobic	NAD(P)H	NA	20.6	Blumel and Stolz (2003)
6	*Rhodobacter sphaeroides* AS1.1737	Aerobic	NADH/NADPH	NA	18.7	Bin et al. (2004)
7	*Enterococcus faecalis*	Aerobic	FMN/NADH	NA	23	Chen et al. (2004)
8	*Pseudomonas aeruginosa*	Aerobic	NADH	35	29	Nachiyar and Rajakumar (2005)
9	*Staphylococcus aureus*	Aerobic	FMN/NADPH	55	85 (tetramer)	Chen et al. (2005)
10	*Bacillus* sp.	Aerobic	NADH	45	23	Ooi et al. (2007)
11	*Pseudomonas aeruginosa* PAO1	Aerobic	NAD(P)H	50 (10 min)	23	Wang et al. (2007)
12	*Pigmentiphaga kullae* K24	Aerobic	NADPH (flavin free)	NA	22	Chen et al. (2010)
13	*Geobacillus stearothermophilus*	Aerobic	FMN/NADH	65 (1 h)	23	Matsumoto et al. (2010)
14	*Bacillus badius*	Aerobic	NADH	60	43	Misal et al. (2011)
15	*Bacillus lentus* BI377	Aerobic	NAD(P)H	70 (3 days)	32	Oturkar et al. (2013)
16	*Aquiflexum* sp.	Aerobic	NADH/NADPH	80	80	Misal et al. (2013)
17	*Pseudomonas putida* MET94	Aerobic	NAD(P)H	NA	21	Gonçalves et al. (2013)
18	*Brevibacillus laterosporus* TISTR1911	Aerobic	FMN/NADH	NA	23	Lang et al. (2013)
19	*Lysinibacillus sphaericus*	Aerobic	NADH (flavin free)	70 (30 min)	29	Misal et al. (2014)
20	*Shewanella xiamenensis* BC0	Aerobic	NAD(P)H	20	26	Zhang et al. (2016)
21	*Halomonas elongata*	Aerobic	NADH	NA	22	Eslami et al. (2016)
22	*Rhodococcus opacus* 1CP	Aerobic	NAD(P)H	40	25	Qi et al. (2016, 2017b)
23	*Clostridium perfringens*	Anaerobic	FMN/NADH	NA	90.4 (tetramer)	Morrison et al. (2012)
24	*Shewanella oneidensis* MR-1	Anaerobic	NADH	25	27	Yang et al. (2013)

NA not available

show a very narrow substrate specificity range (Blumel et al. 2002; Blumel and Stolz 2003). However, the polymeric flavin-dependent and flavin-free azoreductase families efficiently catalyze the wide range of substrates. (Suzuki et al. 2001; Chen et al. 2005; Liu et al. 2008; Misal et al. 2014).

Azoreductase structure and mechanism

Azoreductases identified from various sources were either monomeric or homo-dimeric in nature. Tetrameric form of NADPH-dependent azoreductase from *S. aureus* has also been reported as an exception of flavoprotein (Nakanishi et al. 2001; Chen et al. 2005; Liu et al. 2007). Flavin-free azoreductases were reported to be monomeric in nature (Bürger and Stolz 2010; Misal et al. 2011, 2013, 2015). The FMN-dependent AzoA from *Enterococcus faecalis* exists as a homo-tetramer in solution that is composed of two functional dimers. Each monomer binds with one molecule of FMN as a cofactor and one molecule of substrate methyl red noncovalently (Liu et al. 2007, 2008). The FMN-dependent AzoR from *E. coli* and AzrA from *Bacillus* sp. B29 also exist as homodimers (Fig. 2) (Ito et al. 2008; Yu et al. 2014). Recently, azoreductases were investigated from alkaliphilic and neutrophilic bacterial strains having the monomeric appearance on size-exclusion column chromatography

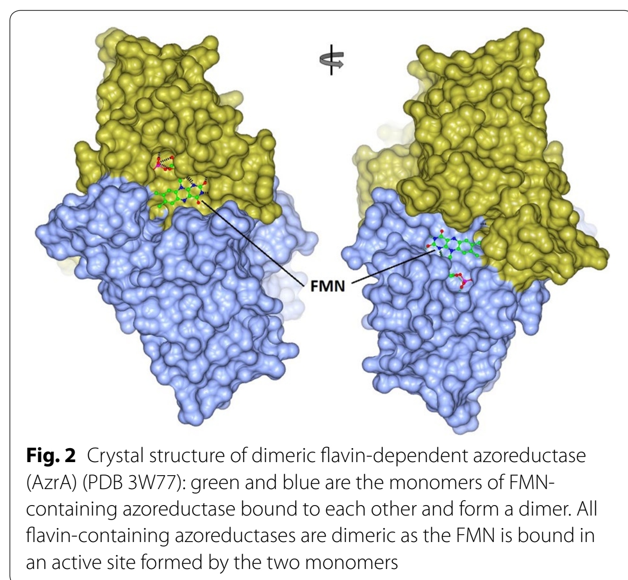

Fig. 2 Crystal structure of dimeric flavin-dependent azoreductase (AzrA) (PDB 3W77): green and blue are the monomers of FMN-containing azoreductase bound to each other and form a dimer. All flavin-containing azoreductases are dimeric as the FMN is bound in an active site formed by the two monomers

and SDS PAGE (Misal et al. 2011, 2013, 2014). Bürger and Stolz (2010) reported a similar form of azoreductase from *Xenophilus azovorans* KF46F.

Aerobic and anaerobic bacterial strains produce the azoreductases that are sensitive or insensitive to oxygen. Different azoreductase enzyme activities with their reduction mechanisms were suggested for each condition. In several reports, it has been hypothesized that the azoreduction is mediated by a flavoprotein in the microbial electron transport chain. This flavoprotein catalyzes the generation of reduced flavins (FMN or FAD) by re-oxidation of reduced NADH or NADPH (Russ et al. 2000). Previously, the anaerobic reduction mechanism of azo dyes was suggested in the *S. xenophaga* BN6. In this mechanism, the reduced flavins transfer electrons to the azo compound (the terminal electron acceptor), thereby reducing the azo bonds and being concurrently re-oxidized. The chemical reaction between the dye and an electron carrier, as well as the occurrence of enzymatic reduction of the electron carrier can both be intracellular and extracellular. However, cofactors like FAD, FMN, NADH, and NADPH, as well as the enzymes reducing these cofactors are located in the cytoplasm (Russ et al. 2000). In another mechanism of aerobic reduction of azo dyes, the azoreductases catalyze the reduction reaction in the presence of molecular oxygen and reducing equivalents as a cofactor (Stolz 2001).

Many azoreductases have been isolated and characterized from variety of microorganisms, but very few of them especially flavin-dependent azoreductases are crystallized and solved for the structure. Structure determination of azoreductase is an initial step toward the elucidation of their molecular mechanism and function (Blumel and Stolz 2003; Ryan et al. 2010a; Misal et al. 2011; Morrison et al. 2012). Liu et al. (2007) explored the structure of FMN-dependent azoreductase (AzoA) from *E. faecalis*. Ito et al. (2008) determined the crystal structure of FMN-dependent NADH-azoreductase (AzoR) from *E. coli* under several conditions to understand the reaction mechanism of the enzyme. It was confirmed that the reduction reaction proceeds via two-cycle ping-pong mechanism (Nakanishi et al. 2001). Further, it became clear from Ryan et al.'s (2010a) report in which they determined the structure of azoreductase and proposed the reduction mechanism. Wang et al. (2007) determined the crystal structure of *P. aeruginosa* azoreductase (paAzoR) with the homology models of paAzoR2 and paAzoR3, and proposed the mechanism for reduction of FMN by NADPH in azoreductases. Further, Wang et al. (2010) determined the structure of *P. aeruginosa* PAO1 (paAzoR1) in complex with the substrate methyl red in order to reveal the role of tyrosine 131 in the active site of the enzyme. Previously, similar mechanism was proposed for reduction of FMN by NADH via hydride ion (Nissen et al. 2008). Yu et al. (2014) supported this existing mechanism with some more details. They determined the crystal structures of AzrA and AzrC complexed with Cibacron Blue (CB), Acid Red 88 (AR88), and Orange I (OI) but were unable to provide the binding of NAD(P)H.

The oxidized FMN at the active site of the enzyme is reduced by NADH. The reduced FMN provides one or two hydrides from N atoms by one or two cycles to the azo bond (Fig. 3). This known azo dye reduction mechanism always involves the reducing equivalents, e.g., FMN, NADH, and NADPH, which can reduce the azo compounds to a hydrazine in the first phase, and further the resulting hydrazines are converted into two amines (Nissen et al. 2008; Wang et al. 2010; Yang et al. 2013; Yu et al. 2014). However, an electron-transfer mechanism during the reduction process in flavin-free azoreductases is not well established by experimental methods.

Potential applications of azoreductases

To date, several microorganisms including bacteria, fungi, and yeast demonstrated efficient degradation of azo dyes and quinones. The degradation or decolorization of azo dyes has always been associated with oxidoreductase enzyme system (Stolz 2001; Bürger and Stolz 2010; Lang et al. 2013). Azoreductases are the major components of the oxidoreductase enzyme system and have demonstrated the ability to reductively cleave the azo bond, and efficiently reduce nitro group in nitro-aromatics at experimental level (Zimmermann et al. 1982; Misal et al. 2014, 2015). The applicability of oxidoreductase enzyme system was first demonstrated by Mendes et al. (2011) who observed maximal decolorization and

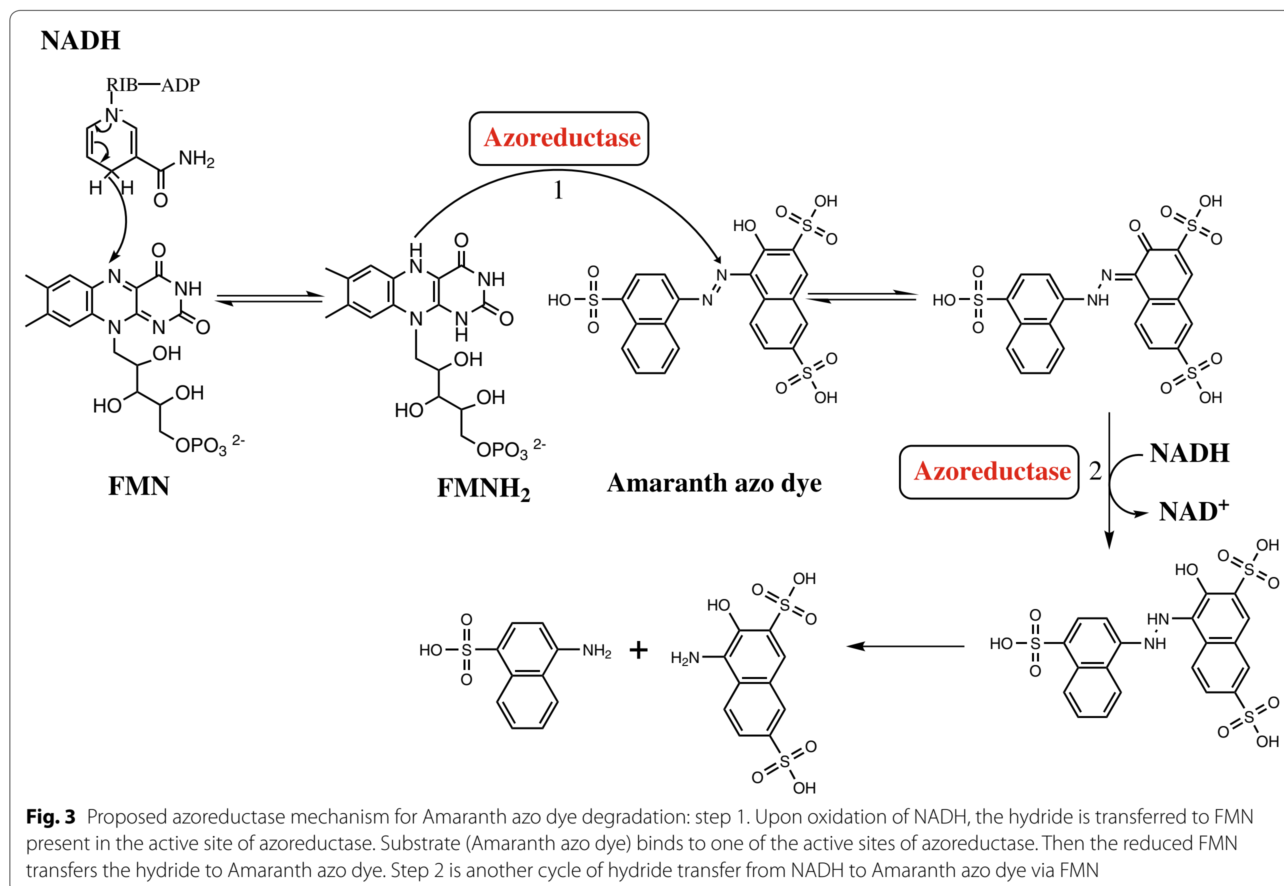

Fig. 3 Proposed azoreductase mechanism for Amaranth azo dye degradation: step 1. Upon oxidation of NADH, the hydride is transferred to FMN present in the active site of azoreductase. Substrate (Amaranth azo dye) binds to one of the active sites of azoreductase. Then the reduced FMN transfers the hydride to Amaranth azo dye. Step 2 is another cycle of hydride transfer from NADH to Amaranth azo dye via FMN

detoxification of 18 azo dyes and three model wastewaters. About 80% decolorization and detoxification of azo dyes by more than 50% show that the oxidoreductase–enzyme system could potentially be used to treat the toxic azo-dye-containing effluent. Vijayalakshmidevi and Muthukumar (2015) reported efficient decolorization and degradation of textile effluent by three bacterial cocultures. They claimed that the increased degradation was due to the oxidoreductase enzymes including laccase, NADH–DCIP reductase, and azoreductase. Recently, Shin et al. (2017) showed the azoreductase/azo–rhodamine reporter system can be efficiently used to monitor the biological events in the live cells at the single cell level. Similar approach was utilized previously by Liu et al. (2015) to track the microbial degradation of azo dyes in the living cells. Moreover, for the clinical purpose, the ability of azoreductase to reduce azo and nitro compounds is exploited to treat inflammatory bowel disease (IBD) and ulcerative colitis (Lautenschlager et al. 2014). The activation of nitrofuran and other nitro-aromatic drug by azoreductase or nitroreductase strategy is commonly used in the treatment of urinary tract infections (Garau 2008).

Conclusions

This review hopefully gives an insight into physical and biochemical properties of known azoreductases with their novel classification scheme. Moreover, this information would be useful in developing effective methods to control and improve the enzymatic degradation process in wastewater-treatment systems as well as in detoxification of carcinogenic azo dyes.

In conclusion, azoreductase enzyme superfamily comprises a large number of enzymes with azo bond-reduction capability. In the coming years, more efforts are essential to characterize azoreductases from environment and human gut bacterial strains, and their properties could be altered in order to enhance their catalytic efficiency, substrate specificity, and enzyme stability. The azoreductases that have optimal activity at wide range of pH, temperature with wide substrate specificity could be potentially important in developing xenobiotic degradation technology. Molecular understanding of azoreductase superfamily and its specific action at each stage of xenobiotic metabolism will also remarkably help to develop the novel azo pro-drugs for cancer chemotherapies and colon diseases.

Abbreviations

NADH: nicotinamide adenine dinucleotide; NADPH: nicotinamide adenine dinucleotide phosphate; FMN: flavin mononucleotide; FAD: flavin adenine dinucleotide; AzoA: azoreductase from *Enterococcus faecalis*; Azo1: azoreductase from *S. aureus*; NQOs: NAD(P)H quinone oxidoreductases; AzoC: azoreductase from *Clostridium perfringens*; AzrA, AzrB, and AzrC: azoreductase A, B, and C from *Bacillus* sp.; AzrG: azoreductase from *Geobacillus stearothermophilus*; AzoR: azoreductase from *E. coli*; paAzoR: azoreductase from *P. aeruginosa*.

Authors' contributions

SAM and KRG conceptualized and wrote the manuscript. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Analysis from the available literature are included in the manuscript.

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