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SYMPOSIUM

Review of Glucose Oxidases and Glucose Dehydrogenases: A Bird's Eye View of Glucose Sensing Enzymes

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Abstract

The evolution from first-generation through third-generation glucose sensors has witnessed the appearance of a number of very diverse oxidoreductases, which vary tremendously in terms of origin, structure, substrate specificity, cofactor used as primary electron acceptor, and acceptable final electron acceptor. This article summarizes our present knowledge of redox enzymes currently utilized in commercially available glucose monitoring systems to promote a fuller appreciation of enzymatic properties and principles employed in blood glucose monitoring to help avoid potential errors.

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Introduction

Ever since Clark and Lyons[1] introduced the first enzyme sensor employing glucose oxidase (GOx) and an oxygen electrode for glucose monitoring, extensive studies have been carried out to develop improved enzyme-based systems for monitoring glycemic levels. First-generation blood glucose monitoring systems employed oxygen as the electron acceptor, determining glucose concentration by following either the consumption of oxygen or the liberation of hydrogen peroxide (**Figure 1**). In second-generation sensors, enzymes transfer electrons to artificial electron acceptors (also referred to as electron mediators or redox dyes) instead of oxygen to avoid interference from other redox species. The reacted (reduced) artificial electron acceptors are monitored colorimetrically or electrochemically. Third-generation sensors employ direct electron transfer to the electrode, thus eliminating toxic artificial electron mediators and avoiding errors due to variations in the concentration of oxygen in blood samples. In addition to the three generations, blood glucose monitoring systems are further categorized based on the type of enzyme employed, whose individual characteristics vary tremendously according to several diverse criteria: reaction toward substrates, availability of electron acceptors, the nonprotein cofactor component (primary electron acceptor), protein structure (from primary to quaternary), presence or absence of glycosylation,

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Abbreviations: (FAD) flavin adenine dinucleotide, (GDH) glucose dehydrogenase, (GMC) glucose/methanol/choline, (GOx) glucose oxidase, (NAD) nicotine adenine dinucleotide, (NADP) nicotine adenine dinucleotide phosphate, (PQQ) pyrroloquinoline quinone, (SMBG) self-monitoring of blood glucose

Keywords: biosensing, glucose dehydrogenase, glucose oxidase, point-of-care testing, self-monitoring of blood glucose

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as well as the host microorganism for the natural or recombinant production of the enzyme.

Oxidoreductases that act on the CH–OH group of donor molecules are classified into the Enzyme Commission group EC 1.1. This group includes several oxidoreductases employed for glucose monitoring, all of which act at the reducing end of glucose, on the first hydroxyl group. These enzymes are divided into two groups, GOxs and glucose dehydrogenases (GDHs), depending on their ability to react with external electron acceptors. Glucose oxidases are defined as oxidoreductases that can utilize oxygen as the external electron acceptor, liberating hydrogen peroxide, and are categorized in the EC 1.1.3 group. Glucose dehydrogenases are defined as oxidoreductases that are unable to utilize oxygen as the electron acceptor and instead transfer electrons to various natural and artificial electron acceptors. Glucose dehydrogenases are further subdivided according to their redox cofactors, which are the essential nonprotein component that act as the primary electron acceptor (**Figure 2**). The GDHs utilizing the cofactor nicotine adenine dinucleotide (NAD) or nicotine adenine dinucleotide phosphate (NADP) are categorized in the EC 1.1.1 group, whereas those utilizing the pyrroloquinoline quinone (PQQ) cofactor belong to group EC 1.1.5. The remaining GDHs belong to the EC 1.1.99 group, corresponding to redox enzymes utilizing "other" electron acceptors and which includes the GDHs utilizing flavin adenine dinucleotide (FAD) as the cofactor. Interestingly, whereas GOxs are able to utilize oxygen as well as a variety of other electron acceptors, FAD-harboring GDHs are unable to utilize oxygen, despite their harboring the same redox cofactor and possessing significant structural similarities with GOx.

This short review summarizes our present knowledge of redox enzymes currently utilized in commercially available glucose monitoring systems, GOxs and GDHs. Our goal is to promote a fuller appreciation of enzymatic

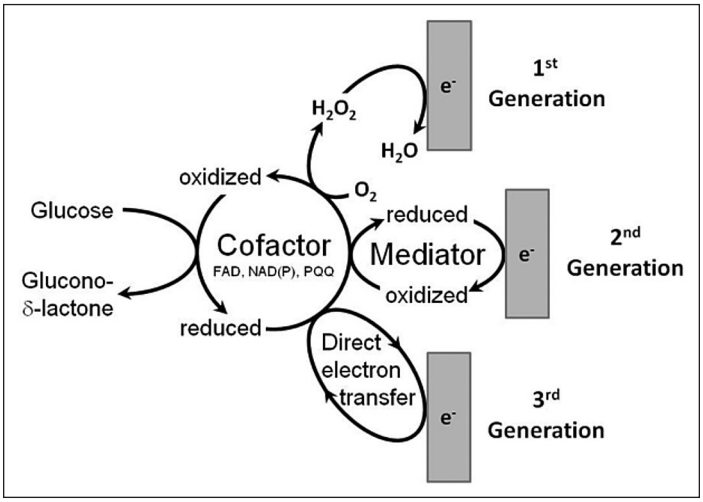

Figure 1. Schematic representation of the principles of first-, second-, and third-generation glucose sensors. Electrons from the glucose oxidation reaction are first taken up by the enzyme's cofactor (primary electron acceptor) and transferred to either oxygen (first generation), an electron mediator (second generation), or directly to the electrode (third generation).

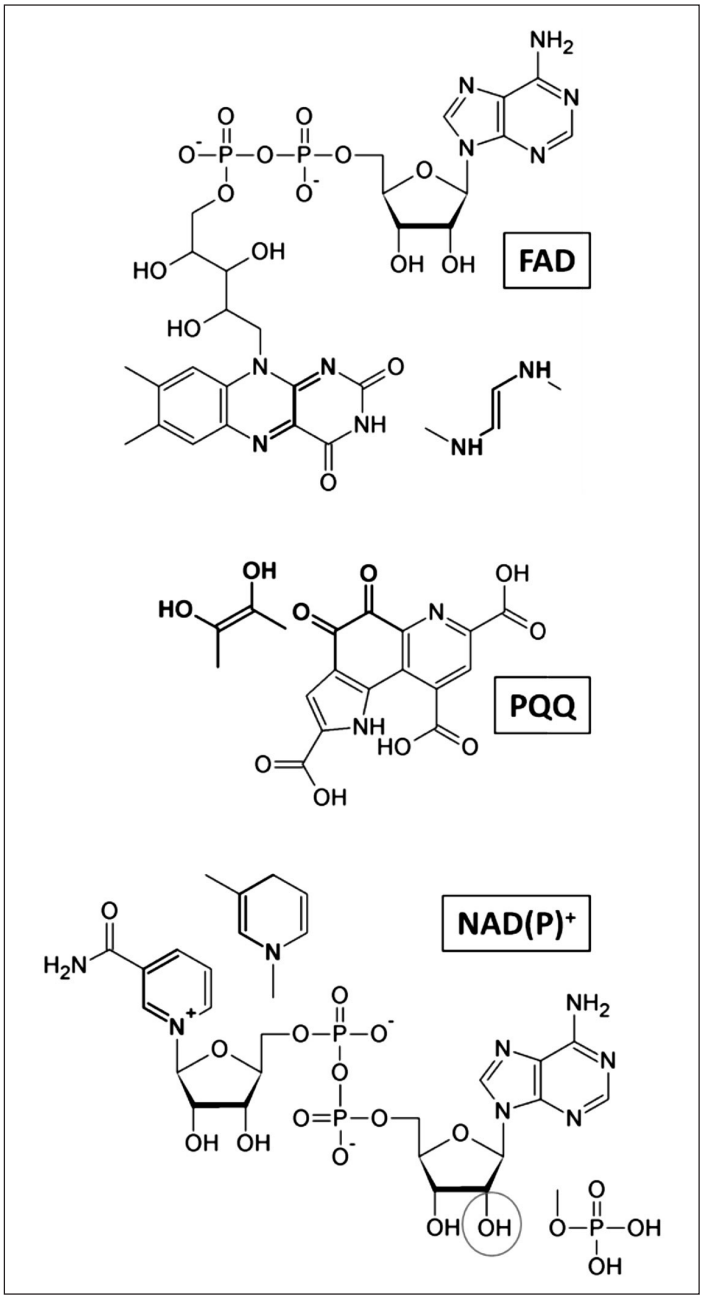

Figure 2. Cofactors used by GDHs. The structures of FAD, PQQ, and NAD are shown in their oxidized state. Regions that undergo reduction are in bold, with the reduced form of the region drawn nearby. The region that distinguishes NAD (OH) and NADP (PO_4H_2) is circled.

properties and principles employed in blood glucose monitoring to help avoid potential errors.

Glucose Oxidase—The Gold Standard of Glucose Sensing

Glucose oxidase (β-D-glucose:oxygen 1-oxidoreductase, EC 1.1.3.4) is a flavoprotein that catalyzes oxidation of β-D-glucose at its first hydroxyl group, utilizing molecular oxygen as the electron acceptor, to produce D-glucono-delta-lactone and hydrogen peroxide. Glucose oxidase is a homodimeric enzyme, with an FAD molecule noncovalently but tightly bound at the active site of each 80 kDa subunit. Glucose oxidase is a representative enzyme of the glucose/methanol/choline (GMC) oxidoreductase family. This large and diverse family includes many industrially important enzymes, especially in the area of diagnostics, such as cholesterol oxidase, alcohol oxidase, amino acid oxidase, and pyranose oxidase. Members of the GMC oxidoreductase family share a homologous structural backbone, including an adenine-dinucleotide-phosphate-binding βαβ-fold close to their amino terminus, as found in other flavoenzymes, and five other segments of conserved sequence dispersed throughout their primary sequences.

Glucose oxidase was originally isolated and produced from the fungus *Aspergillus niger*, which remains the most common source of GOx utilized for glucose monitoring and other industrial applications. Glucose oxidases are commercially available as both reagent grade and diagnostic reagent grade from various enzyme companies. Glucose oxidases have since been isolated from several different fungi as well as a few insects. An important characteristic of *A. niger*-derived GOx is its very high substrate specificity for glucose. Some of the other GOxs have been reported to also oxidize other sugars, including maltose. The high degree of specificity of GOx for glucose and the enzyme's application for measuring glucose in the presence of other sugars was documented in the 1940s.[2]

Natural production of GOx by fungi, usually *A. niger* or *Penicillium*, produces an extracellular enzyme that is over 10% glycosylated. Recombinant production of GOx in yeast, such as *Saccharomyces cerevisiae* and *Hansenula polymorpha*, often results in hyperglycosylation with a concomitant decrease in enzymatic activity.[3] Methylotrophic yeast *Pichia pastoris* was found to be an effective host for the recombinant expression of GOx, producing large quantities of a more moderately overglycosylated enzyme with properties that differed slightly from the native enzyme. Recombinant production in *Escherichia coli* produces inactive GOx in inclusion bodies, which can be solubilized and renatured *in vitro* in low yield to an active unglycosylated enzyme with properties very similar to the native enzyme.[4] Glucose oxidase is inactivated by H_2O_2, which accumulates during the oxidation reaction.[5,6] Reduction in pH, which may accompany the breakdown of D-gluconolactone to gluconic acid, also inhibits the enzyme.[7]

Because native GOx is unable to transfer electrons to conventional electrode surfaces, the enzyme was inadequate for third-generation glucose sensors. Progress has focused on connecting the enzyme's redox center to the electrode. The main approach is by employing various molecular wires, such as osmium complex-linked polymers,[8] artificial cofactor derivatives,[9] thienoviologens,[10] carbon nanotubes,[11] or nanofibers.[12] Alternatively, deglycosylating the enzyme increased the efficiency of direct electron transfer ability,[13] presumably by decreasing the distance between the enzyme's redox center and the electrode, which are believed to be too far apart to transfer electrons in the native glycosylated enzyme.[14]

Pyrroloquinoline Quinone Glucose Dehydrogenase

Pyrroloquinoline quinone was discovered in the late 1970s as the third redox cofactor found in enzymes. Biosynthesis of PQQ has been confirmed only in prokaryotic microorganisms; however, many details of the biosynthetic pathway remain unknown. Unlike NAD or FAD, biogenesis of PQQ is ribosomal, produced through sequential posttranslational modifications of a peptide precursor. Dehydrogenases harboring PQQ are widely distributed, such as GDH, alcohol dehydrogenase, and sorbitol dehydrogenase, all of which have a beta-propeller structure, completely different from the structure of GMC oxidoreductases. The blades of the beta propeller consist of four-stranded antiparallel beta-sheet motifs arranged toroidally around a central axis.

Quinoprotein GDH (EC 1.1.5.2) does not represent one enzyme but instead refers to at least two distinct groups of GDHs harboring PQQ as the redox cofactor: membrane-bound PQQ GDH-A and water-soluble PQQ GDH-B. PQQ GDH-As have been reported from a variety of Gram-negative bacteria, and their substrate specificity, stability, and other features are strain-specific. In bacteria, the physiological role of PQQ GDH-A, which is coupled with the respiratory chain via ubiquinone, is the terminal oxidation of glucose.

PQQ GDH-B has only been reported from the Gram-negative bacterium *Acinetobacter calcoaceticus*, where it is located in the periplasmic space. A soluble PQQ-harboring aldohexose dehydrogenase, with a similar structure to PQQ GDH-B, was identified in *E. coli* by extensive genome database analyses; however, its extremely low enzymatic activity suggests that its true physiological function is not that of an aldohexose dehydrogenase.[15] PQQ GDH-B is not coupled to the respiration chain of *A. calcoaceticus*, and its physiological role remains unclear. PQQ GDH-B has a broader substrate specificity profile than PQQ GDH-A and catalyzes oxidation of glucose, allose, 3-O-methyl-glucose as well as disaccharides lactose, cellobiose, and maltose.[16] Diagnostic reagent-grade PQQ GDH-B is commercially available.

PQQ GDH-B is a homodimeric enzyme, with one PQQ molecule and three calcium ions bound to each subunit. Unlike the eight-bladed beta-propeller PQQ GDH-A, PQQ GDH-B has a six-bladed beta-propeller structure. PQQ resides in a deep, broad, positively charged cleft at the top of the propeller near the six-fold pseudo-symmetry axis.[17] The six blades are labeled 1 to 6, and the four strands of each blade are labeled A to D. The cleft is constructed by the five loop regions 1D2A, 2D3A, 3BC, 4D5A, and 6BC (**Figure 3A**). The polar active site residues form hydrogen bond interactions with the glucose O1 and O2 hydroxyl groups, while hydrophobic interactions form between glucose and the nonpolar active site residues (**Figure 3B**). The amino acid residues reported to bind the substrate are located mainly on loops 1D2A, 2D3A, and 3BC, while residues of loop 6BC have no direct interaction with glucose. Although amino acid residues of loop 4D5A are within Van der Waals distance to the glucose O4 hydroxyl and C5 hydroxymethyl groups, the O4 hydroxyl group does not make hydrogen-bonding interaction with any amino acid.

Among several PQQ-harboring enzymes, PQQ GDHs are the most industrially attractive. Although some PQQ GDH-As have high catalytic efficiency and narrow substrate specificity for glucose, they have never been employed for self-monitoring of blood glucose (SMBG) due to their membrane-binding character, which was unsuitable for recombinant production at the time when industries adopted GDH as a SMBG enzyme. In contrast, recombinant PQQ GDH-B can be conveniently produced in *E. coli*, where it is secreted into the periplasmic space. Due to its several attractive properties, soluble PQQ GDH-B was readily adopted by industries and became a major enzyme used in sensor systems for SMBG. PQQ GDH-B has high catalytic efficiency (over 5–10,000 units/mg protein),

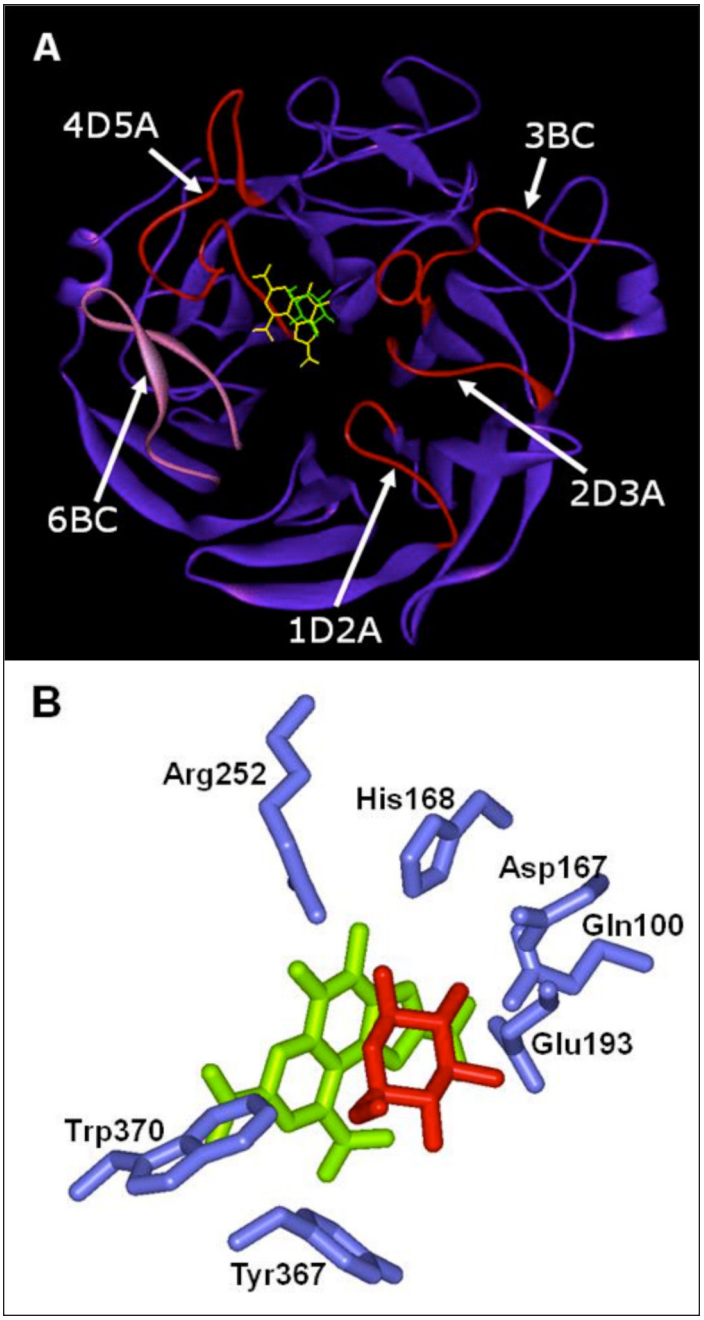

Figure 3. The three-dimensional structure of PQQGDH from *A. calcoaceticus*. **(A)** The overall crystal structure (PDB 1CQ1) with the loop regions interacting with the substrate colored red, the PQQ cofactor colored yellow, and the glucose substrate colored green. **(B)** The active-site region shows the PQQ cofactor (green) and the amino acid residues that interact with the glucose substrate (red).

over 25 times higher activity than GOx, thus enabling rapid glucose sensing. Furthermore, because of the enzyme's inherent dehydrogenase character, its activity is not affected by variations of oxygen content in blood samples, thus providing greater accuracy. Because PQQ is tightly bound to GDH, additional cofactor does not need to be supplied to the reaction, as is the case with NAD(P) GDHs.

Native PQQ GDH-B does have some limitations for glucose sensing applications, especially when compared to GOx. The most significant limitation is in its broad substrate specificity, which can lead to potentially fatal errors in glucose sensing.[18] Protein engineering studies have succeeded in improving its substrate specificity[19–21] and stability.[22–24] There have also been improvements in the recombinant preparation of PQQ GDH-B, such as the secretional production in yeast,[25] and engineering the surface charge of the enzyme to facilitate its purification.[26]

Nicotine Adenine Dinucleotide (Phosphate)-Dependent Glucose Dehydrogenase

Glucose-1-dehydrogenase [NAD(P) GDH, EC 1.1.1.47] represents a large number of enzymes from very diverse origins, from prokaryotes to higher eukaryotes and from mesophiles to extremophiles, such as halophilic and hyperthermophilic archaea. The enzyme catalyzes oxidation of the first hydroxyl group of glucose, utilizing NAD^+ or $NADP^+$ as the primary electron acceptor, and has the systematic name β-D-glucose:$NAD(P)^+$1-oxidoreductase. Unlike GOx or other GDHs, the redox cofactor of this enzyme [NAD(P)] is not bound to the enzyme. Nicotine adenine dinucleotide (phosphate) GDHs are essentially water-soluble cytosolic proteins with homo-oligomeric structures (**Figure 4**).

Investigations of several elucidated three-dimensional structures revealed that NAD(P) GDHs share significant structural features, including their active site, which has made them a representative enzyme group for understanding protein structural stability. Most NAD(P) GDHs are glucose specific; however, some of the enzymes in this group lack recognition of the second hydroxyl group, thus making them reactive toward mannose, 2-amino-2-deoxy-D-glucose, and glucosamine.[27] Some NAD(P) GDHs are also active toward galactose, xylose, and fructose, as well as disaccharides (e.g., maltose) and oligosaccharides.[28]

The NAD(P) GDH from Gram-positive bacterium *Bacillus megaterium* has been utilized for blood glucose monitoring. The enzyme has a homotetrameric structure (4 × 28.2 kDa), with one catalytic site per subunit. NAD(P) GDHs are commercially available as both reagent grade and diagnostic reagent grade from various enzyme companies. Under alkaline conditions, the enzyme reversibly dissociates into inactive monomers.[29] Because the NAD(P) cofactor is not protein bound, it must be supplied exogenously together with specific artificial electron mediators for the electrochemical measurement. Since direct electrochemical oxidation of the reduced NAD(P)H cofactor is known to cause oligomeric inactivation, an applied potential that does not lead to oxidation of NAD(P)H is employed.

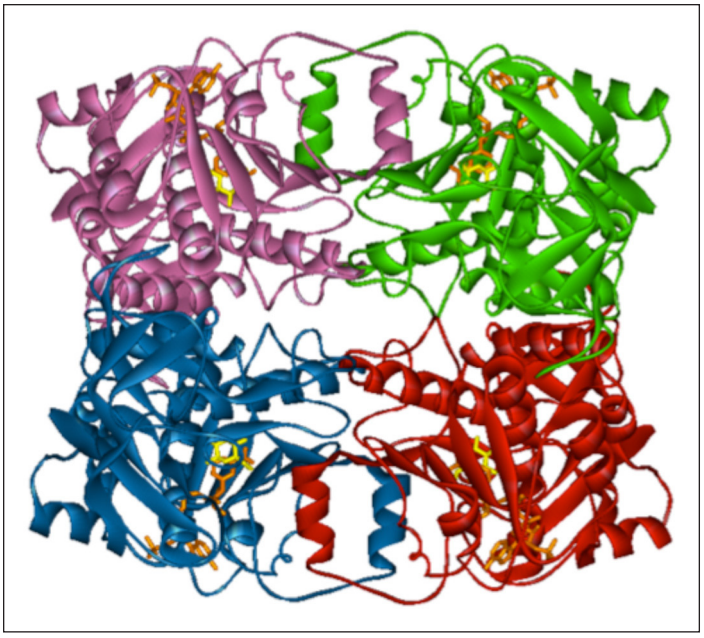

Figure 4. The three-dimensional structure of the *Sulfolobus solfataricus* NAD(P) GDH complexed with NADP and glucose. The crystal structure of NAD(P) GDH (PDB 2CDB) is shown with the NADP cofactor colored orange, glucose colored yellow, and all four protein subunits colored differently to distinguish them.

Flavin-Adenine-Dinucleotide-Dependent Glucose Dehydrogenases

The FAD-dependent GDHs (FAD GDH, EC 1.1.99.10) have received much attention for their potential sensor applications. This group comprises oxidoreductases that catalyze the first hydroxyl group of glucose and other sugar molecules, utilizing FAD as the primary electron acceptor. FAD GDHs utilize a variety of external electron acceptors (but not oxygen), and their systematic name is D-glucose:acceptor 1-oxidoreductase. Although GDHs harboring FAD as the cofactor had been first reported in the 1960s, this group of enzyme remains in the EC 1.1.99 group corresponding to redox enzymes utilizing "other" electron acceptors.

The reported FAD GDHs can be divided into three distinct groups according to their origin. FAD GDHs have been found expressed in the periplasm of Gram-negative bacteria, as extracellular enzymes in fungi, and as cytosolic enzymes in some insects. Both eukaryotic enzyme groups comprise glycosylated enzymes with quaternary structures ranging from monomeric to homo-oligomeric.

Bacterial Flavin Adenine Dinucleotide Glucose Dehydrogenase

Bacterial FAD GDH is a thermostable hetero-oligomeric enzyme complex made up of a catalytic subunit harboring FAD in its redox center, a multiheme cytochrome-complex electron-transfer subunit, and a chaperone-like subunit required for proper folding and secretion of the catalytic subunit. Although the catalytic subunit alone shows high catalytic activity, the electron-transfer subunit facilitates the transfer of electrons between the active-site cofactor and the artificial electron mediator, giving this enzyme a catalytic activity similar to or even higher than that of PQQ GDH. Furthermore, the electron-transfer subunit confers the ability to transfer electrons directly to an electrode. Bacterial FAD GDH's unique direct electron transfer ability eliminates the need for toxic artificial electron mediators and makes this enzyme ideal for third-generation glucose sensors. Although a sensor employing bacterial FAD GDH is on the market, the enzyme is not commercially available.

Native bacterial FAD GDH has broad substrate specificity, catalyzing oxidation of glucose and disaccharides, such as maltose, to the corresponding lactones. Protein engineering studies of the catalytic subunit's active site have succeeded in specifically eliminating the enzyme's activity toward disaccharides, thus making it essentially specific for glucose.[30]

Fungal Flavin Adenine Dinucleotide Glucose Dehydrogenase

An emergent demand for a GDH that is specific for glucose has directed significant attention toward fungi-derived FAD GDHs, focusing on their narrow substrate specificity, especially their lack of activity toward maltose.[31] The earliest report of a fungal GDH dates back to 1937, describing the presence in *Aspergillus* subspecies of an enzyme capable of oxidizing glucose utilizing certain electron acceptors, but not oxygen.[32] In 1967, GDH from *Aspergillus oryzae* was characterized, including the identification of its FAD cofactor.[33] FAD GDH was later studied from *A. niger*[34] and *Aspergillus terreus*.[31] Despite its long history, scientific information describing the fungi-derived FAD GDHs and their availability remains limited.

A screening of a fungal genome database using GOx active-site sequence motifs yielded putative proteins that could be divided into two structurally distinct phylogenetic clades, a GOx clade and a FAD GDH clade.[35] Some members of the FAD GDH clade were successfully expressed in *E. coli* and, despite not having their native glycosylation, showed GDH activity and a high specificity for glucose. Furthermore, the predicted amino acid sequence of one of the putative FAD GDHs was identical to that of a patented FAD GDH. Diagnostic reagent-grade fungal FAD GDHs are commercially available.

The reasons behind the high substrate specificity for glucose exhibited by GOx can be understood by looking at the detailed structure of the enzyme complexed with glucose (**Figure 5**). The cavity forming the substrate-binding site, located in the center of the enzyme, is composed of residues that interact with each of the five hydroxyl groups that characterize glucose, thus preventing any epimer of glucose from binding at the active site. A comparison of GOx active site with a structural model of the active site of fungi-derived FAD GDHs revealed that their similar specificity for glucose was matched by their almost identical configurations around the substrate binding site. In particular, a conserved tyrosine (Tyr68 in *A. niger* GOx) interacting with the fourth hydroxyl group of glucose in both GOx and FAD GDH appears to be a hallmark residue behind the glucose specificity of these two fungal enzyme groups.

Conclusion

The evolution from first-generation through third-generation glucose sensors has witnessed the appearance of a number of very diverse oxidoreductases, which vary tremendously in terms of origin, structure, substrate specificity, primary electron acceptor, and acceptable final electron acceptor (**Tables 1** and **2**). These enzymes belong to four main categories GOx, PQQ GDH, NAD GDH, and FAD GDH, each with their own special characteristics. However, the properties of any particular enzyme also vary depending on protein engineering or post-translational modifications. Some enzymes have been successfully engineered to eliminate certain limitations found in native enzymes. How the different oxidoreductases are produced may have an important impact; for example, the glycosylation level of the enzyme will depend on which host is used for the recombinant production, as well as any possible treatment of the purified enzyme (e.g., enzymatic deglycosylation *in vitro*). These are important points to consider when evaluating an enzyme for glucose monitoring applications.

Of particular importance for glucose sensors is the enzyme's substrate specificity. Patients undergoing peritoneal dialysis or other treatments may have significant quantities of maltose in their blood.[36] Glucose sensors that employ

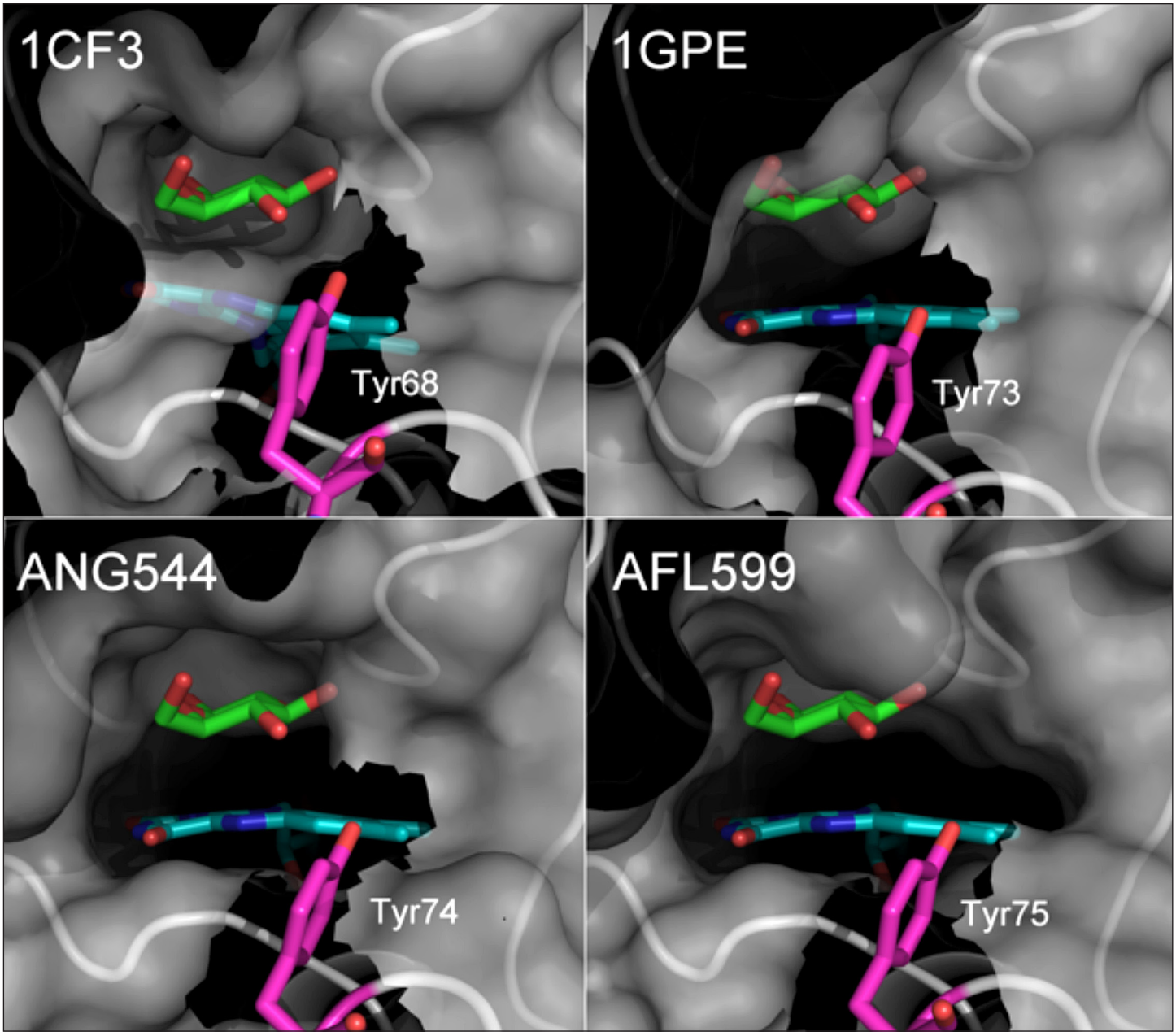

Figure 5. Structure of the GOx and fungal FAD GDH active sites complexed with glucose. The active site structures of the GOxs from *A. niger* (PDB 1CF3) and *Penicillium amagasakiense* (PDB 1GPE) were derived from their crystal structures. The three-dimensional structure models of the FAD GDHs from *A. niger* (ANG544) and *Aspergillus flavus* (AFL599) were constructed by Modeller 9v3 using the structure of *A. niger* GOx as template.[35] The FAD cofactor is colored blue, and D-glucose is colored green. The conserved tyrosine residue interacting with the fourth hydroxyl group of glucose is colored pink.

oxidoreductases with broad specificity may give falsely elevated glucose readings, leading to iatrogenic hypoglycemia. A Food and Drug Administration public health notification[18] warned against potential fatal errors from PQQ-GDH-based sensing technology. Glucose oxidase has long been envied for its high specificity for glucose. However, protein engineering studies on PQQ GDH and bacterial FAD GDH, as well as the increasing use of fungal FAD GDHs, are challenging GOx's historical supremacy.

It is important to understand the basic properties and limitations of the enzymes employed and the principles behind their catalytic activity to avoid potential dangers. When considering the possible suitability of an enzyme for a particular sensor application, one must not only consider the enzyme's main category or conventional name, but should also be aware of other features that may affect the enzymatic properties, such as host strain used for its preparation, engineered mutations, as well as

Table 1.
Properties of Glucose Oxidases and Dehydrogenases

Enzyme	Systematic name	EC number	Cofactor	Microorganism	Structure
Glucose oxidase	β-D-glucose: oxygen 1-oxidoreductase	1.1.3.4	FAD	fungi[a]	homodimer, GMC oxidoreductases family
Glucose dehydrogenase					
PQQGDH-A	D-glucose:ubiquinone oxidoreductase	EC 1.1.5.2	PQQ	Gram negative bacteria[b]	membrane-bound, monomer, 8-bladed β-propeller
PQQGDH-B	D-glucose:ubiquinone oxidoreductase	EC 1.1.5.2	PQQ	*Acinetobacter calcoaceticus*	soluble, homodimer, 6-bladed β-propeller
NAD(P)-GDH	D-glucose:NAD(P)$^+$ 1-oxidoreductase	1.1.1.118 (NAD^+) 1.1.1.119 ($NADP^+$)	NAD(P) (unbound)	Gram positive[c] and negative[d] bacteria, eukaryotes	homooligomer
FADGDH	D-glucose:acceptor 1-oxidoreductase	1.1.99.10	FAD	Gram-negative bacteria[e]	heterotrimer (catalytic, chaperone, cytochrome subunits)
				fungi[f]	monomer-homooligomer
				insects[g]	homodimer

[a] *Aspergillus niger, Aspergillus sp, Penicillium amagasakiense.*
[b] *Erwinia sp., Escherichia coli, Klebsiella aerogenes, Pseudomonas aeruginosa, P. fluorescens, Gluconobacter suboxydans, G. oxydans, Acinetobacter calcoaceticus*
[c] *Bacillus subtilis, Corynebacterium sp., Bacillus megaterium, Haloferax mediterranei*
[d] *Gluconacetobacter xylinus, Sulfolobus solfataricus, Acetobacter xylinum, Pseudomonas putida, Alcaligenes eutrophus, Cryptococcus uniguttulatus, Gluconobacter cerinus, Gluconobacter oxydans, Halococcus saccharolyticus, Pseudomonas fluorescens*
[e] *Burkholderia cepacia*
[f] *Aspergillus sp., A. oryzae, A. niger, A. terreus*
[g] *Drosophila melanogaster, Anopheles gambiae, Apis mellifera, Tribolium castaneum*

Table 2.
Summary of Major Features of Glucose Sensing Enzymes

Enzyme	Advantages	Disadvantages	Sensor applications	Possible error sources
GOx	Glucose specific	Reacts with oxygen; low activity	Electrochemical; colorimetric	Variations of oxygen concentration
PQQ GDH-A	High activity; glucose specific	Membrane bound	Not applied in sensor	
PQQ GDH-B	High activity	Broad substrate specificity[a]	Electrochemical; colorimetric	Maltose in sample[a]
NAD(P) GDH	Glucose specific	Cofactor must be supplied	Electrochemical	
Bacterial FAD GDH	High activity; direct electron transfer (to electrode)	Broad substrate specificity[a]	Electrochemical; colorimetric	Maltose in sample[a]
Fungal FAD GDH	Glucose specific	Low activity; activity toward xylose	Electrochemical; colorimetric	Xylose in sample

[a] Glucose specificity of PQQ GDH-B[19–21] and bacterial FAD GDH[30] have been improved by protein engineering, thus reducing the error from other sugars.

chemical or enzymatic modifications. This short review of oxidoreductases employed in blood glucose sensors has attempted to provide the necessary background to better understand these features and hopefully avoid potential pitfalls.

References:

1. Clark LC Jr, Lyons C. Electrode systems for continuous monitoring in cardiovascular surgery. Ann N Y Acad Sci. 1962;102:29–45.
2. Keilin D, Hartree EF. The use of glucose oxidase for the determination of glucose in biological material and for the study of glucose-producing systems by manometric methods. Biochem J. 1948;42(2):230–8.

3. Romanos MA, Scorer CA, Clare JJ. Foreign gene expression in yeast: a review. Yeast. 1992;8(6):423–88.
4. Witt S, Singh M, Kalisz HM. Structural and kinetic properties of nonglycosylated recombinant *Penicillium amagasakiense* glucose oxidase expressed in *Escherichia coli*. Appl Environ Microbiol. 1998;64(4):1405–11.
5. Kleppe K. The effect of hydrogen peroxide on glucose oxidase from Aspergillus niger. Biochemistry. 1966;5(1):139–43.
6. Bao J, Furumoto K, Fukunaga K, Nakao K. A kinetic study on air oxidation of glucose catalyzed by immobilized glucose oxidase for production of calcium gluconate. Biochem Eng J. 2001;8:91–102.
7. Mirón J, González MP, Pastrana L, Murado MA. Diauxic production of glucose oxidase by Aspergillus niger in submerged culture--a dynamic model. Enz Microb Technol. 2002;31:615–20.
8. Vijayakumar AR, Csöregi E, Heller A, Gorton L. Alcohol biosensors based on coupled oxidase-peroxidase systems. Anal Chim Acta. 1996;327(3):223–34.
9. Katz E, Willner I. Biomolecular architecture: routes to biosensors, bioelectronics and biofuel cells. In: Proceedings of the First International Symposium on Macro- and Supramolecular Architectures and Materials (MAM-01): Biological and Synthetic Systems, Kwangju, South Korea, 10-14 April, 2001, 78–81.
10. Albers MW, Lekkala JO, Jeuken L, Canters GW, Turner AP. Design of novel molecular wires for realizing long distance electron transfer. Bioelectrochem Bioeng. 1997;42:25–33.
11. Tsai YC, Li SC, Chen JM. Cast thin film biosensor design based on a Nafion backbone, a multiwalled carbon nanotube conduit, and a glucose oxidase function. Langmuir. 2005;21(8):3653–8.
12. Vamvakaki V, Tsagaraki K, Chaniotakis N. Carbon nanofiber-based glucose biosensor. Anal Chem. 2006;78(15):5538–42.
13. Courjean O, Gao F, Mano N. Deglycosylation of glucose oxidase for direct and efficient glucose electrooxidation on a glassy carbon electrode. Angew Chem Int Ed Engl. 2009;48(32):5897–9.
14. Wohlfahrt G, Witt S, Hendle J, Schomburg D, Kalisz HM, Hecht HJ. 1.8 and 1.9 A resolution structures of the *Penicillium amagasakiense* and *Aspergillus niger* glucose oxidases as a basis for modelling substrate complexes. Acta Crystallogr D Biol Crystallogr. 1999;55(Pt 5):969–77.
15. Southall SM, Doel JJ, Richardson DJ, Oubrie A. Soluble aldose sugar dehydrogenase from *Escherichia coli*: a highly exposed active site conferring broad substrate specificity. J Biol Chem. 2006;281(41):30650–9.
16. Igarashi S, Ohtera T, Yoshida H, Witarto AB, Sode K. Construction and characterization of mutant water-soluble PQQ glucose dehydrogenases with altered K(m) values--site-directed mutagenesis studies on the putative active site. Biochem Biophys Res Commun. 1999;264(3):820–4.
17. Oubrie A, Rozeboom HJ, Kalk KH, Olsthoorn AJ, Duine JA, Dijkstra BW. Structure and mechanism of soluble quinoprotein glucose dehydrogenase. EMBO J. 1999;18(19):5187–94.
18. U.S. Food and Drug Administration. FDA public health notification: potentially fatal errors with GDH-PQQ glucose monitoring technology. http://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/PublicHealthNotifications/ucm176992.htm. Accessed May 27, 2011.
19. Sode K, Igarashi S, Morimoto A, Yoshida H. Construction of engineered water-soluble PQQ glucose dehydrogenase with improved substrate specificity. Biocatal Biotransform. 2002;20:405–12.
20. Igarashi S, Hirokawa T, Sode K. Engineering PQQ glucose dehydrogenase with improved substrate specificity. Site-directed mutagenesis studies on the active center of PQQ glucose dehydrogenase. Biomol Eng. 2004;21(2):81–9.
21. Hamamatsu N, Suzumura A, Nomiya Y, Sato M, Aita T, Nakajima M, Husimi Y, Shibanaka Y. Modified substrate specificity of pyrroloquinoline quinone glucose dehydrogenase by biased mutation assembling with optimized amino acid substitution. Appl Microbiol Biotechnol. 2006;73(3):607–17.
22. Sode K, Ootera T, Shirahane M, Witarto AB, Igarashi S, Yoshida H. Increasing the thermal stability of the water-soluble pyrroloquinoline quinone glucose dehydrogenase by single amino acid replacement. Enzyme Microb Technol. 2000;26(7):491–6.
23. Igarashi S, Sode K. Stabilization of quaternary structure of water-soluble quinoprotein glucose dehydrogenase. Mol Biotechnol. 2003;24(2):97–104.
24. Tanaka S, Igarashi S, Ferri S, Sode K. Increasing stability of water-soluble PQQ glucose dehydrogenase by increasing hydrophobic interaction at dimeric interface. BMC Biochem. 2005;6:1.
25. Yoshida H, Araki N, Tomisaka A, Sode K. Secretion of water soluble pyrroloquinoline quinone glucose dehydrogenase by recombinant Pichia pastoris. Enzyme Microb Technol. 2002;30(3):312–8.
26. Koh H, Igarashi S, Sode K. Surface charge engineering of PQQ glucose dehydrogenase for downstream processing. Biotechnol Lett. 2003;25(20):1695–701.
27. Giardina P, de Biasi MG, de Rosa M, Gambacorta A, Buonocore V. Glucose dehydrogenase from the thermoacidophilic archaebacterium Sulfolobus solfataricus. Biochem J. 1986;239(3):517–22.
28. Sharma V, Kumar V, Archana G, Kumar GN. Substrate specificity of glucose dehydrogenase (GDH) of Enterobacter asburiae PSI3 and rock phosphate solubilization with GDH substrates as C sources. Can J Microbiol. 2005;51(6):477–82.
29. Pauly HE, Pfleiderer G. Conformational and functional aspects of the reversible dissociation and denaturation of glucose dehydrogenase. Biochemistry. 1977;16(21):4599–604.
30. Yamaoka H, Yamashita Y, Ferri S, Sode K. Site directed mutagenesis studies of FAD-dependent glucose dehydrogenase catalytic subunit of *Burkholderia cepacia*. Biotechnol Lett. 2008;30(11):1967–72.
31. Tsujimura S, Kojima S, Kano K, Ikeda T, Sato M, Sanada H, Omura H. Novel FAD-dependent glucose dehydrogenase for a dioxygen-insensitive glucose biosensor. Biosci Biotechnol Biochem. 2006;70(3):654–9.
32. Ogura Y, Nagahisa M. Untersuchungen über die Atmung und die Dehydrasesysteme von Aspergillus oryzae. Bot Mag Tokyo. 1937;51(606):597–612.
33. Bak TG. Studies on glucose dehydrogenase of *Aspergillus oryzae*. II. Purification and physical and chemical properties. Biochim Biophys Acta. 1967;139(2):277–93.
34. Müller HM. Gluconic acid forming enzymes in *Aspergillus niger*. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg. 1977;132(1):14–24.
35. Mori K, Nakajima M, Kojima K, Murakami K, Ferri S, Sode K. Screening of Aspergillus-derived FAD: -glucose dehydrogenases from fungal genome database. Biotechnol Lett. July 12, 2011. Epub ahead of print.
36. Janssen W, Harff G, Caers M, Schellekens A. Positive interference of icodextrin metabolites in some enzymatic glucose methods. Clin Chem. 1998;44(11):2379–80.