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Electrochemical biosensors for the detection and study of α-synuclein related to Parkinson's disease – A review

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HIGHLIGHTS

- Parkinson's disease and α-synuclein are introduced.
- Electrochemical detection of α-synuclein using antibodies and aptamers is reviewed.
- Electrochemical detection of α-synuclein using tyrosine oxidation is reviewed.
- Novel nanomaterials for improved biosensor performance are discussed.

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ABSTRACT

Parkinson's disease (PD) is a long-term degenerative disorder that affects predominately dopaminergic neurons in the substantia nigra, which mainly control movement. Alpha-synuclein (α-syn) is a major constituent of Lewy bodies that are reported to be the most important toxic species in the brain of PD patients. In this critical review, we highlight novel electrochemical biosensors that have been recently developed utilizing aptamers and antibodies in connection with various nanomaterials to study biomarkers related to PD such as α-syn. We also review several research articles that have utilized electrochemical biosensors to study the interaction of α-syn with biometals as well as small molecules such as clioquinol, (−)-epigallocatechin-3-gallate (EGCG) and baicalein. Due to the significant advances in nanomaterials in the past decade, electrochemical biosensors capable of detecting multiple biomarkers in clinically relevant samples in real-time have been achieved. This may facilitate the path towards commercialization of electrochemical biosensors for clinical applications and high-throughput screening of small molecules for structure-activity relationship (SAR) studies.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor symptoms such as akinesia (loss or impairment of voluntary movement), bradykinesia (slowness of movement), tremors, rigidity, and postural instability [1], along with non-motor symptoms such as cognitive impairment, hallucination, depression, and apathy [2]. Studies have shown that in industrialized countries, the prevalence of people suffering from PD is 0.3% amongst the general population, and 1% for people above the age of 60 [3].

Given the estimated increase in the population of people over 50 years old, the prevalence of PD is anticipated to increase from approximately 400 thousand, PD patients in 2010 to 770 thousand, PD patients by the year 2040 in the United States alone [4,5]. Therefore, there is an increase in demand for faster and more accurate diagnosis of PD in patients. Furthermore, it is difficult to accurately diagnose PD. Studies have shown that about a quarter of patients diagnosed with PD are later diagnosed with a different disorder post-mortem [6]. Clinically, PD is defined by the presence of bradykinesia along with at least one other motor disorder [7]. Furthermore, non-motor symptoms such as sleep cycle dysregulation, cognitive impairment, as well as mood disorders are some of the many symptoms that can help predict PD [8]. Several clinical diagnostic tests are employed to accurately diagnose PD [9] such as positron emission tomography (PET) to visualize striatal dopamine depletion with ^{18}F-labelled L-DOPA [10], or the use of single photon emission computed tomography (SPECT) to visualize presynaptic nigrostriatal terminal dysfunction using ^{123}I-ioflupane [11,12]. Functional magnetic resonance imaging studies have also been

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used to analyze specific changes in the basal ganglia and infratentorial structures [13]. However, although the imaging techniques mentioned may be used to diagnose some patients with PD, the clinical heterogeneity in disease presentation, differences in response to dopaminergic medication, and overlap in symptomatology can make diagnosis of PD difficult in some cases [14].

One way to improve diagnostic methods for PD is to employ detection methods for biomarkers associated with PD. There are several biomarkers that can be used to diagnose PD. One example is orexin, a neuropeptide hormone produced by neurons in the dorsolateral hypothalamus that regulates the sleep-wake cycle, hypertension, and heart rate [15]. Patients with PD have been shown to exhibit lower concentrations of orexin when compared to healthy individuals [16]. Another candidate that can be used as a PD biomarker is 8-hydroxy-2′-deoxyguanosine, which is a product of reactive oxygen species (ROS) oxidizing DNA [15]. Previous research has shown that in rat models, the stage of PD can be correlated to the concentration of 8-hydroxy-2′-deoxyguanosine and therefore has been proposed to be used as a severity marker [17]. However, given that 8-hydroxy-2′-deoxyguanosine is a product of DNA damage, it is important to note that this biomarker is not specific to PD. Dopamine and its related precursor L-DOPA are also potential candidates to be biomarkers for PD as it has been shown that the loss of dopaminergic neurons in the midbrain and substantia nigra leads to the depletion of dopamine in PD brains [15].

However, the potential biomarker that has attracted the most attention is α-syn [15], as the concentration of α-syn has been shown to be lower in PD patients versus Alzheimer's disease patients and controls [18]. A review conducted in 2016 has shown that cerebral spinal fluid (CSF) α-syn levels have shown the most promising results for using it as a biomarker for PD [19]. It has been shown that there is a significantly lower concentration of CSF α-syn in PD patients compared to control patients when measured using ELISA, Luminex™ assays, and mass spectrometry [19]. However, given the invasiveness of obtaining a CSF sample, researchers have been moving towards detecting α-syn in plasma and serum, although the results for these remain to be confirmed. Although psychological and motor symptoms are currently the main technique used to diagnose patients afflicted with PD [9], biosensors offer a potential diagnostic platform that is both cost-effective and easy-to-use [20] and can potentially be used for early diagnosis. Although there is currently no cure for PD, there are treatments available for symptomatic relief. However, these treatments are limited by the extent of neurodegeneration of dopaminergic neurons at the time of diagnosis. Therefore, establishing a biomarker that can be correlated to the progression of PD will not only assist in clinical diagnosis but may also help follow disease progression, assist with personalized treatment, and improve the quality of life of patients and their care-takers [21–23].

Recent advances in electrochemical biosensors have been used to develop commercially available sensors such as Elecsys® β-amyloid immunoassay by Roche Diagnostics (Basel, Switzerland), which utilizes electrochemiluminescence to quantitatively determine amyloid-β concentrations in patients afflicted with Alzheimer's disease [24,25]. Therefore, it is not farfetched to imagine that one day, a similar biosensor may be developed to measure the concentrations of α-syn for PD. There have been several biosensors that have been developed to detect and measure the concentration of α-syn in recent years. Furthermore, biosensors have also been used to study the aggregation kinetics of α-syn as well as the interactions with small molecules to understand structure-activity relationships (SARs). In this review, we highlight electrochemical biosensors that have been recently developed to detect α-syn and anti-α-syn antibodies as well as to study SAR with other small molecules of interest.

2. α-syn

2.1. Background

The main protein implicated in PD is α-syn, a14 kDa peptide [26,27] that has been found to aggregate into Lewy Bodies in PD patients [28,29]. It was first isolated from non-amyloid component of plaques (NACP) found in patients afflicted with Alzheimer's disease [27], and therefore it was thought to be implicated in Alzheimer's disease, as well [27,30]. α-syn comprises mainly of three regions. An amphipathic region at the N-terminus (amino acids 1–60) that is comprised of an apolipoprotein binding motif which is rich in lysine residues. This region forms amphiphilic α-helical structures able to bind to membrane components. The NACP region (amino acids 61–95) which is mainly comprised of hydrophobic residues. This region is believed to play a central role in α-syn aggregation, and it has been shown that if this region is knocked out, aggregation of the peptide is significantly inhibited. The C-terminus of the peptide (amino acids 96–140) has a high negative charge due to acidic residues, which is implicated in the binding of the peptide to metal cations and small molecules. The region is also implicated in the regulation of the nuclear localization of α-syn [26,31]. Moreover, α-syn contains four unevenly distributed tyrosine residues, one at Y39 near the N-terminus and three (Y125, Y133, and Y136) near the C-terminus, which have been used for biosensors based on voltammetric techniques due to the redox activity of tyrosine [32]. α-syn has no tryptophan residues, which would have contributed to the electrochemical oxidation signal on carbon surfaces.

The mechanism in which α-syn exhibits its pathology also remains unclear. Researchers assume that the accumulation of α-syn is controlled by the synthesis, uptake and degradation of the protein [26]. One factor that may affect this is the multiplication of the SNCA gene which encodes α-syn, resulting in an increase in α-syn expression and hence its accumulation in the cell [33], however, this is only implicated in PD. Furthermore, in the presence of biometals (Fe(III), Cu(II), Zn(II)) [34–38], or neurotransmitters such as dopamine [39], α-syn can also be triggered to aggregate.

2.2. Electrochemical immunosensors and aptasensors

In recent literature, α-syn autoantibodies have been reported as an important biomarker in PD development. The progression of PD can result in an elevation of α-syn autoantibodies [40]. Though there are mixed opinions of whether it is directly related to pathological stages of PD, it still serves as an interesting starting point and important target for sensor platform design. Bryan et al. [41] reported a novel biosensor to detect anti-α-syn antibodies in sera. On a gold surface, a self-assembled monolayer of thiolated-polyethylene glycol (PEG)-COOH was immobilized. Using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) activation, wild-type α-syn was covalently attached onto the self-assembled monolayer surfaces. This recognition platform was exposed to α-syn autoantibodies which were detected using electrochemical impedance spectroscopy (EIS) (Fig. 1). The charge-transfer resistance (R_{ct}) would increase when the increasing concentrations of autoantibodies were bound to the surface-anchored α-syn proteins in the analyte sample. In the presence of bovine serum albumin (BSA), the interference showed approximately 5% increase in R_{ct} indicating negligible non-specific adsorption issues on these surfaces. This detection system could be regenerated for a new cycle of measurements. They observed a limit of detection (LOD) of 55 ± 3 pM. In a follow-up study from the same group, they further challenged their detection system with human serum samples [42]. Their results demonstrated that

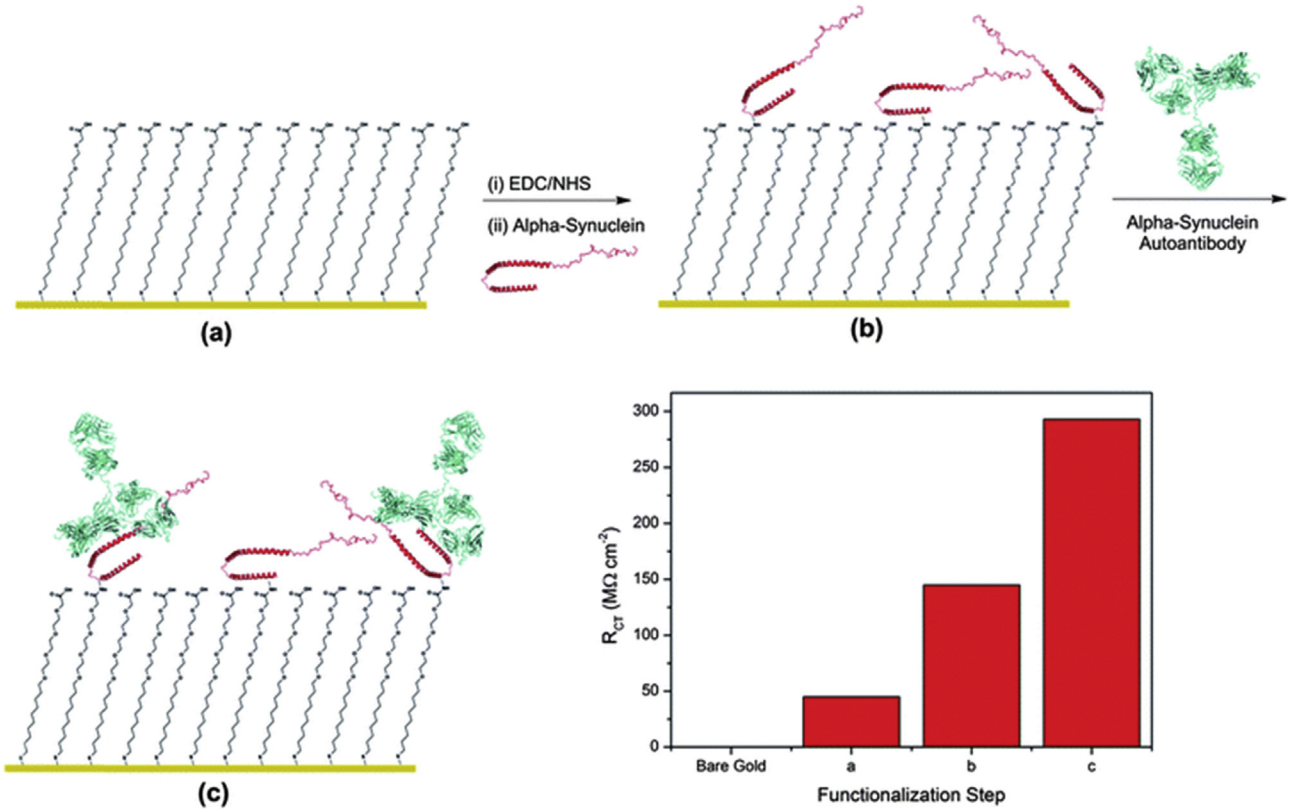

Fig. 1. Schematic illustration of the electrochemical biosensor interface for the detection of α-syn autoantibodies. (a) Gold electrode surface was modified with thiolated-PEG-COOH monolayer, (b) Covalent immobilization of α-syn, (c) Detection of α-syn autoantibodies. The bar graph shows the R_{ct} values that were calculated by fitting the data in Nyquist plots with an equivalent Randles circuit for EIS-based measurements of each surface functionalization step [Adapted from 41]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

autoantibodies seem to be in correlation with the disease development stage of PD, and they were able to clearly determine the concentration of α-syn autoantibodies in healthy serum samples as well as the ones collected from PD patients [42]. When the biosensor was utilized to analyze samples from healthy controls and PD patients, non-specific adsorption studies should have been diligently reported. For this reason, the authors suggested that this proof-of-concept study should be further optimized to demonstrate the selectivity and specificity of the biosensor in real biological matrices.

Recently, nanomaterials such as gold nanoparticles (AuNPs), carbon nanotubes, and graphene oxide have been incorporated into interface design due to their ability to enhance the electrical properties in electrochemical biosensors. Xu et al. [43] designed a platform incorporating graphene oxide to improve the detection limits of α-syn autoantibodies. On the cysteamine-modified gold surface, graphene oxide was drop-cast and covalently coupled to α-syn (Fig. 2) [43]. This system reported an LOD of 1.2 ± 0.3 pM, which was significantly improved in comparison with 55 ± 3 pM in the previously reported system where no graphene oxide or AuNPs were used to enhance the detection system [41].

However, it is noteworthy that this anti-α-syn antibody-based biosensor was not tested for its selectivity against other common proteins found in blood and CSF samples.

Researchers have also successfully incorporated nanomaterials to detect α-syn utilizing an enzymatic cascade incorporated with AuNPs. The synergistic effect between AuNPs and electrochemical sensing systems have long been recognized [44]. It has been previously established that the shape and size of AuNPs can be easily controlled during synthesis under ambient conditions [45–47]. In the attempt to detect α-syn, electrodes were modified by electrochemical polymerization of a film of *o*-aminobenzoic acid (*o*-ABA) [48]. Then, amine-terminated polyamidoamine (PAMAM) modified AuNPs were attached to the *o*-ABA via an EDC/NHS coupling. Then, thionine was exposed to the surfaces to finish the development of the immunosensor. The immunosensor was then placed in a sample solution containing α-syn. The surface-immobilized α-syn enabled the attachment of monoclonal anti-α-syn antibodies. Finally, the strong affinity between α-syn and monoclonal anti-α-syn antibodies promoted the attachment of a secondary antibody, which is a goat anti-mouse IgG conjugated with horseradish peroxidase (HRP) and AuNPs. The attachment of secondary antibody on the electrode surface resulted in an enzymatic reaction with HRP generating a detectable electrochemical signal with an LOD of 14.6 pg/mL (1.0 pM) [48]. Despite a rather complex surface modification procedure, with each incorporation of IgG modified with HRP and AuNPs, the linear range of detection was extended further. This enzymatic cascade system was also shown to have a temporal stability over a 1-month period maintaining a current response of 92.8%. In our opinion, the stability of this immunosensor and its wide linear range of detection are impressive and can serve as a template for future development of immunosensors. However, the experimental protocol might be time-consuming and challenging for clinical applications by non-skilled operators and thus, may cause variability in the results.

Recently, indium tin oxide (ITO) electrodes doped with AuNPs have been modified with poly-glutamic acid for the quantitative detection of α-syn [49]. The sensor was constructed by first electrodepositing AuNPs on hydroxylated ITO electrodes using cyclic voltammetry (CV). The electrodes were then further modified by

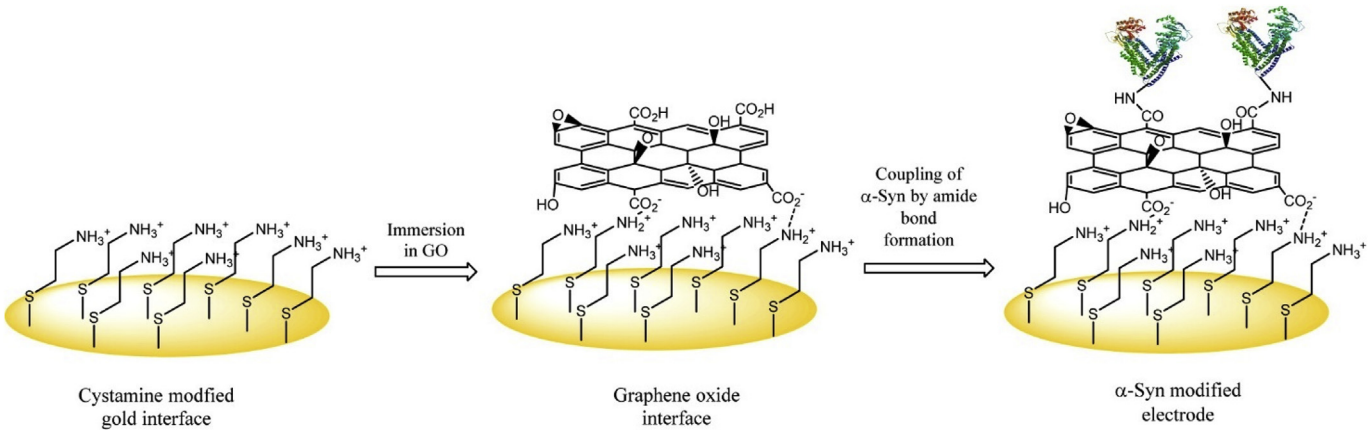

Fig. 2. Graphene oxide-modified biosensor for the electrochemical detection of α-syn autoantibodies [Adapted from 43].

electropolymerization of glutamic acid on AuNPs. This was followed by incubating the electrodes in EDC/NHS solution to activate the carboxyl terminal of poly-glutamic acid in order to covalently attach anti-α-syn antibodies onto the electrode surface. Finally, the antibody-modified electrodes were incubated in BSA solution in order to prevent non-specific interactions and block any unoccupied space followed by exposure to sample solutions containing various concentrations of α-syn. After allowing the attachment of α-syn with anti- α-syn antibodies, the modified surfaces were analyzed in an equimolar (10 mM) solution of $[Fe(CN)_6]^{3-/4-}$ containing 1 M KCl using EIS and square wave voltammetry (SWV). EIS studies established an excellent linear relationship between α-syn concentrations and the R_{ct} in the range of 0.3 pM–143 pM with a remarkable LOD of 0.01 pM [49]. The electrode response was also tested against nonspecific proteins such as Tau-441, HSP-70, and RACK-1 using the same concentrations as α-syn. Negligible changes in the R_{ct} values were observed for these proteins establishing the specificity of modified electrodes. Studies were also performed in order to determine the storage stability of the modified electrodes over time at 4 °C. The results showed that the modified electrodes were stable for up to 6 weeks of storage time retaining 100% of their electrochemical response. Lastly, real sample studies were performed using standard addition method with CSF from healthy individuals and PD patients. CSF samples were spiked with solutions containing α-syn at different concentrations. The % recovery values were calculated and were found to be between 96% and 102% [49].

Recent advances in chemical biology and synthetic biology led to the development of a wide variety of biorecognition layers for modification of biosensor surfaces. For instance, systematic evolution of ligands by exponential enrichment (SELEX) process can generate a high yield of oligonucleotides and short peptides coined as “aptamers” that have specific recognition ability toward targeted ligands [50]. Therefore, the use of aptamers as the recognition layer in biosensors has gained significant interest to detect a wide variety of small chemical and biological molecules. Recently, Sun et al. [51] presented a highly selective system using aptamers to detect α-syn in connection with EIS. On a gold electrode, the thiolated DNA-based aptamers that could selectively bind to α-syn, were immobilized. Upon exposure to α-syn, a complex would form between α-syn and the aptamers. This would prevent the subsequently introduced AuNPs to interact with the aptamer, resulting in an increase in the R_{ct} value. This system was further challenged with 2% (v/v) serum samples spiked with α-syn oligomers. The analytical performance of biosensor was well maintained in 2% (v/v) serum samples, while reporting an LOD of α-syn at 1 pM [51]. In addition, this aptamer-based biosensor displayed a high selectivity towards α-syn oligomers in the presence of α-syn monomers and fibrils in phosphate buffer solutions as well as in human serum samples [51]. In a similar study, a well-described intercalator, methylene blue, was adopted as the redox probe [52]. Aptamers that were previously shown to bind to α-syn oligomers selectively [53], were allowed to complex with their complementary oligonucleotide strands. This structure was then exposed to exonuclease I. In the presence of α-syn oligomers, the aptamers detached from the hybridized form and complexed with the α-syn oligomers. With the cleaved structure, terminal deoxynucleotidyl transferase (TdT) was then introduced into the system along with its substrate 2′-deoxythymidine 5′-triphosphate (dTTP), which could be polymerized on the cleaved DNA strand that was immobilized on the electrode surface. The poly-thymidine (polyT) served as attachment sites for methylene blue that led to the generation of methylene blue-based electrochemical signals. In the absence of α-syn oligomers, the aptamer structure would not be cleaved. The presence of aptamer on the surface then served as an even larger dTTP proliferation site, creating an abundance of polyT chains, which methylene blue could bind easily in turn creating a high redox signal on the electrode surface. This enzymatic cascade approach provided a linear detection range of 60 pM–150 nM, with an LOD of 10 pM [52], which was comparable to the previously reported biosensor by Sun et al. [51]. Furthermore, the aptasensor demonstrated a selectivity for α-syn oligomers in the reported interference study. In a standard addition assay of α-syn in serum samples, the reported recovery range was 95.3–107% demonstrating that this aptamer-based biosensor was robust enough to allow α-syn detection in real samples.

This section has addressed several novel electrochemical approaches to study the pathological elements of PD. It is noteworthy that α-syn autoantibodies could be utilized to determine the stages of PD progression. The incorporation of AuNPs into the biosensor design provided remarkable electrochemical signal increase due to the possible enhancement of electron transfer kinetics. Aptamers have also been shown to contain high selectivity for α-syn. These developments suggest that a new generation of electrochemical biosensors for PD studies are poised to provide promising detection platforms.

2.3. Electrochemical detection of interaction between α-syn and small molecules

In the past decade, several techniques have been used to study the aggregation kinetics of α-syn as well as its interactions with other small molecules [54,55]. For example, Chan et al. [56]

developed a novel electrochemical technique to study the effect of Cu(II) ions and baicalein. Cu(II) ions have previously been shown to accelerate α-syn aggregation by other research groups [35–38]. Baicalein (Fig. 3A), is a flavonoid that has metal chaperone properties [57] that has been shown to inhibit, and even reverse α-syn aggregation [57–59], using electrochemistry. Lopes et al. [54] also took advantage of the four tyrosine residues that α-syn possesses at positions Y39, Y125, Y133, and Y136 and monitored the changes in the oxidation peak current signals of tyrosine as an indication of α-syn fibril formation [55,56].

Using screen-printed carbon strip (SPCS) electrodes, α-syn was incubated in the presence and absence of baicalein and Cu(II). When only α-syn was incubated, a low-current was observed due to the gradual fibrillization. This was attributed to the tyrosine residues becoming progressively buried in the fibril structure (Fig. 4C) and therefore not being accessible to be oxidized as would be the case in the monomers and oligomers (Fig. 4A and B). A fluctuating signal over time was also observed, which was due to the varying accessibility of tyrosine residues to the surface of the electrode. In the presence of Cu(II) ions, the voltammograms showed a decrease in the current in the early stages of fibril formation associated with an increase in the rate of fibrillation (Fig. 4C). Cheng et al. [60] later developed a gold nanostructured indium tin oxide (Au-ITO) electrode using nanolithography to study the aggregation of α-syn in the presence of Cu(II) ions using differential pulse voltammetry (DPV). They reported that after linking α-syn to the electrode and incubating it with Cu(II) solution for 48 h, an increase in the current was observed using DPV. This difference in results from the previous work may be due to multiple reasons, as in the first study, α-syn was incubated on a SPCS electrode [56] while in the second study, α-syn was immobilized on the Au-ITO surface and the surface was blocked with Tris-HCl and 1,1-unmercaptodecanol (MU) [60]. Furthermore, instead of using the tyrosine oxidation peak as an indication of the extent of aggregation, $[Fe(CN)_6]^{3-/4-}$ was used as the probe. Cheng et al. [60] hypothesized that the increase in current responses could be attributed due to the Cu(II) ions binding with the α-syn resulting in a facilitation of charge transport to the Au-ITO surface.

An opposite analytical response was observed, when baicalein was allowed to interact with α-syn in solution [56]. A significant increase was observed in current response. This increase was ascribed to the oxidation of baicalein itself which overlapped with the oxidation potential of tyrosine residues at approximately 0.550 V (vs. Ag/AgCl). However, the increase in current responses subsided after further incubation due to the autooxidation of baicalein with the appearance of a new peak appeared at 0.650 V (vs. Ag/AgCl), which was attributed to a new species formed from the interaction of baicalein with α-syn oligomers [56].

Interestingly, in the study where Cheng et al. [60] used Au-ITO surfaces, they observed that (−)- epigallocatechin-3-gallate (EGCG) (a well-studied flavonoid found in green tea, Fig. 3B), displayed the opposite effect when analyzed using DPV [60]. α-syn in the presence of EGCG showed a decrease in current responses after 48 h of incubation, which they reported to be due to the formation of unstructured amorphous α-syn aggregates [60]. Moreover, it took six years after this study to show *in vivo* that baicalein inhibited the formation of α-syn fibrils in a rotenone mouse model [61]. These promising results point to the possible applications of electrochemical biosensors as a screening technique to analyze and predict biomolecular interactions in a cost-effective format [62].

Recently, DPV-based assays were used in a proof-of-concept study to determine the effect of single mutation on Cu(II)-binding-site of α-syn [63]. This was done by immobilizing three different variants of α-syn (wild-type α-syn, and two pathological mutants, H50Q and G51D), onto the surface of gold electrodes using a thiolated-polyethylene glycol (HS-PEG) linker. Subsequently, the thiolated PEG_3 was used to block the unoccupied gold surfaces to suppress non-specific adsorption issues. Then, different concentrations of Cu(II) solutions were exposed onto the modified electrodes followed by a stringent wash with Cu(II)-free ammonium acetate solution. Compared to the control electrodes which only had the thiolated PEG on surfaces, the modified electrodes with different α-syn peptides displayed a cathodic current peak for Cu(II) ions (Fig. 5). Li et al. [63] reported that with increasing concentrations of Cu (II) ions, the cathodic peak current increased, as well. Furthermore, they demonstrated that the G51D variant displayed the strongest interaction with Cu(II) ions, while the H50Q variant displayed the weakest interaction with Cu(II) ions. This varying trend in the interaction of G51D and H50Q peptides with Cu(II) was also reported using sophisticated analytical techniques such as nuclear resonance spectroscopy [64,65].

In addition, Fe(III) and Fe(II) ions have also been shown to interact with α-syn using electrochemistry as reported by Peng et al. [66]. The interaction between Fe(II) and α-syn was also studied spectroscopically and shown to have a stoichiometry of 1:1, and a binding affinity of $5.8 \times 10^3\ M^{-1}$ [67], however, the binding affinity between Fe(III) and α-syn could not be elucidated due to the ease by which Fe(III) is hydrolyzed at physiological pH [66]. Using

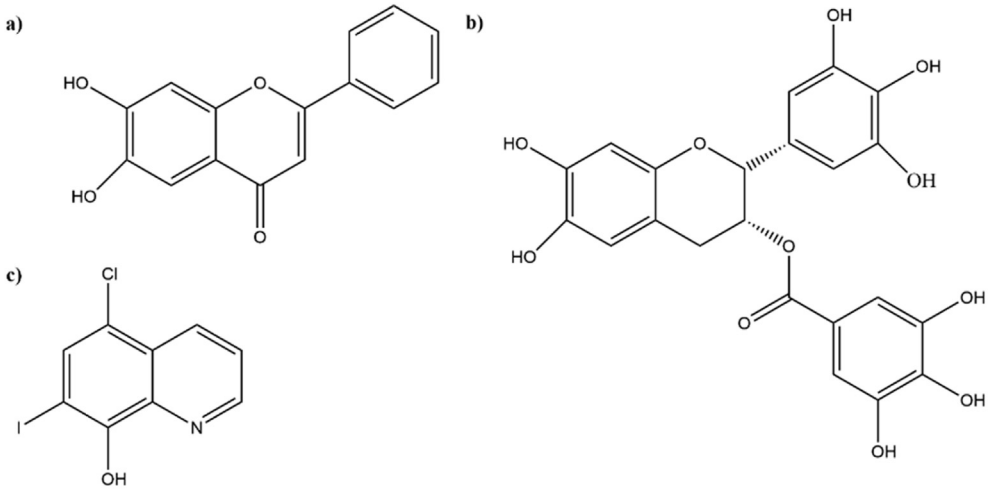

Fig. 3. Chemical structures of (a) baicalein, (b) EGCG and (c) clioquinol.

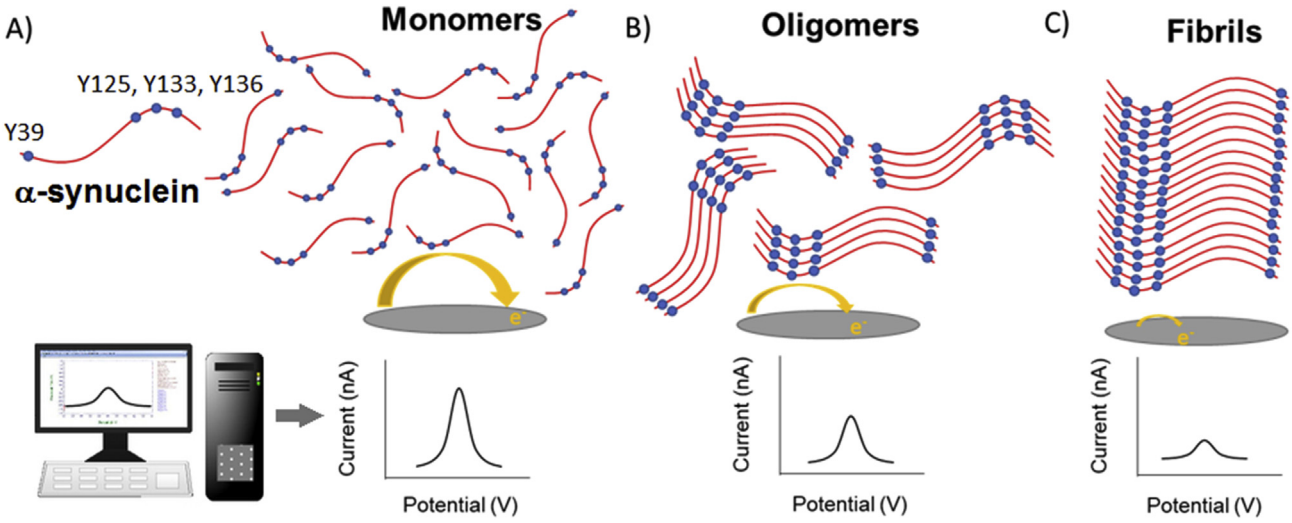

Fig. 4. Schematic illustration showing how the aggregation of α-synuclein progressively causes a significant decrease in the current observed for tyrosine oxidation. (A) Monomeric α-syn possesses four tyrosine residues that are readily accessible for oxidation and therefore, a high current is observed. (B) Oligomeric α-syn possesses less available tyrosine residues, as they are buried within the protein structure and therefore, a relatively lower current is observed. (C) α-syn in the fibril form have a low number of tyrosine residues exposed to the surface and hence, a significantly low current is detected in comparison with the ones obtained with monomeric and oligomeric forms of α-syn.

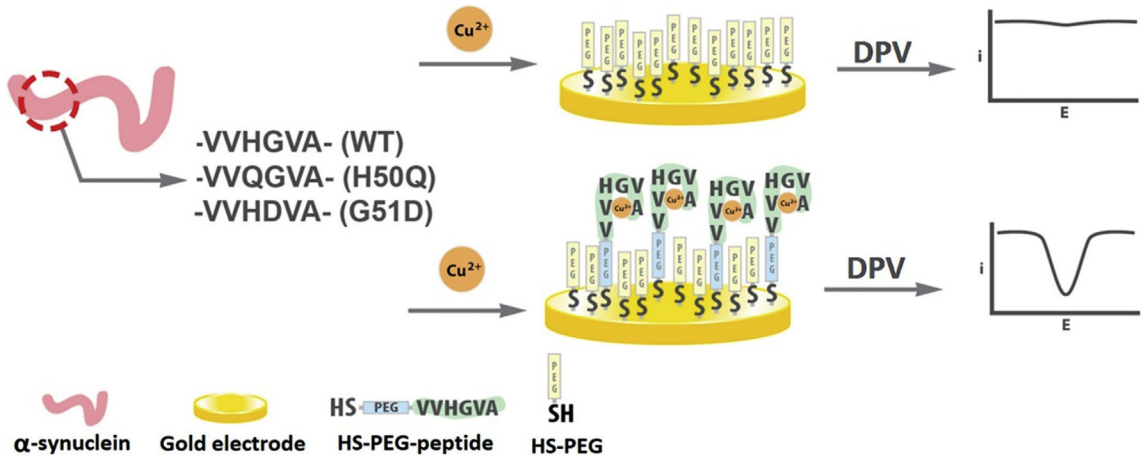

Fig. 5. Modification of the gold electrode surface using the thiolated-PEG (HS-PEG) and the HS-PEG-α-syn peptides. Due to the binding event between Cu(II) ions and α-syn, a cathodic peak current was observed after the exposure of Cu(II) ions to the HS-PEG-α-syn-modified electrodes versus the HS-PEG-modified ones. The inset shows the hexamer representing the 48–53 amino acid sequence for the wild-type α-syn, as well as the H50Q and G51D variants [63]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

CV, they determined the shift of the Fe(III)/Fe(II) redox potential when iron was bound to α-syn, and showed that Fe(III) and α-syn had a reduction potential of 0.025 V vs Ag/AgCl, and a binding affinity of $1.2 \times 10^{13}\,M^{-1}$ which they acknowledged as relatively large. However, they calculated that the product of hydrolysis, $[Fe(III)]_{eq}[OH^-]^3$ would still be significantly more favourable and hence, at physiological conditions, Fe(III) that is bound to α-syn, would likely dissociate and hydrolyze to form $[Fe(III)]_{eq}[OH^-]^3$. In the presence of O_2, Fe(II)–α-syn complexes were oxidized to Fe(III)-α-syn complexes and produced H_2O_2 as a side product [66]. Using these results, it was hypothesized that α-syn might be acting as a potential Fe(II)-chaperone *in vivo*, however they reserved concluding this until more clinical evidence could be obtained.

Clioquinol (Fig. 3C) is an anti-fungal compound which possesses metal chelation properties [68,69]. It has been shown to curtail amyloid-β aggregates in Alzheimer's disease [70]. Cheng et al. [71] showed that clioquinol also inhibited the aggregation process of α-syn using an electrochemical approach. In the absence of clioquinol, α-syn proceeded to aggregate, while decreasing the tyrosine oxidation signal using DPV. In the presence of clioquinol, due to the fibrillation inhibition ability, α-syn proteins were unable to aggregate, thus, tyrosine residue retained its accessibility to interact and exchange electrons with the electrode surface. These results supported the hypothesis that clioquinol possessed anti-fibrillogenic properties towards α-syn.

This section discussed several electrochemical studies describing the interaction of α-syn with other small molecules including baicalein, EGCG and clioquinol, as well as metals such as Cu(II) and Fe(II)/(III) which are hypothesized to play a role in the pathology of PD [72–74]. We believe these studies demonstrated how useful electroanalytical chemistry can be in studying *in vitro* the biomolecular interactions related to PD. Future investigations should be aimed to facilitate the miniaturization of these electrochemical detection systems to provide new platforms for high-throughput screening of small molecules that target α-syn.

3. Conclusion

In this review, electrochemical biosensors that target α-syn as the hallmark biomarker of PD. Techniques developed to detect α-syn utilized antibodies and aptamers as the biorecognition layer on electrode surfaces. Additionally, electrochemical techniques used to study the interaction of α-syn with small molecules have also been discussed. Research efforts can be devoted towards developing a high-throughput multiplexed system that can analyze the interaction that α-syn with a large library of drug candidates to facilitate structure-activity relationship (SAR) studies. Furthermore, in order to develop a biosensor that can be used to diagnose PD in a clinical setting, a few key challenges about biofouling of electrode surfaces must be considered. With the exponential growth in the development of electrochemical biosensors, we envisage that diagnostic devices and drug-screening platforms might be produced in the near future.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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