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Review

Recent development of interaction of transition metal complexes with DNA based on biosensor and its applications

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ABSTRACT

In this article interaction of transition metal complexes with DNA and its applications in electrochemical DNA biosensors as hybridization indicator or electroactive marker of DNA are reviewed. Special emphasis has been given to the efforts for the development of new transition metal complexes and their interaction to DNA. DNA and polymers covalently conjugated with transition metal complexes were also reviewed.

Contents

Abbreviations: DNA, deoxyribonucleic acid; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; bpy, 2,20-bipyridine; phen, 1,10-phenanthroline; bipy, 2,20-bipyridine; PYNI, 2-(20-pyridyl)naphthoimidazole; CT-DNA, calf thymus DNA; dta, 3-(pyrazin-2-yl)-*as*-triazino[5,6-*f*]acenaphthylene; dpt, 3-(pyrazin-2-yl)-*as*-triazino[5,6-*f*]phenanthrene; ddt, 3-(pyrazin-2-yl)-5,6-diphenyl-*as*-triazine; CD, circular dichroism; CNOIP, 2-(2-chloro-5-nitrophenyl)imidazo[4,5-*f*][1,10]phenanthroline; HPIP, 2-(2-hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline; DPPZ, dipyrido[3,2:α-2′-3′:*c*]-phenazine; TAPTP, 4,5,9,18-tetraazaphenanthreno-[9,10-*b*]triphenylene; PMIP, 2-(4-methylphenyl)imidazo[4,5-*f*]1,10-phenanthroline; Dmp, 2,9-dimethyl-1,10-phenanthroline; OBIP, 2-(2-bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline; PBIP, 2-(4-bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline; Me_4phen, 3,4,7,8-tetra-methyl-1,10-phenanthroline; ip, imidazo[4,5-*f*][1,10]phenanthroline; pip, 2-phenylimidazo[4,5-*f*][1,10]phenanthroline; hpip, 2-(2-hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline; Dip, 4,7-diphenyl-1,10-phenanthroline; qdppz, naphtha[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine-5,18-dione; Hqdppz, 5,18-dihydroxynaphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine; py, pyridine; dppz, dipyrido[3,2-*a*:2′,3′-*c*]phenazine]; FID, fluorescent intercalator displacement; Hcmbpy, 4-carboxy-4′-methyl-2,2′-bipyridine; Dafone, 4,5-diazafluorene-9-one; DPV, differential pulse voltammetry; HIV, human immunodeficiency virus; bbt, bis(*N*-benzyl-benzotriazole)dichloro; PVP, poly(4-vinylpyridine); PVP-$[Os(5,6\text{-dmphen})_2Cl]^{2+}$, 5,6-dmphen = 5,6-dimethyl-1,10-phenanthroline; Os, osmium; PEM, polyelectrolyte multilayer; Au, gold; GCE, glassy carbon electrodes; Os-PVP, $[Os(bpy)_2Cl]^{2+}$-PVP; AA, ascorbic acid; phen-dione, 1,10-phenantroline-5,6-dione; DA-bpy, 4-diamino-2,2-bipyridine; DPPZ, dipyrido[3,2-*a*:2,3-*c*]phenazine; Im, imidazole; Teph, terephthalate; Mim, 1-methylimidazole; NCS, thiocyanate; CV, cyclic voltammetry.

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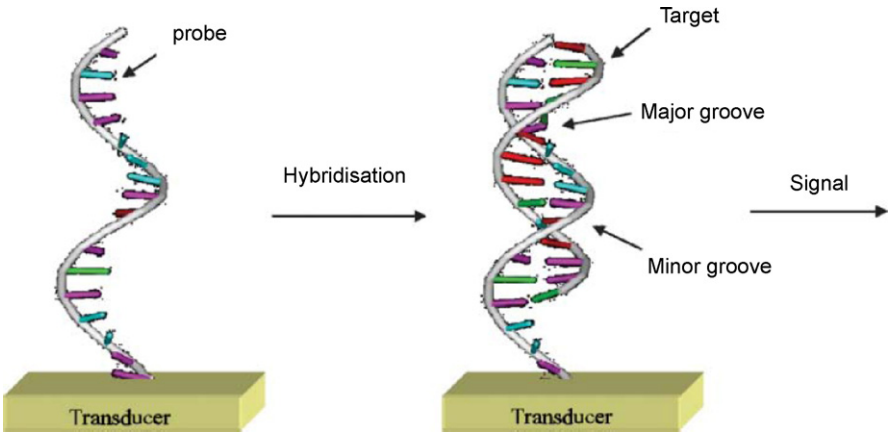

Fig. 1. Recognition interface consisting of the immobilized DNA probe and steps in the detection of a specific DNA target strand (reproduced from Ref. [7], Copyright (2007) with permission from Royal Society Chemistry).

1. Introduction

Deoxyribonucleic acid (DNA) offers chemists a very powerful tool. The detection of specific DNA sequences provides the fundamental basis for monitoring a wide variety of genetic diseases, viral infections and infectious diseases. Moreover, an understanding of how small molecules interact with DNA is potentially useful in the design of new drugs and diagnostic reagents. DNA biosensors based on nucleic acid recognition processes have received considerable attention in rapid and inexpensive DNA assays [1,2]. Different detection methods including fluorescence, surface plasmon resonance, quartz crystal microbalance and electrochemistry have been employed to study DNA hybridization or its interactions with various molecules. Amongst them, electrochemical transducers offer powerful tools for interfacing DNA recognition at the molecular level and converting the hybridization event into an analytical signal [3–6]. Due to attractive advantages such as high sensitivity, low cost and minimal power requirements, electrochemical DNA biosensors represent a dynamically developing field in the fields of clinical diagnosis, environmental monitoring and drug analysis.

Electrochemical DNA biosensors rely on the conversion of DNA base pair recognition events into a useful electrical signal. In such devices, the sensitive recognition layer was coupled to a signal transducer. Detection based on the use of electroactive hybridization indicators is still of attractive significance. In brief, single-stranded DNA (ssDNA) probe is immobilized onto different electrodes to recognize its complimentary target sequence via base pairing. The conversion of hybridization/recognition event into measurable electrochemical signals was achieved by using an electroactive compound [2,4]. Nowadays, transition metal complexes have been widely used as electrochemical hybridization indicators or electroactive markers of DNA. Moreover, it has been reported that many transition metal complexes have activity of chemistry nuclease, such as splitting DNA with specificity. Development of new transition metal complexes and utilization of their interaction with DNA will be potentially useful in clinical diagnosis for the design of new drug or reactive probes.

2. Electrochemical hybridization indicators and electroactive markers of DNA

Fig. 1 illustrates the recognition interface consisting of the immobilized DNA probe and steps in the detection of a specific DNA target strand [7]. Electrochemical DNA biosensors rely on the conversion of the DNA base pair recognition events into a useful electrical signal. Electrochemical hybridization indicators are usually electroactive compounds with small molecular weight and different affinities for ssDNA relative to double-stranded DNA (dsDNA). Binding of small molecules to DNA are normally defined as three modes including electrostatic interaction, groove binding, and intercalation. It is well known that grooves are formed only within the double helix. As a result, groove-binders have greater affinity for dsDNA than ssDNA, whereas, electrostatic binders have the poorest duplex-specific affinity. For intercalators, they usually have much higher duplex affinity and binding stability.

The desired properties [7] of an ideal electrochemical hybridization indicator can be summarized as follows:

(1) Stable reduced and oxidized forms.
(2) Duplex-specific affinity was highlighted and ssDNA affinity should be negligible or, at least, as low as possible.
(3) Fast and reversible electron transfer between the electrochemical hybridization indicator and electrode.
(4) Redox potential should be outside those for the oxidation and reduction processes of the nucleobases. Low redox potentials are preferred.
(5) Ease of preparation, low toxicity and cost.

In summary, hybridization was detected by redox-active complexes that associated selectively and reversibly with hybrid duplex. As a result, the electrochemical signal (peak current) corresponding to the indicator increases in proportion to the amount of hybrid duplex formed, which relates to the amount of complementary target sequence.

Redox label of DNA using transition metal complexes is outstanding over the past few years, despite interaction with dsDNA formed after hybridization. The covalent attachment of metal complexes probes to DNA or polymers have been reported recently [8,9]. The combination of redox characteristics and photophysical properties of transition metal complexes might meet the multifunctional analysis of DNA and possess potential application in biochip device.

3. Interaction of transition metal complexes with DNA and their application as hybridization indicator

Nowadays, transition metal complexes show potential and have been successfully applied as the mostly used electroactive hybridization indicators for DNA analysis. In general, transition metal complexes usually consist of one or several transition metal ion(s) as center ion(s) and several organic heterocycles as ligands.

Cationic metal complexes that interact in a different way with ssDNA and dsDNA are usually applied. Transition metal complexes used as electrochemical hybridization indicators could be classed based the center transition metal ions.

3.1. Ruthenium(II) complexes

Ruthenium(II) complexes was useful for studying DNA binding with transition metal complexes owing to their attractive characteristics including ease of preparation, intense optical absorption and emission, and inertness towards substitution and racemization. Ruthenium(II) complexes containing mixed ligands are usually investigated [10,11]. The structure blocks commonly used for the synthesis of ruthenium(II) complexes included 2,20-bipyridine (bpy), 1,10-phenanthroline (phen), 2,20-bipyridine (bipy) and their derivatives.

Ji group synthesized asymmetric bidentate ruthenium(II) complex and systematically investigated their interaction with DNA [10]. Ruthenium(II) complex $[Ru(bpy)_2(PYNI)]^{2+}$ (PYNI = 2-(20-pyridyl)naphthoimidazole) could bind to calf thymus DNA (CT-DNA) in an intercalative mode. Using asymmetric ligands, 3-(pyrazin-2-yl)-*as*-triazino[5,6-*f*]acenaphthylene (dta) and 3-(pyrazin-2-yl)-*as*-triazino[5,6-*f*]phenanthrene (dpt), three ruthenium(II) complexes $[Ru(bpy)_2(L)][ClO_4]_2$ (L = ddt (complex 1), dta (complex 2) and dpt (complex 3), ddt = 3-(pyrazin-2-yl)-5,6-diphenyl-*as*-triazine) were also synthesized and investigated [11]. Results showed that the planar extension of intercalative ligand increased the interaction with DNA. They also found that the size and shape of the intercalated ligand had remarkable effect on the strength of interaction. $[Ru(bpy)_2(dta)][ClO_4]_2$, and $[Ru(bpy)_2(dpt)][ClO_4]_2$ could enantioselectively interact with CT-DNA but not $[Ru(bpy)_2(ddt)][ClO_4]_2$, as showed by circular dichroism (CD) signals of the dialysates of the racemic complexes against CT-DNA. It was interesting that $[Ru(bpy)_2(PYNI)]^{2+}$, $[Ru(bpy)_2(dta)][ClO_4]_2$, and $[Ru(bpy)_2(dpt)][ClO_4]_2$ could promote cleavage of plasmid pBR 322 DNA from the supercoiled form I to the open circular form II upon irradiation.

A series of enantiomeric polypyridyl ruthenium(II) complexes including $[Ru(bpy)_2CNOIP](PF_6)_2$ (CNOIP = 2-(2-chloro-5-nitrophenyl)imidazo[4,5-*f*][1,10]phenanthroline), $[Ru(bpy)_2HPIP](PF_6)_2$ (HPIP = 2-(2-hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline), $Ru(bpy)_2DPPZ](PF_6)_2$ (DPPZ = dipyrido[3,2:α-2′-3′:*c*]-phenazine), $[Ru(bpy)_2TAPTP](PF_6)_2$ (TAPTP = 4,5,9,18-tetraazaphenanthreno-[9,10-*b*]triphenylene) were also prepared by Ji group [12]. Though all the enantiomers bound to DNA through intercalative mode, the binding affinity of each chiral complex to DNA was different due to the different shape and planarity of the intercalative ligand. Upon irradiation at 302 nm, $[Ru(bpy)_2HPIP](PF_6)_2$, $Ru(bpy)_2DPPZ](PF_6)_2$, and $[Ru(bpy)_2TAPTP](PF_6)_2$ could promote the cleavage of plasmid pBR 322 DNA from supercoiled form I to nicked form II. Additionally, obvious enantioselectivity was observed on DNA cleavage for the enantiomers of $[Ru(bpy)_2HPIP](PF_6)_2$ and $[Ru(bpy)_2TAPTP](PF_6)_2$. PMIP (PMIP = 2-(4-methylphenyl)imidazo[4,5-*f*]1,10-phenanthroline) was also used to synthesize three Ru(II) complexes, including $[Ru(bpy)_2PMIP]^{2+}$, $[Ru(phen)_2PMIP]^{2+}$ and $[Ru(dmp)_2PMIP]^{2+}$ (dmp = 2,9-dimethyl-1,10-phenanthroline) [13]. The theoretical calculations using density functional theory (DFT) on the level of B3LYP/LanL2DZ basis were used to investigate the binding strength or binding constants (K_b). Results showed that $[Ru(bpy)_2PMIP]^{2+}$ and $[Ru(phen)_2PMIP]^{2+}$ could bind to CT-DNA through intercalation, while $Ru(dmp)_2PMIP]^{2+}$ via partial intercalative mode. Ancillary ligands of polypyridyl Ru(II) complexes had significant effects on the spectral properties and the DNA binding behaviors. They [14] also synthesized two polypyridyl ligands with substituent Br at different positions in the phenyl ring, including OBIP (OBIP = 2-(2-bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline) and PBIP (PBIP = 2-(4-bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline). $[Ru(dmp)_2(OBIP)]_2$ bound to CT-DNA via a semi-intercalative mode while $[Ru(dmp)_2(PBIP)]_2$ strongly bound to CT-DNA through intercalation. Some experimental regularities or trends were reasonably explained by the theoretical results.

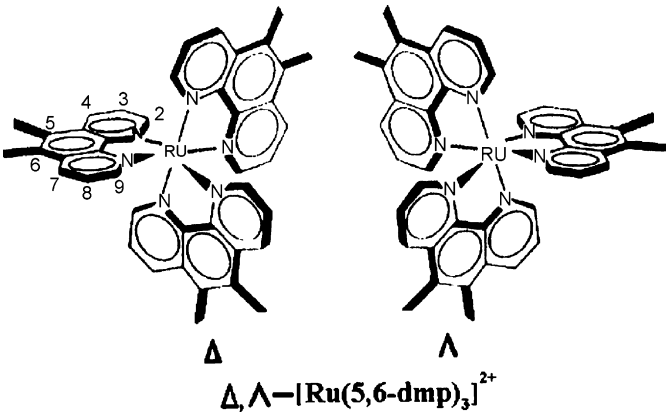

Fig. 2. Schematic view of *rac*-$[Ru(5,6\text{-}dmp)_3]^{2+}$ (reprinted with permission from Ref. [18]. Copyright (2006) American Chemical Society).

Palaniandavar group synthesized a serial of Ru(II) mixed ligand complexes including $[Ru(NH_3)_4(L)]^{2+}$, L = bipy, phen, 5,6-dmp, 4,7-dmp, 22,9-dmp, 3,4,7,8-tetra-methyl-1,10-phenanthroline (Me_4phen), imidazo[4,5-*f*][1,10]phenanthroline (ip), 2-phenylimidazo[4,5-*f*][1,10]phenanthroline (pip), 2-(2-hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline (hpip), 4,7-diphenyl-1,10-phenanthroline (dip), naphtha[2,3-*a*]dipyrido [3,2-*h*:2′,3′-*f*]phenazine-5,18-dione (qdppz), and 5,18-dihydroxynaphtho[2,3-*a*]dipyrido[3,2-*h*:2′,3′-*f*]phenazine (hqdppz) [15,16]. Those complexes interacted with DNA through their diimine face, which was supported by the hydrogen bonding of the ammonia co-ligands. When the diimines in these complexes are different as methyl-substituted 1,10-phenanthrolines [15] and modified 1,10-phenanthrolines [16], the DNA binding affinities were higher than those with phen and bpy co-ligands. The interaction between CT-DNA and Ru(II) complexes with 5,6-dmp as primary ligand and phen, bpy, pyridine (py) and NH_3 as co-ligands was also investigated [17]. The DNA binding constants decreased in the order of tris(5,6-dmp)Ru(II) > bis(5,6-dmp)Ru(II) > mono(5,6-dmp)Ru(II). The phenomenon that they depend upon the number of 5,6-dmp ligands revealed hydrophobic interaction between 5,6-dimethyl groups and DNA surface. For the bis(5,6-dmp)Ru(II) complexes, those with monodentate py or NH_3 as co-ligands showed slightly higher DNA binding affinities than the bpy and phen analogues. The phenomena suggest that the complexes interact with DNA through the co-ligands while 5,6-dmp ligand interacted with the exterior of the DNA surface. Two DNA binding modes including surface/electrostatic and strong hydrophobic/partial intercalative DNA interaction were given for such mixed-ligand complexes. Interaction of *rac*-$[Ru(5,6\text{-}dmp)_3]^{2+}$ with CT-DNA was also investigated [18]. The X-ray crystal structure of the complex *rac*-$[Ru(5,6\text{-}dmp)_3]Cl_2$ revealed a distorted octahedral coordination geometry with the Ru–N bond distances shorter than its phen analogue. Schematic view of *rac*-$[Ru(5,6\text{-}dmp)_3]^{2+}$ was given in Fig. 2. The K_b for the interaction between CT-DNA and the complex was $(8.0 \pm 0.2) \times 10^4\ M^{-1}$, which was much more strong compared with its phen analogue. Moreover, the emission intensity of the 5,6-dmp complex was dramatically enhanced when bound to DNA, which was also higher than that of the phen analogue. The ^1H NMR

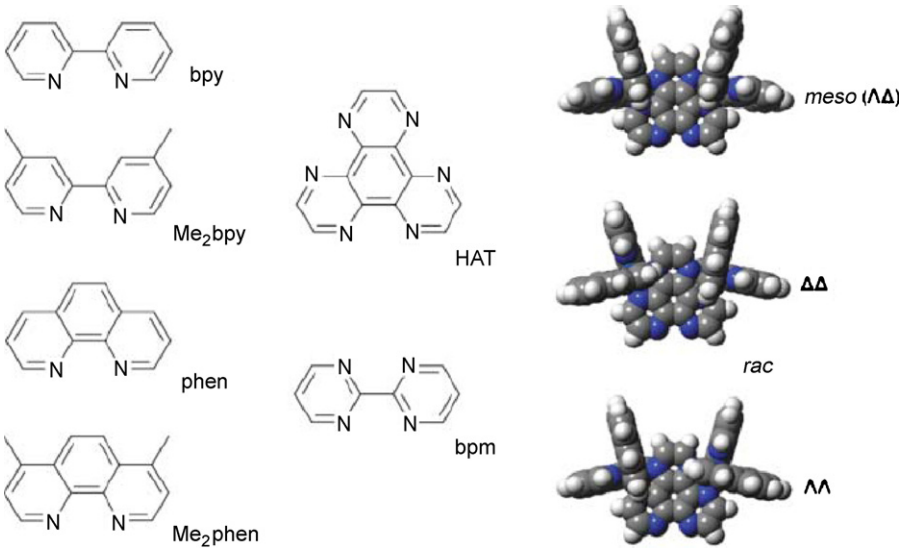

Fig. 3. Structures of the ligands used and models of the three stereoisomeric forms of the compound $[\{Ru(phen)_2\}_2(\mu\text{-HAT})]^{4+}$ (reproduced from Ref. [20], Copyright (2006) with permission from Royal Society Chemistry).

spectra of *rac*-$[Ru(5,6\text{-dmp})_3]^{2+}$ bound to poly $d(GC)_{12}$ revealed that the complex closely interacted in the major grooves of DNA. Electrochemical studies showed that 5,6-dmp complex stabilized CT-DNA from electrocatalytic oxidation of its guanine base.

The preferential binding of the Δ-enantiomer of mixed-ligand complex, *rac*-$[Ru(5,6\text{-dmp})_2(dppz)]Cl_2$ [dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine], to the right-handed CT-DNA was found [19]. The 5,6-dmp complex exhibited preferential binding to $[d(AT)_6]_2$ over $[d(GC)_6]_2$ and the complex aggregated formed with six $[Ru(5,6\text{-dmp})_2(dppz)]^{2+}$ cations per base pair of $[d(AT)_6]_2$. For $[Ru(phen)_2(dppz)]^{2+}$, however, only one cation per base pair was involved in DNA binding.

Smith et al. [20] used ^1H NMR spectroscopy and fluorescent intercalator displacement (FID) assays to investigate the DNA binding abilities of two series of dinuclear polypyridyl ruthenium(II) complexes including $[\{Ru(L)_2\}_2(\mu\text{-BL})]^{4+}$ {L = bpy, 4,4′-dimethyl-2,2′-bipyridine (Me_2bpy), phen, or 4,7-dimethyl-1,10-phenanthroline (Me_2phen); BL = 2,2′-bipyrimidine (bpm) or 1,4,5,8,9,12-hexaazatriphenylene (HAT)}. Structures of the ligands used in the investigation and models of the three stereoisomeric forms of $[\{Ru(phen)_2\}_2(\mu\text{-HAT})]^{4+}$ were given in Fig. 3. *Meso*-$[\{Ru(phen)_2\}_2(\mu\text{-HAT})]^{4+}$ was proven to be a high-affinity DNA hairpin probe.

Vrabel et al. [21] prepared purines bearing phenanthroline or bipyridine ligands and their Ru(II) complexes in position 8. The complexes were investigated as model compounds for electrochemical DNA labeling. Using carbon paste and mercury electrodes, the electrochemistry of the modified purines and the Ru(II) complexes was studied by cyclic or square-wave voltammetry. The experimental redox potentials of such compounds were compared with quantum chemical calculations. A very good agreement between experiment and theory was obtained. Several compounds of this series exhibited considerable cytostatic effect and activity against the hepatitis C virus (HCV).

Gaudry et al. [22] used surface enhanced resonance raman scattering (SERRS) to study the interaction between DNA and $Ru(bpy)_2(Hcmbpy)(PF_6)_2$ (Hcmbpy = 4-carboxy-4′-methyl-2,2′-bipyridine). In the presence of DNA, the intensity of SERRS spectra for such complexes reduced in particular for the specific bands assigned to the Hcmbpy ligand. As a result, such Ru complex bound to DNA through the Hcmbpy moiety via an intercalation mode. DNA binding with enantiopure Ru complex was more efficient than with the corresponding racemic complexes.

Steichen et al. [23] proposed an electrochemical detection of DNA hybridization method based on electrostatic interactions of $[Ru(NH_3)_6]^{3+}$ cations with DNA. Peptide nucleic acid (PNA) molecules, which contained no anionic phosphate groups, were firstly immobilized as capture probes on the gold substrate. $[Ru(NH_3)_6]^{3+}$ adsorbed onto the anionic phosphate backbone of target DNA after hybridization. Consequently, a clear hybridization detection signal was observed. Very good discrimination against the single-base mismatch A2143G, internal to the 23S rRNA gene of *Helicobacter pylori*, was obtained.

3.2. Copper(II) complexes

Palaniandavar group [24] investigated DNA binding properties of mixed-ligand copper(II) complexes of iminodiacetic acid. The complexes were Cu(imda)(phen)(H_2O) (H_2imda = iminodiacetic acid, and phen = 1,10-phenanthroline), Cu(imda)(5,6-dmp) (5,6-dmp = 5,6-dimethyl-1,10-phenanthroline) and Cu(imda)(dpq) (dpq = dipyrido-[3,2-*d*:20,30-*f*]-quinoxaline). K_b for binding of such three complexes with CT-DNA was $0.60 \pm 0.04 \times 10^3\ M^{-1}$, $3.9 \pm 0.3 \times 10^3\ M^{-1}$, and $1.7 \pm 0.5 \times 10^4\ M^{-1}$, respectively. Cu(imda)(phen)(H_2O) and Cu(imda)(dpq) bound to DNA through partial intercalation while Cu(imda)(5,6-dmp) was involved in groove binding. All the three complexes showed cleavage of pBR322 supercoiled DNA in the presence of ascorbic acid and showed hydrolytic DNA cleavage activity in the absence of light or a reducing agent. Three linear unsymmetrical tridentate ligands *N*-methyl-*N*′-(pyrid-2-ylmethyl)ethylenediamine (L1), *N*,*N*-dimethyl-*N*′-(pyrid-2-ylmethyl)ethylenediamine (L2) and *N*,*N*-dimethyl-*N*′-((6-methyl)pyrid-2-ylmethyl)ethylenediamine (L3) were used to prepare complexes of Cu(L1)Cl_2, Cu(L2)Cl_2 and Cu(L3)Cl_2 [25]. A covalent binding mode was revealed with coordination of most possibly guanine N7 nitrogen of CT-DNA to form a CuN_4 chromophore. The complexes could also induce the cleavage of pBR322 plasmid DNA and Cu(L1)Cl_2 was more efficient. DNA-fiber EPR study of the orientation of Cu(II) complexes of 1,10-phenanthroline ($[Cu(phen)]^{2+}$) and its derivatives ($[Cu(phen)_X aa]^{n+}$ (*X* standed for an aa-amino acid) bound to DNA was performed by the same group [26]. The phenanthroline moiety

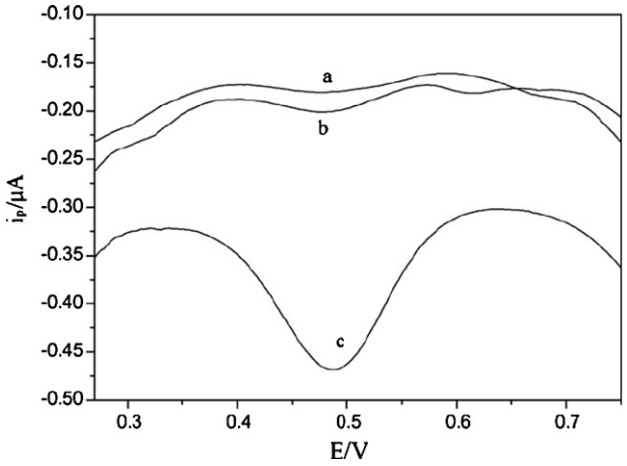

Fig. 4. Differential pulse voltammograms of [Cu(dmp)(H_2O)Cl_2] on the electrode (a) of target ssDNA immobilized, (b) after hybridization with complementary sequence, and (c) after hybridization with mismatched sequence (reproduced from Ref. [29], Copyright (2007) with permission from Elsevier).

intercalated to DNA. The amino acids in the ternary complexes of glycine, leucine, serine, threonine, cysteine, methionine, and asparagine were partly substituted with some coordinating groups in DNA, whereas the ternary complexes of lysine, arginine, and glutamine remained intact on DNA.

Jiao group [27] synthesized a new copper complex of [Cu(bpy)(pba)$_2H_2O$]0.5H_2O (pba = *p*-methylbenzoate). The complex bound to DNA through intercalation mode via 2,20-bipyridine ring into DNA base pairs. Such copper complex could cleave pBR322 DNA effectively in the presence of ascorbic acid. Zhang group [28] prepared copper complex of 4,5-diazafluorene-9-one (dafone) and bromine ligands ([Cu(dafone)$_2$]Br_2). Its interaction with salmon sperm DNA was studied. The intercalation mode was revealed. Using Cu(dafone)$_2Br_2$ as a new electrochemical DNA indicator, detection of human hepatitis B virus (HBV) DNA fragment by differential pulse voltammetry (DPV) was achieved with a detection limit of 3.18×10^{-9} M. They also developed an electrochemical DNA biosensor using [Cu(dmp)(H_2O)Cl_2] as a new hybridization indicator [29]. Numerous factors affecting the probe immobilization, target hybridization, and indicator binding reactions were optimized to maximize the sensitivity and speed the assay time. Signal of [Cu(dmp)(H_2O)Cl_2] was observed after hybridization with complementary sequence in DPV, as shown in Fig. 4. A sequence of HBV could be detected with a detection limit of 7.0×10^{-8} M. [CuL(H_2O)$_2$](ClO_4)$_2$(H_2O)$_2$, (L = *N*-(5-sulfosalicylidene) 4′-bromoaniline) [30], [CuL($C_6H_5N_2O_2$)(ClO_4)$_2H_2O$, CuL) (L = *N*-(5-sulfosalicylidene)-4′-nitraniline) [31], tetraploid (imidazole) Copper(II) Terephthalate [32], and mononuclear copper(II), nickel(II) and cobalt(III) tetracoordinate macrocyclic complexes [33] were also prepared by the same group. Interactions of the complexes with DNA were also systematically investigated.

3.3. Cadmium(II) and cobalt(II)complexes

Zhang et al. [34] developed DNA biosensor using diaquabis[*N*-(2-pyridinylmethyl)benzamide-κ^2N,O]-cadmium(II) dinitrate, {[CdL_2(H_2O)$_2$](NO_3)$_2$, L = *N*-(2-pyridinylmethyl) benzamide}, as a new electroactive indicator for detecting of HBV DNA. The binding ratio between [CdL_2]$^{2+}$ and salmon sperm DNA was calculated to be 2:1 and the binding constant was 25.56 $M^{-1/2}$. Target HBV DNA could be quantified ranged from 1.01×10^{-8} to 1.62×10^{-6} M with a detection limit was 7.19×10^{-9} M. Nucleic acid biosensor for the detection of short sequence related to HBV using bis(benzimidazole)cadmium(II) dinitrate as electrochemical indicator was also developed [35]. They [36] also obtained the crystal of three hexakis(imidazole)metallo complexes of Cd, Co and Ni. Such complexes all interact with DNA mainly by intercalation.

Table 1
Examples of transition metal complexes used for the biosensing of DNA hybridization.

Metal atom	Indicator	Detection mode transducer	Electrode	Refs.
Os	[Os(bpy)$_3$]$^{3+}$	DPV	Gold	[42]
Os	[Os(phen)$_3$]$^{3+}$	DPV	Gold	[42]
Os	[Os(bpy)$_2Cl_2$]$^+$	CV	Gold	[43]
Os	[Os(bpy)$_2Cl_2$]$^+$	CV	GCE	[44]
Os	[Os(bpy)$_2$(phe-dione)]$^{3+}$	DPV	Gold	[45]
Os	[Os(DA-bpy)$_2$DPPZ]$^{2+}$	DPV	Gold	[46]
Mn	([Mn(Im)$_6$](teph)·4H_2O)	DPV	GCE	[47]
Fe	{[Fe(phen)(H_2O)$_3$]$_2O$}(SO_4)$_2$	DPV	Gold	[48]
Ni	Ni(NCS)$_2$(Mim)$_4$	CV	GCE	[49]

Zhang group [37] studied the interaction mechanism between 1,10-phenanthroline cobalt(II) complex [Co(phen)$_2ClH_2O$]Cl and salmon sperm DNA. An intercalative mode was identified. Electrochemical DNA biosensor was developed by covalent immobilization of probe ssDNA related to HIV on the activated glassy carbon electrode (GCE). With [Co(phen)$_2ClH_2O$]$^+$ being the novel electrochemical hybridization indicator, selective detection of complementary ssDNA was achieved using DPV. The complex [Co(phen)$_2$IP]·2ClO_4·3H_2O (IP = imidazo[*f*] phenanthroline) was also synthesized by the group [38]. The binding ratio between [Co(phen)$_2$IP]$^{2+}$ and salmon sperm DNA was calculated to be 1:1 and the binding constant was 3.74×10^5 M^{-1}. A HIV DNA biosensor was developed by covalent immobilizing of single-stranded HIV DNA fragments to a modified GCE. Using [Co(phen)$_2$IP]$^{2+}$ as electrochemical indicator, a detection limit of 27 pM was achieved using DPV. Karadeniz et al. [39] also investigated the using of the intercalator, [Co(phen)$_3$]$^{3+}$, for the detection of DNA hybridization.

Erdem et al [40] accomplished the detection of hybridization using Co(III) complex, [Co(phen)$_3^{3+}$] as indicator species. The reversible electroactivity of such complex and strong association with the immobilized dsDNA segment lead to significantly enhanced voltammetric signals.

Electrochemical and spectroscopic studies on the interaction between tetracoordinate macrocyclic cobalt(III), copper(II) and nickel(II) complexes and DNA were also performed by Zhang et al. to find highly efficient ligands for hepatic asialoglycoprotein receptor(ASGPR) [33]. Four cluster galactosides with different scaffolds were synthesized. Results showed that trivalent cluster galactosides behaved better than divalent analogues. The cluster galactosides with aryl groups on their scaffolds presented better binding affinity than those with aliphatic chain scaffolds. Jiao et al. [41] synthesized a new bis(*N*-benzyl-benzotriazole)dichloro (bbt) Co(II) complex (Co(bbt)$_2Cl_2$). The interaction of such complex with fish sperm DNA was studied. The binding of DNA with the complex contained electrostatic binding and intercalation.

3.4. Other metal complexes

Transition metal complexes used for the biosensing of DNA were given in Table 1. As shown, the central metal atom included Os, Mn, Fe and Ni. The ligands contained bpy, phen, 1,10-phenantroline-5,6-dione, 4,4-diamino-2,2-bipyridine, imidazole, 1-methylimidazole, terephthalate and thiocyanate [42–49].

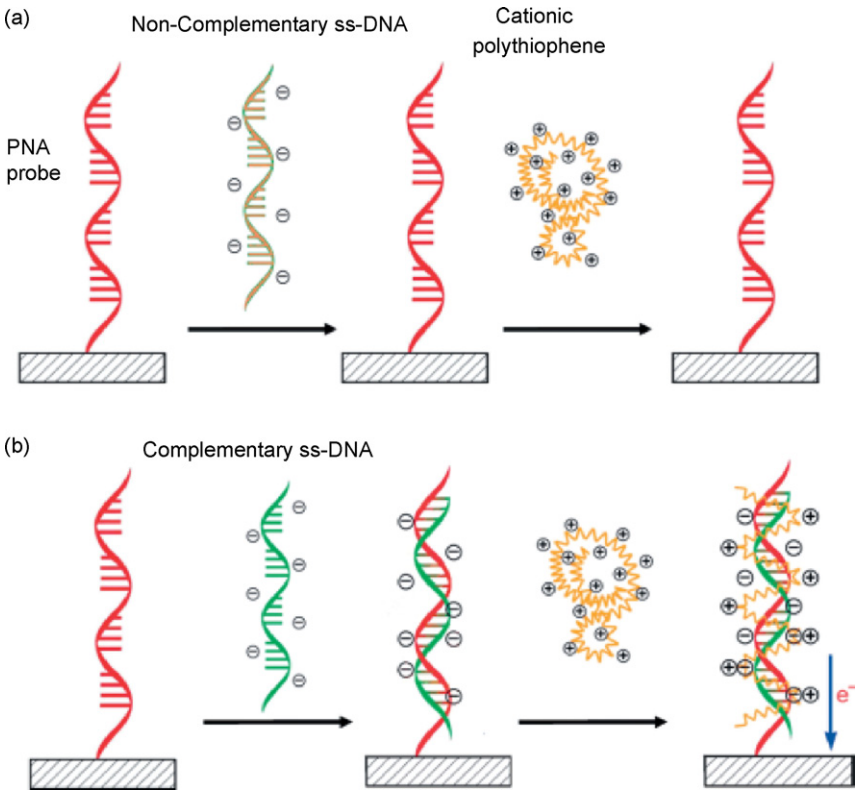

Fig. 5. Schematic description of the electrochemical detection of unlabelled DNA targets, (a) without and (b) with complementary ssDNA (reproduced from Ref. [8], Copyright (2005) with permission from Wiley-VCH Verlag GmbH & Co. KGaA).

4. Transition metal complexes as electroactive marker of DNA

Attachment of electroactive probes based on transition metal complexes to a nucleobase via conjugate linkers should increase the efficiency of charge transfer and thus enhance sensitivity. There are many examples of such probes connected to pyrimidine nucleobases. The attached probe could be used to probe DNA hybridization and charge transfer through DNA. The strategy also possesses potential application in multifunction analysis of DNA by combining the redox characteristic and photophysical properties of transition metal complexes. The ferrocenyl group was well suited for the development of redox-active oligonucleotides owing to its stability, diverse substitution chemistry and amenable stereochemistry. There have some reports on terminal attachment of ferrocenyl groups to oligonucleotides. The attachment of other redox-active metal complexes, e.g. polypyridine Ru(II) and Os(II) complexes, was also attempted.

Hurley and Tor [50] reported a general and versatile method for site-specific incorporation of polypyridine Ru(II) and Os(II) complexes into DNA oligonucleotides using solid-phase phosphoramidite chemistry. Novel nucleosides were synthesized by covalent attachment of $[(bpy)_2M(3\text{-ethynyl-}1,10\text{-phenanthroline})]^{2+}$ (M = Ru, Os) to 5-position in 2′-deoxyuridine. A well-defined $M^{2+/3+}$ wave could be obtained in electrochemical experiments. Photophysical experiments showed that the Ru(II) nucleoside exhibits a rather long-lived excited state in phosphate buffer at pH 7.0 (τ = 1.08 μs) associated with a relatively high emission quantum efficiency (ϕ = 0.051). CD spectra confirmed that the global conformation of the double helix was not altered by the presence of these polypyridyl complexes in the major groove.

Vrabel et al. [51] synthesized 2′-deoxyadenosine nucleosides bearing bipyridine-type ligands and their Ru-complexes in position 8 through cross-coupling reactions. The ligand for modification 2′-deoxyadenosine was bipyridine, phenanthroline or terpyridine ligands.

5. Transition metal complexes as electroactive marker of polymer

Polymers covalently conjugated with transition metal complexes have also enabled the transduction of hybridization events into an electrical signal without labeling of the DNA target. In addition, the combination with other techniques, e.g. layer-by-layer self-assembly could be easily achieved and their potential application in biochip device is promising.

Le Floch et al. [8] reported specific and sensitive label-free electrochemical detection of unlabeled target DNA at room temperature through ferrocene-functionalized cationic polythiophene. The schematic description of such label-free electrochemical detection using gold-bound peptide nucleic acid (PNA) probes and such ferrocene-functionalized cationic polythiophene was shown in Fig. 5. The PNA probe was neutral. The electrochemical detection occurred when the PNA probe hybridized with a complementary DNA target. Afterwards, the electrostatic interaction with the cationic polythiophene transducer was achieved.

Liu et al. [9] prepared a poly(4-vinylpyridine) (PVP) derivative bearing redox-active osmium complexes, PVP-$[Os(5,6\text{-dmphen})_2Cl]^{2+}$ (5,6-dmphen = 5,6-dimethyl-1,10-phenanthroline), as shown in Fig. 6. It was employed as a hybridization indicator for electrochemical DNA sensors. Due to polymeric effects, such polymeric indicator exhibited similar to 1000 times higher sensitivity than the corresponding monomeric analogue ($[Os(5,6\text{-dmphen})_2Cl]^{2+}$) for DNA determination. The detection limit of the present sensor was 0.5 aM. Moreover, the redox potential using

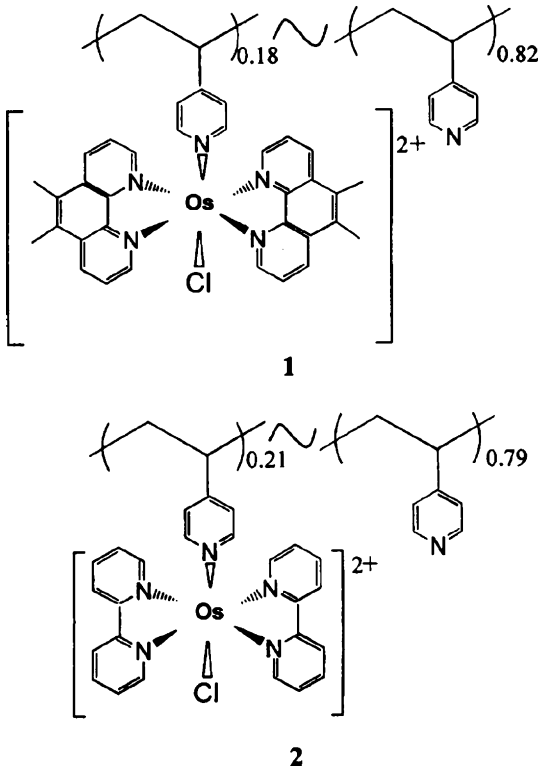

Fig. 6. Chemical structures of polymic indicators PVP-$[Os(5,6\text{-dmphen})_2Cl]^{2+}$ (reprinted with permission from Ref. [9]. Copyright (2004) American Chemical Society).

such polymeric indicator was +360 mV (vs. Ag/AgCl), which was significantly lower than that reported for the monomeric analogue (+672 mV). The polymeric indicator can be repeatedly used by removing from the electrode surface via rinsing the electrode in a high-temperature buffer for 6 min. Polyelectrolyte multilayer (PEM) films were also constructed on the surface of gold (Au) electrodes using a layer-by-layer self-assembly method by the same group. The PEM films contained polycationic Os bipyridyl (bpy) complex-attached PVP derivative, $[Os(bpy)_2Cl]^{2+}$-PVP (Os-PVP) and polyanionic calf thymus CT-DNA [52]. For such PEM layer, a diffusion-free electron transfer from the Os complex moieties in the films to the electrode surface was observed. Electrocatalytic oxidation of ascorbic acid (AA) by this DNA-containing PEM film-covered electrode was also obtained.

Le Floch et al. [53] proposed label-free electrochemical detection of protein based on a ferrocene-bearing cationic polythiophene and aptamer. Human alpha-thrombin could be detected by using a water-soluble, ferrocene-functionalized polythiophene transducer and a single-stranded oligonucleotide aptamer probe. The approach using ferrocene-functionalized polythiophene was a direct method in which the recorded current decreased upon the addition of targeted protein.

Farre et al. [54] investigated electropolymerization of pyrrole-ferrocene derivatives. The monomer was functioned with ferrocene group and the obtained poly(pyrrole) derivatives had potential application in biochip device.

6. Conclusions

The interaction of small molecules with DNA and electrochemical DNA analysis remains a dynamically developing field in clinical diagnosis, environmental monitoring and drug analysis. Application of transition metal complexes achieved significant success in electrochemical DNA biosensors as hybridization indicator or electroactive marker of DNA. Development of new transition metal complexes with high sensitivity are still of attractive significance. Polymers covalently conjugated with redox metal complexes are outstanding. Such functional polymers enable the combination with other techniques such as layer-by-layer self-assembly. Their potential application in biochip device is promising. For labeling of DNA using transition metal complexes, the attachment of metal complexes probes to nucleobase possesses potential application in multifunction analysis of DNA by combination the redox characteristic and photophysical properties of transition metal complexes.

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