DOI 10.5162/15dss2021/P3.8

Novel electrochemical probes used to monitor structure-specific behaviour of oligonucleotides on charged electrode surface

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Introduction

In the field of DNA structure study, a lot of interest is focused on the alternative DNA conformations known as G-quadruplexes (G4). These four-stranded structural motifs are present in cells as critical regulatory elements and are also of interest as potential targets in cancer therapy or nanotechnology building blocks. G4 are formed in nucleic acid sequences containing tracts of guanines that self-associate into square planar G-quartets stabilised by Hoogsteen hydrogen bonds. Individual G-quartets interact with each other via π-π stacking, and the final G4 structure is further stabilised by the presence of cations in its central cavity [1, 2].

The development of analytical tools for the recognition and study of G4 is of increasing relevance due to the growing interest in non-canonical DNA structures. Electroanalytical methods have been long established as practical tools for studying conformational changes of DNA molecules utilising intrinsic redox, as well as non-faradaic signals of DNA mainly obtained by voltammetry on mercury-based electrodes [3]. For example, the structural transition between double- and single-stranded DNA can be monitored by differential pulse or square wave voltammetry through a considerable increase of a cathodic peak corresponding to the reduction of nucleobases cytosine and adenine upon denaturation. This increase was attributed to better accessibility of reduction sites of these bases adsorbed at the electrode surface for the reduction in the denatured, single-stranded state.

However, direct electrochemical analysis of short G4-forming oligodeoxynucleotides (ODNs) using a similar approach has proven difficult due to complex electrode processes related to guanine reduction [4, 5].

In this contribution, an alternative methodological approach for the distinction of G4 structure is proposed, based on voltammetric signals of novel electrochemical probes, the cationic metalloporphyrin *meso*-5,10,15,20-tetrakis(4-(*N*-methyl-pyridinium-4-yl)phenyl)porphyrinato copper(II) (Cu-TMPy2PP, Fig. 1a) and 7-(3-nitrophenyl)-7-deazaguanosine triphosphate (dG^{NP}TP, Fig. 1b). on hanging mercury drop electrode (HMDE). Signals of these probes were used to monitor changes of accessible HMDE surface upon adsorption and/or selective desorption of various ODNs. It was shown that ODNs with the propensity to form G4 assemble into less dense layers at the electrode surface and are more prone to desorption by negative applied potential compared to single- and double-stranded ODNs, which can be used for their recognition.

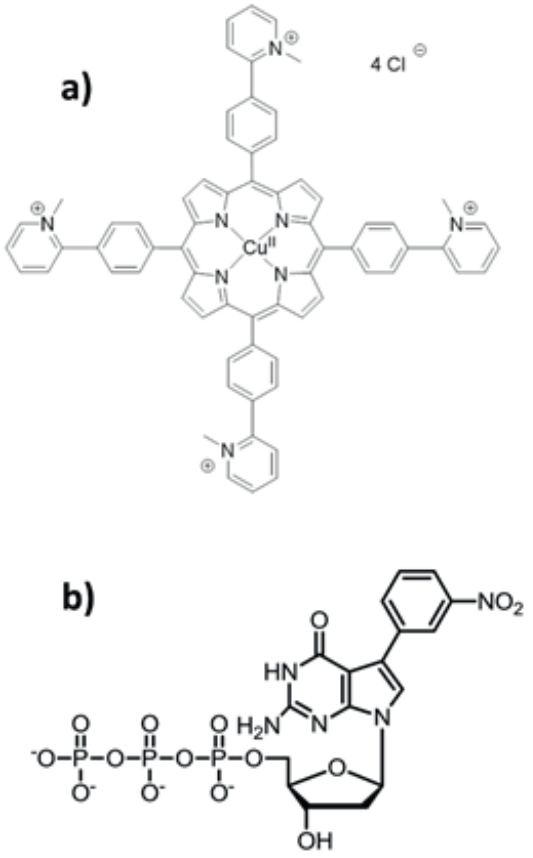

Fig. 1: Structures of the used electrochemical probes Cu-TMPy2PP (a) and dG^{NP}TP (b)

Experimental

Material

Cu-TMPy2PP was synthesised as chloride salt and stored as 100 µM stock solution in DMSO at room temperature. Synthetic ODNs used are listed in Tab. 1. and were purchased from Eurofins Genomics (Germany). T4 polynucleotide kinase was purchased from New England Biolabs (USA). All other chemicals were purchased from Sigma-Aldrich and were of

analytical grade. ODNs were prepared as 1 µM solution in 0.3 M KCl and were denatured at 95°C for 5 min and subsequently slowly cooled down to room temperature to facilitate the formation of possible secondary structures.

Electrochemical methods

Electrochemical measurements were carried out using potentiostat PGSTAT128N controlled by software NOVA 2.1. (Metrohm Autolab) in a standard three-electrode system using HMDE or mercury pool electrode (MPE) as working electrode, Ag/AgCl/3M KCl reference electrode and glassy carbon rod as the auxiliary electrode. Cyclic voltammetry (CV) was performed in the potential window (0.0 V; -1.85 V) unless stated otherwise at scan rate 1 V s^{-1}.

ODNs were adsorbed at HMDE surface from 3 µl of their solution for 60s. Such modified HMDE was then transferred to the solution of base electrolyte (0.05 M sodium phosphate/0.3 M ammonium formate (AFP) buffer pH 6.9) with or without the used electrochemical probe where the analytical CV was performed.

Tab. 1: Sequences of used oligodeoxynucleotides

Name	Length	Sequence
hut-T2	22	$AG_3T_2AG_3T_2AG_3T_2AG_3$
hut-com	22	$C_3TA_2C_3TA_2C_3TA_2C_3T$
odn15	15	$G_3TACG_2CG_3TAC$
odn15-com	15	$GTAC_3GC_2GTAC_3$
T30	30	T_{30}

Polyacrylamide gel electrophoresis (PAGE)

PAGE was used to detect any ODNs present in the base electrolyte due to desorption from the electrode surface during the CV scan. The electrolyte solution was collected and desalted using QIAquick spin columns (QIAGEN, Germany). The volume of the samples was further reduced by water evaporation at 95°C. The samples were incubated with T4-polynucleotide kinase and γ-^{32}P-ATP for 30 min at 37°C, resulting in 5'-^{32}P labelling of the ODNs. The samples were loaded on 12.5% polyacrylamide gel in 1x TBE buffer, separated for 45 min at 150 V and visualised by autoradiography.

Results and discussion

Use of Cu-TMPy2PP as an electrochemical probe

Using cyclic voltammetry (CV) under present conditions, Cu-TMPy2PP produces two cathodic peaks at −0.95 V and −1.21 V at bare HMDE surface. When HMDE surface is first covered in adsorbed ODNs and left to incubate for 60 s in Cu-TMPy2PP solution in AFP buffer before the CV measurement, the peak at −0.95 V (labelled peak I) undergoes a potential shift by approximately 0.2 V towards more negative potentials. This shift reflects the change in charge transfer properties when Cu-TMPy2PP is reduced through the layer of adsorbed ODNs compared to the bare electrode surface. However, this shift was observed in the

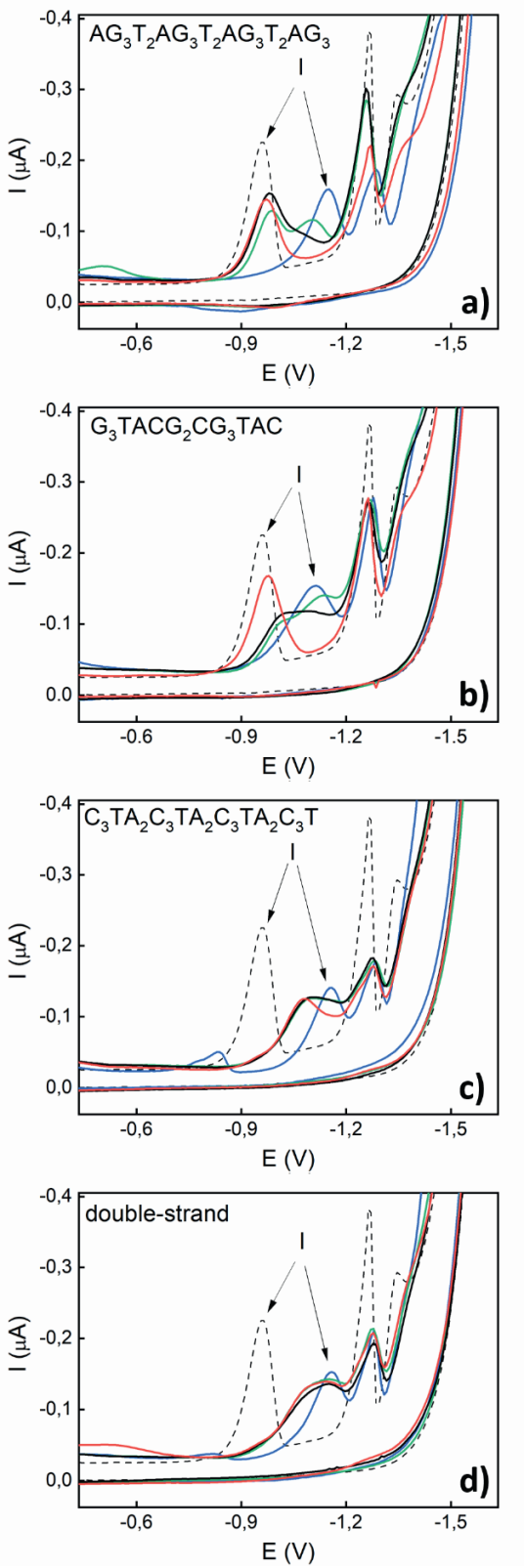

Fig. 2: CV of 0.4 µM Cu-TMPy2PP in AFP buffer measured at bare HMDE (dash line) or with adsorbed: hut-T2 (a), odn15 (b), hut-com (c) and hut-ds (d) after: 0 (blue), 0.5 (green), 1 (black) and 3 (red) potential cycles from 0.0 V to −1.85 V performed in clean AFP buffer before measurement.

same extent for all ODNs studied, and while the height of peak I differed for individual ODNs, it was found not to be structure-specific [6].

When the G4-forming ODNs hut-T2 and odn15 adsorbed on the HMDE surface are subjected to potential cycling in the potential window from 0.0 V to −1.85 V in clean AFP buffer before the transfer of thusly treated electrode to Cu-TMPy2PP solution, the peak I observed in the subsequent CV measurement gradually shifts with the increasing number of the applied potential cycles to the position corresponding to Cu-TMPy2PP reduction on bare HMDE (Fig. 2a). However, this effect was not observed when the described procedure was performed with single-stranded ODNs hut-com (Fig. 2c) or odn15-com (not shown). The double-stranded ODN hut-ds (obtained by mixing equimolar amounts of the complementary strands hut-T2 and hut-com) also did not exhibit the described behaviour (Fig. 2d). This behaviour appears to be characteristic for G4 and can be attributed to higher proneness of these structures to desorption in the highly negative potential region during the potential cycling. G4 are most likely adsorbed by the single-stranded regions in loops and overhangs [7]. Therefore, their adsorption is notably weaker compared to the linear structural forms. With the increasing number of potential cycles, more G4 ODNs are desorbed, and consequently, more space is exposed on the HMDE surface for direct reduction of Cu-TMPy2PP. This is reflected in the gradual shift of peak I.

Use of dG^{NP}TP as an electrochemical probe

The modified nucleotide dG^{NP}TP used as an electrochemical probe can provide higher sensitivity to changes of the ODN-modified surface of the HMDE compared to Cu-TMPy2PP due to its smaller size and minimal interaction with the adsorbed ODNs.

This compound offers a single reduction peak of the nitro group at −0.47 V. Coverage of HMDE with ODNs causes a decrease of this signal compared to bare HMDE since the access of dG^{NP}TP to the electrode surface is hindered (grey bars vs black bar in Fig. 3). The observed decrease is more significant for single-stranded ODNs hut-com and odn15-com compared to the G4-forming hut-T2. This suggests that a denser adsorbed layer is formed by the single-stranded ODNs. Exposure of ODN-modified HMDE to potential cycling before transfer to the solution of dG^{NP}TP causes an increase of the signal with increasing vertex potential of the cycling from −1.30 V to −1.90 V (white bars in Fig. 3). This increase is gradual for single-stranded ODNs through the whole studied range of vertex potentials. Still, in the case of G4-forming ODNs, a sharp increase was observed between vertex potentials −1.70 V and −1.80 V, suggesting extensive, structure-specific desorption of G4 at potentials more negative than −1.70 V and confirming the results obtained with Cu-TMPy2PP. Nevertheless, using the more sensitive probe dG^{NP}TP, it was shown that even the single-stranded ODNs are desorbed to a lesser degree. The described differences in surface behaviour of ODNs could not be detected by the commonly used redox probe $[Ru(NH_2)_6]Cl$, which due to its small size, was insensitive to the surface changes related to ODN adsorption and desorption.

Homo-ODN T30 behaves like G4 forming ODNs

The homothymidine 30-mer T30 is expected to be in an unstructured single-stranded state in solution, which was also confirmed by spectral data [8]. However, its behaviour on the electrode surface observed

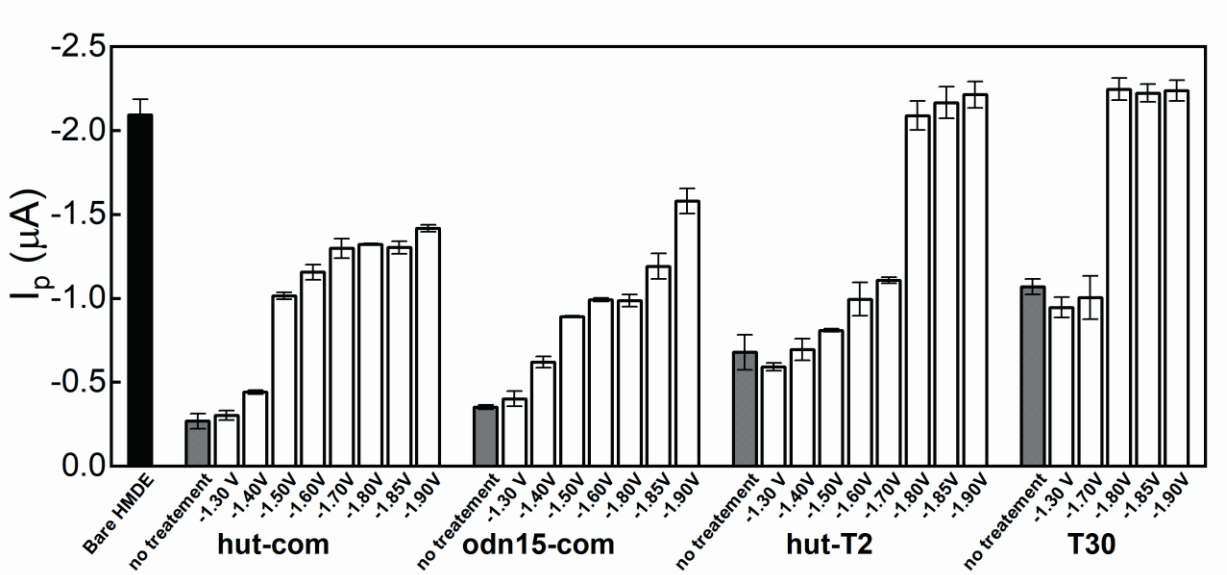

Fig. 3: Peak height of 50 µM dG^{NP}TP measured with bare HMDE (black), with HMDE modified with individual ODNs with no treatment (grey) or after three potential cycles with vertex potentials labelled on the x-axis (white) performed in clean AFP buffer before measurement.

in this work is unlike the other tested single-stranded sequences and is instead very similar to the G4-forming ODNs. An example of this is shown in Fig. 3, which indicates that T30, like the G4-forming hut-T2, forms more permeable layers than the single-stranded ODNs and undergoes significant desorption between −1.70 V and −1.80 V.

Since a formation of a folded conformation similar to G4 in T30 in solution is unlikely, the cause of this peculiar behaviour remains unclear. It is probably related to the interaction of T30 with HMDE surface, where the formation of covalently linked T-Hg-T base pairs is possible [9]. This possibility will be investigated more in the future.

Detection of desorbed ODNs in base electrolyte

In order to confirm that the above-described effects were caused by structure-specific desorption of ODNs from the electrode surface, the desorbed ODNs were successfully detected in the AFP buffer solution after potential cycling. The detection was done by the highly sensitive radioactive labelling of ODNs by ^{32}P in combination with native PAGE (Fig. 4). Because the amount of ODNs desorbed from a single HMDE was expected to be very low, a mercury pool electrode (MPE) with a significantly bigger surface on which a larger amount of ODNs can be adsorbed was used as the working electrode.

When three potential cycles were performed between −0.00 V and −1.85 V, even the single-stranded ODN hut-com was detected in the base electrolyte, although in considerably lesser quantity compared to hut-T2 and T30. These observations agree with the results obtained with the probe dG^{NP}TP, which also showed partial desorption of hut-com by potential cycling. Furthermore, from the selected ODNs, the highest quantity was determined for T30, which further demonstrate its curious G4-like behaviour in the adsorbed state.

Conclusion

In this contribution, a method for studying ODN structures in adsorbed state at HMDE was developed, based on their relative strength adsorption using selected electrochemical probes Cu-TMPy2PP and dG^{NP}TP. ODNs with the propensity to form the G4 structure were shown to be significantly more prone to desorption from the HMDE surface at highly negative potentials compared to single- or double-stranded ODNs. Additionally, unique behaviour of homo-ODN T30 was shown, similar to that of G4-forming ODNs and which will be studied more in the future. The obtained results confirmed the perspective application of novel electrochemical probes in the studies of ODN secondary structures (specifically G4 formation) in adsorbed state and can be utilised in the future development of analytical tools for the study of DNA structures.

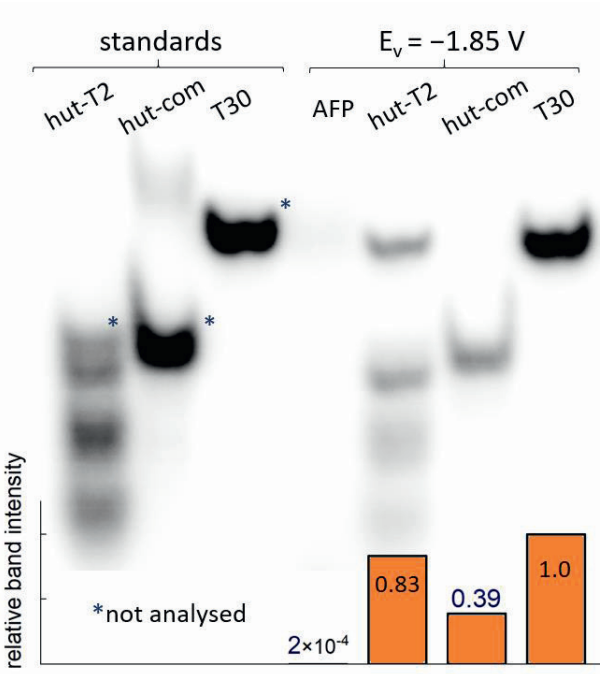

Fig. 4: Native PAGE of selected ODNs desorbed from MPE surface during three potential cycles with vertex potential (E_v) = −1.85 V, cycling with bare MPE in AFP buffer was used as negative control (lane AFP). Intensities of the analysed bands are evaluated below the autoradiogram normalised for the highest intensity (T30).

Acknowledgement

This work was supported by the project SYMBIT reg. no.:CZ.02.1.01/0.0/0.0/15_003/0000477 funded from European Regional Development Fund (EFRD). Cu-TMPy2PP used in this study was kindly donated by Prof. G. Pratviel (Laboratories of Coordination Chemistry, CNRS, Toulouse, France) and dG^{NP}TP by Prof. M. Hocek (Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic).

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