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Nucleoside–metallacarborane conjugates for multipotential electrochemical coding of DNA

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Abstract

Metallacarboranes as electrochemical labels are proposed. The electrochemical properties of nucleoside conjugates, derivatives of thymidine (T), 2′-deoxycytidine (dC), 2′-deoxyadenosine (dA) and 2′-deoxyguanosine (dG), containing metallacarborane complex of cobalt or iron are described. A multielectrochemical detection using specific metallacarborane tags is shown. The proposed labelling of nucleosides lays the foundations for electrochemical coding of DNA with metallacarborane complexes and simultaneous detection of several DNA targets.

Keywords: Metallacarboranes; Nucleosides; DNA labelling; Electrochemical detection

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1. Introduction

The detection of DNA hybridization is of central importance to the diagnosis and treatment of genetic and infectious diseases. Recent activity has focused on the development of hybridization assays based on various optical coding systems and allowing simultaneous detection of multiple DNA targets. Electrochemical detection of nucleic acids is an attractive alternative to established fluorescence and others optical coding technologies, with advantages including cost, sensitivity, and direct electronic readout [1]. Various approaches have been explored, including conjugation of DNA oligonucleotides with electroactive reporters, use of soluble electroactive mediators or intercalators, redox enzyme mediation, and measurement of direct label-free electrochemical processes [1–3]. Among the known methods, explicit electroactive labelling has the combined advantage of a positive detection signal, low background and the ability to introduce several electrochemically distinguishable tags [4,5]. Redox active metal complexes covalently linked to DNA are most frequently used as reporters, in particular ferrocene is the best known example [6]. Such a small molecule is selected as a label since it possesses reversible and tunable redox properties. However, in spite of the fact that many metallocenes with different central metals are available [7], none of them was used till now as DNA-electrochemical label due to air- and/or moisture sensitivity [8]. The only alternative to ferrocene as covalently linked, electrochemically active labels for electrochemical detection of nucleic acids described so far are metallacarboranes [9].

The electrochemical behaviour of the cobalta- and ferradicarbollides $[(1,2\text{-}C_2B_9H_{11})_2M]^{n-}$ and their functionalised derivatives is long known [10–12]. Cobaltacarboranes can shuttle the sequence CoIV/CoIII/CoII/CoI, whereas ferracarboranes can display the sequence FeIII/FeII/FeI, the different members of the two sequences being more or less

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accessible as a function of the experimental conditions. Even though a number of bio-active functionalized carboranes and metallacarboranes have been characterized [13], to the best of our knowledge no pertinent electrochemical data are available. Herein we report the electrochemical studies of metallacarborane–nucleoside conjugates and the successful HPLC electrochemical detection of typically labeled nucleosides.

2. Experimental section

2.1. Chemicals

Compounds **1**a–c, **2**, **3**Co, **3**Fe, **4**a–c have been obtained as described [9,14]. Detailed synthesis of dinucleotides **5**Co and **5**Fe will be described elsewhere. Briefly, first **3**Co or **3**Fe was transformed into suitable 5′-*O*-dimethoxytrityled 3′-*O*-(H-phosphonate), next H-phosphonate monomer was coupled with 3′-*O*-protected thymidine and oxidised yielding **5**Co and **5**Fe, respectively. [8-Hydroxy-3-cobalt bis(dicarbollide)]$^-$ ion (**6**) was prepared as described by reductive acetoxylation of [3-cobalt bis(1,2-dicarbollide)]Cs followed by hydrolysis of the intermediate [15]. [8-(5-Hydroxy-3-oxa-pentoxy)-3-cobalt bis(1,2-dicarbollide)]$^-$ (**7**) was prepared *via* dioxane ring opening in [8-dioxane-3-cobalt bis(dicarbollide)]^0 under alkaline conditions [16], [3-iron-bis(1,2-dicarbollide)]$^-$ ion (**8**) was obtained as described [17].

2.2. Electrochemical measurements

Materials and apparatus for electrochemistry and spectroelectrochemistry have been described elsewhere [18]. The electrochemical tests have been carried out at $T = -10\,°C$, in DMF solution containing [NEt$_4$][ClO$_4$] (0.1 mol dm^{-3}) as supporting electrolyte, using either platinum, or gold, or glassy carbon working electrodes and the Ag/AgCl reference electrode. Under such experimental conditions the one-electron oxidation of ferrocene occurs at +0.52 V. Extrapolation of the potential values on passing from DMF to aqueous solutions are based on the following room temperature experiments: [Fe(C$_5$H$_5$)$_2$][PF$_6$] reduces at +0.41 V, vs. SCE in DMF/[NEt$_4$][ClO$_4$] (0.1 mol dm^{-3}), whereas it reduces at +0.16 V, vs. SCE in H$_2$O/NaCl (1 mol dm^{-3}).

2.3. HPLC electrochemical experiments

Sample preparation: Stock solution of compound **1c** was prepared by dissolving **1c** (0.88 mg, 1.0 μmol) in a mixture of acetonitrile/water (60:40 v/v, 1 mL) containing ammonium acetate (0.05 M, pH 5.5). Aliquot of resulting solution (10 μL) containing 10 nmol of **1c** was then applied to RP-HPLC column. HPLC-conditions: HPLC analysis was performed with electrochemical detector Coulochem II (Esa, Inc., USA) equipped with isocratic pump (Dionex Corporation, Sunnyvale, USA) and amperometric analytical cell Model 5040 (Esa, Inc., USA), respectively at analytical cell potential = 1.7 V. Altech Econosil C18 5 μm, 4.6 × 250 mm column was applied. As eluent a mixture of acetonitrile/water (60:40 v/v, 1 mL) containing ammonium acetate (0.05 M, pH 5.5) was used under isocratic conditions. Flow rate 1 mL/min.

3. Results and discussion

The conjugates of all four canonical nucleosides: thymidine (T), 2′-deoxycytidine (dC), 2′-deoxyadenosine (dA), 2′-deoxyguanosine (dG) and metallacarborane bearing cobalt (Scheme 1), were obtained according to the original procedure based on reaction between metallacarborane-dioxane adduct and suitably protected, base activated nucleosides [9,14]. The conjugates containing metallacarborane bearing iron have been obtained according to the same methodology.

At a glassy carbon as well as at a gold electrode, most of the present Co(III) complexes exhibit two separate reduction processes featuring chemical reversibility in the cyclic voltammetric time scale. As a typical example, Fig. 1a shows the voltammetric profile of complex **3**Co.

Analysis of the first reduction with scan rates progressively increasing from 0.02 V s^{-1} to 2.00 V s^{-1} testifies to the chemical and electrochemical reversibility of the process, in that: (i) the current ratio i_{pc}/i_{pa} is constantly equal to one; (ii) the current function $i_{pa} \cdot v^{-1/2}$ remains substantially constant; (iii) the peak-to-peak separation tends to increase from 65 mV to 130 mV, a trend quite similar to that exhibited by the ferrocene/ferrocenium oxidation [7].

Attempts to determine the number of electrons involved in each step by controlled potential coulometry were unsuccessful because of the continuous reoxidation probably triggered by trace of air entering the nominally deaerated solution. This drawback is rather common in the case of processes occurring at negative potential values. In agreement with the known behaviour of [(1,2-C$_2$B$_9$H$_{11}$)$_2$Co]$^-$, we confidently assign the two processes to the sequence Co(III)/Co(II)/Co(I). It is noticed that at a platinum electrode the second reduction is masked by the solvent discharge.

As far as the anodic path is concerned, no oxidation process was detected up to the anodic discharge of the DMF solvent ($\leqslant +1.6$ V). It is observed also that under the actual experimental conditions no well defined redox processes are exhibited by thymidine, 2′-deoxycytidine and 2′-deoxyadenosine. Only in the case of 2′-deoxyguanosine a well defined, irreversible oxidation was detected ($E_p = +1.17$ V) which agrees with the known electrochemical activity of guanine base [3]. This finding further supports the cobalt-centred nature of the above mentioned cathodic processes.

As shown in Fig. 1b, the Fe(III) analogue **3**Fe exhibits only a single (coulometrically measured) one-electron reversible reduction. An irreversible oxidation occurs at high potential values ($E_p = +1.43$ V). The spectroelectrochemical profile recorded in a OTTLE cell upon the step-