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Electrochemical evaluation of rhodium dimer-DNA interactions

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Abstract

The interaction of rhodium dimers, including the carboxylates (acetate, propionate, butyrate, trifluoroacetate, citrate and gluconate), amidates (acetamidate and trifluoroacetamidate) and carboxamidate (Doyle catalyst *S*) with DNA was investigated by electrochemical methods. Differential pulse voltammetry measurements showed, in agreement to literature data, that most of rhodium carboxylates have a higher affinity for adenine than guanine residues. Some differences of reactivity may be correlated with the compound structures and these were helpful in understanding the influence of equatorial ligands on axial coordination mechanisms. The preliminary results might be extended for further studies on quantitative structure activity relationship approaches, highlighting electrochemical methods as a tool for this purpose. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: DNA-interactions; Rhodium complexes; Bioelectrochemistry; Structure–activity relationship; Carbon fiber electrode

1. Introduction

The biological importance of compounds that can bind to the DNA molecule has led to numerous studies describing chemical–DNA interactions [1–3]. These interactions can occur through three different modes: (i) by electrostatic interactions with the negative charged sugar-phosphate DNA structures, without selectivity; (ii) by interactions with the minor and major grooves of the DNA double helix; and (iii) by intercalation between the stacked base pairs of the double helix structure. The first decreases the repulsive forces between the near phosphate groups, resulting in a stabilization of the double helix. On the other hand, due to the disruption of hydrogen bonds or hydrophobic and van der Walls forces involved in base stacking [3,4], interactions through the bases decrease the stability of the double helix, besides the possible decrease in the repulsive forces between phosphate groups.

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Several, biochemical and physicochemical methods (e.g. linear dichroism, RMN and fluorescence spectroscopy, chromatography, mass spectrometry, crystallography, immunoassays and thermodynamic methods) have been applied to study drug–DNA interaction [1–9]. However, these methods are generally time-consuming and very expensive.

Considering that DNA modified electrodes have been developed and applied for detection of nucleic acid hybridizations [10] and used to study DNA-interactions with nitroimidazoles [11], carboplatin [12], mitomycin [13], quinacrine [14], daunomycin [15] and thiotepa [16]. In the case of drugs that act on DNA, the suppression of guanine or adenine oxidation current peaks was used as one of the electrochemical approaches [11–17]. Rhodium compounds have attracted considerable interest due to their recognizable structure, reactivity [18,19], and potential antineoplastic action [18–22]. These compounds are able to coordinate on the axial position (L) through donor molecules containing –S, –N or –O. The mechanism proposed for biological activity of these compounds [18–22] is the axial coordination with RNA and DNA polymerases as well with N7 of adenine residues. Based on the electrochemical behavior, it could be a good way to investigate the interactions between rhodium complexes and DNA with some sites more specifically. This information could be very important to aid in understanding the mechanism of the biological activity of the rhodium complexes. Acetate, trifluoroacetate, propionate, butyrate [18], citrate [21] and gluconate [22] can be cited among rhodium carboxylates with biological activity in tumor cell cultures. Amidates such as trifluoroacetamidate [23] are also active in in vitro assays (Fig. 1). The biological activity order for some of these carboxylates is as follow: acetate < propionate < butyrate [18,24]. Although this order may reflect mainly, the correlation between lipophilicity and membrane permeation, other parameters could also be important in the interaction with macromolecules, as previously mentioned. Electronic and steric properties may be involved in this mechanism [18,25].

The electrochemical behavior of the rhodium dimers depends on the electron density of Rh–Rh bond, so the electronic properties of their ligands will affect the redox potentials [25] of the dimers. Moreover, DNA-complex interactions can lead to the suppression or shift the adenine and guanine oxidation peak potentials. In this paper, this electrochemical approach was used as a strategy to evaluate the DNA interactions with rhodium dimers that have biological activity in tumor cell cultures. Acetamidate [26,27] and Doyle catalyst [28,29] have not been tested yet in vitro, but they were already investigated, from the viewpoint of reactivity, because their structures are very interesting and permit extension of this approach to other ligands.

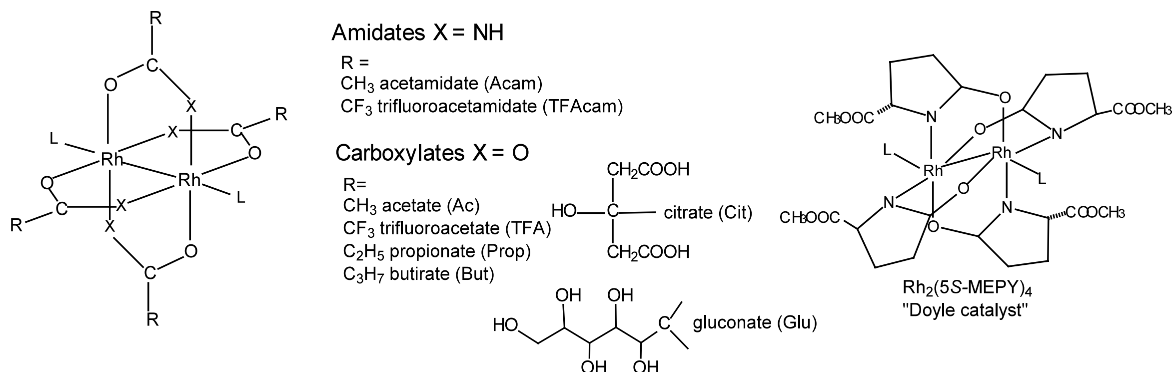


Fig. 1. Potential anticancer rhodium dimers with different ligands.

2. Experimental

2.1. Chemicals

Doyle catalyst (Rh_2(5*S*-MEPY)$_4$) and calf thymus DNA were purchased from Sigma (St. Louis, MO, USA). The carbon fiber was acquired from Toray Ind. Corporation (Japan). The aqueous salt solutions used for the electrochemical studies were analytical grade reagents. The rhodium carboxylates, as well as the amidates, were synthesized and characterized as described in the previous papers [18–25,27].

2.2. Instrumentation

For the electrochemical experiments a potentiostat/galvanostat from Princeton Applied Research (PAR), 273A model, was used. The differential pulse voltammetry measurements were carried out with a three-electrode system. The working electrode consisted of a bundle of about 50 fiber of T-800 sized carbon fiber. A commercial saturated calomel electrode (SCE) was used as reference electrode and a platinum wire as counter.

2.3. Stability evaluation of the anodic peaks

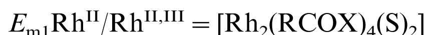
For this procedure, 5 ml of 0.2 mg ml^{-1} DNA solution and 5 ml of 10^{-4} mol l^{-1} rhodium complexes were independently submitted to ten repeated oxidation process in the range from 0 up to 1.6 V, by differential pulse voltammetry, at a scan rate of 5 mV s^{-1}, pulse amplitude of 25 mV and pulse width of 50 ms.

2.4. Studies of DNA-complex interaction

These studies were carried out in the range from 0 up to 1.6 V, by differential pulse voltammetry, at a scan rate of 5 mV s^{-1}, pulse amplitude of 25 mV and pulse width of 50 ms. The effects of the dimer concentrations were evaluated step-by-step of the additions of 200 μl of 1×10^{-3} mol l^{-1} [$Rh_2(XOCR)_4$] to an electrochemical cell containing 5 ml of 0.2 mg ml^{-1} DNA solution. All solutions were prepared in a 5×10^{-2} mol l^{-1} KCl solution at pH 4.5.

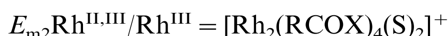
3. Results and discussions

The synthesis and characterization of rhodium compounds were carried out according to the previous papers [18–28]. The redox potential measurements of these dimers are generally obtained in organic solvents [25–29] and the $Rh^{II/III}$ metal centered redox process occurs in two steps according to the equations:



$$E_{m1} Rh^{II}/Rh^{II,III} = [Rh_2(RCOX)_4(S)_2]$$

$$\rightleftarrows [Rh_2(RCOX)_4(S)_2]^{+} + 1e^{-}$$



$$E_{m2} Rh^{II,III}/Rh^{III} = [Rh_2(RCOX)_4(S)_2]^{+}$$

$$\rightleftarrows [Rh_2(RCOX)_4(S)_2]^{2+} + 1e^{-}$$

In aqueous solutions the second redox process can be recorded only for amidates [25–28]. In other compounds the potential of this second redox process is very high and the solvent often limits its appearance. The midpoint potentials (E_m) for these complexes are directly proportional to the electron donor character of their ligands. Furthermore, the $\Delta E_m(E_{m1} - E_{m2})$ is inversely proportional to the length of the Rh–Rh bond, which is also ligand dependent. Therefore, for rhodium acetate, in which the Rh–Rh bond length is 2.38 Å, a sharp separation between both redox couples is observed, which decreases for rhodium acetamidate (2.41 Å) being almost null for rhodium carboxamidate (2.46 Å) [28,29]. Table 1 shows the anodic peak potential obtained by differential pulse voltammetry for dirhodium(II) complexes. These data express the electron withdrawing character of the ligands and could be associated to the electronic parameters in further quantitative structure activity relationship (QSAR) studies.

3.1. DNA-dimer interactions study

The electrochemical study of DNA is usually carried out in acetate buffer at pH 4.5 with low ionic strength [17]. However, this electrolyte was substituted by 0.05 mol l^{-1} KCl (pH 4.5) to avoid

Table 1
Oxidation peak potentials for rhodium dimers obtained with a carbon fiber electrode in 0.05 mol l^{-1} KCl solution vs. SCE

Compound	E_{m1} (V)	E_{m2} (V)
$Rh_2(Ac)_4$	0.973	–
$Rh_2(Prop)_4$	0.951	–
$Rh_2(But)_4$	0.949	–
$Rh_2(Cit)_4$	1.084	–
$Rh_2(Glu)_4$	1.086	–
$Rh_2(TFA)_4$	1.180	–
$Rh_2(Acam)_4$	0.234	0.463
$Rh_2(TFAcam)_4$	0.889	–
$Rh_2(5S\text{-MEPY})_4$	0.350	–

an acetate reaction with the equatorial ligands (R) [18,27]. Additionally, KCl decreases the redox potentials of the rhodium complexes and improves the electrochemical signals, as previously reported [27].

The anodic peak potentials and currents for guanine and adenine, obtained from an average of ten experiments carried out in optimized conditions, were 885 (±10) mV; 1252 (±10) mV; 14 (±2) μA and 23 (±3) μA, respectively. Stable signals with good resolution were observed and the results are in agreement with the other DNA oxidation studies [17]. In addition, the anodic peaks for 10^{-4} mol l^{-1} rhodium dimers solution (Table 1) also showed good stability even after repeated cycles, even after one week in contact with the solution, if kept under argon atmosphere. Only after careful investigation about the stability and repeatability the interactions studies were carried out. In most cases, the effects of rhodium dimers on DNA signals were more intense on the peak currents than the peak potentials of the purinic bases from DNA (Fig. 2). This behavior suggests that the complex interacts with some purinic bases blocking the redox process, at least in the working potential range. Evidence that it occurs was confirmed in the experiments carried out mixing the simple purinic base (adenine) and rhodium complexes (data not shown).

Table 2 shows the anodic current suppression of guanine (G) and adenine (A) residues in DNA solution at higher levels of rhodium dimer. It seems that rhodium carboxylates interact with

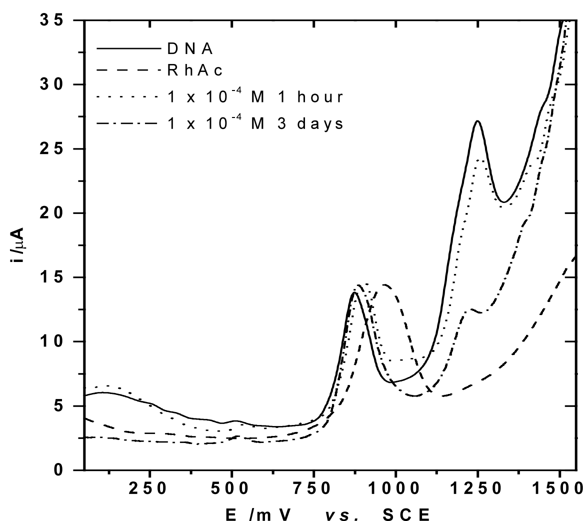


Fig. 2. Differential pulse voltammograms obtained for rhodium acetate–DNA interaction studies (carbon fiber electrode, 10 mV s^{-1}, 0.2–1.55 V vs. SCE, pulse of 25 mV and pulse width of 50 ms, 0.2 mg ml^{-1} DNA solution, 1×10^{-4} mol l^{-1} $Rh_2(Ac)_4$ both in 0.05 mol l^{-1} KCl solution at pH 4.5).

adenine in a more intense and selective way than those observed for amidate dimers. Furthermore, the suppression of the peak current for the most hindered ligands was lower, showing the influence of the steric effects on the interactions between DNA and rhodium dimer. Higher electron density along the Rh–Rh bond axis (z-axis) must improve

Table 2
Immediate effect on guanine and adenine oxidation current peaks of 0.2 mg ml^{-1} DNA solution with 1×10^{-4} mol l^{-1} $Rh_2(XOCR)_4$ levels

Compound	Guanine peak suppression (%)	Adenine peak suppression (%)
$Rh_2(Ac)_4$	0	15
$Rh_2(Prop)_4$	2	30
$Rh_2(But)_4$	5	25
$Rh_2(Cit)_4$	0	8
$Rh_2(Gli)_4$	3	4
$Rh_2(TFA)_4$	0	10
$Rh_2(Acam)_4$	10	3
$Rh_2(TFAcam)_4$	1	6
$Rh_2(5S\text{-MEPY})_4$	0	0

(I_{DNA} = 100%) I = peak currents of guanine and adenine (0.2 mg ml^{-1} DNA solution).

Table 3
Suppression effects of anodic peaks of DNA bases as a function of incubation time (0.2 mg ml^{-1} DNA solution with 1×10^{-4} mol l^{-1} $Rh_2(XOCR)_4$)

Compound	Guanine peak suppression (%)			Adenine peak suppression (%)		
	1 h	72 h	360 h	1 h	72 h	360 h
$Rh_2(Ac)_4$	0	0	7	17	81	100
$Rh_2(Prop)_4$	0	22	100	53	65	100
$Rh_2(But)_4$	19	49	95	30	54	92
$Rh_2(Cit)_4$	7	8	53	11	32	61
$Rh_2(Gli)_4$	11	11	44	6	8	18
$Rh_2(TFA)_4$	1	22	75	15	36	65
$Rh_2(Acam)_4$	29	51	100	39	57	77
$Rh_2(TFAcam)_4$	2	50	78	7	47	90
$Rh_2(5S\text{-MEPY})_4$	0	0	0	0	0	0

the axial reactivity, especially by π back donation [18], decreasing the selectivity for adenine interactions. This fact is in agreement with the lower oxidation peak potentials observed for rhodium acetamidate (see Table 1). In the acetate, propionate and butyrate series, an inverse correlation between rhodium dimers weight (size chain) and adenine selectivity was observed. The effects were more evident in the studies of interaction along the incubation time (Table 3).

Table 3 shows that rhodium acetate has no affinity for guanine residues, while compounds with higher molecular weight have some. However, the hypothesis that it could be a consequence of some lower reactivity of rhodium acetate is rejected by the direct comparison with rhodium butyrate. This latter one was not more reactive than rhodium acetate but presented a higher affinity for guanine.

Influences of the length of flexible chains in structure–activity relationships are very useful and increase the steric hindrance and van der Waals interactions. These two opposite effects can compensate for the decreasing of the differences between adenine and guanine in the suppression of the peak currents. Although, the increase in the steric hindrance and the poor selectivity of the van der Waals interactions can affect both bases equally, these effects are relatively more significant for guanine than for adenine. Furthermore, the heavier dimers, such as gluconate, citrate and Doyle catalyst, showed lower levels of suppression for guanine and adenine residues, even at long incubation times. Besides the steric hindrance, these three compounds are able to make strong hydrogen bonds across OH, COOH and COOR functional groups. As a consequence, they can interact with extra sites (e.g. deoxyribose; phosphate groups or bases), which decrease the suppression that should be observed from the base oxidation.

The electronic properties also affect the selectivity for adenine in the acetamidate, trifluoroacetamidate and trifluoroacetate series (Table 3). Complexes with higher electron density over the Rh–Rh bond, as well as ligands with longer chains, were less selective for adenine than guanine residues, at least after the longest incubation times. The higher selectivity for adenine found for rhodium acetate agrees with other methods described in the literature [3], in which the rhodium acetate–DNA interaction occurred through N7 of this purine base. These data show the potentialities of electrochemical techniques as a new tool to help or be an alternative to other techniques like mass spectrometry [30], EPR [31] and NMR [32] in QSAR approaches. Although, this procedure is not a form to measure quantitatively the interaction degree between the rhodium complexes and some DNA sites, information about the interaction strength correlating to the nature of the complex can be obtained. This electrochemical procedure may give in many cases the same information like mass spectrometry, EPR, etc. in a cheaper way.

4. Conclusions

The results show that the complex interactions of the DNA through the guanine and adenine residues promote the suppression of the oxidation peak current of the purinic bases in different form, and these effects can be associated with the steric hindrance and electronic properties of the rhodium dimers. This electrochemical approach is a promising tool for future structure activity relationship (QSAR) studies. Presumably the same information could be obtained with other powerful techniques such as EPR, NMR, mass spectrometry, but they require instruments that are very expensive in comparison to the electrochemical. The electrochemical technique might have advantages in experiments in vitro giving more realistic information about the system. However, the electrochemical techniques may be more difficult for data interpretation and less quantitative in many cases.

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