Proof of Concept of the Electrochemical Sensing of 3-iodothyronamine (T_1AM) and Thyronamine (T_0AM)

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Abstract (925/1000 characters)

It is shown in recent studies that besides the well-recognized T_3 and T_4 there are other relevant thyroid hormones circulating in the human body. In particular this is the case for 3-iodothyronamine (T_1AM) and thyronamine (T_0AM). One of the reasons for the lack of studies showing its precise importance is the absence of analytical methodologies available. Herein, for the first time, T_1AM and T_0AM are electrochemically characterized. T_0AM was sensed by means of a glassy carbon electrode; interestingly T_1AM was sensed both with a graphitic surface (oxidatively) as well as with mercury (reductively). With both compounds it was possible to, subsequently to oxidation, to observe the reversible redox reaction concerning the couple benzoquinone/hydroquinone, thus increasing the specificity of the electroanalysis. *Ergo*, this work provides the basis for an 'at-point-of-use' electrochemical strips test for T_1AM and T_0AM.

Keywords (max. 5)

Clinical analysis
Cyclic voltammetry
Electrochemistry
Hormones
Thyroid

The biological relevance of a hormone stems from its definition: it is a chemical messenger that transports a signal from one cell to another. The thyroid, anatomically situated in the neck produces several hormones, particularly 3',5',3,5-L-tetraiodothyronine (T_4) and 3´,5',3-L-triiodothyronine (T_3) (Figure 1). Given their importance in several physiological functions, these thyroid hormones are routinely quantified to help diagnose and assess several pathologies.[1]

Thyroid hormones have crucial effects on metabolism and thermogenesis, on processes involving muscular contraction, growth, reproduction, immune and antiviral defense as well as defense against free radicals.[1] These functions are not specific to the human species, since exactly the same molecules produce similar effects in most vertebrates.[1] In human plasma, unbound T_4 and T_3 are in the picomolar range, while T_4 and T_3 bound to thyroid-binding-proteins (mainly thyroxine-binding globulin, transthyretin and albumin) is regulated in the nanomolar range.[1]

Less than ten years ago a previously unsuspected thyroid hormone, T_1AM (3-iodothyronamine), was unveiled as a new biologically active thyroid hormone derivative (Figure 1).[2] Later, experiments in small mammals showed that systemic T_1AM and T_0AM (thyronamine) produced hypothermia, a cardiac reversible dose-dependent negative inotropic effect[3] and a rapid increase in blood glucose.[4] These data support the notion that these two hormones, like T_3 and T_4, play a role in the regulation of metabolism.

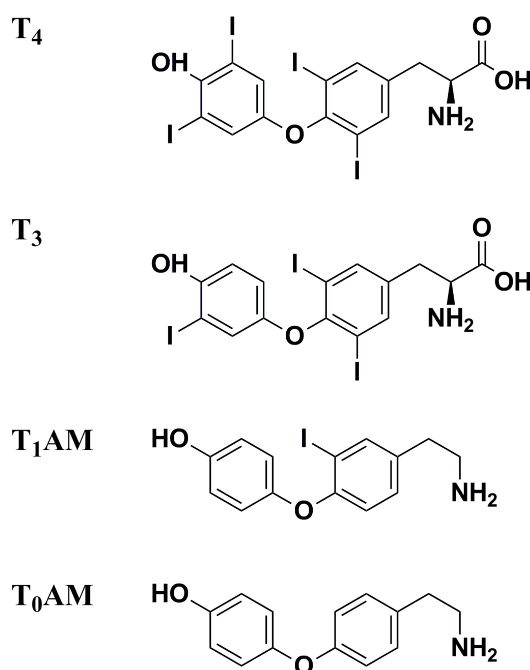

Figure 1 – Molecular structures of T_4, T_3, T_1AM and T_0AM.

This leads to compelling, as yet unanswered, questions such as: how much T_1AM and T_0AM circulate in the human body?; how are they distributed?; which metabolic programs are influenced by them?; in which concentrations are they physiological or pathological?; can they be used to treat any pathological process?; can they help in difficult endocrinological differential diagnosis?

Answering these questions requires a simple and reliable way to quantify T_1AM and T_0AM.[5] So far, their detection and quantification has been based on liquid chromatography with tandem mass spectrometry detection (LC-MS/MS)[6] and immunoassay methodologies[7], which are laborious and costly. This manuscript advocates the case that electroanalysis may be a viable and low-cost alternative.

Although T_4 and T_3 have been electroanalyzed,[8] to the best of our knowledge, neither T_1AM nor T_0AM were ever electrochemically studied before this work. Herein, T_1AM and T_0AM are successfully analyzed with a glassy carbon electrode (GCE) (Figure 2), in an oxidative electrode reaction, and also T_1AM with hanging mercury drop electrode (HMDE) (Figure 3), in a reductive electrode reaction.

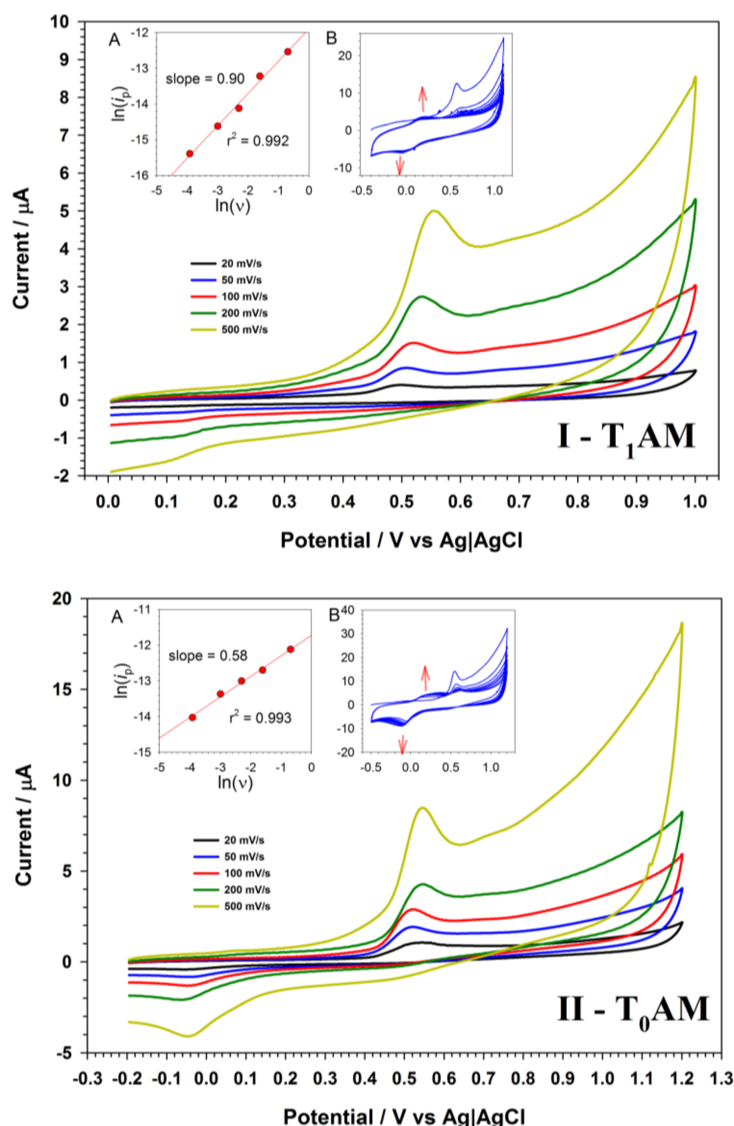

Figure 2 – I – Cyclic voltammograms of aqueous phosphate buffer solution (pH 7) containing 0.174 mM of T_1AM on a GCE, cyclic voltammetry was run between 0.0 and +1.0 V vs. Ag|AgCl at different scan rates. Inlay A: logarithm of scan rate vs. logarithm of peak current. Inlay B: Sequential cyclic voltammograms at 1000 mV/s showing the appearance of a reductive and an oxidative peak around 0.0 and +0.2 V vs. Ag|AgCl, respectively. II – Cyclic voltammograms of aqueous phosphate buffer solution

94 *(pH 7) containing 0.153 mM of T_0AM on a GCE, cyclic voltammetry was run between -0.2 and +1.2 V vs.*
95 *Ag|AgCl at different scan rates. Inlay A: logarithm of scan rate vs. logarithm of peak current. Inlay B:*
96 *Sequential cyclic voltammograms at 1000 mV/s showing the appearance of a reductive and an oxidative*
97 *peak around +0.0 and +0.2 V vs. Ag|AgCl, respectively.*

98

99 The oxidative processes in a GCE electrode, at pH 7, gives origin to voltammetric signals
100 with peak potential of ca. +0.5 V vs Ag|AgCl.

101 Considering that a fully irreversible diffusion-only system follows the Randles–Ševčík
102 equation:[9]

103

104 $$i_p = 0.496\sqrt{\alpha + n'}FnAC^*\sqrt{\frac{FDv}{RT}} \quad (1)$$

105

106 where i_p is peak current, v is the scan rate, n' is the number of electrons transferred
107 before the rate determining step, n is the total number of electrons transferred, A is the area of
108 the electrode surface, α is the Tafel coefficient (or transfer coefficient), D is the diffusion
109 coefficient of the species, C^* is the bulk concentration of the species, F is the Faraday constant,
110 R is the ideal gas constant, T is the temperature.[10] Note that equation utilizes the *multi-electron*
111 form of the Randles–Ševčík equation[11] and is consistent with recent IUPAC recommendations
112 on the definition of the transfer coefficient.[10]

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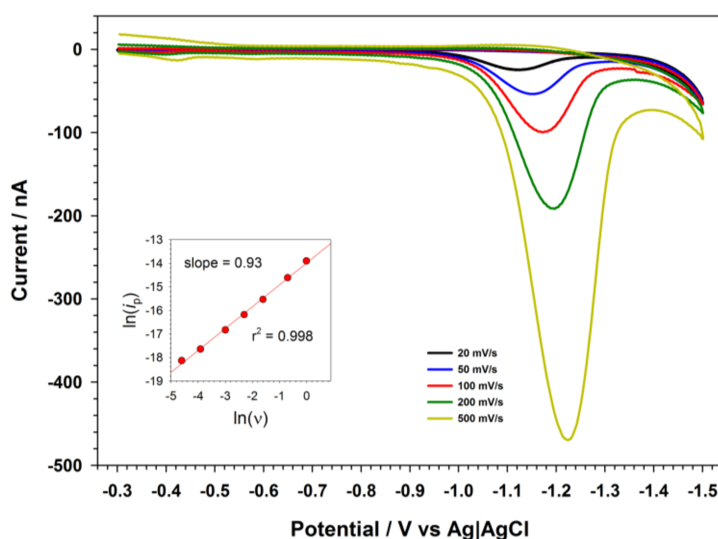

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116 *Figure 3 – Cyclic voltammograms of aqueous phosphate buffer solution (pH 7) containing 0.174 mM of*
117 *T_1AM on a HMDE, cyclic voltammetry was run between -0.3 and -1.5 V vs. Ag|AgCl at different scan*
118 *rates. Inlay: logarithm of scan rate vs. logarithm of peak current.*

119

120 For an irreversible surface bound species, i_p vs. v is given by the following equation:[11]

121

122 $$i_p = \frac{(\alpha + n')nF^2}{2.718RT}vA\Gamma \quad (2)$$

123

124 where Γ is the surface coverage. Surface and diffusional controlled voltammetric
125 processes can be distinguished on their scan rate dependence. A direct dependence on the
126 voltage scan rate, v, corresponds to the former, whereas a square root dependence signals the
127 latter.[12] This means that a logarithm of peak current ($\ln|i_p|$) vs. logarithm of scan rate ($\ln|v|$)
128 will give rise to a slope close to 0.5 in case of a fully diffusional process and a slope close to 1.0
129 for a fully adsorptive process, both apply to either a spherical or a plane macro-electrode. As
130 can be seen in the inlay A of Figure 2 - I, a slope ca. 0.9 ± 0.1 suggests an adsorptive process,
131 which could be expected considering T_1AM molecular structure, a π-π overlapping could occur
132 between T_1AM and the graphitic surface. However, considering that for T_0AM (inlay A of Figure
133 2 - II) a slope closer to 0.5 leads the authors to speculate it is the highly polarizable iodine atoms
134 that indeed promote adsorption. Plots showing the dependence of the peak current on scan
135 rate and the square root of scan rate are shown in the Supporting Information.
136 For the oxidative reaction (i.e. using the GCE) considering a peak potential vs. pH slope
137 of 62 mV (Figure 4), i.e. ca. 59 mV,[11] the electrochemical reaction up to the rate determining
138 step should have involved an equal number of protons and electrons. It is herein suggested that,
139 first there is the electrochemically reversible withdraw of 1 electron 1 proton, then, second a
140 chemically irreversible step where another electron and another proton are removed along with
141 addition of a water molecule. This cleavages the ether linkage forming *p*-benzoquinone and an
142 hydroxyl group is introduced in an *ortho* position to the iodine atom (reaction A of Figure 5). By
143 means of using a fast scan rate, immediately after the oxidative electrochemical reaction one
144 can observe the benzoquinone/hydroquinone reversible redox reaction at potential around 0.1
145 V vs. Ag|AgCl (inlay B of Figure 2). A cyclic voltammogram of benzoquinone in the media using
146 the same voltammetric conditions gave origin to a similar peak with similar peak potential thus
147 confirming such assumption (data shown in the Supporting Information).
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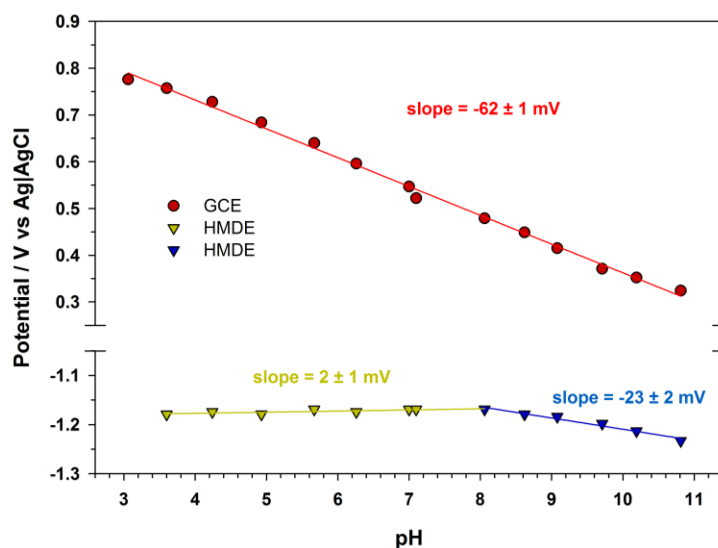

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150
151 *Figure 4 – pH studies, performed on universal buffer solution of a Britton-Robinson type, containing 0.12*
152 *mM of T_1AM, with a GCE (the section above) and a HMDE (the section below). Similar results were*
153 *obtained for T_0AM, 0.10 mM, for the case of the GCE (data not shown).*
154

155 The reductive process, for T_1AM, in a HMDE electrode, at pH 7, gives rise to a
156 voltammetric signal with peak potential of ca. -1.2 V vs Ag|AgCl (Figure 3). A similar behavior to
157 that obtained with the GCE was also noticed with the HMDE electrode (inlay of Figure 3), a slope
158 of almost 1 in the logarithm plot of peak potential vs. scan rate ascribes to an adsorptive
159 behavior.
160 Concerning the reductive reaction, the rate determining step should be a first electron
161 transfer – an α value of 0.41 ± 0.05, i.e. ca. 0.5, in the Tafel analysis agrees with such assumption
162 – followed by the cleavage of the carbon-iodine bond releasing an iodide anion, and subsequent
163 protonation of the carbon to which the iodine was bond (reaction B of Figure 5). Although in
164 overall this would be a 2 electron 1 proton uptake, the first steps (the first electron transfer plus
165 carbon-iodine cleavage) is rate determining which leads to the observed negligible pH
166 dependence (Figure 4). However, when plotting peak potential vs. pH, there is a considerable
167 slope above pH 8.2 (Figure 4), which might be explained as an extra removal of a proton from
168 the reaction molecule. Considering this reductive mechanism for T_1AM where iodine plays part,
169 one was not expecting to obtain a similar voltammetric signal for T_0AM. Such was the case, no
170 signal was obtained in an effort to electrochemically reduce T_0AM in the HMDE.
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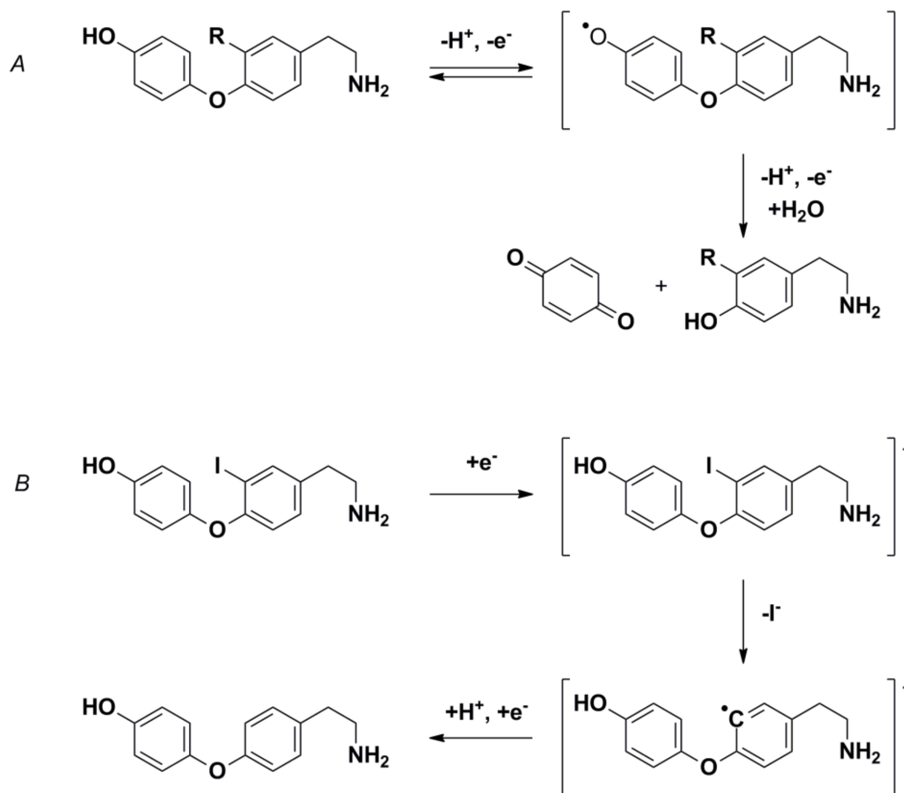

172
173
174 *Figure 5 – Reaction mechanisms. Reaction A – oxidative reaction on a GCE, Reaction B – reductive*
175 *reaction on a HMDE.*
176
177 These results not only show that is it possible to electroanalyze T_1AM and T_0AM but,
178 furthermore, the analyses of T_1AM can be done with several different surfaces, since it can be
179 performed either in a reductive or in an oxidative way. These results are proof-of-concept that
180 may ultimately pave the way to the creation of low-cost reliable analytical methodologies for
181 the quantitative analysis of T_1AM and/or T_0AM from biological and clinical samples, and

therefore provide the scientific and clinical community with better tools to understand the full scope of their importance in human physiology.

Experimental section

All reagents used were of analytical grade and were used without further purification. pH studies were performed with a universal buffer solution (of a Britton-Robinson type) composed of 0.1 mol L^{-1} sodium phosphate, 0.1 mol L^{-1} sodium acetate and 0.1 mol L^{-1} sodium borate. The pH was adjusted to the intended value with 6 mol L^{-1} hydrochloric acid or 4 mol L^{-1} sodium hydroxide. All aqueous solutions were prepared using ultrapure water with resistivity not less than 18.2 MΩ cm at 298 K.

All voltammetric measurements were performed using a Metrohm 663 VA voltammetric stand using a Ag|AgCl (KCl, 3 mol L^{-1}) reference electrode and platinum as the counter-electrode. Two working electrodes were used: a HMDE (drop size ca. 0.52 mm^2) and a GCE (area ca. 3.14 mm^2).

The system was connected to a µAutolab II voltammetric system operated by the software GPES v 9.4. All measurements were performed at room temperature. Solutions were deoxygenated with water-saturated nitrogen for 10 min.

T_1AM and T_0AM were synthesized according to a previously published method.[13]

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