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

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Recent studies have shown that, besides the well-recognized T_3 and T_4 hormones, 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 their 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; furthermore, T_1AM was sensed both with a graphitic surface (oxidatively) as well as with mercury (reductively). For both compounds, after oxidation, it was possible to observe the reversible redox reaction concerning the benzoquinone/hydroquinone couple, thus increasing the specificity of the electroanalysis. Therefore, this work provides the basis for an 'at-point-of-use' electrochemical strip test for T_1AM and T_0AM.

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]

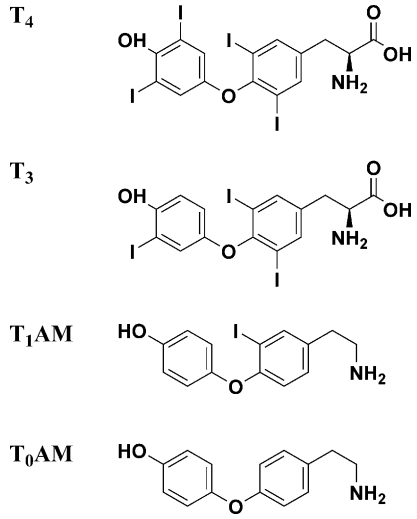

Figure 1. Molecular structures of T_4, T_3, T_1AM, and T_0AM.

Thyroid hormones have crucial effects on metabolism and thermogenesis, on processes involving muscular contraction, growth, reproduction, immune and antiviral defenses, as well as defense against free radicals.[1] These functions are not specific to the human species, as 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, whereas T_4 and T_3 bound to thyroid-binding proteins (mainly thyroxine-binding globulin, transthyretin, and albumin) are 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 symptoms, 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.

This leads to compelling, and 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] neither T_1AM nor T_0AM have been electrochemically studied before this work, to the best of our knowledge. Herein, T_1AM and T_0AM are successfully analyzed with a glassy carbon electrode (GCE) (Figure 2) in an oxidative electrode reaction, and T_1AM is analyzed with a hanging mercury-drop electrode (HMDE) (Figure 3) in a reductive electrode reaction.

The oxidative processes in a GCE electrode, at pH 7, give origin to voltammetric signals with peak potential of approximately +0.5 V versus Ag|AgCl.

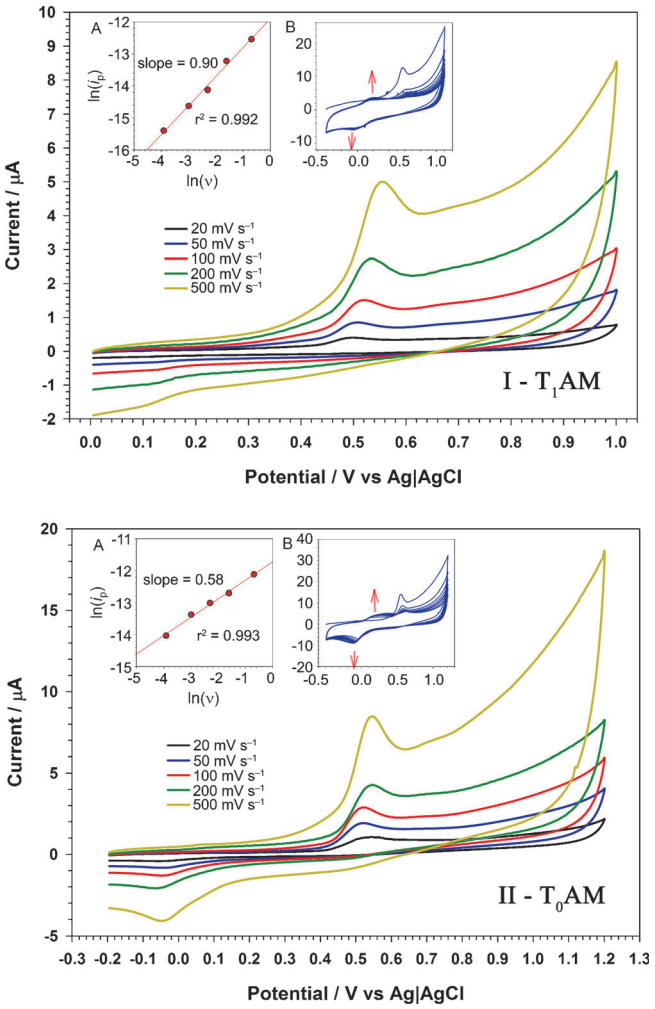

Figure 2. I) Cyclic voltammograms of aqueous phosphate buffer solution (pH 7) containing 0.174 mmol L^{-1} T_1AM on a GCE, run between 0.0 and +1.0 V versus Ag|AgCl at different scan rates. Inset A: logarithm of scan rate versus logarithm of peak current. Inset B: sequential cyclic voltammograms at 1000 mV s^{-1}, showing the appearance of a reductive and an oxidative peak around 0.0 and +0.2 V versus Ag|AgCl, respectively. II) Cyclic voltammograms of aqueous phosphate buffer solution (pH 7) containing 0.153 mmol L^{-1} T_0AM on a GCE, run between −0.2 and +1.2 V versus Ag|AgCl at different scan rates. Inset A: logarithm of scan rate versus logarithm of peak current. Inset B: sequential cyclic voltammograms at 1000 mV s^{-1}, showing the appearance of a reductive and an oxidative peak around +0.0 and +0.2 V versus Ag|AgCl, respectively.

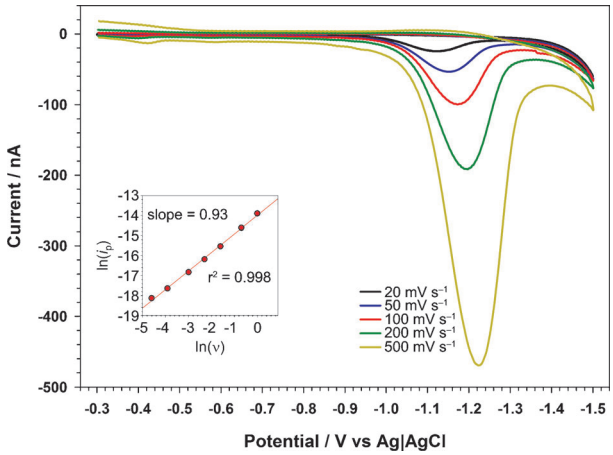

Figure 3. Cyclic voltammograms of aqueous phosphate buffer solution (pH 7) containing 0.174 mmol L^{-1} T_1AM on a HMDE, run between −0.3 and −1.5 V versus Ag|AgCl at different scan rates. Inset: logarithm of scan rate versus logarithm of peak current.

A fully irreversible diffusion-only system follows the Randles–Ševčík equation [Eq. (1)]:[9]

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

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

For an irreversible surface-bound species, i_p versus ν is given by Equation (2):[11]

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

where Γ is the surface coverage. Surface and diffusion-controlled voltammetric processes can be distinguished by their scan-rate dependence. A direct dependence on the voltage scan rate, ν, corresponds to the former, whereas a square-root dependence indicates the latter.[12] This means that a logarithm of peak current ($\ln|i_p|$) versus the logarithm of the scan rate ($\ln|\nu|$) will give rise to a slope close to 0.5 in the case of a fully diffusional process and a slope close to 1.0 for a fully adsorptive process, both apply to either a spherical or a plane macroelectrode. As can be seen in inset A of Figure 2I , a slope approximately 0.9±0.1 suggests an adsorptive process, which could be expected considering the molecular structure of

T_1AM, that is, π–π overlapping could occur between T_1AM and the graphitic surface. However, considering that, for T_0AM (inset A of Figure 2 II), the slope is closer to 0.5, we speculate that it is the highly polarizable iodine atoms that promote adsorption. Plots showing the dependence of the peak current on the scan rate and the square root of scan rate are shown in the Supporting Information.

For the oxidative reaction (i.e. using the GCE), considering a peak potential versus pH slope of 62 mV (Figure 4), that is, approximately 59 mV,[11] the electrochemical reaction up to the rate-determining step should involve an equal number of protons and electrons. We suggest that, first, there is the electrochemically reversible withdrawal of one electron and one proton and, second, a chemically irreversible step where another electron and another proton are removed together with the addition of a water molecule. This cleaves the ether linkage, forming *p*-benzoquinone and it introduces a hydroxyl group in the position *ortho* to the iodine atom (reaction A of Figure 5). By using a fast scan rate immediately after the oxidative electrochemical reaction, we observe the benzoquinone/hydroquinone reversible redox reaction at potential around 0.1 V versus Ag|AgCl (inset B of Figure 2 I and Figure 2 II). A cyclic voltammogram of benzoquinone in the media, using the same voltammetric conditions, gave origin to a similar peak with similar peak potential, thus confirming such assumption (data shown in the Supporting Information).

The reductive process for T_1AM on a HMDE electrode, at pH 7, gives rise to a voltammetric signal with peak potential of approximately −1.2 V versus Ag|AgCl (Figure 3). A similar behavior to that obtained with the GCE was also noticed with the HMDE electrode (inlay of Figure 3), and a slope of almost 1 in the logarithmic plot of peak potential versus scan rate ascribes to an adsorptive behavior.

Concerning the reductive reaction, the rate-determining step should be a first electron transfer—an α value of 0.41 ± 0.05, that is, approximately 0.5, in the Tafel analysis agrees with such assumption—followed by the cleavage of the carbon–iodine bond, releasing an iodide anion, and subsequent protonation of the carbon to which the iodine was bond (reaction B of Figure 5). Although this would, overall, be a two electron–one proton uptake, the first steps (the first electron transfer plus carbon–iodine cleavage) is rate determining, which leads to the observed negligible pH dependence (Figure 4). However, when plotting peak potential versus pH, there is a considerable slope above pH 8.2 (Figure 4), which might be explained as the removal of an extra proton from the reaction molecule. Considering this reductive mechanism for T_1AM, where iodine plays part, we were not expecting to obtain a similar voltammetric signal for T_0AM, and this was the case, as no signal was obtained in an effort to electrochemically reduce T_0AM at the HMDE.

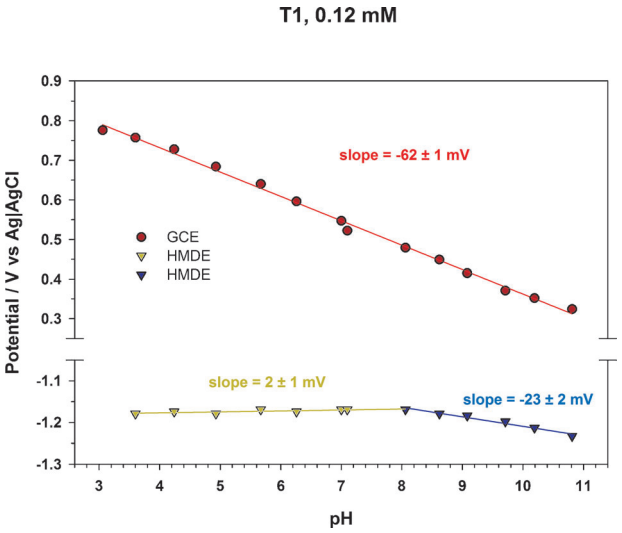

Figure 4. pH studies performed with universal buffer solution of a Britton–Robinson type, containing 0.12 mmol L^{-1} T_1AM, with a GCE (top line) and a HMDE (bottom line). Similar results were obtained for T_0AM (0.10 mmol L^{-1}) for the case of the GCE (data not shown).

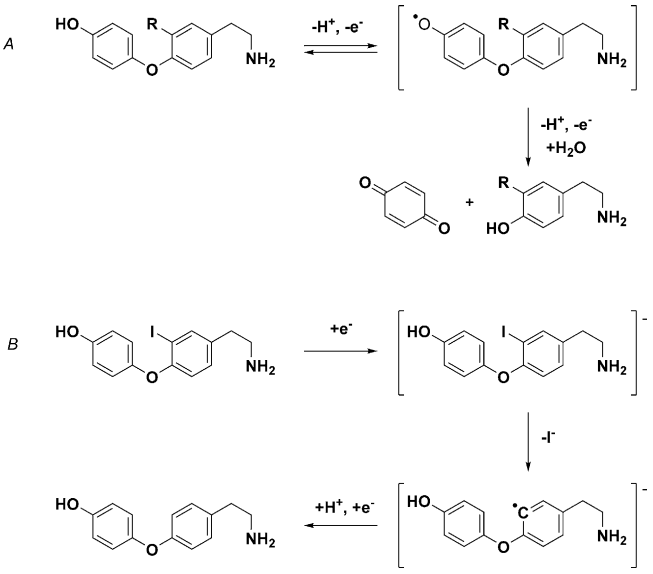

Figure 5. Reaction mechanisms for the oxidative reaction on a GCE (A) and the reductive reaction on a HMDE (B).

These results not only show that is it possible to electroanalyze T_1AM and T_0AM, but they also show that the analyses of T_1AM can be performed with several different surfaces, as it can be performed either in a reductive or in an oxidative way. These are proof-of-concept results that may ultimately pave the way to the creation of low-cost and reliable analytical methodologies for the quantitative analysis of T_1AM and/or T_0AM from biological and clinical samples, therefore providing the scientific and clinical community with better tools to understand the full scope of their importance in human physiology.

Experimental Section

All reagents were of analytical grade and were used without further purification. The 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 by using ultrapure water with a resistivity not less than 18.2 MΩ cm at 298 K.

All voltammetric measurements were performed with a Metrohm 663 VA voltammetric stand by 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 GPES v 9.4 software. 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]

Keywords: clinical analysis · cyclic voltammetry · electrochemistry · hormones · thyroid

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