Proof of Concept of the Electrochemical Sensing of 3-iodothyronamine (T₃AM) and Thyronamine (T₀AM)

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Abstract (925/1000 characters)

It is shown in recent studies that besides the well-recognized T₃ and T₄ there are other relevant thyroid hormones circulating in the human body. In particular this is the case for 3-iodothyronamine (T₁AM) and thyronamine (T₀AM). 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, T₁AM and T₀AM are electrochemically characterized. T₀AM was sensed by means of a glassy carbon electrode; interestingly T₁AM 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 T₁AM and T₀AM.

Keywords (max. 5)
Clinical analysis
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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 (T₄) and 3',5',3-L-triiodothyronine (T₃) (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 T₄ and T₃ are in the picomolar range, while T₄ and T₃ 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, T₁AM (3-iodothyronamine), was unveiled as a new biologically active thyroid hormone derivative (Figure 1).² Later, experiments in small mammals showed that systemic T₁AM and T₀AM (thyronamine) produced hypothermia, a cardiac reversible dose-dependent negative inotropic effect³ and a rapid increase in blood glucose.⁴ These data support the notion that these two hormones, like T₃ and T₄, play a role in the regulation of metabolism.

![Molecular structures of T₄, T₃, T₁AM and T₀AM.](image)

This leads to compelling, as yet unanswered, questions such as: how much T₁AM and T₀AM 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 T₁AM and T₀AM. So far, their detection and quantification has been based on liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) and immunoassay methodologies, which are laborious and costly. This manuscript advocates the case that electroanalysis may be a viable and low-cost alternative.

Although T₄ and T₃ have been electroanalyzed, to the best of our knowledge, neither T₁AM nor T₀AM were ever electrochemically studied before this work. Herein, T₁AM and T₀AM are successfully analyzed with a glassy carbon electrode (GCE) (Figure 2), in an oxidative electrode reaction, and also T₁AM 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 T₁AM 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...
(pH 7) containing 0.153 mM of TzAM on a GCE, cyclic voltammetry was run between -0.2 and +1.2 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.

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

Considering that a fully irreversible diffusion-only system follows the Randles–Ševčík equation:

\[
i_p = 0.496\sqrt{\alpha n' F n A C^* \frac{FDv}{RT}}
\]  

(1)

where \(i_p\) is peak current, \(v\) 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, \(\alpha\) 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, \(T\) is the temperature. Note that equation utilizes the multi-electron form of the Randles–Ševčík equation and is consistent with recent IUPAC recommendations on the definition of the transfer coefficient.

For an irreversible surface bound species, \(i_p\) vs. \(v\) is given by the following equation:

\[
i_p = \frac{(\alpha + n') n F^2}{2.718 RT} \sqrt{\nu A \Gamma}
\]  

(2)
where $\Gamma$ is the surface coverage. Surface and diffusional controlled voltammetric processes can be distinguished on their scan rate dependence. A direct dependence on the voltage scan rate, $v$, corresponds to the former, whereas a square root dependence signals the latter.\(^{[12]}\) This means that a logarithm of peak current ($\ln|i_p|$) vs. logarithm of scan rate ($\ln|v|$) will give rise to a slope close to 0.5 in 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 macro-electrode. As can be seen in the inlay A of Figure 2 - I, a slope ca. 0.9 ± 0.1 suggests an adsorptive process, which could be expected considering T1AM molecular structure, a π-π overlapping could occur between T1AM and the graphitic surface. However, considering that for T0AM (inlay A of Figure 2 - II) a slope closer to 0.5 leads the authors to speculate it is the highly polarizable iodine atoms that indeed promote adsorption. Plots showing the dependence of the peak current on 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 vs. pH slope of 62 mV (Figure 4), i.e. ca. 59 mV,\(^{[11]}\) the electrochemical reaction up to the rate determining step should have involved an equal number of protons and electrons. It is herein suggested that, first there is the electrochemically reversible withdraw of 1 electron 1 proton, then, second a chemically irreversible step where another electron and another proton are removed along with addition of a water molecule. This cleavages the ether linkage forming $p$-benzoquinone and an hydroxyl group is introduced in an ortho position to the iodine atom (reaction A of Figure 5). By means of using a fast scan rate, immediately after the oxidative electrochemical reaction one can observe the benzoinone/hydroquinone reversible redox reaction at potential around 0.1 V vs. Ag|AgCl (inlay B of Figure 2). 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).

![Figure 4 – pH studies, performed on universal buffer solution of a Britton-Robinson type, containing 0.12 mM of T1AM, with a GCE (the section above) and a HMDE (the section below). Similar results were obtained for T0AM, 0.10 mM, for the case of the GCE (data not shown).](image-url)
The reductive process, for T₁AM, in a HMDE electrode, at pH 7, gives rise to a voltammetric signal with peak potential of ca. -1.2 V vs Ag|AgCl (Figure 3). A similar behavior to that obtained with the GCE was also noticed with the HMDE electrode (inlay of Figure 3), a slope of almost 1 in the logarithm plot of peak potential vs. 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, i.e. ca. 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 in overall this would be a 2 electron 1 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 vs. pH, there is a considerable slope above pH 8.2 (Figure 4), which might be explained as an extra removal of a proton from the reaction molecule. Considering this reductive mechanism for T₁AM where iodine plays part, one was not expecting to obtain a similar voltammetric signal for T₀AM. Such was the case, no signal was obtained in an effort to electrochemically reduce T₀AM in the HMDE.

These results not only show that it is possible to electroanalyze T₁AM and T₀AM but, furthermore, the analyses of T₁AM can be done with several different surfaces, since it can be performed either in a reductive or in an oxidative way. These results are proof-of-concept that may ultimately pave the way to the creation of low-cost reliable analytical methodologies for the quantitative analysis of T₁AM and/or T₀AM 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 M\(\Omega\) 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.

\(T_4\)AM and \(T_6\)AM were synthesized according to a previously published method.\(^{[13]}\)

References