Journal of Pharmaceutical and Biomedical Analysis 61 (2012) 156–164

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

The site-specific basicity of thyroid hormones and their precursors as regulators of their biological functions

Gergő Tóth, Sándor Hosztafi, Zsuzsanna Kovács, Béla Noszál*

Department of Pharmaceutical Chemistry, Semmelweis University, Research Group of Drugs of Abuse and Doping Agents, Hungarian Academy of Sciences, Budapest H-1092, Hőgyes Endre u. 9, Hungary

ARTICLE INFO

Article history:
Received 29 October 2011
Received in revised form
18 November 2011
Accepted 20 November 2011
Available online 28 November 2011

Keywords:
NMR
Thyroxine
Protonation constants
Thyroid hormone biosynthesis
Microspeciation

ABSTRACT

The complete macro- and microequilibrium analyses of thyroxine, liothyronine, reverse liothyronine and their biological precursors – diiodotyrosine, monoiodotyrosine and tyrosine are presented. Their biosyntheses, receptor- and transport protein-binding are shown to be distinctively dependent on the phenolate basicity. The protonation macroconstants were determined by ^{1}H NMR-pH and/or UV-pH titrations. Microconstants of the minor microspecies were determined by deductive methods, in which O-methylated and carboxymethylated derivatives were synthesized, and the combination of their NMR-pH and UV-pH titration provided the experimental base to evaluate all the microconstants. NMR-pH profiles, macro-, and microscopic protonation schemes, and species-specific diagrams are included.

Biosyntheses of the thyroid hormones take place by oxidative coupling of two iodotyrosine residues catalyzed by thyreoperoxidase in thyreoglobulin. On the grounds of our phenolate microconstants of precursors the thyroxine over liothyronine ratio needs to be 9:1 after their biosynthesis in thyroid gland, which is in good agreement with biochemical data. The microconstants show that the phenolates are in proton donor (–OH) form in liothyronine whereas they occur in proton acceptor ($-O^-$) form in thyroxine at the pH of blood. These facts explain several facts that have previously been empirically known: the affinity of liothyronine for the receptor is higher than that of thyroxine, the affinity of thyroxine for the transport proteins is higher than that of liothyronine and the selectivity of thyroxine for the OATP1C1 organic anion transporter is higher than that of liothyronine.

1. Introduction

Thyroxine (3,5,3′,5′-tetraiodothyronine, T4), liothyronine (3,5,3′-triiodo L-thyronine, T3) and "reverse" liothyronine (3,3′,5′-triiodo L-thyronine, rT3), the thyroid hormones are formed in the human thyroid gland by iodination and coupling reactions of tyrosine (Tyr) [1,2]. T4 contains four iodine atoms, and is formed upon coupling of two diiodotyrosine (DIT) molecules. T3 and rT3 contain three iodine atoms, and are formed by coupling DIT with monoiodotyrosine (MIT). T3 and rT3 are produced during the peripheral metabolism primarily when T4 is converted to T3 or rT3 [3]. The normal daily T4 production is between 70 and 90 mg, while that of T3 is between 3 and 8 mg by the thyroid gland. Eighty percent of the daily production of T3 is produced by deiodination of T4 in extrathyroidal tissues [4].

Following biosynthesis, T3 and T4 bind to transport proteins in blood. The main transport proteins are the thyroxine-binding globulin (TBG), transthyretin (TTR) and human serum albumin (HSA). T4 is bound more tightly than T3 to each of these proteins [5,6]. Only the unbound hormones have biological activity, the amount and ratio of the free T3 and T4 is therefore important. Approximately 0.03% of total T4 and 0.3% of total T3 in serum are circulating in a free form.

The deiodinase enzymes, as well as the receptors are located intracellularly. Thus, the action and conversion of thyroid hormone require transport across the cell membrane. Thyroid hormones are very lipophilic substances (T4: $\log P = 6.14$; T3: $\log P = 5.21$ [7]) they are therefore able to cross the plasma membrane of target cells with passive diffusion. Nevertheless, many active iodothyronine transporters have been identified (e.g. the OATP1C1, OATP14 organic anion transporter and the amino acid transporter LAT-1), and it is now widely accepted that the cellular uptake is effected by energy dependent, carrier-mediated processes [8].

After the membrane transport T3 binds the thyroid hormone receptors (a type of nuclear receptors), and it modulates the transcription of specific genes. T3 is the biologically active hormone, whereas rT3 is assumed to have no biological activity. T4 is believed to be a prohormone, T3 is more active, and the affinity of T3 for the receptor is 5-fold higher than that of T4 [9]. The thyroid hormones are crucial for the normal development of the central nervous

* Corresponding author. Tel.: +36 06 1 217 0891; fax: +36 06 1 217 0891.
E-mail address: nosbel@gytk.sote.hu (B. Noszál).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.11.020

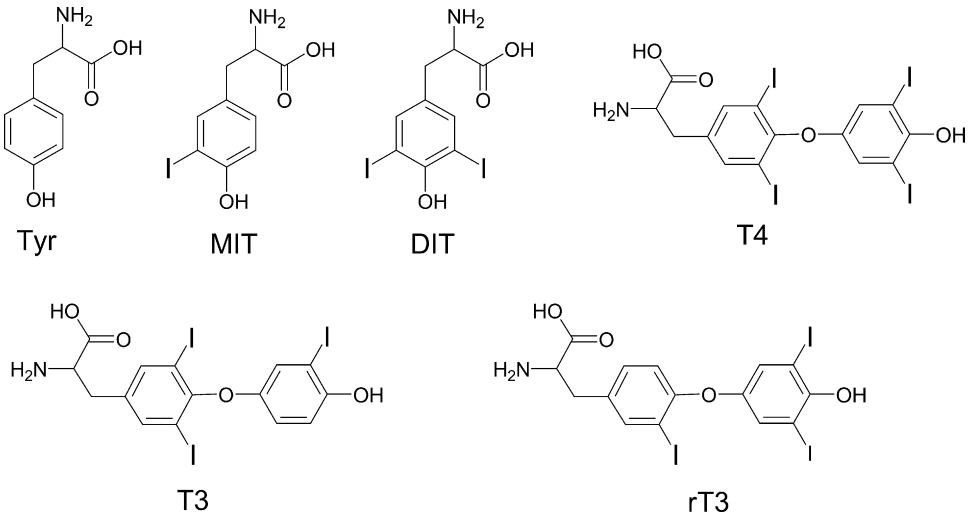

Fig. 1. Constitutional formulas of the thyroid hormones and their precursors studied.

system (CNS) in infants, the skeletal growth in children, and also for the normal function of multiple organ systems in adults [10].

It was our goal to characterize the acid–base properties of the thyroid hormones and their precursors at the site-specific level, and to find possible relationships between the basicities determined and the biological functions of the molecules. The structure of the thyroid hormones and their precursors are in Fig. 1.

Macroscopic equilibrium constants (expressed either as dissociation constants pK_a or protonation constants $\log K$) of multiprotic molecules depict the acid–base properties of the compound as a whole. They hold information on the site of the protonation in exceptional cases only. Site-specific, submolecular basicities can be obtained when microconstants are determined [11]. The site-specific acid–base properties of thyroid hormones and their precursors are therefore of fundamental importance to understand their biosynthesis, and their binding behavior in thyroid gland and in periphery. Knowledge of protonation macro- and microconstant is essential to understand the ADME properties since absorption and distribution are highly affected by the protonation state of the compound.

The thyroid hormones are of very poor solubility in aqueous media and most watermiscible solvents, which is the obvious cause of the controversy in their reported protonation macroconstants [12,13]. The exceptions are the two macroconstants of T4, which were determined by a fast UV-pH titration technique by Comer et al. [14]. The mixed-solvent procedure is a common approach for $\log K$ determination of poorly soluble molecules, and the methanol–water mixture is the most commonly used cosolvent system. This method is based on the determination of apparent protonation constants in solvent mixtures of gradually changing composition, the aqueous $\log K$ is then obtained by extrapolation [15,16]. This method could not be used for $\log K$ determination of our molecules due to the insolubility of the thyroid hormones in several solvent mixtures, including DMSO and methanol.

We therefore used ^{1}H NMR titration in water, applying thousands of transients, especially in the acidic pH region where our compounds exhibit the poorest solubility. Several important molecules have been characterized by NMR-pH titration [17,18]. This method offers a number of advantages. For example, high field NMR can distinguish between peaks of the parent compound and its possible decomposition products and other impurities, and the chemical shift is independent of the concentration. 2–32 transients are typically coadded in a ^{1}H NMR spectrum; but the signal/noise ratio can be improved upon increasing the number of transients. For the thyroid hormone and precursor molecules at low pH about 6000 transients were necessary to discern the proton signals.

NMR-pH titration is ideally suited for the determination of protonation microconstants, because each basic center can usually be followed by the protonation shift of an adjacent NMR nucleus [19]. In our investigated compounds all NMR nuclei are multiple influenced, their chemical shifts reflect to some extent the protonation of every basic center. Thus, in order to quantitate the site-specific protonation, we also introduced model molecules with reduced number of basic centers and/or methods that are selective for the protonation of a particular basic group.

The phenolate protonation could be selectively monitored by UV-pH titration at the absorption maximum near 290 nm (depending on the compound studied). Concerning molecules with minor microspecies of inferiorly low concentration, the contribution of such microspecies to the UV signal can be insignificant. In such cases the deductive method is the only approach to determine the protonation microconstants. Model compound(s) synthesized in the deductive method mimic the minor protonation isomer, and the macroconstant(s) of the auxiliary compound are then introduced into the microscopic protonation scheme of the main compound. It has been shown in many works [20,21] that electronic effects of a carboxylic (–COOH) group and a carboxylic ester (–COOR); and those of an aromatic hydroxyl (–OH) group and a phenol ether (–OR) are virtually identical on the adjoining moieties. We synthesized the carboxylic esters, the phenol ethers, and the O-methyl-carboxymethyl esters of our investigated compounds, and determined the $\log K$ values of all these compounds by NMR-pH titration.

The microspeciation of tyrosine is well-known [22,23]. In order to ensure identical circumstances for direct comparison we also determined all the tyrosine microconstants. Yamazaki et al. determined two macroconstants of monoiodotyrosine and diiodotyrosine, and four microconstants of monoiodotyrosine by spectrophotometric and potentiometric titration [24]. Gemmill and Tata examined the apparent ionization constants of the phenolate of thyroid hormones by UV-pH titration [25,26].

Although the physiological effects and many physicochemical characteristics of thyroid hormones are well known, no previous