Deiodinase 1 (DIO1) activity based on Sandell-Kolthoff (SK) reaction

Thyroid hormones (THs) are important regulators of growth, development, and homeostasis in all vertebrates. The deiodination of thyroid hormones by deiodinases (isoenzymes DIO1, 2 and 3) plays a fundamental role in the regulation of thyroid hormone concentration in peripheral tissues as well as plasma concentration. There are many environmental contaminants that are known to disrupt TH action, yet their mechanisms are only partially understood that lead to a gap in existing methods to detect such chemicals. However, several chemicals were identified (e.g. 10.1093/toxsci/kfy302.*) that can disrupt Deiodinase 1 activity and therefore might consequently alter thyroid hormone homeostasis (Schmutzler and al., 2007).*

References

Renko, K., C. S. Hoefig, F. Hiller, L. Schomburg and J. Köhrle (2012)."Identification of iopanoic acid as substrate of type 1 deiodinase by a novel nonradioactive iodide-release assay." Endocrinology 153(5): 2506-2513.

Renko, K., S. Schäche, C. S. Hoefig, T. Welsink, C. Schwiebert, D.Braun, N.-P. Becker, J. Köhrle and L. Schomburg (2015). "An improved nonradioactive screening method identifies genistein and xanthohumol as potent inhibitors of iodothyronine deiodinases."Thyroid 25(8): 962-968.

Weber, A.G., B. Birk, C. Müller, et al., The thyroid hormone converting enzyme human deiodinase 1 is inhibited by gold ions from inorganic salts, organic substances, and by small-size nanoparticles.Chemico-Biological Interactions, 2021: p. 109709.

In EURL-ECVAM project(a):

Birk, B. and A. G. Weber (2020). Part 1: Reproducibility Assessment for method 4a: DIO1-SK assay, BASF SE. This optimization and standardization of the method will be published shortly (manuscript is in the last review process; Weber et al. 2022, Applied in vitro toxicology) In EURL-ECVAM project(a):

Birk, B. and A. G. Weber (2022). Part 2: Relevance assessment formethod 4a: DIO1-SK assay, BASF SE.

Principle of the method

The objective of this method is to assess the functional capacity of the Iodothyronine Deiodinase type 1 (DIO1) enzyme to deiodinate thyroid hormone in the presence of chemicals.

The DIO1 is thought to possess iodide recycling capacity through the deiodination of the inactive reverse T3 (rT3) but is also capable to deiodinate thyroid hormone substrates towards T3, rT3 or 3,3'-T2 (*figure*). Thereby DIO1 contributes to the T3 production in the thyroid and facilitates the recycling of iodide from thyroid hormone metabolites in excreting organs like the liver and the kidney.

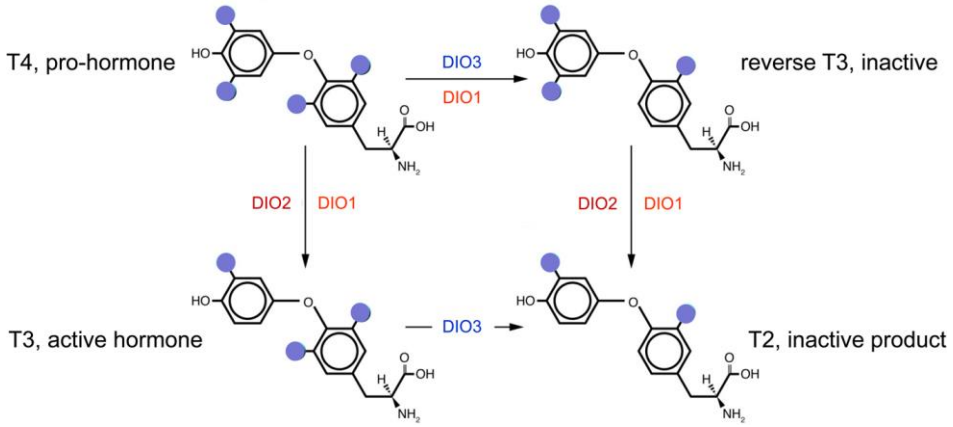

Figure : DIO1 deidonates thyroid hormone substrates towards T3, rT3 or 3,3'-T2

The method, originally published by Renko et al., uses the colorimetric Sandell-Kolthoff (SK) reaction to quantify DIO acitivity:

During an incubation period, iodide is released by the enzymatic activity from the substrate molecule (reverse T3), in the presence or absence of a test compound. After a given timeframe of e.g. 2h, the released iodide is separated from the intact substrate molecules via ion exchange chromatography, using a custom-made 96well column package filled with DOWE50wx2-resin.

For quantification of released iodid, representing the enzymatic activity, the eluted fraction is applied to the Sandell-Kolthoff-reaction.

The reduction of yellow-colored cerium (IV) to colorless cerium (III) by arsenite is dependent on the concentration of iodide which is released from the substrate. The yellow-colored cerium (IV) loses its color after the reduction to cerium (III) which can be quantified through measurement of the optical density (OD) before and after the reaction. The velocity of destaining is a direct measure of free iodide in the incubation mixture, which results from the deiodinating activity of the enzyme.

Readouts : Deiodinase 1 activity, optical density (Sandell Kolthoff reaction)

Necessary equipment and consumables

- **Photometer** for absorbance measurement in 96 well (steady state and kinetic)
- **Centrifuge** with swing-out rotor for microtiter plates (96 well and deep well)
- **Microcentrifuge** tubes 1.5 mL
- **Microplate shaker**
- **Incubator** (or temperature-controlled MP shaker)
- **Analytical balance**
- **pH meter**
- **Fume hood**
- **Filter plates (96 well format)**
- **Assay plates (96 well format)**
- To emphasize a safety relevant aspect: The handling of arsenic during this method requires additional safety measures (e.g. safety operating instructions)