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Radiometric enzyme assays: Development of methods for extremely sensitive determination of types 1, 2 and 3 iodothyronine deiodinase enzyme activities

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We elaborated novel, reliable methods for extremely sensitive radiometric determination of enzyme activities of iodothyronine deiodinases (IDs) of types 1, 2 and 3 in microsomal fractions of different rat and human tissues, as well as in homogenates of cultured mammalian cells. These enzymes catalyze selective 5'- (outer ring) and 5- (inner ring) monodeiodinations of iodothyronines and play crucial roles in the biotransformations of thyroid hormones (TH). The newly developed radiometric assays for IDs were based on the use of appropriate high-specific-radioactivity ¹²⁵I-labeled iodothyronines as substrates; high-performance TLC separation of radioactive products from the unconsumed substrates; film-less autoradiography of radiochromatograms using storage phosphor screens; and quantification of the separated compounds with a BAS-5000 (Fujifilm Life Science Co.) laser scanner. This methodology enabled us to determine IDs enzyme activities in the range as low as 10-18 katals.
For the proper measurement of the individual IDs enzyme activities, we found out first the optimum assay conditions, including the concentrations of the respective radioactively labeled substrates, appropriate concentrations of thiol cofactor, the amount of total protein and enzyme concentration in the incubation mixtures, and suitable incubation times. Further, we demonstrated the applicability of our sophisticated methods by following the alterations of IDs activities induced in cultured rat astroglial cells by a series of purinergic agonists, retinoic acid, and their combination. In the case of ATP as a representative of purinergic agonists, we determined also time-course and dose-response curves to characterize in more details the induction of each type of deiodinase by purines.
The introduced radiometric assays proved to be very sensitive and rapid and, at the same time, reliable and robust.

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