Biotinidase Activity in the Urine of Healthy Subjects

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Dear Sir,

Biotinidase (EC 3.5.1.12) is a multiple hydrolase which mainly hydrolyses biotinyl-amido compounds, such as biocytin [1], biotinyl-4-aminobenzoate [2] and biotinyl-6-aminoquinoline (BAQ) [3]. Although detectable biotinidase activity has been reported in most mammalian tissues and body fluids [4], no enzyme activity in the human urine has been reported to date.

Recently, a highly sensitive and specific HPLC-fluorimetric method for a biotinidase assay with BAQ as substrate has been developed by us [5] and subsequently applied to the enzyme determination in the cerebrospinal fluid [6]. Using a similar method, biotinidase activity has been detected in the urine of the majority (19/25 = 76%) of patients with renal disease associated with protein-uria, whereas it was non-detectable in 40 healthy, age- and sex-matched Japanese controls [7].

In the present study, the investigation on the biotinidase activity in the urine was extended to healthy non-Japanese individuals.

A total of 24 subjects (12 male, 12 female; mean age 25.9 years, range 3.7-44 years) was selected for the present study. The participants were originally from Russia (n = 12), Italy (n = 3), the Czech Republic (n = 3), China (n ≈ 2), the USA (n = 1), South Korea (n = 1), Hungary (n = 1) and Germany (n = 1). At the time of the study, the subjects had been living in Japan, for an average of 15 months (range 1-84 months).

The healthy state was ascertained on the basis of a clinical work-up including detailed history, physical examination and standard laboratory tests on blood and urine. The participants were not receiving drugs known to affect the biotin metabolism. Informed consent for the study was obtained.

Random, as well as 24-hour urine samples were collected. A 200- μ l volume of fresh urine from each sample was filtered (Eki-crodisc 13, pore size 0.2 μ m; Gelman Sciences, Japan) and stored at -80°C until the date of the assay.

Biotinidase activity using BAQ as enzyme substrate was determined by the HPLC-fluorimetric assay, as previously described [5-7]. Enzyme activity was expressed both as pmol·min⁻¹·ml⁻¹ (activity per volume) and pmol·min⁻¹·mg⁻¹ of protein (specific activity). Mean intra- and inter-assay coefficients of variation were 1.2 and 2.6%, respectively. Urine

protein concentrations were determined either using a bicinchoninic acid protein assay kit (Pierce, Rockford, 111, USA) or a protein HPLC assay developed by us [unpubl. data]. All of the urine samples exhibited biotinidase activity. Mean biotinidase activity was $15.2 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mH}^{-1}$ (range 0.93-56.7); biotinidase specific activity was (mean $\pm$ SD) $627 \pm 343 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ of protein, and 24-hour biotinidase activity was $70.9 \pm 21.1 \text{ nmol} \cdot \text{min}^{-1}$. The highest and lowest enzyme activities were detected in the urine of a subject from Hungary and a subject from China, respectively. Mean biotinidase activity was approximately 1/9(11.1%) of the mean reported for human serum [8].

In order to elucidate the enzyme physical-chemical characteristics, biotinidase was purified from the urine. The purified urine biotinidase was shown to be different from the serum enzyme with respect to molecular size (66,000 D of urine enzyme vs. 76,000 D of serum enzyme, as determined by SDS-PAGE), and N-terminal amino acid sequence (up to 19 residues) [unpubl. data].

The data indicate that considerable biotinidase activity is present in the urine of healthy non-Japanese subjects. This enzyme activity appears to be related to a hitherto unreported type of biotinidase, whose tissue origin and biological function remain to be determined.

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