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Stereoselective and manganese-dependent sulfation and urinary excretion of D-form and L-form *meta*-tyrosine *O*-sulfate by Sprague–Dawley rats

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Abstract

In our previous studies, we have demonstrated the stereoselective and manganese-dependent sulfation of tyrosine and Dopa isomers by human monoamine (M)-form phenol sulfotransferase (PST). In the present study, we investigated the occurrence of these phenomena in vivo using Sprague–Dawley rats as an experimental model. Three groups of six male rats were orally introduced with 1 ml of, respectively, 3 mM, 10 mM and 30 mM $MnCl_2$ with a constant level of 0.2 mM DL-*m*-tyrosine per day for 7 days. Their urine was collected and analyzed for the presence of sulfated DL-*m*-tyrosine (DL-*m*-TyrS) by ion-pair HPLC using a C18 reversed-phase column. The level of urinary DL-*m*-TyrS, which was detected in the urine of the $MnCl_2$-treated rats but not control rats, appeared to increase proportionally to the amount of $MnCl_2$ administered. Chiral HPLC was employed to differentiate the D-form and L-form *m*-TyrS present in the urine sample of $MnCl_2$-treated rats. Both D-*m*-TyrS and L-*m*-TyrS were detected, with the D-form being present at significantly higher level than the L-form.

Keywords: Tyrosine *O*-sulfate; Stereoselective; Manganese-dependent; Sulfation; Rat

1. Introduction

Sulfation has been shown to be a major pathway in vivo for the biotransformation and/or excretion of drugs and xenobiotics as well as endogenous compounds such as thyroid and steroid hormones, catecholamines, and bile acids [1–3]. The responsible enzymes, the so-called 'cytosolic sulfotransferases' in mammalian cells, catalyze the transfer of a sulfonate group from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to the hydroxyl groups or amino groups of substrate compounds [4]. In addition to the endogenous compounds mentioned above, our recent studies have revealed that the amino acid form of tyrosine and Dopa can also be used as substrates by some cytosolic sulfotransferases [5–9].

Using HepG2 human hepatoma cells, we have obtained evidence for the enzymatic sulfation of

Abbreviations: TyrS: tyrosine *O*-sulfate; DL-*m*-TyrS: DL-*meta*-tyrosine-*O*-sulfate; TBA: tetra-*n*-butylammonium hydroxide; Mn^{2+}: manganese ion

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L-*p*-tyrosine and its derivatives/isomers including L-Dopa, D-Dopa, L-*m*-tyrosine, D-*m*-tyrosine, and D-*p*-tyrosine [5,6,8]. Metabolic-labeling experiments in the presence or absence of cycloheximide, a protein synthesis inhibitor [10], indicated that the majority of the free TyrS released by HepG2 cells into the culture medium was generated by the direct sulfation of L-*p*-tyrosine [5,6]. We have further demonstrated that the Dopa/tyrosine sulfotransferase activities present in HepG2 human hepatoma cell homogenate co-eluted with the monoamine (M)-form phenol sulfotransferase (PST) upon ion-exchange and hydroxylapatite column chromatography, and displayed properties similar to those of the latter in terms of thermostability and sensitivity to 2,6-dichloro-4-nitrophenol [8]. To investigate whether the M-form PST is truly responsible for the Dopa/tyrosine sulfotransferase activities in HepG2 cells, we have cloned this enzyme and expressed it for functional characterization [9]. The recombinant enzyme, expressed in COS-7 cells, indeed catalyzed the sulfation of not only dopamine and other monoamines, but also Dopa and tyrosine isomers [9]. Interestingly, the enzyme exhibited higher activities toward the D-enantiomer of Dopa or tyrosine isomers. Moreover, addition of 10 mM $MnCl_2$ to the reaction mixture resulted in a dramatic stimulation of the activities catalyzing the sulfation of, particularly, D-form tyrosine isomers [8,9]. We were interested in investigating whether these phenomena, i.e. preference for D-form tyrosine/Dopa as substrates for sulfation and the stimulatory effects of manganese ions, actually occur under physiological conditions.

In the present study, we used Sprague–Dawley rats as a model to investigate the occurrence of and the stimulatory effect of manganese ions on the sulfation of DL-*m*-tyrosine in vivo. We report, in this communication, the results of the quantitative analysis of the differential sulfation and urinary excretion of D-form and L-form *m*-TyrS by the experimental rats.

2. Materials and methods

2.1. Materials

DL-*m*-Tyrosine was product of Sigma Chemical Company. Dowex 50W-X8 cation exchange resin was from Dow Chemical Company. DL-*m*-Tyrosine *O*-sulfate (DL-*m*-TyrS) was synthesized according to the method of Jevons [11]. Potassium dihydrogen phosphate (KH_2PO_4) and tetra-*n*-butylammonium hydroxide (TBA), both of the HPLC grade were purchased from Wako Pure Chemical Industries. All other reagents were of the highest grades commercially available.

2.2. Experimental animals

Twenty 10-week-old male Sprague Dawley rats weighing approximately 300 g each were divided into four groups, (three groups of six, designated Groups 1, 2, and 3, and one group of two for the control). The rats were placed in individual cages and kept in a 24°C room with 12 h light-dark cycle. Foods and water were available ad lib. Following a 4-day adaptation period, food intake and weight of the rats were monitored throughout the experimental period. Groups 1, 2, and 3 were treated with, respectively, 3, 10 and 30 mM $MnCl_2$ with a constant level of 0.2 mM DL-*m*-tyrosine. Due to the low solubility of DL-*m*-tyrosine in water, the compound was first dissolved in 0.1 N HCl and the pH adjusted to 5 with KOH. The control group was treated with 0.1 N HCl only with pH adjusted as described above. Each rat was treated with 1 ml of the respective DL-*m*-tyrosine/$MnCl_2$ solutions for 7 days, and urine samples were collected daily from the day of treatment for 3 weeks. The urine samples were stored at 4°C until the analysis described below.

2.3. Analysis of urine samples for DL-*m*-TyrS

Urine samples collected from the experimental and control rats were centrifuged at 13,500 × *g* for 15 min. The supernatants were individually passed through a Dowex 50W-X8 resin (H^+ form) column and the pH of the eluates adjusted to 5 with 1 N KOH. The samples were lyophilized, dissolved in 3 ml of water, and filtered. The DL-*m*-TyrS constituent of each sample was analyzed by ion-pair HPLC using a Shodex C18 reversed-phase column in conjunction with UV monitoring at 260 nm. The mobile phase employed was 10 mM TBA, 30 mM potassium dihydrogen phosphate and 12% acetonitrile at a flow rate of 0.8 ml/min. The system was operated at 40°C. Stereochemical identification of D-form and L-form *m*-TyrS present in the sample was carried out using a chiral stationary

phase column (150 mm × 0.4 mm, i.d.; packed with CROWNPAK CR (+) (Daicel Chemical Ind., Ltd.)). A solution of 0.098% (v/v, pH 2) perchloric acid was used as the eluent with a flow rate of 0.8 ml/min. D-Form and L-form *m*-TyrS eluted from the column were identified using circular dichroism (CD) spectroscopy. The CD measurements were made using a Jasco-720 WI CD spectropolarimeter at 25°C in water. The eluted fractions corresponding to Peaks 1 and 2 (compare with Fig. 3) from the chiral column were further purified using reversed-phase HPLC as described above.

3. Results and discussion

We have previously demonstrated that human M-form PST can catalyze the sulfation of Dopa and tyrosine isomers [5–9]. Recombinant M-form PST was shown to display stereoselective and manganese-dependent activities toward these substrates [8,9]. The present study aimed to investigate whether these similar phenomena also occur in vivo using Sprague–Dawley rats as an experimental system. DL-*m*-Tyrosine was used in this study based on two considerations. One is that *m*-tyrosine appeared to be a much better substrate than *p*-tyrosine for sulfotransferase enzymes in not only human, but also rat and mouse [7,12], and thus, may provide higher sensitivity for the detection of the sulfated product excreted in urine. The other consideration is that the use of a DL-mixture of the compound will allow us to examine additionally the stereoselectivity and the differential stimulatory effects of manganese ions on this sulfation. HPLC procedures were employed for the studies described below.

3.1. Effects of manganese on the production of DL-*m-TyrS excreted in urine samples of experimental rats*

Ion-pair HPLC using a reversed-phase column was first employed for determining the conditions for analyzing DL-*m*-TyrS. As shown in Part (a) of Fig. 1, when 10 mM TBA was used as the counterion with 30 mM KH_2PO_4 plus 12% acetonitrile at a flow rate of 0.8 ml/min, a distinct peak with a retention time of 16.2 min was detected for the synthetic DL-*m*-TyrS. When the urine sample of the rat treated with $MnCl_2$,

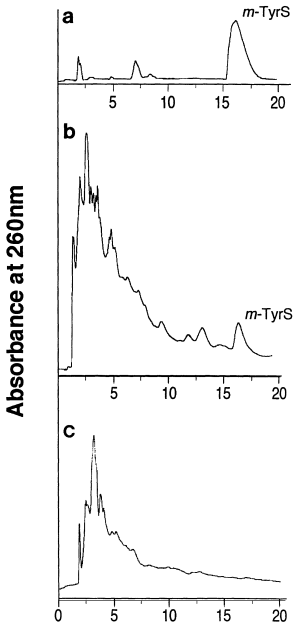

Fig. 1. Ion-pair HPLC chromatograms of (a) synthetic DL-*m*-TyrS and (b) urine sample of the rat treated with 10 mM $MnCl_2$ (c) urine sample of the control rat.

processed as described in Section 2, was similarly analyzed (Fig. 1, Part (b)), a peak with the same retention time was detected. No peak in the same region was detected when the urine samples from the control rats were analyzed (Fig. 1, Part (c)). It is worthwhile mentioning that when the synthetic *m*-TyrS or the urine samples from the rats treated with $MnCl_2$ was subjected to acid treatment with 1 N HCl at 100°C for 15 min, the peak corresponding to the DL-*m*-TyrS was no longer detected (not shown). This latter finding is consistent with the previous report on the acid-labile nature of L-*p*-TyrS [13,14].

As a preliminary attempt to investigate the effects of manganese ions on the sulfation and excretion of DL-*m*-TyrS, two experimental rats were treated with either DL-*m*-tyrosine only or DL-*m*-tyrosine plus 10 mM $MnCl_2$. Urine samples were collected and analyzed by the HPLC procedure as described above. No DL-*m*-TyrS peak was detected for the urine sample collected from the rat treated with DL-*m*-tyrosine only, whereas the urine sample collected from the rat treated with DL-*m*-tyrosine plus $MnCl_2$ showed a distinct DL-*m*-TyrS peak (compare with Fig. 1, Part (b)).

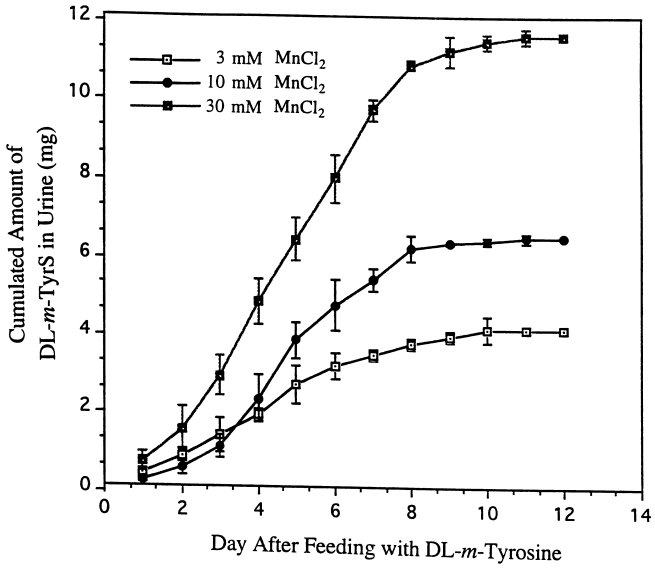

Fig. 2. Amount of DL-*m*-TyrS excreted in the urine samples of the three groups of experimental rats during a 12-day-period time. The data shown represent mean ± S.D.

To investigate further the manganese-dependence of the sulfation and excretion of DL-*m*-TyrS, we treated three groups of experimental rats with, respectively, 3, 10, and 30 mM of $MnCl_2$, with a constant level of 0.2 mM DL-*m*-tyrosine. As shown in Fig. 2, during a 12-day-period monitored, the amount of DL-*m*-TyrS excreted in urine appeared to be proportional to the concentration of $MnCl_2$ fed to the experimental rats. For all three groups of rats tested, the highest level of urinary excretion of DL-*m*-TyrS was observed around day 6, and started leveling off on day 8. Based on the total amounts of DL-*m*-TyrS determined, the calculated percentage of DL-*m*-tyrosine sulfated and excreted in urine for the three groups of experimental rats were, respectively, 4.8, 8.5, and 13.2%.

The functional relevance of the manganese-dependence of the sulfation and excretion of DL-*m*-TyrS remains an unsolved, yet intriguing, issue. Manganese is known to be an essential nutrient and a cofactor for a number of enzymes that are involved in various oxidation–reduction processes [15]. While its deficiency appears to manifest with ataxia, chronic manganese poisoning has been shown to be accompanied by Parkinsonian symptoms observed more frequently among miners [16,17]. It is especially interesting to consider the manganese-dependent sulfation and excretion of DL-*m*-tyrosine from the viewpoint of neurophysiopathology. Although L-*p*-tyrosine is generally known to be the amino acid used for protein synthesis, L-*m*-tyrosine has been shown to be present in vivo [18] and is capable of crossing the blood-brain barrier [19]. Quantitative analysis revealed that L-*m*-tyrosine constitutes a significant amount (2.8%) of the total tyrosine circulating in blood [18]. Using bovine adrenal medulla or rat brain homogenate, L-*m*-tyrosine has been shown to be produced through the *meta*-hydroxylation of L-phenylalanine [20,21]. Furthermore, in vivo studies have shown that L-*m*-tyrosine can be converted to L-Dopa [22–24] or *m*-tyramine [19,25], a decarboxylated product of L-*m*-tyrosine with neurotransmitter activity [25]. In this regard, it is tempting to speculate a possible link to the manganese poisoning reported to occur frequently among miners as mentioned above [16]. It is possible that, for those afflicted with manganese poisoning, the manganese-dependent sulfotransferase enzyme(s) may become activated in vivo. Increasing amounts of L-*m*-tyrosine, and possibly also L-Dopa and L-*p*-tyrosine, which serve indirectly or directly as biosynthetic precursors for catecholamines, may become sulfated and excreted. The lowering of the levels of these Dopa and tyrosine isomers in turn

may result in the decreased production of dopamine and other catecholamines. In line with these hypothetical events, individuals afflicted with manganese poisoning have been reported to display symptoms resembling those of Parkinson's disease [17], and reduction or even elimination of some of these symptoms have been observed for those receiving treatment with L-Dopa [17,26].

3.2. Quantitative analysis of D-form and L-form m-TyrS excreted in urine samples of experimental rats

To differentiate and quantify the D-form and L-form *m*-TyrS excreted in the urine of $MnCl_2$-treated rats, HPLC using a chiral column was employed. As shown in Part (a) of Fig. 3, two distinct peaks (designated Peaks 1 and 2) with retention times of, respectively, 5.1 and 6.8 min were detected when synthetic DL-*m*-TyrS was analyzed. Based on CD spectropolarimeter analysis, the compounds eluted in Peaks 1 and 2 were identified to be, respectively, D-*m*-TyrS and L-*m*-TyrS (data not shown). When the urine sample collected from the experimental rat treated with DL-*m*-tyrosine plus 10 mM $MnCl_2$ was subjected to chiral HPLC analysis, two peaks with the same retention times as those of D-*m*-TyrS and L-*m*-TyrS were detected (Fig. 3, Part (b)). Based on the calculated peak areas, it appeared that D-*m*-TyrS was excreted in considerably larger quantity than L-*m*-TyrS. The higher degree of manganese-dependent sulfation of D-*m*-tyrosine versus L-*m*-tyrosine may reflect the stereoselectivity of the sulfotransferase(s) in vivo, as previously found with the human M-form PST [8,9]. In this regard, it is interesting to note that recent studies have demonstrated that tyrosine residues of human lens protein may gradually undergo racemization forming the D-isomer during the aging process [27]. Since D-tyrosine cannot be used for protein synthesis, it is likely that sulfation may be used as a means for increasing the water solubility and thereby facilitating the excretion of D-tyrosine generated from the turnover of D-tyrosine-containing proteins in vivo.

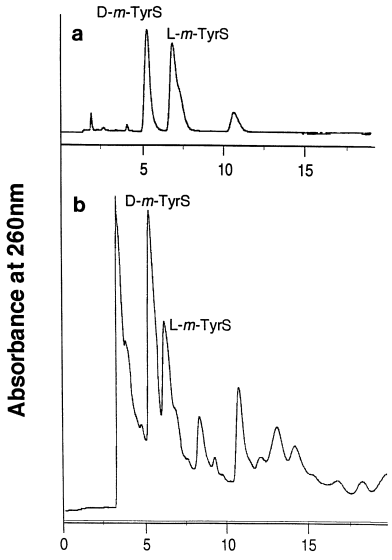

Fig. 3. Chiral HPLC chromatograms of (a) synthetic DL-*m*-TyrS and (b) urine sample of the rat treated with $MnCl_2$.

In conclusion, the results presented in this communication have provided clear evidence for the occurrence of stereoselective and manganese-dependent sulfation of DL-*m*-tyrosine in vivo. More studies are warranted in order to decipher the physiological significance of these interesting phenomena.

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