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ORIGINAL ARTICLE

Identification and quantification of isoguanosine in humans and mice

Allan Weimann[a], George McLeod[b,c], Trine Henriksen[a], Vanja Cejvanovic[a,d] and Henrik E. Poulsen[a,d]

[a]Department of Clinical Pharmacology, Bispebjerg and Frederiksberg HospitalCopenhagen, Denmark;[b]Bruker UK Ltd, Coventry, UK;[c]Owlstone Medical Ltd, Cambridge, UK;[d]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

ABSTRACT

Isoguanine (2-hydroxyadenine), considered to be a non-natural nucleobase has, however, been shown to occur in the croton bean, butterfly wings and a mollusk. For the first time, to the best of our knowledge, we report the identification of isoguanosine (2-hydroxyadenosine), the ribonucleoside, in humans and mouse. Isoguanosine is identified and quantified in RNA from mouse liver samples and in human urine and cerebrospinal fluid. Isoguanine could not be detected as the 2′-deoxyribonucleoside in mouse liver DNA. It could be speculated that the source of isoguanosine was formation from adenosine during oxidative stress in the body. However, the urinary concentrations of isoguanosine and the levels in the liver found here by using isotope dilution liquid chromatography–tandem mass spectrometry are identical to or exceed those of 8-oxo-7,8-dihydro-2′-deoxyguanosine and 8-oxo-7,8-dihydro-guanosine. Guanine is the nucleobase that is oxidized the easiest, so it appears spectacular that the levels of isoguanosine are higher than the levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine and 8-oxo-7,8-dihydro-guanosine. It also appears intriguing that it was only possible to detect the ribonucleoside isoguanosine and not the 2′-deoxyribonucleoside. These observations could indicate that the isoguanosine found is not formed by oxidative stress and could have biological functions.

Abbreviations: 8-oxoGua: 8-oxo-7,8-dihydro-guanine; 8oxodG: 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8oxoGuo: 8-oxo-7,8-dihydro-guanosine; 2-OHdA: 2-oxo-1,2-dihydro-2′-deoxyadenosine; 2-OH-ATP: isoguanosine triphosphate; 2-OHAde: 2-oxo-1,2-dihydro-adenine; 8-oxoGTP: 8-oxo-7,8-dihydro-guanosine triphosphate; 8-oxodGTP: 8-oxo-7,8-dihydro-2′-deoxyguanosine triphosphate

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Introduction

Isoguanine was first synthesized in 1897 by Fischer [1], who predicted that it could occur *in vivo*. In 1932, the ribonucleoside isoguanosine (Figure 1) was found in the toxic croton bean *Croton tiglium* L. [2]; in 1940, isoguanine was reported in butterfly wings [3]; and in 1981, isoguanosine was isolated from the mollusk *Diaulula sandiegensis* [4].

Isoguanine (ribonucleoside names: isoguanosine; 2-hydroxyadenosine; crotonoside; 2-oxy-6-amino-purine-D-riboside or 2-oxo-1,2-dihydroadenosine) can be generated from adenine in a two-step process [5], and as a free nucleotide it seems more susceptible to oxidation by reactive oxygen species than the nucleosides in DNA [6]. Isoguanine can base-pair with isocytosine, which is also considered non-natural, i.e. not occurring in mammals [7]. Isoguanosine triphosphate (2-OH-ATP) is a substrate of the human HTH1 protein, an enzyme that sanitizes the nucleotide pool and has a high pyrophosphatase activity close to that of 8-oxo-7,8-dihydro-2′-deoxyguanosine triphosphate (8-oxodGTP) [8]. The *in vitro* transcription of 2-OH-ATP causes a T → C mutation, whereas 8-oxo-7,8-dihydro-guanosine triphosphate (8-oxoGTP) can cause T → G and T → C mutations [9,10].

Pharmacological doses of isoguanosine produce effects that are similar to those of adenosine [11], and the inhibition of inosine monophosphate-phosphorylase has been demonstrated [12]. It has anti-tumor activity, and in high doses it shows severe toxicity [13]. This modified nucleobase/nucleoside is assumed to be absent from DNA because of its unfavorable physicochemical properties, which result in less-specific pairing compared with the normal DNA bases [14]. Isoguanine has been useful in synthetic nucleic acid research [15].

2-Oxo-1,2-dihydro-adenine (2-OHAde) has previously gained attention since it was reported found in human and mouse chromatin with levels of a few modifications per 10^5 DNA bases [16,17]. The attention has also included terms of formation, assessment of repair and mutagenic features [6,8,10,18–21].

Later it has, however, been reported that 2-oxo-1,2-dihydro-2′-deoxyadenosine (2′-deoxy-isoguanosine) could not be detected by LC–MS/MS in the DNA of human cells with a detection limit of about one modification per 10^7 DNA bases [22]. The discrepancies between the results above is likely due to erroneously overestimated assessment of 2-OHAde in cellular DNA with levels comparable to those of 8-oxo-7,8-dihydro-guanine (8-oxoGua). The levels of a few modifications per 10^5 DNA bases may have been caused by the disputed GC–MS assay where artificial formation of oxidation products have been reported following the required

CONTACT Allan Weimann aweimann@rh.dk Laboratory of Clinical Pharmacology Q7642, Rigshospitalet, Blegdamsvej 9, 2100, Copenhagen, Denmark.

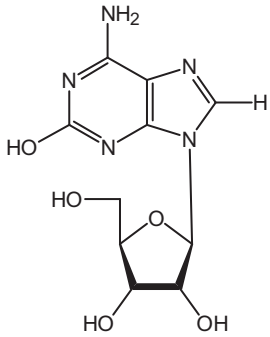

Figure 1. Isoguanosine (2-hydroxyadenosine).

(for GC–MS) acid hydrolysis and derivatization both at elevated temperatures [23–25]. Since the levels of unmodified bases in DNA are about a factor of 10^6 or more higher than the levels of the oxidation products only a very small fraction of the DNA bases has to undergo artificial oxidation during the sample preparation in order to produce erroneous results.

We investigated the presence of isoguanosine in human cerebrospinal fluid (CSF), human urine and mouse livers with ultra-performance liquid chromatography coupled with electrospray ionization isotope dilution tandem mass spectrometry.

Materials and methods

Animal tissue

Mouse livers from healthy untreated mice decapitated for other experiments were excised, immediately placed in a small plastic bag and quickly put into a container with dry ice to prevent oxidation. Thereafter, the livers were stored at −80 °C for later extraction of DNA and RNA.

Biofluids

Human CSF (anonymized excess fluid from diagnostic procedures; Department of Clinical Chemistry, Rigshospitalet) and human urine from healthy anonymized test persons. According to Danish law, it is now allowed to use anonymized excess fluid from diagnostic procedures for method development and testing.

Analytical procedures

Chemicals

Isoguanosine (2-hydroxyadenosine) and 2-hydroxy-2′-deoxyadenosine were obtained from Berry & Associates (Dexter, MI). Adenosine was obtained from Sigma (St. Louis, MO).

$^{15}[N]_5$ isoguanosine was synthesized in-house from $^{15}[N]_5$ adenosine (Cambridge Isotope Laboratories, Inc., Tewksbury, MA).

Synthesis of $^{15}[N]_5$ isoguanosine

$^{15}[N]_5$ isoguanosine was synthesized by the method described by Murata-Kamiya et al. [26]. Briefly, 1 mL of a solution containing 0.5 mM $^{15}[N]_5$ Adenosine and 50 mM sodium phosphate buffer (pH 7.0) were treated with Fe(II)-EDTA (5 mM $FeSO_4$ and 5 mM EDTA) at 37 °C for 30 min. The $FeSO_4$ solution was prepared immediately before adding to the other components.

The synthesis product was purified by UPLC and the identity of the product was confirmed by UPLC–MS/MS.

Sample preparation

Tissue

A slightly modified version of the sample preparation method of Hofer et al. [27] was used for the extraction of DNA and RNA. Approximately, 200 mg tissue was used for each extraction. The concentration of desferoxamine used during the extraction was 30 mM, and hydrolysis to liberate the nucleosides was performed using nuclease P1 and alkaline phosphatase at 37 °C.

Urine

The urine samples were thawed at 0 °C and swirled. Fifty microliters urine were added 50 µL eluent A mixed and heated to 37 °C for 10 min. Then, the sample was cooled to 4 °C and centrifuged at 5000×*g* for 10 min. 12.5 µL urine was added 10 µL of $^{15}[N]_5$ Isoguanosine solution.

Cerebrospinal fluid

The CSF samples were thawed at 0 °C and swirled. Hundred microliters CSF were added 10 µL of $^{15}[N]_5$ Isoguanosine solution and centrifuged through an Amicon 0.5 mL Ultracel – 10 K filter (Merck Millipore, Cork, Ireland) at 20,000 rcf, 4 °C for 10 min. The filter was washed by adding 50 µL eluent A and centrifuged again. The washing step was repeated twice.

Instrumentation

The Waters Acquity UPLC system used consists of an Acquity Sample Manager and an Acquity Binary Pump. The UPLC was connected to a Waters XEVO TQ-s triple quadrupole mass spectrometer with an electrospray ion source and controlled by MassLynx software version 4.1 (Waters, Wexford, Ireland). The column used for the urine and the CSF samples was an Acquity UPLC™ HSS T3 column (2.1 × 100 mm, 1.8 µm) that was protected by an Acquity UPLC HSS T3 VanGuard™ pre-column (2.1 × 5 mm, 1.8 µ). An Acquity UPLC™ BEH Shield RP18 (2.1 × 100 mm, 1.7 µm) column was used for the tissue samples. The columns were obtained from Waters (Wexford, Ireland). Argon was used as collision gas. Nitrogen was used as nebulizer, cone and desolvation gas.

Liquid chromatography–mass spectrometry

The mobile phase was composed of the following:

- Eluent A: 0.5% acetic acid
- Eluent B: acetonitrile.

The LC-flow was diverted to waste for early and late eluting fractions to avoid contamination of the MS ion source.

The column, the injection volume and the gradient were adjusted depending on the matrix analyzed (see Tables 1–3). The column was immersed in a cooling bath with a temperature of 1 °C. This temperature was chosen because nucleosides are better retained at low temperatures. Thus, it was possible to focus the nucleosides on top of the column before the elution started, enabling the use of large injection volumes without generating broad peaks [28]. During chromatography, it is especially important to ensure that adenosine does not co-elute with isoguanosine because a small fraction of adenosine can become oxidized in the ion source and spuriously form either isoguanosine or 8-oxo-7,8-dihydroadenosine (8-oxoAdo), which could bias or interfere with isoguanosine measurements. It seems, however, that less than 1/10,000 adenosine molecules are oxidized in this way, so the separation is expected to only really be critical in tissue samples with an overwhelming amount of unmodified adenosine. Initially when measuring the adenosine and isoguanosine content in mouse liver tissue, the method shown in Table 3 was used directly on the hydrolyzed extracts, but here adenosine and isoguanosine elutes close to one another. To make sure that the isoguanosine that was measured was not artificially formed by oxidation in the ion source, the identification of isoguanosine in the hydrolyzed RNA extracts was confirmed by first pre-fractionation by the same UPLC method as used for the pre-fractionation (separation) of adenosine and isoguanosine in urine (see Table 6). After this the identity of isoguanosine was confirmed by analyzing the collected fractions by the method shown in Table 3. The levels of isoguanosine measured by both the direct method and after pre-fractionation were identical.

Electrospray ionization was always performed in positive ion mode for both isoguanosine and adenosine. For both analytes, the $[M+H]^+$ ion was selected by the first mass filter. After collision activation, the most intense ions were selected by the last mass filter for quantification. The signals corresponding to isoguanosine in urine, liver RNA and CSF were after tuning for maximum sensitivity for each MRM pair sufficiently high to allow the use of one quantifier and

Table 1. Gradient profile used for the quantification of isoguanosine in CSF.

Time (min)	Flow (μL/min)	% *B*
0	200	0
2	200	0
20	200	4.4
32	200	6.1
32.1	200	100
34	200	100
34.1	250	0
43.9	300	0
44	200	0

The column used was an Acquity UPLC™ HSS T3 column that was protected by an Acquity UPLC HSS T3 pre-column. The column temperature was 1 °C. Eluent A was 0.5% acetic acid and eluent B was acetonitrile. The loop volume was 250 μL and the injection volume 130 μL.

Table 2. Gradient profile used for the quantification of isoguanosine in urine.

Time (min)	Flow (μL/min)	% *B*
0	200	0
2	200	0
16	200	12.5
16.1	200	100
18	200	100
18.1	250	0
25.9	300	0
26	200	0

The column used was an Acquity UPLC™ HSS T3 column that was protected by an Acquity UPLC HSS T3 pre-column. The column temperature was 1 °C. Eluent A was 0.5% acetic acid and eluent B was acetonitrile. The loop volume was 250 μL and the injection volume 10 μL.

Table 3. Gradient profile used for the quantification of isoguanosine, adenosine, 2-OHdA and dA in the RNA and DNA from mouse liver.

Time (min)	Flow (μL/min)	% *B*
0	200	0
2	200	0
18	200	4.4
18.1	200	100
20	200	100
20.1	250	0
29.9	300	0
30	200	0

The column used was an Acquity UPLC™ BEH Shield RP18 column. The column temperature was 1 °C. Eluent A was 0.5% acetic acid and eluent B was acetonitrile. The loop volume was 250 μL and the injection volume 50 μL for the quantification of isoguanosine and 2-OHdA and 5 μL for the quantification of adenosine and dA. The DNA and RNA extracts were diluted 1000× before they were injected for the quantification of adenosine and dA.

Table 4. MRM pair ratios for the identification of isoguanosine in human bodily fluids and in mouse liver RNA.

MRM pairs	1 nM isoguanosine	Tolerance [29]	Tolerance range	Urine[a]	CSF[a]	Tissue[b]
284–107	25.3	±50%	12.7–38.0	26.5	29.1[c]	28.2
284–109	40.7	±50%	20.4–61.1	43.7	46.6[c]	44.3
284–135	3.4	±25%	2.6–4.3	3.5	3.2	3.6
284–152	1	–	–	1	1	1

Comparing peak ratios of the significant MRM pairs from isoguanosine in a neat standard and in real samples. The numbers in the table show how many times the peak heights of the individual qualifier MRM pairs are smaller than the peak height of the quantifier (284–152) MRM pair (peak height quantifier MRM pair/peak height qualifier MRM pair).
[a]Ratio calculated as the mean of five samples.
[b]Ratio calculated as the mean of two samples.
[c]Peaks were very small.

Table 5. Mass spectrometric parameters.

Analyte	MRM pairs	Collision energy (eV)	Dwell times (msek)
Isoguanosine	284–152	18	300
	284–135	35	50
	284–109	40	50
	284–107	45	50
Adenosine	268–136	14	50
2-OHdA	268–152	14	300
	268–135	32	50
	268–117	14	50

In addition to the parameters shown in the table, the following mass spectrometric parameters were used: electrospray probe temperature 600 °C, desolvation gas flow 1000 L/h, cone gas flow 150 L/h, capillary voltage 1.0 kV, cone voltage 0 V, source offset 0 V, nebulizer gas pressure 7.0 bar, collision gas flow 0.18 mL/min. Unit resolution was used in both mass filters.

three qualifier MRM pairs. The ratios present in a pure standard correspond to the ones in urine, CSF and RNA, as shown in Table 4. By using four MRM pairs, a total of 7 identification points was obtained for the peaks. Only four identification points are required to confirm the presence of a banned substance in the EU [29].

Table 6. Gradient profile used for the isolation of isoguanosine from urine and extracted RNA.

Time (min)	Flow (µL/min)	% *B*
0	200	0
30	200	25
30.1	200	100
32	200	100
32.1	200	0
50	230	0

The column used was a Cortecs UPLC C18 column (2.1 × 100 mm, 1.6 µ) protected by a guard-column (2.1 × 5 mm, 1.6 µ); both were obtained from Waters (Wexford, Ireland). The column temperature was kept at 1 °C. The mobile phases were: eluent A: 5 mM ammonium formate, pH 5.0 and eluent B: 60% methanol, 40% water and 0.1% formic acid.

The used mass spectrometric settings are shown in Table 5.

For accurate quantification using LC–MS, stable isotopic $^{15}[N]_5$-labeled internal standards were used.

Liquid chromatography–high-resolution mass spectrometry

The identity of the assumed isoguanosine compound was further confirmed by high-resolution mass spectrometry.

Samples and sample preparation

A standard of isoguanosine and a neat urine sample were analyzed using high-resolution mass spectrometry.

In addition, a urine sample was fractionated first to obtain a clear separation of isoguanosine from adenosine, which as mentioned earlier can form isoguanosine in the ion source.

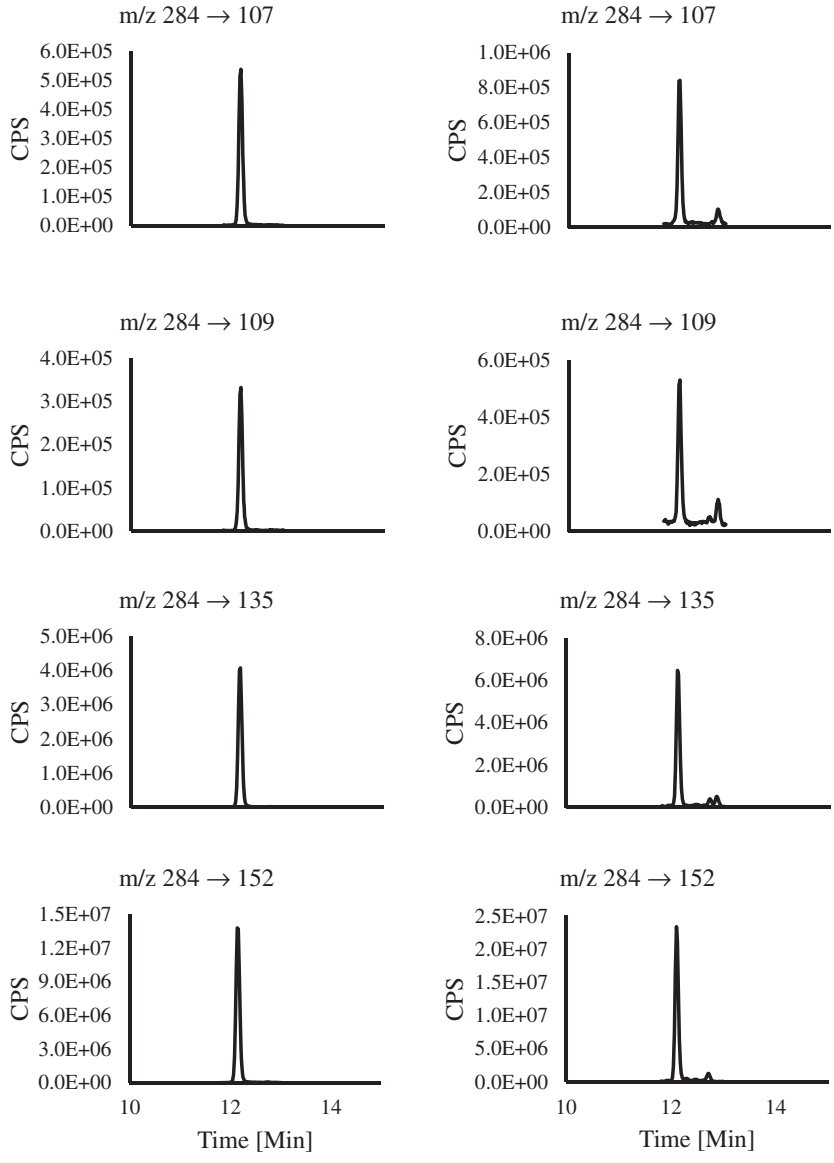

Figure 2. MRM chromatogram of a 100 nM standard of isoguanosine (Left) and an MRM chromatogram of a urine sample containing a high concentration of isoguanosine (Right). Isoguanosine elutes at 12.1 min.

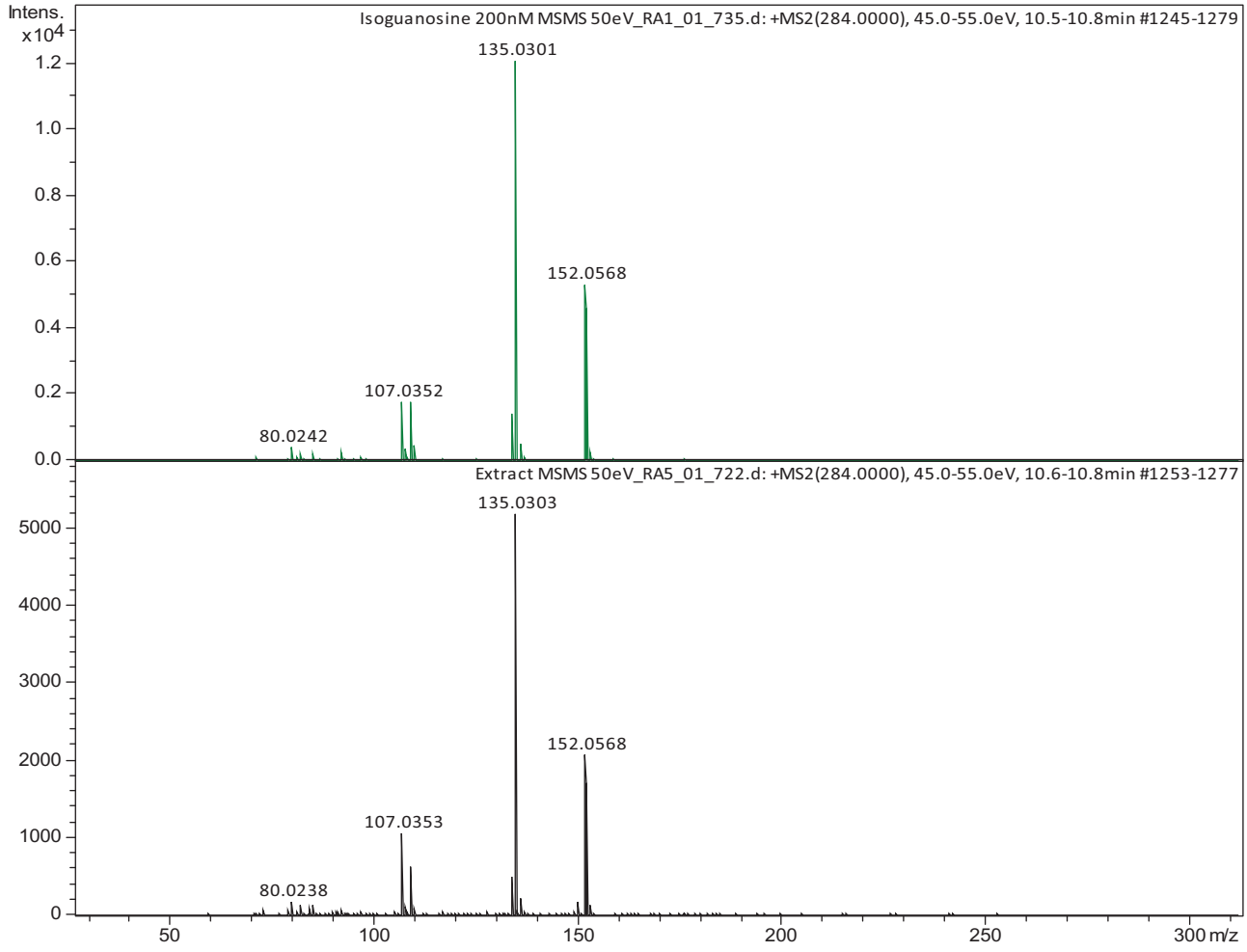

Figure 3. Comparison of the 50 eV collision energy MS/MS spectra of an isoguanosine standard (upper trace) and the unknown peak in the urine extract (lower trace).

The UPLC used for the fractionation was the same as that used for the LC–MS/MS analysis. The UPLC conditions used are as shown in Table 6.

Using this set-up, isoguanosine elutes at approximately 13.8 min and adenosine at 27.5 min so there is a clear separation of isoguanosine and adenosine. The fraction eluting between 13.0 and 17.0 min was collected and evaporated almost to dryness before it was re-dissolved in water.

Apparatus

High-resolution MS was performed on a Bruker impact HD Qq-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) with an attached Dionex U3000 UHPLC system. MS/MS was performed on *m*/*z* 284 at collision energy 50 eV stepped ±10% in-scan and with a data acquisition rate of 2 Hz (digitizer sampling rate 4 GHz). The scan range was *m*/*z* 30–600 and the ions monitored by hrXIC for quantitation were: *m*/*z* 107.0352, 109.0509, 135.0301 and 152.0567.

The collision gas was nitrogen, the ion source temperature 220 °C, the drying gas flow 8 L min^{-1} and the nebulizer gas had a constant pressure of 1.8 bar.

The same LC parameters were used as in the initial LC–MS/MS experiments (see Table 2), except that the column was held at 5 °C.

Results

The initial identification of isoguanosine was based on a comparison of its retention time and the ratios of four different MRM fragments with that of a pure standard (Figure 2 and Table 4). To further confirm the presence of isoguanosine in urine, an LC setup was used where isoguanosine and adenosine do not elute close to one another and thus could be fractionated. (The peaks in that setup are, however, not sharp and thus not good for quantification. This is the reason why other LC conditions are used for quantification and identification.) The isoguanosine fractions collected from multiple injections of urine were pooled and evaporated. This fractionation was introduced to remove adenosine because a small fraction of adenosine may oxidize in the ion source, producing an artificial isoguanosine peak. Next, a pure standard of isoguanosine and the extract were analyzed using high-resolution mass spectrometry for final identification (Figures 3 and 4). The exact masses of the fragments and the precursor ion confirmed the presence of

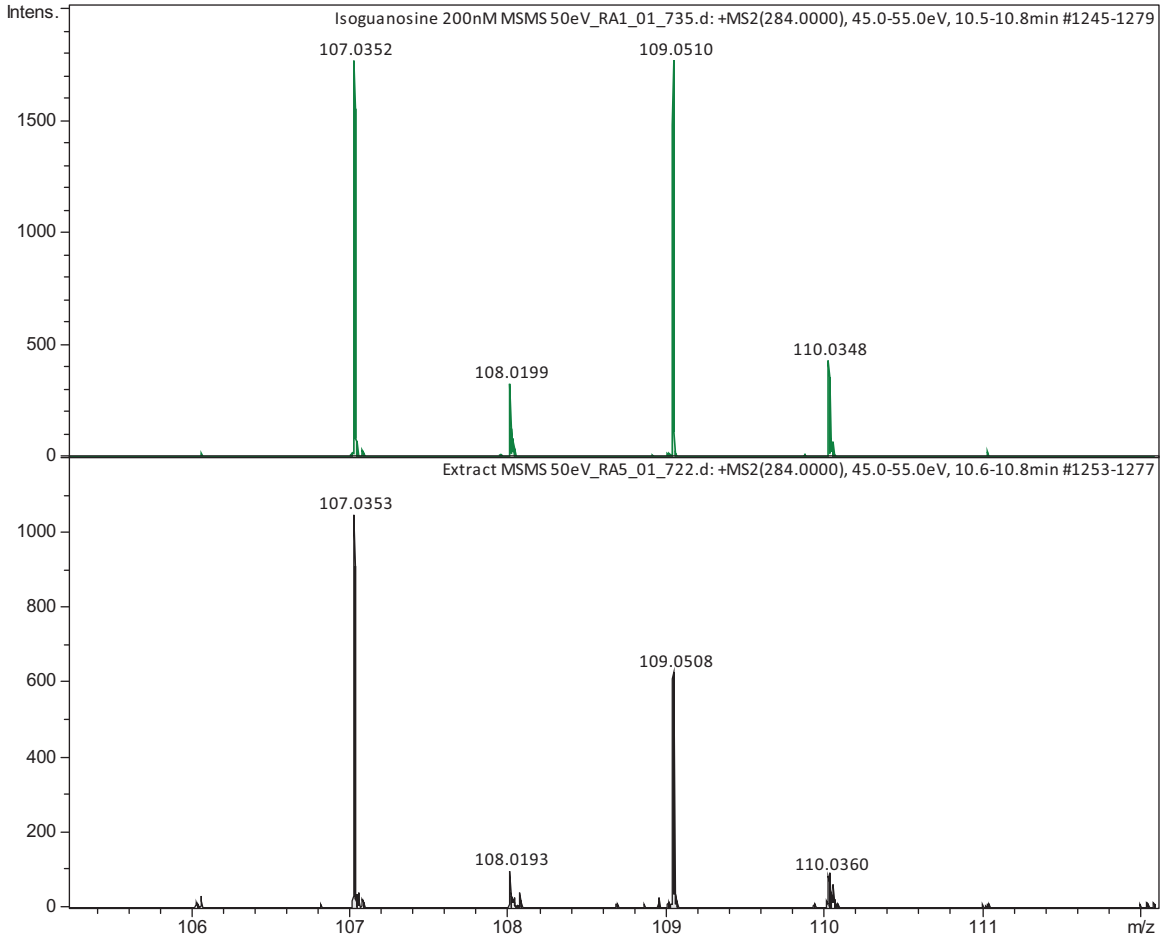

Figure 4. The same as in Figure 3, but zoomed in at a lower mass region.

Table 7. High resolution ion mass measurements for the spectra of isoguanosine and a matching component in neat urine.

Measured *m/z*	Ion formula	Theoretical *m/z*	Err (ppm)	Err (mDa)
Isoguanosine standard				
107.0356	$C_4H_3N_4$	107.0352	−3.5	−0.4
109.0511	$C_4H_5N_4$	109.0509	−1.9	−0.2
135.0296	$C_5H_3N_4O$	135.0301	4.1	0.6
152.0565	$C_5H_6N_5O$	152.0567	1.3	0.2
Urine component				
107.0360	$C_4H_3N_4$	107.0352	−7.1	−0.8
109.0518	$C_4H_5N_4$	109.0509	−8.1	−0.9
135.0302	$C_5H_3N_4O$	135.0301	−0.4	0.0
152.0568	$C_5H_6N_5O$	152.0567	−0.4	−0.1

isoguanosine. The presence of isoguanosine was also confirmed directly in the urine sample, in which a compound eluted at the same time as isoguanosine and produced the same fragment pattern as isoguanosine (Table 7).

On this background, the identification of isoguanosine in human urine seems established. Since a compound with the same retention time as an isoguanosine standard and a matching fragmentation pattern based on the ratio of four MRM pairs was found in human CSF and in mouse liver RNA isoguanosine was also confirmed to be present here.

To determine whether the 2′-deoxyribonucleoside analogous could be found in DNA, DNA was isolated and hydrolyzed from mouse livers and analyzed for the presence of 2′-deoxy-isoguanosine. Urine was also tested for the presence of 2′-deoxy-isoguanosine. The detection limit for 2′-deoxy-isoguanosine was similar to that of isoguanosine in these experiments (this was judged from that the sensitivities for the two analytes were similar upon tuning and when urine was spiked to 10 nM with 2′-deoxy-isoguanosine a huge signal of same magnitude as a pure 10 nM standard was observed), but a signal for 2′-deoxy-isoguanosine could not be detected. This is consistent with previously reported results for human cells and *Escherichia coli* [22]; therefore, we consider it to be established that 2′-deoxy-isoguanosine is either not present in mouse liver DNA and urine or is only present in very low and currently undetectable concentrations. The deoxyribonucleoside was also undetectable in CSF.

The concentrations of isoguanosine measured in human bodily fluids and their ratios to adenosine in mouse liver RNA are shown in Table 8.

A full validation of the used methods was not performed since it was found to be outside the scope of this paper which only was to document the presence and the concentration ranges of isoguanosine in humans and in mice.

The linearity of the three methods used were tested in the ranges covering the concentrations found in the individual sample types: the urine method was tested to be linear in the range 10 nM to 1 μM; the CSF method in the range 10 pM to 2 nM, and the tissue method in the range 0.5–50 nM. The tissue method was also tested to be linear

Table 8. The concentrations of isoguanosine in human bodily fluids and their ratios to adenosine in mouse liver RNA.

	Human urine	Human CSF	Mouse liver RNA
Isoguanosine			
Mean (SD, *N*)	92.8 (47.5, 20) nM	87.5 (22.8, 10) pM	4.7 (0.24, 2[a]) per 10^6 Ado
Range	16.7–224.3 nM	38.5–118.8 pM	4.5–4.8 per 10^6 Ado
8-oxoGuo			
Mean	32 nM[c] [28]	89 pM [30]	2.6 per 10^6 Guo[b] [27]

For comparison, the levels of 8-oxoGuo measured in other publications are included.
[a]Double determination.
[b]Measured in rat liver.
[c]48 nmol/24 h. Corresponding to 32 nM if it is assumed that normal human diuresis is 1.5 L/24 h.

for adenosine in the range 50 nM to 1 μM (here, the samples were diluted 1000 times before injection). It was not tested, if the methods were linear outside these ranges.

The stability of the samples was tested by re-analyzing five urine samples after 5 months. The retrieval of isoguanosine was found to be 98.2% and the relative standard deviation of the retrieval was 7.4%.

Discussion

Increasingly modifications to RNA are being discovered. Modified nucleosides have especially been found in tRNA and mRNA and these modified nucleosides serve specific functions. At the same time, *in vitro* oxidative processes have been reported to produce more than 100 different modifications to nucleic acids [31], all with the potential to serve as mechanistic biomarkers; however, only a small number have been identified *in vivo* e.g. in human urine [32].

The urinary concentrations of isoguanosine and its levels in the liver and biofluids that are reported here (Table 8) are identical to or exceed those of 8-oxoGuo. In addition, only RNA contains this modified base (2-OHAde), whereas DNA, urine and CSF all contains levels of 8-oxodG levels comparable to the levels of 8-oxoGuo in RNA, urine and CSF. Guanine is the nucleobase that is oxidized the easiest, so it appears strange, that the levels of isoguanosine are higher than or equal to the levels of 8-oxoGuo if it was formed by oxidation. It also appears intriguing that it was only possible to detect the ribonucleoside isoguanosine and not the 2′-deoxyribonucleoside.

It has been shown that 2′-deoxy-isoguanosine is a minor product of the oxidation of dA in DNA when exposed to the Fenton reaction by Fe^{2+}/EDTA and H_2O_2. It was reported found in the range of a few percent relative to 8-oxo-7,8-dihydro-2′-deoxyadenosine (8-oxodA) [22]. In the same paper, it is described that 8-oxodA is formed in about 10-fold lower yield than 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) due to preferential oxidation of guanine relative to adenine. It can then be estimated that •OH-mediated generation of 2′-deoxy-isoguanosine in cellular DNA is at best less than 1% of the level of 8-oxodG. This may explain why 2′-deoxy-isoguanosine has not been detected in the DNA of human cells [22] and mouse liver (present study). Why then are isoguanosine found at levels equal to or exceeding the levels of 8-oxoGuo? The most obvious explanation would be that it is not formed by a simple oxidation process, but rather that it is formed by a controlled process. Most post transcriptional ribonucleoside modifications found are formed by methylations, but also several are similar to oxidation products, e.g. 5-hydroxycytidine [33], 5-hydroxymethyluridine [34] and others [35] and could potentially be formed by both oxidation and post transcriptionally modification.

The finding that 2′-deoxy-isoguanosine could not be found in either DNA or as a repair product in urine (analogous to 8-oxo-7,8-dihydro-2′-deoxyguanosine, for example), whereas isoguanosine could be found in relatively high amounts in both urine and RNA suggests that isoguanosine is not mainly formed by oxidation, but rather that it could be formed by a more specific reaction such as e.g. a post transcriptional modification in RNA that may possess novel and unknown functions. As ribosomal RNA is the most abundant form of RNA, we predict that it contains this lesion and that the unspecific pairing properties of isoguanosine [14] may be the basis of its functional role. Base lesions in mRNA possess differential effects on translation [36]: some lesions produce a mixture of full-length and truncated products, whereas others produce only truncated translation products.

Conclusions

The ribonucleoside isoguanosine has been identified and quantified in mouse liver RNA and human urine, and CSF. Although it could stem from oxidation of adenosine in the body, several observations seem to contradict that theory. Instead it could be a posttranscriptional modification, but so far, the question as to why isoguanosine is present in mammals remains open.

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Disclosure statement

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ORCID

Henrik E. Poulsen http://orcid.org/0000-0003-4242-9924

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