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ORIGINAL RESEARCH

Determination of amino acid enantiomers in human urine and blood serum by gas chromatography–mass spectrometry

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ABSTRACT: Amino acid (AA) enantiomers were determined as *N(O)*-pentafluoropropionyl-(2)-propyl esters by chiral gas chromatography–mass spectrometry (GC-MS) in 24 h samples of the urine of three healthy volunteers and in their blood sera. In urine the largest amounts were determined for D-Ser (64–199 μmol/day) and D-Ala (24–138 μmol/day). In blood sera, D-Ala (2.3–4.2 μmol/L) and D-Ser (1.0–2.9 μmol/L) were most abundant. Varying amounts of the D-enantiomers of Thr, Pro, Asx, Glx, Phe, Tyr, Orn and Lys were also found, albeit not in all urines and sera. Further, enantiomers were quantified in urine samples of two volunteers fasting for 115 h. Quantities of renally excreted D-AAs decreased in fasting, although amounts of D-Ser (69 and 77 μmol/L urine) as well as other D-AAs were still detectable. Time-dependent analyses of urine showed that D-AAs are continuously excreted. Copyright © 2001 John Wiley & Sons, Ltd.

INTRODUCTION

Amino acids (AAs) resulting from the digestion of proteinaceous food in the gastro-intestinal tract of human beings are mainly used for the biosynthesis of proteins and peptides. Furthermore, they are catabolized to a major extent in the liver to yield mainly 2-oxo acids and glucose, which are further metabolized, and urea. Low amounts of AAs entering the kidney with the blood stream are neither metabolized nor reabsorbed in the glomerular nephrons of the kidney. On average, quantities of 0.4–1.1 g of free AAs are daily excreted in the urine of healthy adults (Bender, 1985).

The quantities and the chirality of the AAs excreted renally attracted attention when mixtures of DL-AAs (racemates) were administered intravenously to patients for parenteral nutrition. It was shown that parts of the D-AAs applied were renally excreted and parts were metabolized (Bansi *et al.*, 1964). Since conventional amino acid analysis does not distinguish among AA enantiomers, an enzymic method for the determination of D-AAs in urine using D-amino acid oxidase (D-AAO) was developed (Müller, 1963). Using selected ^{15}N-labeled AAs for the parenteral feeding of infants it was shown that, for example, of the D-enantiomers of Val, Ala, Leu and Phe amounts of 44%, 53%, 58% and 72%, respectively, were renally excreted (Heine *et al.*, 1983). Disfunction of the kidney led to an increase of D-AAs in the blood plasma of patients (Nagata *et al.*, 1987; Brückner and Hausch, 1993).

From an analytical point of view use of D-AAO has the disadvantage that the enzyme exerts different selectivity on various D-AAs. By contrast, advanced chromatographic separation and monitoring techniques have the potential to separate and quantify complex mixtures of D- and L-AAs.

Using ligand-exchange chromatography and a mobile phase containing Cu^{2+} and aspartame (L-Asp–L-Phe–OMe), excretion of both D- and L-pipecolic acid (Pip) in the urine of a patient with hyperlysinemia was detected (Lam *et al.*, 1984). After derivatization with FMOC-Cl, varying amounts of the enantiomers of Pip were also detected in the urine of normals and of patients by use of a β-cyclodextrin-bonded stationary phase for the chiral discrimination of enantiomers (Armstrong *et al.*, 1993). Using a two-dimensional technique comprising an achiral octadecylsilyl (ODS) and silica bonded chiral crown ether for separation, and *o*-phthaldialdehyde together with mercaptoethanol for post-column derivatization, low amounts of the D-enantiomers of the hydrophobic AAs Phe, Tyr, Trp and Leu were found in the urine of healthy individuals (Armstrong *et al.*, 1991, 1993). A

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modification of this method was also used for the determination of these D-AAs in the blood plasma of patients with chronic renal failures and normals and for the determination of urinary losses of the enantiomers of Tyr and Phe in non-dialyzed patients (Young *et al.*, 1994). A similar two-dimensional liquid chromatographic system comprising an ion exchange column and a silica-bonded chiral crown ether enabled the determination of D-Asp, D-Ser and D-Ala in the urine of human normals as well as in other biological samples (Van de Merbel *et al.*, 1995).

For the liquid chromatographic determination of DL-AAs in urine and other biosamples, automated precolumn derivatization with OPA together with the chiral thiols *N*-isobutyryl-L-cysteine and *N*-isobutyryl-D-cysteine followed by separation of the resulting diastereomeric isoindol derivatives on an ODS stationary phase was reported (Brückner *et al.*, 1994a). After derivatization or urine AAs with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) the resulting NBD-derivatives of Ser and Ala were micropreparatively isolated using HPLC on a non-chiral reversed-phase column. The quantification of the respective AA-enantiomers was achieved by HPLC on chiral Pirkle-type columns (Huang *et al.*, 1998). Using gas chromatography–mass spectrometry (GC-MS) on the chiral stationary phase Chirasil-L-Val and a flame ionization detector, high relative amounts of various D-AAs were detected in the urine of a patient with short bowel syndrome and high relative amounts of D-Ala in the urine of healthy controls (Ketting *et al.*, 1991). Chiral GC also revealed that D-Ser and D-Ala were present in the dialysate from a chronic dialysis patient (Brückner and Hausch, 1990). These D-AAs were also the most abundant excreted in random urine samples of healthy adults, accompanied by lower amounts of other D-AAs (Brückner *et al.*, 1994b). For further aspects of the analytical chemistry, biochemistry and nutritional considerations of D-AAs we refer to recent reviews and the references cited therein (Friedman, 1999; Imai *et al.*, 1996).

In continuation of our previous studies, in the following we describe the gas chromatographic determination of AA enantiomers in 24 h urine of healthy volunteers and, for completion, in their blood sera. Further, AAs were determined in the urine of two volunteers fasting for several days. Finally, we determined the time-dependent excretion of D-AAs in the urine of volunteers after consumption of yogurt.

The aim of our study was (i) to demonstrate the applicability of chiral GC-MS for the determination of D-AAs in urine (or physiological fluids in general); (ii) to measure quantities of daily excreted D-AAs in healthy adults; (iii) to determine whether D-AAs are still excreted after a fasting period; and (iv) to estimate the amounts of D-AAs in urine following stimulation by food intake.

EXPERIMENTAL

Instrumental

Absolute amounts of (D + L)-AAs were determined by conventional amino acid analysis (AAA), which does not distinguish among AA enantiomers, on a Model LC 6001 amino acid analyzer (Biotronik-Eppendorf, Hamburg, Germany) using a standard program for physiological AAs and post-column derivatization with ninhydrin according to Spackman *et al.*, (1958).

Chiral resolution and determination of relative amounts of D-AAs was performed by GC of *N(O)*-pentafluoropropionyl amino acid (2)-propyl esters on Chirasil-L-Val (*N*-propionyl-L-valine-*tert*-butylamide polysiloxane; Chrompack, Middelburg, The Netherlands) and detection by selected ion monitoring mass spectrometry (SIM-MS) as described previously (Schieber *et al.*, 1999).

Quantitation of amino acid enantiomers

From the absolute amounts of (D + L)-AAs (μmol/L or μmol/day) determined by conventional AAA and the ratios of D- and L-AA determined by GC-SIM-MS, quantities of L- and D-AA were calculated. Relative amounts of D-AAs (%D) were calculated from

Table 1. Absolute and relative (%) amounts of D-amino acids (D-AA) in 24 h urine samples of volunteer nos 1, 2 and 3

	D-AA (μmol/day)						% D					
	1a	1b	2a	2b	3a	3b	1a	1b	2a	2b	3a	3b
Ala	98	84	66	138	54	24	21.9	39.4	28.0	35.9	19.1	22.6
Thr	0.8	n.d.	n.d.	0.3	3.0	1.3	0.6	—	—	0.5	1.0	1.2
Pro	0.4	n.d.	n.d.	0.6	1.7	0.9	2.1	—	—	0.9	5.0	1.6
Ser	168	81	79	64	199	116	49.5	51.4	44.7	44.3	33.5	43.8
Asx	21	12	11	11	3.3	0.9	25.8	24.0	26.5	31.2	14.2	11.5
Phe	1.7	0.1	4.4	1.2	6.6	1.8	1.2	0.1	4.4	1.7	3.0	2.3
Glx	23	21	25	20	22	20	3.3	5.7	7.8	5.6	2.6	4.7
Tyr	n.d.	n.d.	n.d.	0.9	n.d.	0.9	—	—	—	0.5	—	0.56
Orn	4.9	7.9	3.5	6.5	7.7	1.8	21.1	24.1	30.4	25.3	32.3	17.1
Lys	18	7.0	63	8.0	80	94	4.4	5.0	49.5	7.7	17.2	43.3

'a' refers to first and 'b' to second urine sample of volunteer nos 1–3; n.d. (in all tables) = not detected or absolute amounts not determinable. Standard amino acids are abbreviated according to three-letter nomenclature: Asx = Asp + Asn; Glx = Glu + Gln; creatinine (g/day); 1b (1.7); 2b (2.1); 3b (0.9).

Table 2. Absolute and relative (%) amounts of D-amino acids (D-AA) determined in blood sera of volunteer nos 1, 2, and 3 taken when the second urine samples were collected

	D-AA (μmol/L)			% D		
	1	2	3	1	2	3
Ala	3.0	4.2	2.3	0.6	0.4	0.3
Val	0.3	0.4	0.3	0.1	0.1	0.1
Pro	0.9	0.9	0.9	0.4	0.4	0.4
Ser	1.9	1.0	2.9	1.7	0.9	1.3
Asx	0.7	0.6	1.1	1.2	1.6	0.9
Phe	n.d.	n.d.	0.3	n.d.	n.d.	0.4
Glx	0.6	n.d.	1.5	0.2	n.d.	0.3
Tyr	n.d.	n.d.	0.4	n.d.	n.d.	0.2
Orn	0.1	0.2	0.4	0.3	0.3	0.2
Lys	0.2	0.3	1.4	0.2	0.2	0.3

n.d. = not detected.

Table 3. Absolute and relative amounts (%D) of D-amino acids (D-AA) determined in 24 h urine of volunteer 4 at the beginning of fasting (sample no. 4a), urine of the same individual after 115 h fasting (sample no. 4b), and urine of volunteer 5 after 115 h fasting (sample no. 5)

	D-AA (μmol/L) 4a	%D 4a	D-AA (μmol/L) 4b	%D 4b	D-AA (μmol/L) 5	%D 5
Ala	51	15.3	4.4	6.5	3.9	20.6
Val	1.0	1.9	0.2	0.9	n.d.	—
Thr	0.9	0.6	0.5	1.5	0.8	2.5
Pro	n.d.	—	n.d.	—	0.5	7.3
Ser	197	50.3	77	57.8	69	68.5
Asx	8.5	18.4	9.2	22.6	11	29.8
Phe	2.3	1.9	n.d.	—	n.d.	—
Glx	39	4.6	29	9.6	3.4	6.2
Orn	0.7	4.9	n.d.	3.7	1.1	8.7
Lys	8.7	2.1	2.9	3.2	2.1	5.5

n.d. = not detected.

Table 4. Time-dependent excretion of D-AA in urine of volunteer no. 6

	0 min		60 min		120 min		180 min	
	D-AA (μmol/L)	%D	D-AA (μmol/L)	%D	D-AA (μmol/L)	%D	D-AA (μmol/L)	%D
Ala	37	9.2	30	9.3	61	14.6	65	19.1
Thr	3.9	1.8	4.8	1.6	2.5	1.1	3.7	1.6
Val	1.6	1.9	3.2	2.6	1.7	1.9	0.8	0.9
Pro	n.d.	0.4	0.1	0.2	n.d.	0.5	0.04	0.5
Leu	3.2	2.2	2.6	1.4	1.5	1.1	0.9	0.7
Ser	221	40.9	252	41.6	170	36.6	163	38.2
Asx	14	22.7	35	21.6	15	17.3	19	20.0
Phe	2.8	1.3	1.8	0.7	1.3	0.8	1.0	0.6
Glx	20	2.4	32	3.4	18	3.4	28	4.2
Tyr	1.4	0.5	1.5	0.4	1.0	0.3	0.5	0.2
Orn	1.0	5.3	1.7	5.1	0.8	4.5	1.5	7.2
Lys	11	5.4	13	5.4	6.6	3.5	7.6	4.3

n.d. = not detected or absolute amounts not determinable.

peak areas of enantiomers according to the equation %D = 100 D/(D + L). Data are calculated from single analyses or average of two measurements. Absolute and relative amounts of D-AAs are given in Tables 1–5. Amounts of L-AAs can be calculated therefrom if required. In some cases absolute amounts of AAs could not be determined by conventional AAA as a result of interfering compounds or low ninhydrin response. Relative amounts (%D) of these AAs, however, could be determined owing to the higher

Table 5. Time-dependent excretion of D-amino acids (D-AA) in urine of volunteer no. 7

	0 min		60 min		120 min		180 min	
	D-AA (μmol/L)	%D	D-AA (μmol/L)	%D	D-AA (μmol/L)	%D	D-AA (μmol/L)	%D
Ala	17	12.7	51	12.0	88	12.7	19	18.3
Thr	0.3	0.9	1.3	0.8	1.8	0.4	0.5	0.8
Val	n.d.	—	0.7	1.3	n.d.	—	n.d.	—
Pro	n.d.	0.4	n.d.	0.5	n.d.	—	n.d.	—
Leu	0.2	0.8	1.6	1.2	1.4	0.9	n.d.	—
Ser	26	37.1	99	33.4	143	21.6	32	32.8
Asx	2.3	19.0	7.7	17.8	10	10.1	1.7	15.0
Phe	0.4	1.6	0.8	0.9	0.7	0.4	0.1	0.4
Glx	n.d.	5.2	n.d.	4.5	64	6.2	13	8.3
Tyr	0.1	0.3	0.5	0.2	0.4	0.1	0.1	0.1
Orn	n.d.	9.6	0.2	6.1	1.5	11.2	0.2	5.5
Lys	1.8	4.8	5.3	3.9	7.0	2.2	1.1	2.4

n.d. = not detected or absolute amounts not determinable.

selectivity and sensitivity for GC-MS. In tables 'n.d.' refers to not detected or not determinable.

Chemicals

The cation exchanger (Dowex 50W X8, particle size 0.037–0.075 mm, H^+ form) was from Serva, Heidelberg, Germany. 2-Propanol (2-PrOH), analytical grade and 5-sulfosalicylic acid were from Merck, Darmstadt; pentafluoropropionic acid (PFPA) anhydride was from Pierce, Rockford, IL, USA.

Physiological samples

Urine samples were provided by seven healthy adults (volunteer nos, 1–7) living on their normal diets: no. 1 [male (m), 51 years old, 68 kg]; no. 2 (m, 40 years old, 83 kg); no. 3 [female (f), 41 years old, 57 kg]; no. 4 (f, 23 years old, 53 kg); no. 5 (f, 25 years, 50 kg); no. 6 (m, 27 years old, 78 kg); and no. 7 (m, 27 years old, 83 kg).

Collection and treatment of urine and sera

Urine of volunteer nos 1–3 was collected for 24 h and stored at 4°C. A few milliliters of toluene were added as a preservative. Aliquots of urine were frozen and thawed immediately before analysis.

Volunteer nos 1–3 also provided 24 h samples of urine collected 8 weeks later and donated also fasting blood at the time of collecting the second set of 24 h urine samples. Volumes of 24 h urines were 1.20 L (sample 1a), 0.90 L (sample 1b), 2.90 L (sample 2a), 2.97 L (sample 2b), 3.34 L (sample 3a), and 4.44 L (sample 3b); 'a' and 'b' refer to the first and second samples of volunteer nos 1–3.

Spontaneous urine samples were collected from volunteer nos 7 and 8 before (sample nos 7 and 8; $t = 0$ min) and after consumption ($t = 60$, 120 and 180 min) of 500 g portions of a commercial mild yogurt. Volunteers had free access to mineral water after ingestion in order to stimulate renal excretion.

Preparation of a standard and analysis of physiological samples

Standard. A qualitative standard mixture of 14 DL-amino acids (ratio D:L ca. 1:2) and γ-aminobutyric acid (Gaba) was converted into *N*(*O*)-pentafluoropropionyl (PFP)-(2)-propyl esters. Derivatives were analyzed by GC-SIM-MS using a Model GC-17 A gas chromatograph and a QP 5000 mass spectrometer (Shimadzu, Kyoto, Japan). The derivatization procedures and temperature and pressure programs of the GC have been described previously in detail (Schieber *et al.*, 1999).

Urine. Aliquots of urine (2.5–10 mL) were adjusted to pH 2 by addition of 4 M HCl and passed through glass columns filled with cation exchanger (bed volume 5–10 cm length and 1.5 cm diameter). After washing with bidistilled water until pH 7 of the eluate, AAs adsorbed were desorbed with 4 M aqueous ammonia. The eluate was evaporated to dryness on a rotary evaporator and the residues were dissolved in 0.1 M HCl (0.5–1.0 mL). Lithium citrate buffer of pH 2.2 (0.5–3 mL) was added and AAs quantified by conventional AAA using the ninhydrin procedure.

For the determination of the ratios of AA enantiomers by GC, 100–200 μL aliquots were transferred into glass vials and evaporated to dryness in a stream of nitrogen at ambient temperature. Then the residues were converted into their PFP-2-propyl esters and analyzed by GC-SIM-MS as described previously (Schieber *et al.*, 1999).

Blood serum. Fasting blood of volunteers nos 1–3 was collected and stored overnight at 4°C. To aliquots of the serum (0.2–2 mL), 10% aqueous 5-sulfosalicylic acid (100–200 μL) was added and samples were centrifuged at 1600 × ***g*** for 15 min. The supernatants were passed through columns filled with cation exchanger (bed volume 5 cm length and 0.5 cm diameter) and the eluants were treated and analyzed as described above for urine samples.

RESULTS

As described above, amounts of (D + L)-AAs were

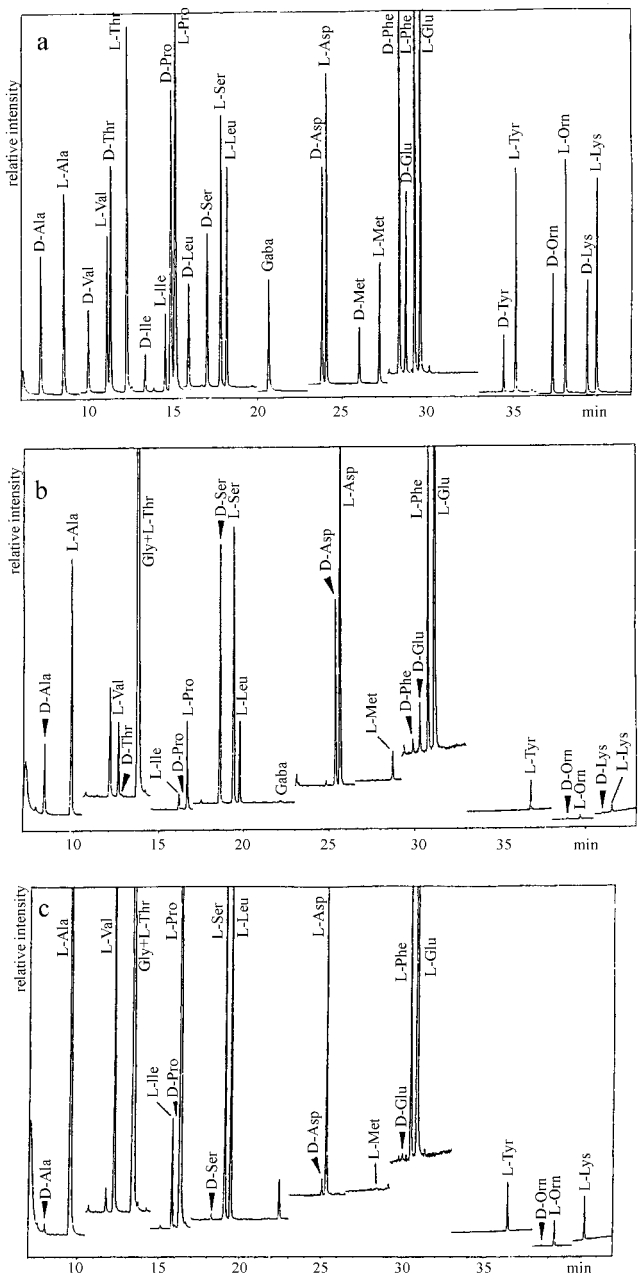

Figure 1. GC-SIM-MS [PFP-(2)-propyl esters on Chirasil-L-Val] of amino acids of (a) a standard of 14 DL-amino acids (ratio D:L ca. 1:3) and γ-aminobutyric acid (Gaba); Gly not included in standard, (b) of the 24 h urine sample no. 1a of volunteer no. 1, and (c) of the blood serum of volunteer no 1.

quantified by conventional AAA and relative amounts were determined by GC-SIM-MS. Amounts of L-AAs can be calculated therefrom, if required. For the chromatogram of a standard see Fig. 1a. Absolute and relative amounts of D-AAs are presented in Tables 1–5. Quantities of AA enantiomers in the pooled 24 h urines of two male volunteer nos 1 and 2 and one female volunter no. 3 are shown in Table 1. Urine samples of these volunteers were designated nos 1a, 2a and 3a, respectively, and those collected 8 weeks later nos 1b, 2b and 3b in Table 1. In the second urine samples (nos 1b, 2b and 3b) creatinine was also determined. Amounts of 0.9–2.1 g/day (cf. Table 1) indicate the normal renal functions of volunteers. Amounts of D-AAs renally excreted per day and the relative amounts of D-AAs calculated are presented in Table 1. Since Asn and Gln are hydrolyzed to Asp and Glu under the conditions required for GC-MS analysis, these AAs are designated Asx and Glx in the tables. In the figures, however, these AAs are designated Asp and Glu as actually determined by GC-MS. As can be seen, the D-enantiomers of Ala, Ser, Asx, Glx, Phe, Orn and Lys were detected in all urine samples, whereas those of Thr, Pro and Tyr were not present in all samples. For the chromatogram of urine sample 1a of volunteer no. 1 see Fig. 1b.

In four out of six urine samples of 24 h urines, highest absolute amounts among D-AAs were determined for D-Ser, in one sample D-Ser and D-Ala were about equal, and in one sample D-Ala was about twice the amount of D-Ser. Quantities of D-Ser ranged from 64 to 199 μmol/day, of D-Ala from 24 to 138 μmol/day, and of D-Lys from 7 to 94 μmol/L. Relative amounts were also highest for D-Ser (34–51%) and D-Ala (19–39%) and D-Lys amounted to 43% and 50% in two urine samples. In two urine samples large relative amounts of 30% and 32% D-Orn were measured, however the absolute amounts were low. From the data of Table 1 it can also be calculated that about 2–12 mg D-Ala and 7–21 mg D-Ser were excreted in 24 h urines.

Quantities of D-AAs in the sera of the three volunteers at the time of collecting the second set of urine samples were also determined and the absolute and relative amounts of D-AAs are given in Table 2. The D-enantiomers of Ala, Val, Pro, Ser, Asx, Orn and Lys were detected in all sera, whereas D-Phe and D-Tyr were detected in one serum and D-Glx in two sera. Among the D-AAs, D-Ala (2.3–4.2 μmol/L) and D-Ser (1.0–2.9 umol/L) were the most abundant. For a chromatogram of AAs in the blood serum of volunter 1 see Fig. 1(c).

In order to investigate the influence of fasting on the excretion of D-AAs, spontaneous urine samples were provided by two female volunteers, and analyzed for AA enantiomers. Data from volunteer no. 4 at the beginning and at the end of the 115 h fasting period, and of volunteer no. 5 at the end of of the fasting period, are presented in Table 3 (the urine of the second volunteer was not analyzed at the beginning of fasting).

In the urine of volunteer no. 4 the highest absolute amounts were determined for D-Ser and D-Ala at the beginning of fasting. Their amounts, as well as those of other D-AAs, had decreased after fasting, but D-enantiomers were still detectable. Relative amounts of D-Ser increased from 50% to 58%, whereas relative amouts of D-Ala decreased from 15% to 7%.

In the urine of fasting volunteer no. 5, at the end of fasting the highest absolute and relative amounts of D-Ser

among D-AAs were determined. Notably, absolute amounts of D-enantiomers were very close to those of volunteer no. 4 at the end of fasting, with the exception of D-Glx.

Amounts of D-AAs excreted in the urine of volunteers nos 6 and 7 after consumption of yogurt, serving as an easily digestable source of milk proteins, are presented in Tables 4 and 5, respectively. It can be seen that D-AAs are steadily excreted renally. The largest absolute amounts in most cases were determined for D-Ser and D-Ala besides other D-AAs. It appears that there is an increase of excreted D-AAs 1–2 h after intake of food. However, since the volunteers had free access to mineral water in order to stimulate renal excretion this needs further investigation. Relative amounts of D- and L-AAs did not change very much.

DISCUSSION

Analysis of D-amino acids in urine and blood serum

The results demonstrate that GC-SIM-MS is a sensitive and specific method for the quantification of AA enantiomers in urine and sera. The data establish that, in general, the highest amounts of D-Ser and D-Ala are excreted in urine, accompanied by other D-AAs.

From an analytical point of view GC using chiral capillary columns together with SIM-MS in comparison to other methods for the determination of of AA enantiomers has the advantage of excellent selectivity and sensitivity. Use of a flame ionization detector (Brückner and Hausch, 1993) is more prone to baseline noise resulting from column bleeding and impurities from physiological matrices. A direct gas chromatographic quantification of D- and L-AAs is also possible, but requires use of an external standard, determination of response factors of derivatives and periodical calibration of the instrument (Erbe and Brückner, 1998).

A disadvantage of the GC method is that derivatization of AA enantiomers under acidic conditions leads to the conversion of Asn and Gln to Asp and Glu, respectively, and to a partial decomposition of Trp. The derivatives of the basic AAs Arg and His require special treatment, otherwise they are not eluted from quartz capillary columns. Although methods have been described to overcome the problems related to these AAs, applications are less suitable for routine analyses. Further, Met, Trp and Cys are sensitive to oxidative degradation and low amounts of the respective D-enantiomers thus may remain undetected. The derivatives of Gly and L-Thr eluted together under the chromatographic conditions used. These analytical limitations should be taken into account. It should also be noted that the urines were not analyzed for uncommon AAs.

Significant amounts of most of the D-AAs detected in urine could also be determined in blood sera of volunteer nos 1–3 (Table 2). The highest absolute amounts were also found for D-Ala and D-Ser. From the data of Table 2 it is also obvious that the relative amounts of D-enantiomers were much lower in sera as compared to urine samples.

The results are in agreement with and extend those reported previously (Brückner and Hausch, 1993; Brückner *et al.*, 1994b) and also confirm a report focusing on the urinary excretion of D-Ser and D-Ala of humans in relation to their age (Huang *et al.*, 1998).

Possible origin of D-amino acids in physiological samples

It has been discussed that D-AAs might be formed in human proteins as a result of time-dependent *in vivo* racemization (epimerization) and that they are released on catabolism. This is not likely since AAs in long-lived proteins such as dentin or eye lens protein are preferably susceptible to racemization. Further, if formed, bonded D-AAs are not, or at least not easily, cleaved by the body's proteases and peptidases which show stereospecifity towards peptide bonds of L-amino acids.

In contrast, microorganisms which are an integral and important part of the gastro-intestinal tract of human beings, contain various D-AAs in their peptidoglycans. Various more or less specific microbial AA racemases and epimerases have been characterized which are capable of converting all L-AA common in physiological samples into D-AAs. D-AAs have been detected in the cytoplasma of bacteria which are not constitutents of the respective peptidoglycans. Thus microbial enzymic conversion of free L-AAs, which are abundant in the digestive tract, as well as release of D-AAs on autolysis of bacteria, might be considered as one possible source of D-AAs detected in urine and blood sera. Further, D-AAs are taken up in particular with fermented foods and beverages (Brückner *et al.*, 1995a).

Most free D-AAs in physiological samples are oxidized by D-amino acid oxidases (D-AAO) localized in the kidney, liver and tissues. Enzymic oxidation leads to the formation of the corresponding 2-oxo acids which can be reaminated to yield L-AAs. However, as kinetics for various D-AAs are different, certain amounts of D-AAs might not be oxidized by D-AAO. Since reabsorption of D-AAs in the glomerular tubuli is lower in comparison to L-AAs (Silbernagl, 1988), this can lead to an increase of D- relative to the L-enantiomers in the urine.

D-amino acid levels in urine of fasting volunteers

From the data shown in Table 3 it is evident that D-AAs are still excreted renally at the end of fasting, although

quantities decreased in comparison to those determined at the beginning of fasting. Total amounts of determinable D-AAs in urine from volunteer no. 4 (123 μmol/L at the end of fasting) are close to those of volunteer no. 5 (92 μmol/L). D-Ser is excreted most abundantly. Remarkably, although absolute amounts of D-Ser decreased in the urine of volunteer no. 4, relative amounts did not change very much and, after fasting, very roughly were comparable to those of volunteer no. 5.

Since no food was taken and only mineral water was drunk 12 h before collecting the urine samples, an endogenous origin of D-AAs has to be considered. It is known that digestive enzymes as well as serum albumin are secreted into the intestinal lumen, and that proteins from the shed cells of the intestinal mucosa are metabolized in the intestinal lumen (Bender, 1985). It is assumed that intestinal microorganisms and their racemaces are still active in fasting and capable of racemizing at least parts of the free L-AAs resulting from protein catabolisms. Furthermore, the detection of an endogenous serin racemase in glial cells of rat brain also indicates the presence of racemases in Mammalia not dependent on microorganisms (Wolosker *et al.*, 1999). However, nonenzymic racemization mechanisms contributing to the pool of D-AAs in physiological fluids have also to be considered (Erbe and Brückner, 2000).

In summary, the mechanisms outlined above in concerted action might account for the amounts of D-AAs which are renally excreted continuously by all mammalia (Brückner *et al.*, 1995b; Brückner and Schieber, 2000) and which are also detectable in organisms such as insects (Corrigan, 1969) and marine invertebrates (Preston, 1987).

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