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Gas chromatographic characterization of free D-amino acids in the blood serum of patients with renal disorders and of healthy volunteers

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ABSTRACT

A capillary gas chromatographic method, using the chiral stationary phase Chirasil-L-Val, after treatment and isolation with Dowex 50W X8 cation exchanger and conversion into trifluoroacetyl-1-propyl esters or pentafluoropropionyl-1 (or 2)-propyl esters, has been developed for the determination of the relative amounts of free D-amino acids in the blood serum of eighteen patients with renal failure (continuous ambulatory peritoneal dialysis (CAPD), $n = 11$; hemodialysis, $n = 5$; nephrotic syndrome, $n = 2$) and compared with data obtained from healthy volunteers ($n = 5$). Significant amounts of D-Ala (0.5–13%) and D-Asx (1.5 7.7%; Asx = Asp + Asn) were found in all serum samples. D-Ser was detected in the serum of all patients with renal disorders and, in addition, D-Pro (0.6–2.5%) was found in the serum of all patients undergoing hemodialysis and with nephrotic syndrome. D-Ser (2.9–3.1%) and D-Pro (0.6–0.9%) were also found in the samples of three volunteers. D-Leu (1.2–1.7%) was present in three patients with CAPD, and D-Glx (0.3–1.3%; Glx = Glu + Gln) was present in eight of eighteen patients with renal malfunction. Linear regression analysis of the relative amounts of D-amino acids and the serum creatinine levels of all donors revealed positive correlation factors for D-Asx ($r = 0.748$) and D-Ser ($r = 0.667$), but not for D-Pro and D-Ala. Remarkably high amounts of D-Ser (12.1 and 19.8%) were found in two hemodialysates investigated. Participation of intestinal bacteria and nutrition are discussed as possible sources of serum D-amino acids. An increase of some D-amino acids in the serum of patients with renal diseases might be explained, in part, by decreased activity of renal D-amino acid oxidase.

INTRODUCTION

Despite the overwhelming presence and importance of L-amino acids in organisms, certain free D-amino acids (D-AA) have been detected in microorganisms [1], insects [2], crustaceae [3], as well as in marine invertebrates [4] and bivalves [5]. Significant amounts of D-AA were also found in the serum of guinea-pigs and mice [6], in human blood plasma [7], in fetal blood [8] and in human urine [9].

Using capillary gas chromatography (GC) on the chiral stationary phase Chirasil-Val [10], we have shown that free D-AA are ubiquitous in fermented foods and beverages [11,12] and have discussed their microbial origin and possible nutritional aspects with regard to their metabolization and renal excretion [13–15]. Application of the GC method to the dialysate of a chronic dialysis patient revealed the presence of relatively high amounts of D-Ser (19.8%) and D-Ala (2.5%) [14]. These data prompted us to investigate the enantiomer patterns of amino acids in the serum of patients with chronic renal failure, in comparison with those of healthy volunteers.

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EXPERIMENTAL

Chemicals

Picric acid and solvents were of analytical grade from Merck (Darmstadt, Germany). Trifluoroacetic anhydride, acetyl chloride and 2,6-di-*tert.*-butyl-*p*-cresol (BHT) were from Fluka (Buchs, Switzerland). Pentafluoropropionic anhydride was from Pierce (Rockford, IL, USA). Dowex 50W-X8 cation exchanger (H^+ form, 200–400 mesh, practical grade from Serva, Heidelberg, Germany) was thoroughly washed prior to use with 2 *M* NaOH and 4 *M* HCl until the effluents were colorless. The water used was purified by reversed osmosis (Milli-Q water purification system; Millipore, Bedford, MA, USA). Standard amino acids were from Sigma (St. Louis, MO, USA) or from Serva or Fluka. Blanks were analysed regularly by GC in order to monitor possible contamination of resin, chemicals, and solvents by D-AA.

Blood serum samples

The blood samples were collected at the Department of Nephrology of the Robert Bosch Hospital (Stuttgart, Germany) and originated from eighteen patients with renal failure. Blood samples were taken from the veins by means of plastic syringes and, after 1 h at room temperature, centrifuged at 2000 *g* for 10 min. Then the sera were immediately frozen and stored at −20°C until analysis. Five healthy volunteers of the Department of Nephrology (one female, four males, age 36–59 years) served as controls. Serum creatinine levels were determined with an enzymic assay using the Kodak Ektachem 700 single-slide method [16].

Serum treatment for amino acid analysis

Immediately after thawing, picric acid (5.0 ml of a saturated solution in water) was added to serum (1 ml) with stirring. After 5 min, 0.1 *M* HCl (5.0 ml) was added, and the solution was filtered into a separating funnel and extracted twice with 20.0-ml portions of light petroleum (b.p. 50–70°C)–diethyl ether (1:1, v/v). The aqueous phase was passed through a column packed with Dowex 50W-X8 cation exchanger (bed size 5 cm × 1 cm), the resin was washed with water (50 ml), amino acids were eluted with 2 *M* aqueous ammonia (30 ml), and the effluent was evaporated to dryness at 40 mbar using a rotatory evaporator. For the determination of the amino acid enantiomers by GC, the residue was dissolved in 1 ml of 0.1 *M* HCl, the solution was transferred to a 1 ml Reacti-vial (Wheaton, Millville, MA, USA) and evaporated to dryness in a stream of nitrogen. The antioxidant BHT (*ca.* 0.5 mg) and 1-propanol in acetyl chloride (8:2, v/v; 200, μl) were added, the vial was securely closed with a PTFE-lined screw-cap, and the solution was heated for 1 h at 100°C for esterification. Solvents were removed in a stream of nitrogen. Dichloromethane (200 μl) and trifluoroacetic anhydride (or pentafluoropropionic anhydride, each 50 μl) were added, and the mixture was heated for 20 min at 100°C for acylation. Solvents were removed in a stream of nitrogen, and the residue was dissolved in 10–20 μl of dichloromethane.

In the cases of hemodialysates (records of patients not available), samples (1 l) were concentrated by lyophilization. Acetonitrile (10 ml) was added to 5-ml samples of the viscous residues, and the mixtures were stirred for 5 min and then centrifuged for 15 min at 1600 *g*. The supernatants were evaporated to dryness in a rotatory evaporator. The residues were dissolved in 0.1 *M* HCl (10 ml), and the solutions were subjected to Dowex 50W-X8 treatment and derivatization as described above.

Gas chromatography

A Model HRGC 5160 gas chromatograph (Carlo Erba, Milan, Italy) was used, with a flame ionization detector, a Model C-R3A integrator (Shimadzu, Kyoto, Japan) and Chirasil-L-Val columns (25 m × 0.25 mm I.D.) (column A from Macherey-Nagel, Düren, Germany; column B from C.G.C. Analytic, Mössingen, Germany). The carrier gas was hydrogen at an inlet pressure of 45 kPa, and the temperature of the injector and detector was set to 250°C. Samples of 0.6–1.0 μl were injected in the split mode; the splitting ratio was *ca.* 1:15. The temperature programmes are shown in the captions to Figs. 1–4.

Ion-exchange chromatography (IEC)

IEC for quantitative amino acid analysis was performed on a Model LC 6001 autoanalyser (Biotronik, Maintal, Germany) with a standard lithium citrate buffer programme for physiological fluids and post-column derivatization of amino acids with ninhydrin reagent.

Calculations

As a consequence of the limited volumes of serum available, work-up of samples as described above was performed only once. In order to confirm the presence of a certain D-AA found, the effluents from Dowex columns were split in two parts and trifluoroacetyl (TFA)-1-propyl esters and pentafluoropropionyl (PFP)-2-propyl esters were prepared and investigated by GC on columns A and B, respectively. Aliquots of derivatized amino acids were analysed four to eight times by GC, and average values and standard deviations are given in Tables I–IV. Relative amounts of D-AA were calculated from the equation $\%D = 100 \cdot D/(D + L)$.

RESULTS

A typical GC elution profile of derivatized amino acid enantiomers on a Chirasil-L-Val capillary column from the serum of a patient undergoing continuous ambulatory peritoneal dialysis (CAPD) is shown in Fig. 1, to demonstrate the performance of the column.

Sections of interest of the chromatograms of samples from a patient undergoing hemodialysis (Fig. 2), a patient with nephrotic syndrome (Fig. 3) and of a healthy volunteer (Fig. 4) are shown. The elution positions of D-AA are indicated by arrows.

Tables I–IV list the calculated relative amounts of D-AA, together with the respective serum creatinine concentrations (mg per 100 ml serum) of patients and of healthy volunteers. Because the acid-catalysed esterification of Gln and Asn, if they are present in the plasma, causes alcoholysis of the carboxamide group, no differentiation between these amides and Glu and Asp is possible under the derivatization conditions used. These

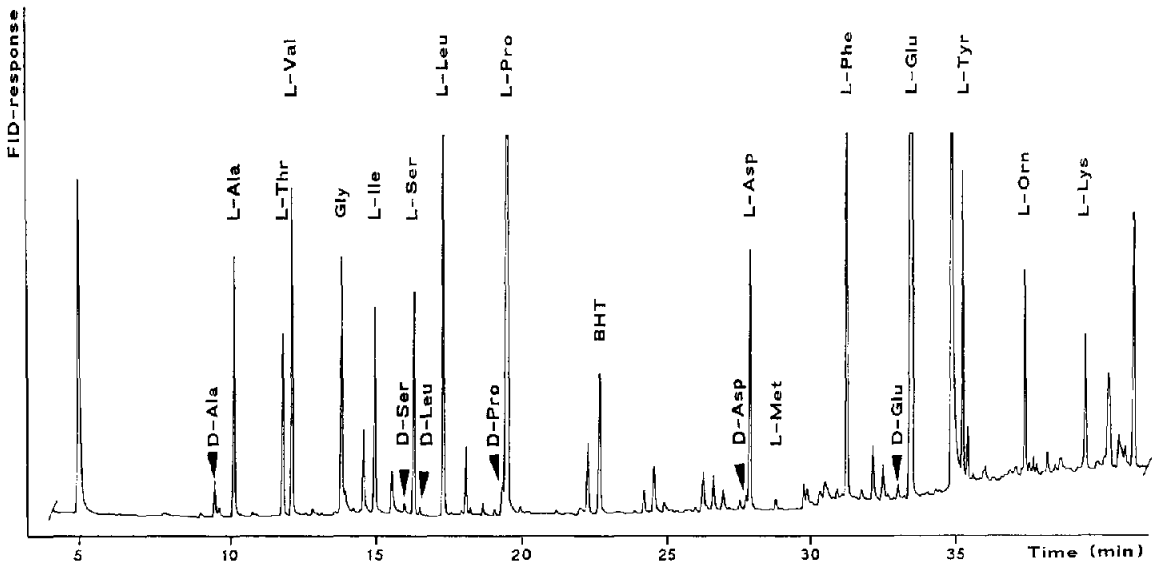

Fig. 1. GC analysis of free amino acids of the serum of a patient (No. 1 in Table I) undergoing CAPD. The derivatives were the PFP-1-propyl esters. Column A was used, with the following temperature programme: 5 min at 80°C; 3.5°C/min to 128°C; 3 min at 128°C; 3.5°C/min to 155°C; 4.5°C/min to 195°C; 10 min at 195°C.

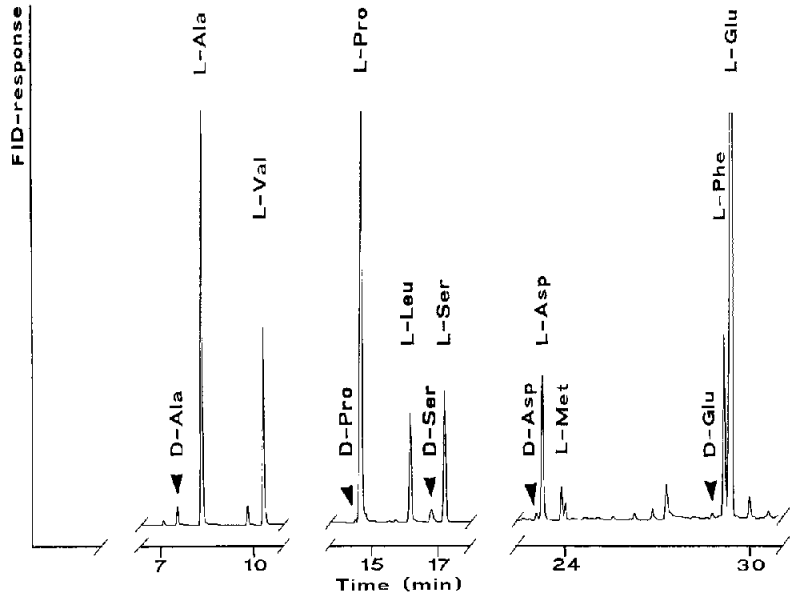

Fig. 2. Sections of a gas chromatogram of the serum amino acids of a patient (No. 2 in Table II) undergoing hemodialysis. The derivatives were the PFP-2-propyl esters. Column B was used, with the following temperature programme: 5 min at 80°C; 4°C/min to 112°C; 3 min at 112°C; 3.5°C/min to 162°C; 5°C/min to 195°C; 5 min at 195°C.

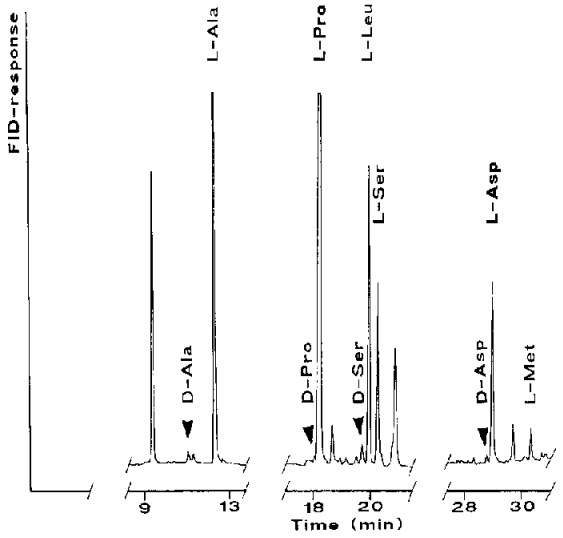

Fig. 3. Sections of a gas chromatogram of the serum amino acids of a patient (No. 1 in Table III) with nephrotic syndrome. The derivatives were the TFA-1-propyl esters. Column B was used, with the following temperature programme: 5 min at 80°C; 3.5°C/min to 124°C; 2 min at 124°C; 3.5°C/min to 161°C; 6°C/min to 195°C; 15 min at 195°C.

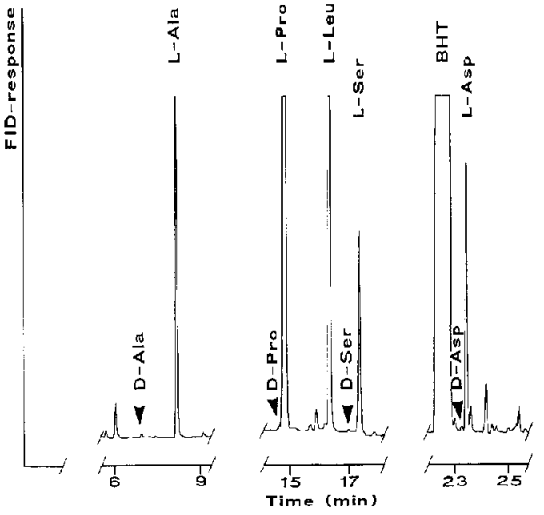

Fig. 4. Sections of a gas chromatogram of the serum amino acids of a healthy volunteer (No. 2 in Table IV). The derivatives were the PFP-2-propyl esters. Column B was used, with the following temperature programme: 5 min at 80°C; 4°C/min to 112°C; 3 min at 112°C; 3.5°C/min to 162°C; 5°C/min to 195°C; 15 min at 195°C.

TABLE I

RELATIVE AMOUNTS OF D-AMINO ACIDS IN THE BLOOD SERUM OF PATIENTS UNDERGOING CONTINUOUS AMBULATORY PERITONEAL DIALYSIS

Patient No.	Age (years)	Sex	Serum creatinine (mg/100 ml)	D-Amino acid	%D[a] (mean ± S.D.)	n
1	78	M	9.1	D-Ala	13.01 ± 0.137	6
				D-Asx	5.85 ± 0.147	7
				D-Glx	0.70 ± 0.066	4
				D-Leu	1.66 ± 0.022	5
				D-Pro	4.99 ± 0.284	5
				D-Ser	3.70 ± 0.209	6
2	72	M	12.0	D-Ala	1.70 ± 0.124	5
				D-Asx	4.60 ± 0.112	5
				D-Glx	1.34 ± 0.082	6
				D-Pro	1.28 ± 0.115	5
				D-Ser	7.78 ± 0.224	7
3	70	M	6.3	D-Ala	1.94 ± 0.089	8
				D-Asx	2.81 ± 0.172	5
				D-Glx	1.22 ± 0.035	6
				D-Pro	1.75 ± 0.075	6
				D-Ser	4.25 ± 0.136	5
4	65	M	13.1	D-Ala	4.78 ± 0.133	8
				D-Asx	3.64 ± 0.186	6
				D-Pro	6.53 ± 0.269	6
				D-Ser	7.83 ± 0.143	6
5	64	M	11.6	D-Ala	2.73 ± 0.064	6
				D-Asx	6.38 ± 0.294	7
				D-Leu	1.37 ± 0.106	6
				D-Ser	8.23 + 0.182	5
6	58	F	5.9	D-Ala	1.67 ± 0.061	6
				D-Asx	6.12 ± 0.264	5
				D-Pro	1.00 ± 0.066	5
				D-Ser	6.53 ± 0.172	6
7	58	M	8.0	D-Ala	2.50 ± 0.168	5
				D-Asx	5.13 ± 0.256	6
				D-Glx	0.52 ± 0.032	6
				D-Leu	1.20 ± 0.152	4
				D-Ser	8.14 ± 0.306	6
8	57	F	7.6	D-Ala	1.63 ± 0.101	7
				D-Asx	5.12 ± 0.245	6
				D-Pro	1.33 ± 0.059	5
				D-Ser	7.47 ± 0.140	6
9	55	M	10.2	D-Ala	1.31 ± 0.099	5
				D-Asx	6.28 ± 0.292	7
				D-Pro	1.94 ± 1.83	6
				D-Ser	5.17 ± 0.123	6
10	53	M	5.8	D-Ala	1.74 ± 0.098	7
				D-Asx	3.68 ± 0.115	6
				D-Glx	0.46 ± 0.012	4
				D-Ser	4.57 ± 0.195	4
11	33	M	10.0	D-Ala	0.53 ± 0.025	5
				D-Asx	1.45 ± 0.123	7
				D-Ser	0.90 ± 0.045	5

[a] %D = 100 · D/(D + L).

TABLE II

RELATIVE AMOUNTS OF D-AMINO ACIDS IN THE SERUM OF PATIENTS UNDERGOING HEMODIALYSIS

Patient No.	Age (years)	Sex	Serum creatinine (mg/100 ml)	Amino acid	%D[a] (mean ± S.D.)	n
1	73	M	11.3	D-Ala	2.35 ± 0.183	4
				D-Asx	6.13 ± 0.248	8
				D-Pro	1.66 ± 0.065	5
				D-Ser	9.01 ± 0.157	5
2	72	M	9.7	D-Ala	4.38 ± 0.172	8
				D-Asx	6.10 ± 0.249	7
				D-Glx	0.72 + 0.100	5
				D-Pro	0.85 ± 0.030	6
				D-Ser	10.95 ± 0.386	7
3	66	F	11.5	D-Ala	1.74 ± 0.083	5
				D-Asx	6.45 ± 0.130	7
				D-Pro	0.73 ± 0.049	4
				D-Ser	8.74 ± 0.359	7
4	55	F	4.4	D-Ala	4.10 + 0.147	6
				D-Asx	6.69 ± 0.243	4
				D-Pro	2.52 ± 0.111	4
				D-Ser	14.50 ± 0.715	5
5	51	F	10.6	D-Ala	1.96 ± 0.180	8
				D-Asx	7.66 ± 0.279	5
				D-Glx	0.86 ± 0.062	5
				D-Pro	0.56 ± 0.084	6
				D-Ser	3.84 ± 0.203	6

[a] %D = 100 · D/(D+L).

TABLE III

RELATIVE AMOUNTS OF D-AMINO ACIDS IN THE SERUM OF PATIENTS WITH NEPHROTIC SYNDROM

Patient	Age (years)	Sex	Serum creatinine (mg/100 ml)	D-Amino acid	%D[a] (mean ± S.D.)	n
1	50	M	6.0	D-Ala	1.31 ± 0.101	6
				D-Asx	5.79 ± 0.160	5
				D-Pro	0.72 ± 0.045	6
				D-Ser	8.44 ± 0.109	5
2	28	M	1.1	D-Ala	0.96 ± 0.022	6
				D-Asx	1.47 ± 0.095	6
				D-Glx	0.34 ± 0.031	5
				D-Pro	0.57 ± 0.037	6
				D-Ser	2.89 ± 0.100	6

[a] %D = 100 · D/(D+L).

TABLE IV

RELATIVE AMOUNTS OF D-AMINO ACIDS IN THE SERUM OF HEALTHY VOLUNTEERS

Volunteer No.	Age (years)	Sex	Serum creatinine (mg/100 ml)	D-Amino acid	%D[a] (mean ± S.D.)	n
1	59	M	0.8	D-Ala	0.45 ± 0.049	5
				D-Asx	1.59 ± 0.102	6
2	51	M	0.9	D-Ala	1.48 ± 0.170	5
				D-Asx	1.71 ± 0.061	6
				D-Pro	0.57 ± 0.010	5
				D-Ser	3.14 ± 0.156	6
3	51	M	0.9	D-Ala	0.52 ± 0.099	4
				D-Asx	2.08 ± 0.119	5
				D-Glx	0.32 ± 0.028	4
				D-Pro	0.91 ± 0.069	5
				D-Ser	3.67 + 0.132	6
4	42	M	0.8	D-Ala	1.79 ± 0.147	6
				D-Asx	1.63 ± 0.111	7
5	36	F	0.9	D-Ala	1.36 ± 0.113	6
				D-Asx	2.19 ± 0.120	5
				D-Pro	0.87 ± 0.039	5
				D-Ser	2.86 ± 0.070	7

[a] %D = 100 · D/(D+L).

amino acids are therefore described as Glx and Asx in the tables. In the chromatograms they are designated as Glu and Asp as they are determined. The basic amino acids, Arg and His, could not be determined under the derivatization conditions and analyses using fused-silica columns. The reducing agent BHT was added to the samples prior to derivatization. D-AA in serum samples have been characterized as follows.

Patients undergoing continuous ambulatory peritoneal dialysis (CAPD) (Table I, Fig. 1)

D-Asx (Asx = Asp + Asn), D-Ala and D-Ser were found in the sera of all eleven patients. D-Pro was detectable in seven cases, small amounts of D-Glx were found in five cases and D-Leu was found in three samples. Patient 1 showed an exceptionally high amount of D-Ala (13%). Noteworthy are the relatively high amounts of 3.7–8.2% D-Ser, with the exception of patient 11 who only exhibited 0.9% D-Ser. Patients 1 and 4 showed high amounts of D-Pro, 5 and 6.5%, respectively. Serum creatinine levels ranged from 5.9 to 13.1 mg per 100 ml serum, indicating renal insufficiency. The gas chromatogram of the serum of patient 1 is shown in Fig. 1.

Patients undergoing hemodialysis (Table II, Fig. 2)

D-Asx, D-Ala, D-Ser and D-Pro were present in the sera of all five patients and low amounts of D-Glx were found in the sera of two patients. The high amount of D-Ser (14.5%) in the serum of patient 4 is noteworthy. Serum creatinine levels ranged from 4.4 to 11.5 mg per 100 ml serum, again indicating renal insufficiency. Sections of a chromatogram of the amino acids from patient 2 are displayed in Fig. 2. The hemodialysates of two patients were also analysed. Patient 1 contained on average 6.9% D-Asx, 2.1% D-Ala, 12.1% D-Ser and 1.0% D-Pro, and patient 2 contained 2.5% D-Ala and an exceptionally high amount of D-Ser (19.8%) [14] (for a chromatogram see ref. 14).

Patients with nephrotic syndrome (Table III, Fig. 3)

D-Asx, D-Ala, D-Ser and D-Pro were detected in both samples. There were surprisingly high amounts of D-Ser (8.4%) in the serum of patient 1. Serum creatinine levels were 6.0 and 1.1 mg per 100 ml plasma. The chromatogram of the amino acids from patient 1 is shown in Fig. 3.

Healthy volunteers (Table IV, Fig. 4)

Significant amounts of D-AA were detected. D-Asx and D-Ala were present in the sera of all five volunteers; D-Ser and D-Pro were found in three samples, and a small amount of D-Glx was found in one sample. Sections of a chromatogram of the amino acids from volunteer 2 are shown in Fig. 4. Serum creatinine levels were within the normal range and amounted to 0.8–0.9 mg/100 ml serum.

DISCUSSION

Using an enzymic assay, Nagata *et al.* [7] have shown that significant amounts of free D-AA occur in human blood plasma, and Dunlop *et al.* [8] reported the liquid chromatographic detection of free D-Asp in the blood of human foetuses and adults.

Nagata *et al.* [17], again using the enzymic method, found a positive correlation between the plasma D-AA content and the age of the donors. Further, positive correlations between the amount of D-AA in plasma of patients with glomerulonephritis and the serum levels of creatinine, β_2-microglobulin, and to the glomerular filtration rate were reported [17]. However, this enzymic assay, based on the oxidation of D-AA by D-amino acid oxidase (DAO) isolated from porcine kidney, does not distinguish between individual D-AA and shows a very low affinity for D-Asp and D-Glu [18]. Our results show that capillary GC employing a chiral stationary phase (Chirasil-L-Val) is a highly suitable method for the resolution and determination of the relative amounts of individual amino acid enantiomers in human blood serum. After the samples had been deproteinized the amino acids were isolated by cation-exchange treatment before conversion into volatile derivatives for GC analysis.

Application of the method to the sera of eighteen patients suffering from renal insuffiency revealed that D-Asx and D-Ala were present in all cases and that D-Ser, D-Pro and D-Glx were detectable in certain sera. The, in part, relatively high amounts of certain D-AA (see tables) in the sera of patients with renal diseases is noteworthy. Healthy volunteers also exhibited D-Asx and D-Ala in all cases and D-Pro and D-Ser in three instances. In order to evaluate a possible correlation between the relative amounts of D-AA in the sera and the creatinine levels, which serve as an indicator of renal malfunction, the relative amounts of D-Ala, D-Pro, D-Asx and D-Ser were subjected to linear regression analysis. The D-AA contents of all serum donors were used, with the exception of those mentioned in Figs. 5 and 6. Linear regression analysis leads to positive correlation factors for D-Asx ($r = 0.748$, Fig. 5) and D-Ser ($r = 0.667$, Fig. 6) with statistical significance at the 95% confidence level (see legends to Figs. 5 and 6). No positive correlation could be found for D-Pro ($r = 0.185$) and D-Ala ($r = 0.451$). Further, using IEC, the absolute amounts

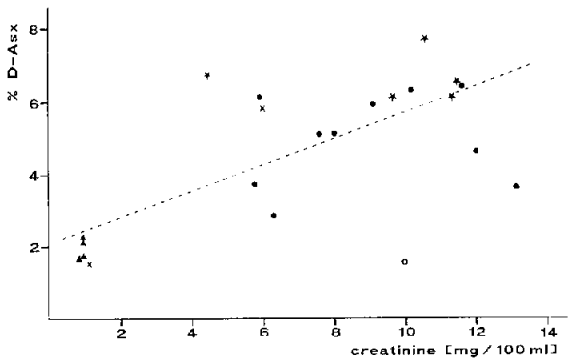

Fig. 5. D-Aspartic acid (Asx = Asp + Asn) *versus* creatinine content of human blood sera; the dotted line represents the fitted regression line: $y = 2.13 + 0.36x$, $r = 0.748$; $n = 22$. Symbols are means of multiple measurements (see tables) of patients undergoing CAPD (●), undergoing hemodialysis (★), with nephrotic syndrome (×), and of healthy volunteers (▲); (○) not within the 95% confidence limit and was not used for calculation.

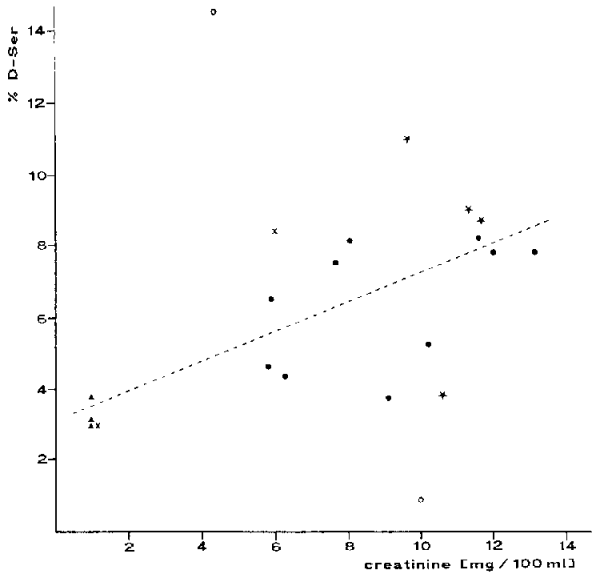

Fig. 6. D-Ser *versus* creatinine content of human blood sera; the dotted line represents the fitted regression line: $y = 3.11 + 0.41x$, $r = 0.667$; $n = 19$. Symbols are means of multiple measurements (see tables) of patients undergoing CAPD (●), undergoing hemodialysis (★), with nephrotic syndrome (×), and of healthy volunteers (▲); (○) not within the 95% confidence limit and was not used for calculation.

of all amino acids in the sera of three patients undergoing CAPD were determined, and the absolute amounts of D-AA were calculated from the relative amounts as determined by GC (*cf.* Table I). For patient 2, 52.2 mg of free AA per 100 ml of serum were found, and a value of 0.46 mg of free D-AA per 100 ml of serum (40.3 nmol/ml serum) was calculated. For patient 7, 46.2 mg of free AA per 100 ml of serum were found, and a value of 0.54 mg of free D-AA per 100 ml of serum (50.7 nmol/ml) was calculated. For patient 11, 52.8 mg of free AA per 100 ml of serum were found, and a value of 0.07 mg of free D-AA per 100 ml (6.6 nmol/ml serum) was calculated. These values are similar to those reported by Nagata *et al.* [17] for patients with glomerulonephritis (3.6–52.6 nmol D-AA per ml plasma).

The results show that the application of the GC method described allows the characterization of D-AA in the blood serum of healthy donors and patients suffering from renal diseases. It appears that a positive correlation exists between the amounts of D-Asx and D-Ser and the serum creatinine level in patients with renal malfunction. One might speculate that a reduced renal function and excretion, and the probably not fully active D-AA oxidase systems, result in an increase of the relative amounts of D-AA which are, however, also present in healthy individuals. It should be stressed that only a limited number of patients were available and therefore no correlation of serum creatinine levels with respect to the age or sex of the donors could be done, in contrast to the work of Nagata *et al.* [17]. Further, with respect to the activity and presence of DAO, not only renal malfunction has to be taken into account, because DAO and D-aspartate oxidase also occur in human liver and other tissues [18,19] and have been proposed by Hamilton and Buckthal [20] to be present in virtually all cells. These authors have also reported that certain metabolites and drugs inhibit mammalian DAO [20]. In our investigations, with one exception, the patients undergoing CAPD were also being treated with various drugs, such as diuretics, analgetics, cardiacs, antihistamines and insulin. These data were not available for the patients on hemodialysis or suffering from nephrotic syndrome. Further, the composition of the food ingested by the donors was not controlled, although this certainly has an influence on the intestinal flora (see below).

Possible origin of D-amino acids in human blood serum

The release of free D-AA, in particular D-Asp, from long lived proteins, such as tooth enamel, human lenses, human brain and human myelin basic protein (see ref. 21 and the literature cited therein), is not considered to be a likely source for the D-AA found in serum. Intramolecular conversion of L-Asp into D-Asp in proteins is attributed to a temperature- and age-dependent, chemically induced isomerization process, and the D-Asp formed has to be released from the protein by total acidic hydrolysis. The exogenous uptake of free D-AA with foodstuffs, which

might result in the intake of several hundred milligrams when certain cheeses are consumed, has already been reported, and nutritional aspects have been discussed; we therefore refer to a recent publication [15] and the references cited therein.

Besides food, the gastrointestinal microbial flora in humans, the most common genera being *Streptococci*, *Lactobacilli* and *Enterobacteriaceae* [22–24], are suspected as possible sources for D-AA found in blood. The D-enantiomers of Ala, Asp and Glu are typical constituents of the peptidoglycan-forming bacterial cell walls [25], and these as well as other D-AA have been found in the cytoplasma of bacteria [1]. Bacterial amino acid racemases that catalyse the conversion of almost all physiological L-AA into their D-enantiomers have been described, including those that catalyse the inversion of L-Ser and L-Pro into their D-enantiomers, as well as D-proline reductases that catalyse the conversion of 5-aminovalerate into D-Pro [26]. From these data it appears that D-AA originate as metabolites or through autolysis and digestion of intestinal bacteria. They are, therefore, after permeation through the mucosa [27], a possible source of the D-AA found in serum, or at least a contribution. This view is indirectly supported by the appearance of ^{15}N-labelled intestinal microbial amino acids in the venous blood of the pig colon [28]. An extension of this concept would be that bacterial infection of tissues might also be a source for D-AA under certain circumstances, as indicated by a report of Ueda *et al.* [29]. However, in contrast to this view, Nagata and Akino [30] also found free D-AA in the kidney, liver, serum, and urine of germ-free mice and no significant differences were observed in comparison with normally raised controls. Therefore these authors exclude a microbial origin of free D-AA in mice, and question an earlier report by Hoeprich [6] who found no D-Ala in the sera of germ-free guinea-pigs and mice. Furthermore, free D-Asp has been found in various tissues of 36-h-old rats and human foetuses [8].

Taking all the data into account, a partial endogenous formation of certain D-AA in humans cannot be excluded. These findings might explain the as yet not fully understood presence of DAO and D-aspartic acid oxidase in a multitude of human and animal cells (for a discussion of the role of DAO see refs. 18–20 and the references cited therein). Finally, the presence of free D-Ser and D-Pro in most human blood sera is of interest, because toxic effects of D-Ser [31,32], and D-Pro [33] have been reported in rats, and kidney homogenates of rats show a high oxidation rate for D-Pro [34].

ADDENDUM

After this paper was submitted for publication Nagata *et al.* [35] published a paper on the liquid chromatographic determination of individual D-AA in human plasma, in which they come to similar conclusions.

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