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Journal of Chromatography B, 864 (2008) 29–37
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of the oxido-redox status of plasma albumin in hemodialysis patients

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Received 2 October 2007; accepted 21 January 2008
Available online 31 January 2008

Abstract

The oxido-redox status of plasma albumin in patients treated with hemodialysis was characterized with LC–ESI-MS/MS and was compared with models of oxidative stress. Oxidised albumin was characterized by sulfonation (SO_3^-) of the SH at Cys 34, unfolding and acidification of the molecule. Albumin in hemodialysis patients presented, instead, only intermediate oxidation products such as sulfenic (SO_2), sulfonic (SO) and methionine sulfoxide ($C_5H_9NO_2S$) involving Cys 165–269 and Met 329–548 but did not present SO_3^- at Cys 34. Absence of charge and structural alterations compared to the oxidised templates was also confirmed with electrophoretic titration and calorimetry. In conclusion, the oxido-redox status of plasma albumin in hemodialysis patients lacks the hallmarks of the advanced oxidation products. LC–ESI-MS/MS was crucial to characterize albumin in conditions of oxidation stress; surrogate techniques can mirror conformational changes induced by oxidation.

Keywords: Plasma albumin; Oxidation stress; Oxidation markers; Hemodialysis

1. Introduction

Reactive oxygen species play tremendous impact in human livings. Small amounts of oxygen radicals are physiologically generated in mitochondria during oxidative phosphorylation and are integrated by specialized anti-oxidant defences. They are also generated by polymorphonuclear leukocytes (neutrophils and monocytes) during the oxidative burst [1] and are released in plasma where glutathione and anti-oxidant enzymes are less concentrated and probably inadequate to blunt a severe stress [1]. At this site, proteins are target of oxygen radicals and may function as scavengers of the majority of reactive compounds [2–6]. Owing to their stability, oxidation products of plasma proteins can retain the fingerprint of the initial modification and be utilized as markers of the oxidative stress. Unfortunately, only few data are available in the literature on the chemistry of protein oxidation products that have been obtained utilizing adequate techniques and most of our knowledge derives from the characterization of proteins in artificial oxidation models.

The first and unique 'in vivo' study was done on albumin in patients with renal lesions for focal segmental glomerulosclerosis (FSGS) by Musante et al. [7,8] who proposed a critical role of oxidation in the pathogenesis of this disease. Other groups described the presence of elevated levels of advanced oxidation products, also known as AOPPs, in several diseases including conditions which present or develop renal lesions

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doi:10.1016/j.jchromb.2008.01.035

such as diabetes mellitus and IgA glomerulonephritis. When present, AOPPs [9–12] predict the progression of renal lesions, atherosclerotic cardiovascular events [9,13,14] and death. In spite of these important pathologic implications, we still lack a clear structural characterization of AOPPs that is an essential step to understand the pathogenesis of diseases and for devising specific analytical methodology. Utilizing indirect techniques, Capeillere-Blandin et al. [15] identified albumin as the main AOPPs product in plasma and confirmed previous data indicating albumin as the major target of oxidant stress in uremia [11,12,16]. However, structural data showing specific oxidation products of albumin in uremia are not available.

The indication of albumin as the most important plasma component undergoing oxidation seems the logical consequence of the fact that it retains a myeloperoxidase activity and is, for this reason, vulnerable to oxidation by hypochlorous acid (HOCl). So far, the only paper attempting a spectroscopic definition of oxidised albumin have been recently published by Musante et al. [8] showing specific sulfonation of Cys 34 of albumin in patients with FSGS. We extend now the structural analysis of albumin to uremic patients. Data here presented put the basis for developing specific analytical techniques for the characterization of oxidised albumin in screening studies.

2. Experimental

2.1. Patients

Twenty-four patients who were following a stable dialytic program had been enrolled in the study (Table 1). They were subdivided into three groups according to the age and the duration of the dialytic treatment: (a) 10 patients were over 40 years and had followed a dialytic program for more than 10 years; (b) 10 patients were over 40 years and had less than 1 year of dialysis; (c) 4 patients were less than 30 years and had less than 1 year of dialysis. There was a prevalence of males (18 vs. 6) and almost all were following the same dialytic scheme with bicarbonate dialysis, utilizing different filters. The dialysis parameter *Kt*/*V* (amount of plasma cleared of urea divided by the volume of urea distribution) and nPCR (normalized protein catabolic rate) were comparable. In most cases, the origin of chronic renal failure was not clearly defined since renal histology was unclear (terminal kidney) or, in some cases, not available at all. Two patients had diabetic nephropathy, two had dominant polycystic kidneys, two had chronic pyelonephritis or reflux nephropathy and two were reported as chronic glomerulonephritis.

Five people of the laboratory staff (four males, one female) were taken as normal controls and four patients with FSGS (three males, one female) were utilized as positive samples for albumin oxidation [7,8].

2.2. Purification of albumin

Albumin was purified from plasma of healthy donors and uremic patients by preparative continuous mono-dimensional PAGE electrophoresis (*T* 4–12%) in native conditions as described by Musante et al. [8]. Briefly, 1 ml of serum was applied to gel and electrophoresis was run in Tris–Borate–EDTA (80/90/2.5 mM) for 12 h with 16 mA at 12 °C. Albumin was desorbed from acrylamide by gentle pest and was maintained in PBS at 4 °C for 24 h with two changes of the solution. The recovery was in all cases >95%.

2.3. 'In vitro' oxidation

Healthy plasma albumin (500 μg) was oxidised 'in vitro' by exposure to 10 mM H_2O_2 in 50 mM Tris–HCl buffer pH 8.8 at 20 °C for 30 min.

2.4. LC–ESI-MS/MS for tryptic digest characterization

After purification, alb was first delipidated in a methanol:acetone:tributyl-phosphate (1:12:1) with gentle agitation at room temperature overnight and was then digested by trypsin. Trypsin was added at an enzyme substrate ratio of 1:30 (w/w) in a solution of 100 mM ammonium bicarbonate and 1 mM $CaCl_2$, pH 8.5. After overnight incubation at 37 °C, the reaction was stopped with formic acid at pH 2. All mass spectrometric measurements were performed using a LTQ linear ion trap mass spectrometer (Thermo Electron, San Jose, USA) coupled to a HPLC Surveyor (Thermo Electron) and equipped with a Jupiter C18 column 250 mm × 1 mm (Phenomenex). Peptides were eluted from the column using an acetonitrile gradient, 5% B for 6 min followed by 5 to 90% B within 109 min (eluent A: 0.1% formic acid in water; eluent B: 0.1% formic acid in acetonitrile) at flow-rate of 50 μl/min. The column effluent was directed into the electrospray source. The spray voltage was 5.0 kV. The ion trap capillary was kept at 200 °C and the voltage at 2.85 V. Spectra were obtained in automated MS/MS mode: each full MS scan (*m*/*z* 400–1800) was followed by five MS/MS of the most abundant ions. The ions analyzed this way, were automatically excluded for 30 s. Computer analysis of peptide MS/MS spectra was performed using Bioworks software, version 3.2, from Thermo Electron (San Jose, USA) and searched against a ALB protein database. Peptide MS/MS assignments were filtered according to the following criteria: $X_{corr} \geq 1.9$ for the singly charged ions, $X_{corr} \geq 2.2$ for doubly charged ions, and $X_{corr} \geq 3.7$ for triply charged ions, peptide probability ≤0.001, Delta Cn ≥0.1 and percent ions ≥30%, for all protein searches two missed cleavages was allowed.

2.5. Electrophoretic titration curves

For electrophoretic titration curves we utilized total plasma in ACD (130 mM citric acid–152 mM sodium citrate–112 mM glucose). Titration was performed according to Bruschi et al. [17]. Both methodology for electrophoresis runs and calculations have been described in previous papers [18]. Briefly, square gel slabs (145 mm × 145 mm × 1.5 mm) were cast to contain acrylamide (%*T* 5.00, %*C* 2.67) and 2.5% w/v carrier ampholytes, pH 3.5 ± 10. The first-dimensional separation was run at 100 V for 5 min, 200 V for 30 min, 300 V for 30 min

Table 1
Clinical features of 24 patients who had been treated with hemodialysis for variable periods

	Sex	Age (years)	Renal disease	Months of dialysis	Type of dialysis	Filter/surface	*Kt/V*	PCRn
Pts >40 years—more than 10 years of dialysis								
1	M	79	Diab. nephropathy	156	Bicarb. dial.	Cell mod/1.9	1.81	0.89
2	F	46	Chronic pyelonephritis	396	Bicarb. dial.	PMMA/1.6	1.76	1.12
3	M	69	ADPKD	170	AFB	AN 69/1.9	1.44	0.92
4	M	81	Terminal kidney	194	Bicarb. dial.	Polycarbonate	1.53	0.97
5	M	87	Terminal kidney	154	Bicarb. dial.	Sint	1.36	0.65
6	M	74	ADPKD	202	Bicarb. dial.	Polycarbonate	1.37	0.84
7	M	76	Terminal kidney	142	Bicarb. dial.	Polycarbonate	1.48	0.84
8	F	79	n.d.	208	Bicarb. dial.	Cell mod	1.71	1
9	M	69	Terminal kidney	235	Bicarb. dial.	Sint	1.36	1.03
10	M	41	Terminal kidney	329	Bicarb. dial.	Sint	1.73	1.16
Pts >40 years—less than 1 year of dialysis								
1	M	70	n.d.	8	Bicarb. dial.	Polycarbonate/2.1	1.21	1.11
2	M	63	Diab. nephropathy	8	Bicarb. dial.	Polycarbonate/2.1	1.13	1.74
3	M	71	Terminal kidney	7	AFB	Polycarbonate/2.1	1.32	1
4	F	63	n.d.	7	Bicarb. dial.	Polycarbonate/2.1	1.15	0.88
5	F	86	n.d.	8	Bicarb. dial.	Sint/1.9	1.48	0.7
6	M	68	Chronic glomerul.	7	Bicarb. dial.	Sint/1.9	1.2	1
7	F	72	n.d.	7	Bicarb. dial.	Sint/1.9	1.27	0.82
8	M	79	n.d.	8	AFB	AN 69/1.9	1.44	0.96
9	M	55	Chronic glomerul.	6	Bicarb. dial.	Polycarbonate/2.1	1.33	0.88
10	F	82	n.d.	8	Bicarb. dial.	Polycarbonate/1.7	1.37	0.97
Pts <40 years								
1	M	23	Terminal kidney	6	Bicarb. dial.	Polysulfone	1.54	1.09
2	M	29	n.d	12	Bicarb. dial.	Polysulfone	1.49	1,25
3	M	26	Reflux nephropathy	4.5	Bicarb. dial.	Polysulfone	1.26	1.4
4	M	15	n.d.	19	Bicarb. dial.	Polysulfone	1.41	1.41

Both type and duration of the dialytic treatment is reported in the table. Abbreviations: n.d., not determined; ADPKD, autosomal dominant polycystic kidney; PMMA, Diab. Nephropathy, diabetic nephropathy; AFB, acetate free bio-filtration.

and then 400 V for 30 min, until the pH gradient was fully developed and the system reached a steady state. After that electrode gel layers were cut away, gels were turned 90° and the trench was filled with serum (200 μg of protein). After that new electrode strips had been overlaid onto the gel electrophoresis was performed perpendicular to the first dimension at 200, 300 and 400 V for 5, 10, and 30 min, respectively, at 13 °C.

2.6. 'In gel' determination of free SH

Free SH group titration in plasma albumin was done with the maleimide-PEO_2-biotin (biotinyl-3-maleimidopropionamidyl-3,6-dioxactanediamine) assay (Pierce, Rockford, IL) according to the manufacturer's instructions [8]. After the reaction with maleimide-PEO_2-biotin at pH 6.5 for 2 h at 37 °C, plasma proteins were first separated in mono-dimensional polyacrylamide gels ($T = 5$–16%; $C = 2.67\%$) performed according to Laemmli [19] without mercaptoethanol. Biotin was revealed with streptavidin conjugated with Horseradish peroxidase utilizing the EZ™ Biotin Quantitation Kit (Pierce) at 500 nm with correction for the amount of albumin as determined by Coomassie R-250. A calibration curve consisting in four dilutions of the same serum with known concentration of albumin was utilized as standard. Mean error of repeated analysis at several dilution was <5%. Specificity of the maleimide dye for the free SH group of Cys 34 was demonstrated by preventing the binding with methylmethanethiosulfonate that specifically binds this group at pH 5.

2.7. Differential scanning calorimetry

Degassed samples with a concentration ranging from 0.5 mg/ml to 1.0 mg/ml were performed with a MicroCalorimeter VP-DSC and curves have been analyzed with Origin software (MicroCal, Northampton, MA, USA). The scan heating rate were 30, 60 and 90 °C/h.

Independent instrumental baselines were carried out by scanning 50 mM phosphate buffer at pH 7.4, at the correspondent heating rate and subtracted from experimental runs.

3. Results

Complete spectrometric analysis of six albumin purified from patients undergoing hemodialysis was done by LC–ESI-MS/MS. Albumin from four patients with FSGS and from six normal subjects was evaluated as positive and negative control of oxidation and 'in vitro' oxidised albumin was utilized as template (Table 2). All the following chemical groups (NO/NO_2, $Br/2Br^-$, $Cl^-/2Cl^-$, $SO/SO_2{}^-/SO_3{}^-$) potentially involved in any oxidative stress upon Tyr, Trp, Met and Cys were checked [5,6].

Table 2
LC–ESI-MS/MS of plasma albumin purified from patients of different ages who had been treated with hemodialysis for variable periods from less than 1 year to more than 10 years

Without Signal peptide a.a. no.	Healthy	pt 1 HD > 10	pt 2 HD > 10	pt 3 HD > 10	pt 4 HD > 10	pt 5 Diabetic HD > 10	pt 6 Diabetic HD < 1	pt 7 Diabetic HD < 1	pt 9 FSGS	pt 10 FSGS	pt 11 FSGS	pt 12 FSGS	In vitro oxidation
C34									SO_3	SO_3	SO_3	SO_3	SO_3
C75												SO_3	SO_3
C101												SO_3	SO_3
C124												SO_3	SO_3
C168						SO							
C169			SO_2	SO_2		SO							
C265							SO_2	SO_2			SO_2	SO_3	
C278												SO_3	SO_3
C279												SO_3	SO_3
C289												SO_3	SO_3
C360													SO_3
C361													SO_3
C392												SO_3	SO_3
C448												SO_3	SO_3
C461												SO_3	SO_3
C476												SO_3	
C477												SO_3	
C487												SO_3	
C514												SO_3	SO_3
C567												SO_3	SO_3
M123				SO		SO						SO	SO
M298												SO	SO
M329	SO	SO	SO	SO	SO	SO	SO	SO			SO	SO	SO
M446												SO	SO
M548	SO	SO	SO	SO	SO	SO	SO	SO	SO	SO	SO	SO	SO

The change relative to oxidation of SH groups of cysteines are reported. SO indicate the formation of a sulfenic derivate, SO_2 indicates the presence of a sulfinic derivate, SO_3– indicates the presence of a stable sulfonic derivate. Addition of 1 molecule of oxygen in S of methionine produces a methionine sulfoxide ($C_5H_9NO_2S$) residue.

3.1. LC–ESI-MS/MS of albumin in FSGS and 'in vitro' oxidation

Albumin oxidised 'in vitro' presented extensive sulfonation (SO_3^-) of the free sulfhydryl group of 13 Cys (C34, C75, C101, C124, C265, C278, C279, C289, C360, C361, C392, C448, C461, C514, C567); in parallel, the S residue of several methionone (M123, M298, M329, M446, M548) was transformed into methionine sulfoxide ($C_5H_9NO_2S$). Examples of chromatograms and MS/MS spectra relative to $^{34}SO_3^-$ and ^{548}SO that are common to several patients are reported in Fig. 1. The search for nitro, chlorine and bromine derivatives was negative in all cases. This finding suggest conformational derangement with rupture of disulfide bonds that was confirmed by differential calorimetry (see below). Albumin purified from one of the patients with FSGS presented comparable extensive oxidation of sulfhydryl groups (FSGS 4, Table 2). A major finding common to all FSGS samples and the 'in vitro' produced template was the presence of SO_3^- in place of SH at Cys 34, that is the unique free SH residue of albumin whereas, in conditions of normal enthalpy, the other 34 of the sequence are linked in 17 disulfide bridges. Once again thermodynamic parameters demonstrated unfolding of the molecule.

3.2. LC–ESI-MS/MS of albumin in hemodialysis patients

Spectrometric analysis of albumin purified from the seven patients on hemodialysis showed the presence of intermediate products of oxidation such as sulfenic (SO^-) and/or sulfinic (SO_2^-) derivates at Cys 169 and Cys 265 besides a $C_5H_9NO_2S$ group at Met 329 and Met 446. The same groups were also detected in normal albumin and probably represent a signal of physiologic aging of the protein. Notably, Cys 34 was always unchanged in all albumin samples from normal subjects and hemodialysis patients.

3.3. Albumin Cys 34 determination

Albumin Cys 34 detection was evaluated with the PEO-maleimide staining after electrophoresis, in 24 plasma of patients in hemodialysis and for comparison in normal subjects and in patients with FSGS [8]. The results are shown in Fig. 2 indicating that the Cys 34 is titrated in all hemodialysis patients and in normal subjects but not in FSGS patients and in the 'in vitro' oxidised sample. This finding shows that the SH group is substituted by other groups in the 'in vitro' template and in FSGS patients while is still free in hemodialysis patients.

3.4. Electrical charge

The presence of SO_3^- can also be detected by techniques that determine the charge of albumin since, when present, this group introduces 1 negative charges and makes the albumin more anionic in the pH range between 4.5 and 7 that is the pH range of ionization of SO_3^-. The electrical charge of albumin was evaluated in all patients with electrophoretic titration curves [17,20] showing no change (Fig. 3a and b) in respect to normal albumin. At variance, and as known, albumin from FSGS patients presented a more anionic charge in the range of pH between 4.5 and 7 that is the range of ionization of the sulfonic residue.

3.5. Differential scanning calorimetry (DSC)

The DSC curve of purified albumin was evaluated in hemodialysis patients with Cys 169 and Cys 265 SO (pt 3, 5, 6) and in three with FSGS (pt 9, 10, 11). The calorimetric traces after subtraction of the baseline was done according to Freire and Biltonen [21] showing that the experimental profiles of DSC curves of hemodialysis patients and healthy albumin are very similar (Fig. 4). On the contrary, FSGS profiles presented a thermal stabilization in respect to albumin purified from healthy people and hemodialysis patients. This observation clearly indicates that the albumin folding status in FSGS patients is significantly different to the other two groups.

4. Discussion

Plasma proteins are massively exposed to oxidants, mainly HOCl and chloramines that are generated by polymophonuclear leukocytes (neutrophils and monocytes) during the oxidative burst [1]. Oxidation of proteins determine profound structural alterations that involve conformational changes and degradation [5,6]. Several data on oxidation of albumin 'in vitro' and few observation in humans support this possibility. The recent report by Musante et al. [8] in children with FSGS is the first demonstration based on spectroscopic techniques of the existence of oxidised albumin 'in vivo'. A few other reports based on indirect techniques proposed the existence of oxidised albumin in patients undergoing hemodialysis under the acronymous AOPPs but lack of chemical structural completeness did not allow any conclusion. There is much emphasis, however, in considering a role of AOPPs in atherosclerotic cardiovascular events in patients with renal failure and it is, more in general, believed that oxidised proteins may contribute to the inflammatory process that is associated with uremia and hemodialysis. AOPPs are also proposed as predictors of survival in these patients [9–13,22]. A clear chemical and structural characterization of oxidised albumin (and more in general of AOPPs) in patients undergoing hemodialysis is, for the reasons above, critical to understand the overall mechanisms involved in cardiovascular pathology and to the development of adequate preventive strategies in patients with uremia. We have, therefore, characterized with LC–ESI-MS/MS plasma albumin in different cohorts of patients undergoing hemodialysis and compared salient chemical and structural features with albumin from children with FSGS. Albumin oxidised 'in vitro' was utilized as template for the molecular analysis following our own experimental flow-chart and considering the relevant literature on 'in vitro' oxidation models. The clinical categories included non-diabetic and diabetic patients of different ages and with different dialysis time from less than 1 to more than 10 years. The results were highly repetitive and on this basis the extension of the study to other patients was considered unnecessary.

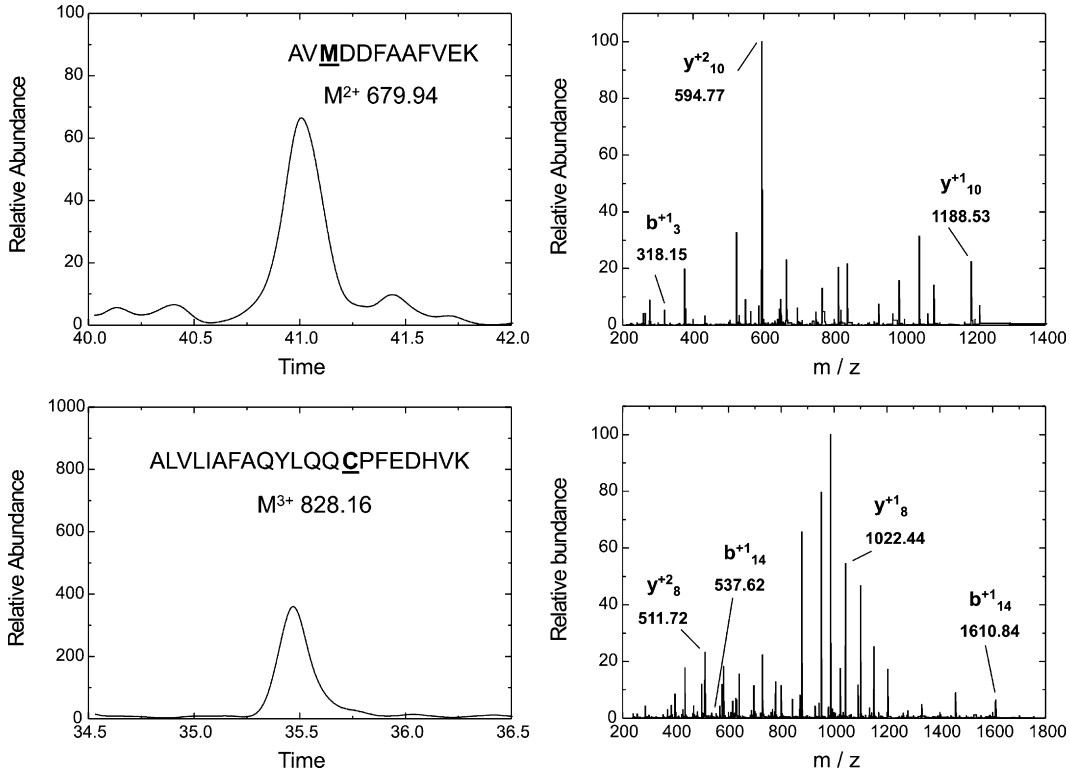

Fig. 1. Chromatograms and MS/MS spectra. Chromatograms and MS/MS spectra of $^{34}SO_3^-$ and ^{528}SO residues that are common to albumin purified from several patients. The first one has been found in all patients with FSGS; the second was found in all samples.

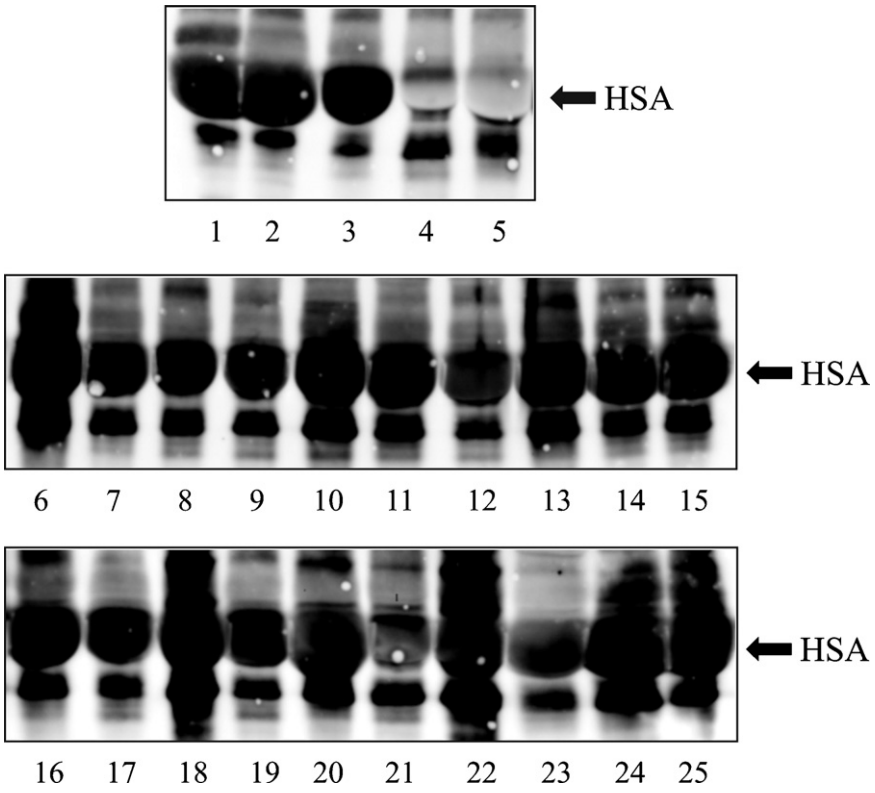

Fig. 2. Titration of the Cys 34 free SH groups of albumin. Titration of alb free SH of Cys 34 by 'in gel' labelling with PEO_2-maleimide-biotin. Albumin purified from healthy people (samples 1–3) and from hemodialysis patients (samples 6–25) showed PEO_2-maleimide staining corresponding to albumin (see arrows) whereas FSGS patients (samples 4–5) had not staining indicating absence of free SH groups. Specificity of the maleimide staining for the free SH was proved by selected inhibition of the binding with methyl-methanethiosulfonate (not shown).

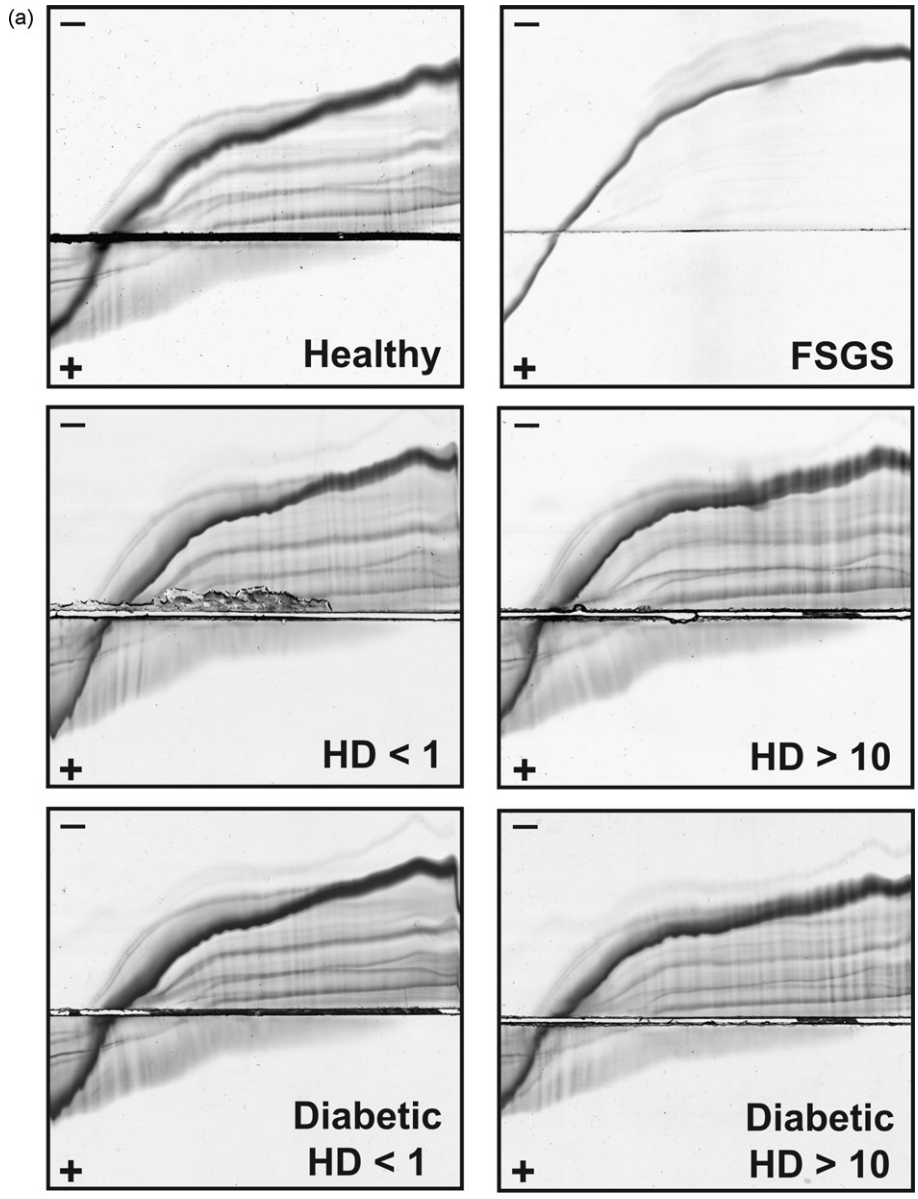

Fig. 3. Electrophoretic titration curves for N–A transition. (a) Example of electrophoretic titration curve of a plasma samples from healthy people, one patient with FSGS and patients undergoing hemodialysis for different periods. In the case of healthy and hemodialysis patients albumin migrates as single and homogeneous band throughout the pH range between 4 and 9. Albumin from a FSGS patient presented a more acidic charge in the pH range between 4.5 and 7 that is consistent with the presence of a sulfonic group. (b) Theoretical electrophoretic curve of alb in which a sulfonic group replaces a free sulfhydril residue. According to the Lindestrom-Lang theory, this acid charge shift fits with the introduction of a sulfonic acid group in the molecule. Determination of alb charge along a stable pH gradient was done according the procedure described by Bruschi et al. [17,20].

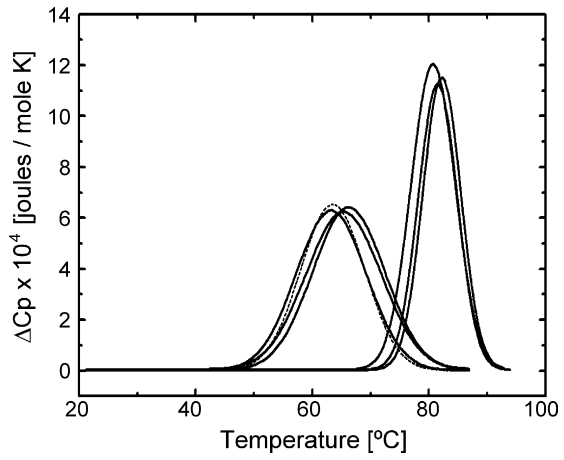

Fig. 4. Differential scanning calorimetry curves. Temperature dependence of partial heat capacity of (a) healthy albumin (T_m = 64.6 °C), (b) albumin from several patients in hemodialysis (pt 7 T_m = 64.1 °C, pt 3 T_m = 65.1 °C, pt 5 T_m = 66.6 °C) and (c) FSGS patients (pt 10 T_m = 81.2 °C, pt 11 T_m = 82.3 °C, pt 12 T_m = 83.6 °C). The spectra were corrected by subtraction of baseline and calculated with Origin 7.5 software.

The analysis of several samples of normal plasma albumin indicated the constant presence of a methionine sulfoxide ($C_5H_9NO_2S$) residues at Met 329 and Met 548 that are to be considered as physiologic signals of aging of the protein whose half-life is 19 days. Besides sulfonation of the two Met residues above, plasma albumin in patients treated with hemodialysis presented only two reversible sulfenic (SO_2^-) derivates of Cys 169 and Cys 265, one methionine sulfoxide residue at Met 123 in two cases and in one case Tyr 401 presented bromine (Br). It is important to stress that Cys 34, that is the unique free SH group of albumin out of other 34 linked in 14 disulfide bridges in conditions of normal enthalpy, is not modified in albumin of patients undergoing hemodialysis and that the structural characteristics of the molecule such as electrical charge and conformation appears unmodified. Therefore, there are striking differences with oxidised albumin (in vitro model and patients with FSGS) that presented sulfonation (SO_3^-) of the free Cys 34 in all cases. The analysis of thermodynamic parameters measured by differential scanning calorimetry confirmed the structural data above and showed important modification of the swelling and the unfolding of albumin in patients with FSGS that are instead irrelevant in patients undergoing hemodialysis. In the former case, structural alterations are so profound that cannot readily be justified by the presence of only few hydrogen bonds as the simulation of molecular dynamic as suggested by Kawakami et al. [4]. These data strengthen therefore the relevance of oxidation of the unique free SH groups of Cys 34 for conformation of albumin, in analogy with what already reported after sulfonation of free SH in other proteins.

Our conclusion is in partial agreement with data deriving from studies on albumin oxidised 'in vitro' reported by other authors who established the rate of reactions of HOCl and chloramines with different reactive groups showing that thiol groups are only partially involved in the reactivity of albumin with HOCl (probably for the initial formation of a sulfenyl intermediate that is converted into SH by ascorbate) [2,3,5]. The unique free thiol group of albumin (Cys 34) is instead extremely reactive with chloramines that achieve its transformation into the un-reactive end-product sulfonic acid [2].

The present study provides, therefore, several basic clues for a correct interpretation of the oxidative stress in uremia. Mass spectrometry is the technique of choice for a clear characterization of oxidation products. The direct determination of free SH at Cys 34 obtained by its alkylation with PEO-maleimide and the evaluation of electrical charge of the protein are simple technical surrogates that can be utilized in screening studies.

In conclusion, the present study defines, for the first time, the oxido-redox status of plasma albumin in patients undergoing hemodialysis showing modifications restricted to few intramolecular cysteines but lacks modification of the free Cys 34. Conformation and charge of the protein is accordingly maintained. The analysis of albumin oxidation in large cohorts of patients should consider the determination of free SH at Cys 34 by labelling the protein by PEO-maleimide and/or electrophoretic titration curves.

Acknowledgements

This work was done with the financial support of the Italian Ministry of Health and of a grant from the Renal Child Foundation. Authors also acknowledge Fondazione Mara Wilma e Bianca Querci for the financial support to the project "Nuove evoluzioni sulla multifattorialità della sindrome nefrosica". The manuscript was revised by Miss Capurro.

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