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Quantitation of N^{ϵ}-(dichloroacetyl)-L-lysine in proteins after perchloroethene exposure by gas chromatography–mass spectrometry using chemical ionization and negative ion detection following immunoaffinity chromatography

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Abstract

An antibody specific to N^{ϵ}-(dichloroacetyl)-L-lysine (DCA-Lys) was immobilized to immunoaffinity columns for the use in selective enrichment of dichloroacetylated proteins. These result from the reaction with dichlorothioketene the β-lyase cleavage product of the perchloroethene metabolite *S*-(trichlorovinyl)-L-cysteine. Dichloroacetylated proteins from rat kidney mitochondria, rat plasma and human blood plasma were isolated after exposure to 40 ppm tetrachloroethene (PER) for 6 h. After acid hydrolysis of the protein fraction, DCA-Lys was derivatized with 1,3-dichloro-1,1,3,3-tetrafluoroacetone using N^{ϵ}-(trifluoroacetyl)-L-lysine as internal standard. Recovery of dichloroacetylated reference proteins from immunoaffinity columns was about 73%. Samples were analyzed by GC–MS with chemical ionization and negative ion (NCI) detection showing DCA-Lys in proteins with 2.26 (±0.02) pmol/mg protein in male rat kidney mitochondria and 1.92 (±0.05) pmol/mg total mitochondrial protein in female rats. In rat plasma 0.47 (±0.006) pmol DCA-Lys/mg protein in male and 0.34 (±0.02) in female animals were found. DCA-Lys could not be detected in blood plasma of human volunteers exposed to PER with a detection limit of 20 fmol for the DCA-Lys derivative 2,2-bis(chlorodifluoromethyl)-4-(1-dichloroacetamido)-butyl-1,3-oxazolidine-5-one. Immunoaffinity chromatography with specific antibodies provides a powerful tool for the enrichment of minor quantities of dichloroacetyled proteins in biological samples for GC–NCI-MS analysis of the modified amino acid lysine having broad utility in the biomonitoring of PER exposure. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Derivatization, GC; Immunoaffinity chromatography; Amino acids; Dichloroacetyl lysine; Proteins; Tetrachloroethene

1. Introduction

Perchloroethene (tetrachloroethene, PER) (Fig. 1, **1**) is extensively used in industry as a metal degreasing solvent and as dry cleaning agent. Due to its volatility and resistance to degradation, it is a widely distributed environmental air pollutant and groundwater contaminant [1].

Long-term exposure of rodents to PER has been shown to increase the incidence of liver tumors in male mice and to result in a small but significant increase in the incidence of renal tumors in male rats [2]. The chronic toxicity of PER is most likely mediated by bioactivation reactions. PER is known to be metabolized by both cytochrome P450 and glutathione-dependent biotransformation pathways

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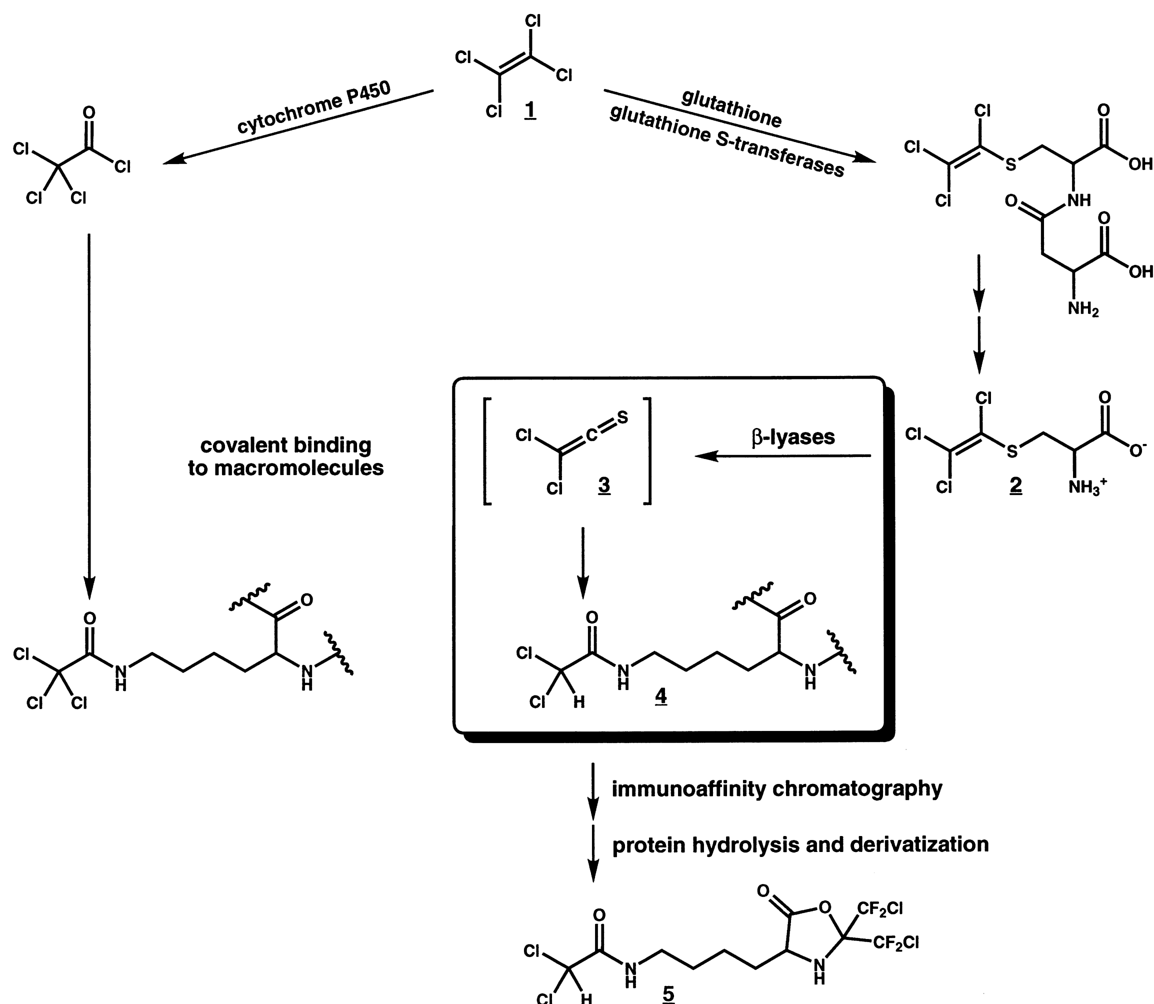

Fig. 1. Protein adduct formation in PER metabolism related to different bioactivation pathways.

leading to the generation of reactive metabolites which may covalently bind to cellular macromolecules (Fig. 1). Cytochrome P450 oxidation of PER results in formation of trichloroacetyl chloride which reacts with amino groups in macromolecules [3,4]. In addition, glutathione conjugation of PER followed by cysteine conjugate β-lyase-mediated activation of *S*-(trichlorovinyl)-L-cysteine (Fig. 1, 2) to the ultimate metabolite dichlorothioketene 3 likely responsible for the nephrotoxicity and possible renal tumorigenicity of PER [5–8]. Both N^{ϵ}-(dichloroacetyl)-L-lysine (Fig. 1, 4) and N^{ϵ}-(trichloroacetyl)-L-lysine have been identified as modified amino acids in proteins by GC–MS in the liver and kidneys of rats treated with PER [4].

Up to now, the main difficulty in the identification and detection of protein modifications linked to chemical or metabolite interactions in tissues are the large amounts of unmodified proteins and the enrichment of sufficient quantities for instrumental analysis. Separation of modified proteins from native proteins is the most challenging problem. In the recent years several immunochemical methods have become available to detect and quantify trace levels of protein modifications after chemical exposure. Antibodies to reactive metabolite-induced protein

alterations have been developed to selectively enrich the covalent adducts by immunoaffinity chromatography [9,10]. In combination with radioimmunoassays a more sensitive detection as compared to classical methods, i.e. liquid chromatographic separation using fluorescence detection or GC–MS, was achieved. The detection of minor quantities of protein adducts by such methods using monoclonal antibodies against even small protein modifications after reaction with nitrogen oxides were reported [11]. We recently developed monospecific antibodies to N^{ϵ}-(dichloroacetyl)-L-lysine and to N^{ϵ}-(trichloroacetyl)-L-lysine in proteins [12]. The anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody was used for immunoaffinity enrichment of N^{ϵ}-(dichloroacetyl)-L-lysine containing proteins for quantitation of N^{ϵ}-(dichloroacetyl)-L-lysine by GC–negative ion chemical ionization (NCI) MS after electrophore derivatization in the kidney and in blood of rats and in blood of humans exposed to PER. The obtained data comparatively quantify biologically effective doses of PER and provide further evidence for a much lower extent of β-lyase-dependent bioactivation of PER-metabolites in humans as compared to rats.

2. Experimental

2.1. Chemicals

PER (99% purity) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride were obtained from Aldrich (Deisenhofen, Germany). Keyhole limpet hemocyanin (KLH), rabbit serum albumin (RSA), human serum albumin, 1,3-dichloro-1,1,3,3-tetrafluoroacetone (DCTFA) and ethanolamine were from Sigma (Deisenhofen, Germany). HiTrap *N*-hydroxysuccinimide-activated agarose columns and Sephadex PD-10 columns were obtained from Pharmacia Biotech (Freiburg, Germany). Horseradish peroxidase-conjugated goat anti-rabbit IgG was from Amersham Life Science (Braunschweig, Germany). Enhanced chemiluminescence reagents were purchased from Pierce (Rockford, IL, USA). All other chemicals used were reagent, electrophoresis, or gradient grade as commercially available. N^{ϵ}-(dichloroacetyl)-L-lysine and N^{ϵ}-(trifluoroacetyl)-L-lysine were synthesized as described previously [4]. Chemically modified RSA was synthesized by coupling with dichloroacetic acid using the carbodiimide method [13]. Briefly, 2.5 ml of a methanolic solution of dichloroacetic acid (60 mmol) were added to 1-ethyl-3[3-(dimethylamino)-propyl]carbodiimide hydrochloride (288 mg, 1.5 mmol) in 7.5 ml of 20 m*M* sodium phosphate buffer (pH 5.0). After 2 min, the reaction mixtures were mixed with a solution of 40 mg of RSA in 20 ml of 160 m*M* sodium phosphate buffer (pH 8.0) and left overnight at room temperature. The obtained conjugates were dialysed three times against 3 l of bidistilled water. Anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody was raised in rabbits against *N*-(dichloroacetyl)-4-aminobenzoic acid coupled to KLH and isolated from crude antisera by immunoaffinity purification on N^{ϵ}-(dichloroacetyl)-L-lysine coupled to affinity columns. The antibody was highly specific to the desired epitope (dichloroacetyl moiety) and did not recognize unmodified RSA, trichloroacetylated proteins or native human albumin [12]. However, the antibody recognized the dichloroacetyl modification in rat and human serum albumin.

2.2. Gel filtration chromatography

Buffer exchange of all protein containing samples to 20 m*M* Tris–HCl, pH 8.0, was performed by gel filtration chromatography on Sephadex PD10 columns. The columns were equilibrated with 20 ml of the buffer and 2.5 ml of the sample was applied. Proteins were then eluted with 3.1 ml of the Tris-buffer and stored at −20°C prior to further analysis.

2.3. Immunoaffinity chromatography

All steps were carried out at 4°C and all solutions and samples were filtered through 0.45-μm filters (Millipore, Eschborn, Germany) before application to the affinity column. Anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody was coupled to an NHS-activated agarose column (Pharmacia, Freiburg, Germany). The column was rinsed with 6 ml icecold 1 m*M* HCl, then the antibody (10 mg in coupling buffer comprising 0.2 *M* $NaHCO_3$ at pH 8.3) was circulated through the column with a peristaltic pump (P1, Pharmacia, Freiburg, Germany) for 1 h. After the

coupling of the ligand, the column was rinsed with 0.5 *M* ethanolamine at pH 8.3 to deactivate any residual hydroxysuccinimide groups. For binding of antibody-reactive proteins, the column was equilibrated by rinsing with 10 ml of 20 m*M* Tris–HCl, pH 8.0. Rat kidney mitochondrial and rat or human plasma samples were recirculated on the immunoaffinity-column at 4°C for 12 h in 20 m*M* Tris–HCl, pH 8.0. Not or non-specifically bound proteins were eluted from the column with 10 ml of the same buffer. Specifically bound proteins were then eluted with 10 ml of an acidic buffer (20 m*M* glycine, 0.5 *M* NaCl adjusted to pH 2.7 with concentrated HCl). After elution of immunoreactive proteins, the columns were immediately rinsed with Tris–HCl buffer containing 0.1% sodium azide for storage at 4°C. Eluted proteins were concentrated by ultracentrifugation (molecular mass cut-off, 10 000) using Ultrafree (Millipore, Eschborn, Germany) devices. Recovery of chemically dichloroacetylated RSA as a reference protein was performed in the same manner. One mg of protein was applied to immunoaffinity chromatography and recovered from the column. This experiment was also performed in the presence of 100 mg RSA in the sample solution for evaluation of the enrichment capability.

2.4. Protein hydrolysis and derivatization of modified L-lysine residues

Reference proteins (chemically modified RSA) or protein fractions isolated by immunoaffinity chromatography were lyophilized and hydrolyzed with 500 μl of 6 *M* HCl at 60°C for 16 h after addition of the internal standard N^{ϵ}-(trifluoroacetyl)-L-lysine. After addition of 500 μl water, samples were lyophilized and redissolved in 200–500 μl acetonitrile–pyridine (99:1, v/v). Derivatization was performed after adding 30 μl of 1,3-dichloro-1,1,3,3-tetrafluoroacetone at 60°C for 30 min in a closed reaction vial [14]. Reaction products generated for GC–MS analysis by reaction with 1,3-dichloro-1,1,3,3-tetrafluoroacetone were 2,2-bis(chlorodifluoromethyl)-4-(1-dichloroacetamido)-butyl-1,3-oxazolidine-5-one as derivative of N^{ϵ}-(dichloroacetyl)-L-lysine and 2,2-bis(chlorodifluoromethyl)-4-(1-trifluoroacetamido)-butyl-1,3-oxazolidine-5-one as derivative of N^{ϵ}-(trifluoroacetyl)-L-lysine [4].

2.5. GC–MS analysis

GC–MS analyses were performed on a Fisons MD 800 quadrupole mass spectrometer coupled to a Carlo Erba 8000 series GC and equipped with an AS 800 autosampler (Fisons Instruments, Mainz, Germany). For separations, a DB5-MS (J&W Scientific, Folsom, CA, USA) fused-silica capillary column (20 m×0.18 mm I.D., 0.1 μm film thickness) with helium as carrier gas was used. Quantitation of N^{ϵ}-(dichloroacetyl)-L-lysine was performed with chemical ionization and negative ion detection (NCI-MS) in the single ion monitoring mode. Characteristic fragments (*m/z* 209, *m/z* 211 and *m/z* 342) were monitored for the quantitation of the derivatives of N^{ϵ}-(dichloroacetyl)-L-lysine and N^{ϵ}-(trifluoroacetyl)-L-lysine. Quantitation of N^{ϵ}-(dichloroacetyl)-L-lysine was performed relative to the content of the internal standard and referenced to calibration curves with authentic material. For gas chromatographic separation, a linear temperature program (10°C/min) from 55 to 300°C was applied; injector temperature was 280°C and transfer line temperature was 280°C. The ion source temperature was adjusted to 140°C and methane was used as reactant gas.

Mass spectra of DCA-Lys and TFA-Lys after derivatization with 1,3-dichloro-1,1,3,3-tetrafluoroacetone were: DCA-Lys: *m/z* (^{35}Cl)=436 (4 Cl) $[M]^-$, 356 (3 Cl) $[M-CO_2-HCl]^-$, 209 (2 Cl) $[M-C(CF_2Cl)_2-CO_2-H]^-$. TFA-Lys: *m/z* (^{35}Cl)=422 (2 Cl) $[M]^-$, 342 (1 Cl) $[M-CO_2-HCl]^-$, 231 $[M-C(CF_2Cl)_2-CO_2+Cl]^-$.

2.6. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting

Reference protein (dichloroacetylated RSA) and proteins isolated after immunoaffinity chromatography were diluted with SDS sample buffer (0.125 *M* Tris–HCl, pH 6.8, containing 10% (w/v) SDS, 20% (v/v) glycerol, 0.002% (w/v) bromophenol blue and 10% (v/v) 2-mercaptoethanol) to a final concentration of 1 mg/ml. Samples were heated to 95°C for 10 min prior to redissolving aliquots (10 μg) as described by Laemmli with 12 and 4.5% polyacrylamide gels at room temperature [15]. Proteins were separated for 90 min at 30 mA from 8.4×7.0

mm minigels of 0.75 mm thickness using Bio-Rad MiniProtean II gel electrophoresis equipment. Running buffer consisted of 438 m*M* glycine, 57 m*M* Tris, 4 m*M* SDS and 2 m*M* ethylenediaminotetraacetic acid in water without pH adjustment. Separated proteins were transferred to nitrocellulose sheets (Bio-Rad, TransBlot pure nitrocellulose 0.45 μm) by tank blotting using 25 m*M* Tris-buffer containing 192 m*M* glycine and 10% (v/v) methanol at pH 8.3 for 200 V·h. After protein transfer the sheets were blocked for 1 h at room temperature with phosphate-buffered saline (PBS; containing 10 m*M* Na_2HPO_4, 3 m*M* KH_2PO_4 and 137 m*M* NaCl, pH 7.4), containing 1% (w/v) casein and 0.02% (w/v) thimersal. After washing the sheets with three quick changes of PBS-T (PBS, containing 0.05% Tween 20), then once for 15 min and twice for 5 min with PBS-T, sheets were incubated with anti-DCA antibody (1:1 000 in PBS-T) overnight at 4°C. After additional washing steps as described above the goat anti-rabbit horseradish peroxidase-conjugated secondary antibody was incubated (1:5000 in PBS-T) for 1 h at room temperature. After washing visualization of recognized bands was performed by enhanced chemiluminescence detection on Hybond-enhanced chemiluminescence (ECL) film using Pierce luminescence reagents.

2.7. Blood samples of rats and volunteers exposed to PER

To simulate working place conditions, three healthy female (age 25–38 years, body masses between 50 and 58 kg) and three healthy male volunteers (age 38–72 years, body masses between 75 and 84 kg) and three male (250–300 g) and female (200–250 g) Wistar rats from Harlan Winkelmann (Borchen, Germany) were exposed to 40 ppm PER for 6 h in a dynamic exposure chamber [16]. The study was carried out according to the Declaration of Helsinki, after approval by the Regional Ethical Committee of the University of Würzburg, Germany, and after written consent by the volunteers. The design of the chamber and the generation of the PER–air mixtures has been described previously [16–19]. Blood samples of rats and volunteers were collected 24 h after the end of the exposure.

2.8. Protein determination

Protein concentrations in liquid samples were determined by the method described by Bradford [20] basing on the formation of a protein–dye complex with Coomassie blue G-250.

3. Results and discussion

3.1. GC–NCI-MS determination of N^{ϵ}-(dichloroacetyl)-L-lysine as its 1,3-dichloro-1,1,3,3-tetrafluoroacetone derivative

Reaction of N^{ϵ}-modified L-lysine conjugates with 1,3-dichloro-1,1,3,3-tetrafluoroacetone yield reaction products generated by a condensation reaction of the carboxy and the α-amino group of lysine with the derivatization reagent confirming the modification of the ϵ-amino group. This additional electrophore labeling in the compounds 2,2-bis(chlorodifluoromethyl)-4-(1-dichloroacetamido)-butyl-1,3-oxazolidine-5-one (Fig. 1, **5**) as derivate of N^{ϵ}-(dichloroacetyl)-L-lysine and 2,2-bis(chlorodifluoromethyl)-4-(1-trifluoroacetamido)-butyl-1,3-oxazolidine-5-one as derivate of N^{ϵ}-(trifluoroacetyl)-L-lysine provides excellent gas chromatographic properties and high sensitivity using GC–MS with chemical ionization and negative ion detection (Fig. 2). Using authentic material, the detection limit of N^{ϵ}-(dichloroacetyl)-L-lysine was 20 fmol/2 μl solution injected monitoring the m/z 209 fragment. Response was linear over a range from 20 to 2000 fmol/2 μl solution injected if normalized against the internal standard N^{ϵ}-(trifluoroacetyl)-L-lysine ($r^2=0.997$).

3.2. GC–MS quantitation of N^{ϵ}-(dichloroacetyl)-L-lysine in proteins after immunoaffinity chromatography and protein hydrolysis

Quantitation of N^{ϵ}-(dichloroacetyl)-L-lysine as the modified amino acid in dichloroacetylated proteins was performed after acidic hydrolysis of the entire protein fraction. Using rabbit serum albumin modified with dichloroacetyl groups under controlled conditions, the release of N^{ϵ}-(dichloroacetyl)-L-lysine could be demonstrated after hydrolysis. Moreover, synthetic N^{ϵ}-(dichloroacetyl)-L-lysine was re-

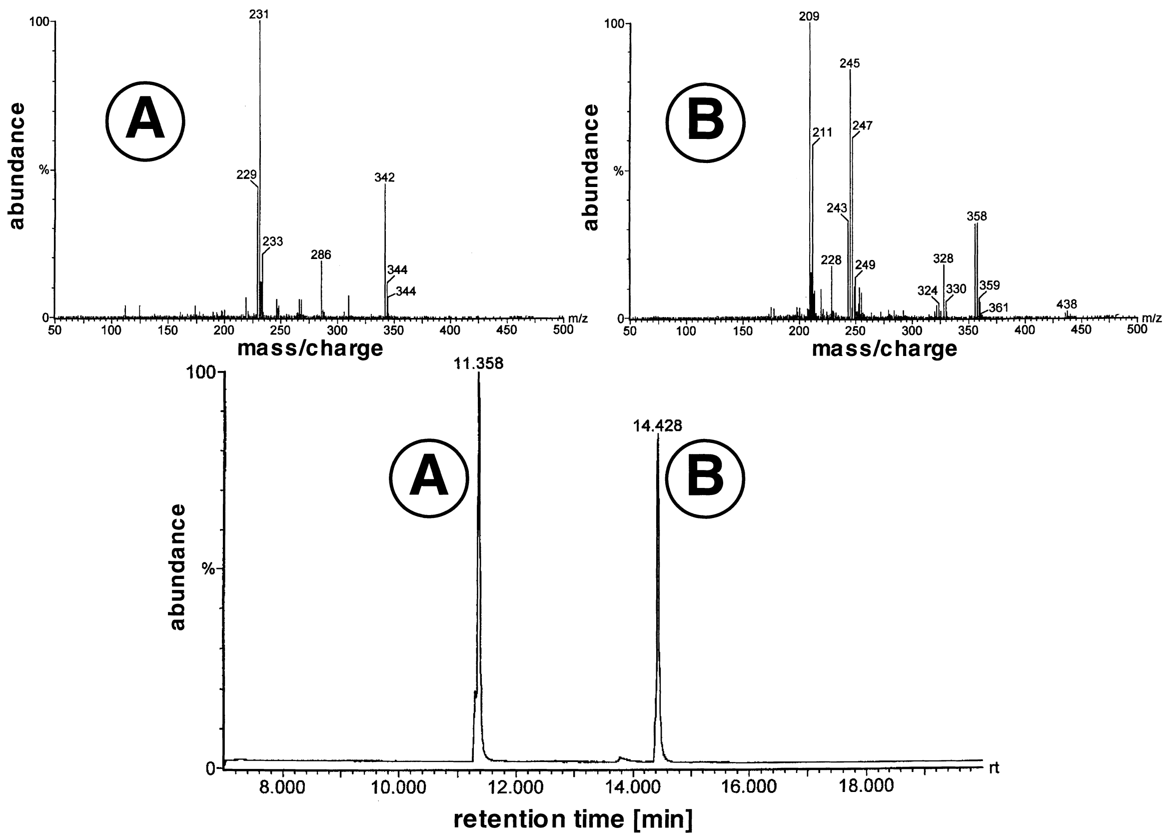

Fig. 2. Gas chromatographic separation and corresponding mass spectra of the internal standard N^{ϵ}-(trifluoroacetyl)-L-lysine (A) and N^{ϵ}-(dichloroacetyl)-L-lysine (B) after derivatization with 1,3-dichloro-1,1,3,3-tetrafluoroacetone.

covered with a yield of >85% when treated under identical conditions (Table 1). To obtain an enrichment of modified proteins in blood from perchloroethene exposed individuals to be able to quantify modified amino acids after protein hydrolysis by GC–MS, the anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody was coupled to affinity columns. The enrichment capability of the immunoaffinity column coupled with the anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody was demonstrated by recovering chemically

Table 1
Recovery of N^{ϵ}-(dichloroacetyl)-L-lysine as its 1,3-dichloro-1,1,3,3-tetrafluoroacetone derivative in GC–MS analysis after different steps in the quantitation procedure[a]

	N^{ϵ}-(Dichloroacetyl)-L-lysine	Dichloroacetylated rabbit serum albumin
Recovery after hydrolysis (%)	>85	–
Recovery after immunoaffinity chromatography (%)	–	76±7.4% (Reference only) 73±11.2% (Reference in presence of excess of native RSA)

[a] Values are given as recovery (%±standard deviation, n=3) compared to the GC–MS analysis of an identical sample without treatment.

modified dichloroacetylated rabbit serum albumin by comparison with the hydrolysis and derivatization of an identical sample without immunoaffinity chromatography. Even in an excess amount of unmodified rabbit serum albumin (100-fold), the recovery of dichloroacetylated reference protein was not significantly affected (Table 1).

3.3. Detection and quantitation of N^{ϵ}-(dichloroacetyl)-L-lysine in biological samples after perchloroethene exposure of individuals

Administration of perchloroethene to rats has been shown to induce dichloroacetyl protein modifications predominantly in mitochondrial proteins due to a high activity of metabolizing β-lyase enzymes forming the electrophilic compound dichlorothioketene. This highly reactive intermediate reacts with proteins present at the side of formation to yield dichloroacetylated L-lysine residues. Rat kidney mitochondrial proteins and rat and human plasma samples were passed through the immunoaffinity columns, unmodified proteins which were not or non-specifically bound to the immobilized anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody were eluted. Elution of the modified proteins specifically bound to the immobilized antibody were eluted with a salt containing acidic buffer forcing conformational changes in the antibody structure and protein release. Efficient enrichment of immunoreactive proteins was demonstrated by comparing the intensities of protein bands of samples (10 μg of total protein each) before and after immunoaffinity purification. With the anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody, an increased concentration of immunoreactive proteins was detected in rat blood samples. In human blood samples, immunoreactive proteins remained below the limit of detection in immunoblot analysis even after immunoaffinity chromatography (Fig. 3).

Comparative quantitation of modified amino acids was performed using GC–MS using chemical ionisation and negative ion detection after protein hydrolysis and electrophore derivatization. In blood samples and kidney subcellular fractions from rats exposed to PER by inhalation N^{ϵ}-(dichloroacetyl)-L-lysine could be detected monitoring the m/z 209 mass fragment in the single ion monitoring mode. In human blood plasma no N^{ϵ}-(dichloroacetyl)-L-lysine could be detected by this method or by immunoblotting even after immunoaffinity chromatography of large amounts of plasma proteins (Fig. 4). The results of the comparative quantitation of modified dichloroacetylated proteins by this procedure are shown in Table 2. In the kidney mitochondrial proteins of rats exposed to 40 ppm PER, 1.9–2.2 pmol N^{ϵ}-(dichloroacetyl)-L-lysine/mg protein were present and 0.35–0.48 pmol N^{ϵ}-(dichloroacetyl)-L-lysine/mg protein were present in blood proteins. Again, in human blood samples from PER exposed volunteers, N^{ϵ}-(dichloroacetyl)-L-lysine concentrations were below the detection limit (0.01 pmol/mg). Quantitation of N^{ϵ}-(trichloroacetyl)-L-lysine in human and rat protein samples could not be performed by this procedure since the formed derivative is not stable and decomposes rapidly during derivatization for gas chromatography.

In human blood, the anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody failed to detect this modification. Moreover, immunoaffinity enrichment of N^{ϵ}-(dichloroacetyl)-L-lysine-containing proteins was also unsuccessful as indicated by negative results using antibody detection and gas chromatography–mass spectrometry. Since N^{ϵ}-(dichloroacetyl)-L-lysine-containing human serum albumin was recognized by the antibody and retained by the immunoaffinity column, these data indicate a much lower rate of formation of reactive intermediates by the glutathione conjugation/β-lyase pathway for PER in humans as compared to rats.

Protein modifications are important markers in biomonitoring since they represent biochemical endpoints believed to be closely related to events resulting in a toxic response. Therefore, many methods have been developed to quantify protein adducts. These methods include liberation of the protein bound xenobiotic by hydrolysis, quantitation of the modified amino acid after protein cleavage or use of antibodies with specific affinity to the protein adducts of interest. The needed sensitivities for monitoring of human populations may often only be reached by a combination of different techniques. For these studies, we used a combination of immunoaffinity chromatography to isolate protein adducts from blood of exposed rats and human volunteers and GC–MS using chemical ionisation and negative ion detection after protein hydrolysis for

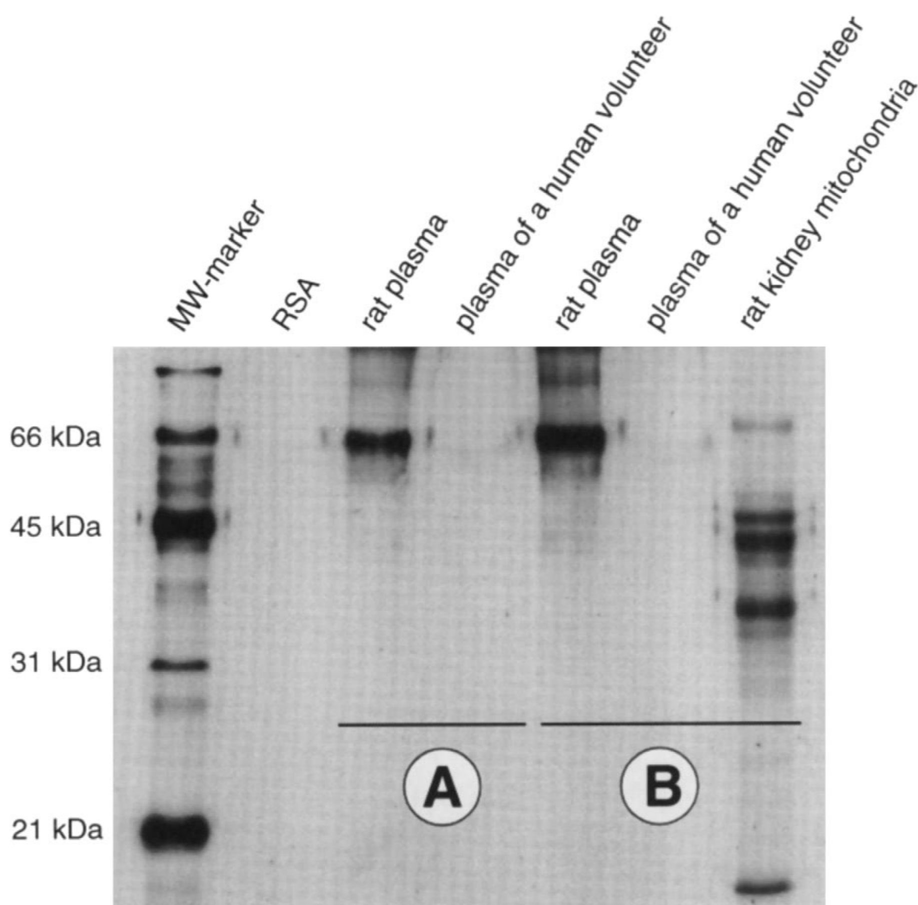

Fig. 3. Immunoblot analysis of protein fractions prior to (A) and after immunoaffinity enrichment (B) of dichloroacetylated proteins. Ten μg of total protein were analysed per lane and modified proteins were detected using the anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody. M_r, molecular mass; kDa, kilodalton.

quantitation. A specific antibody to the chemically induced amino acid modification within proteins, N^{ϵ}-(dichloroacetyl)-L-lysine, was used to selectively extract modified proteins from blood samples. After this enrichment step, isolated proteins were hydrolysed in presence of a structurally related internal standard. The liberated lysine derivatives were then reacted with an electrophore label, 1,3-dichloro-1,1,3,3-tetrafluoroacetone, to yield volatile cyclic oxazolidinone derivatives and analysed by GC–NCI-MS. With N^{ϵ}-(dichloroacetyl)-L-lysine, the method was very sensitive and permitted excellent reproducibility including a recovery of greater than 85% after immunoaffinity chromatography. Using the combination of selective sample enrichment and the very sensitive GC–NCI-MS measurement after electrophore-labelling the detection and characterization of modified rat blood proteins after inhalation of working place concentration of perchloroethene could be performed.

4. Conclusion

A GC–NCI-MS method for separation and detection of N^{ϵ}-(dichloroacetyl)-L-lysine using the chemically related compound N^{ϵ}-(trifluoroacetyl)-L-lysine as internal standard has been developed after

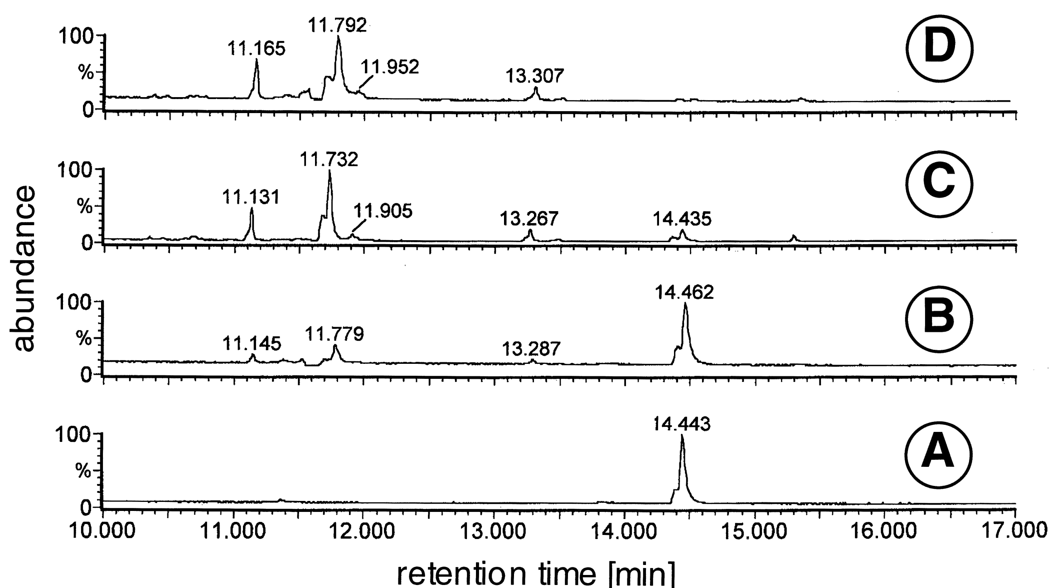

Fig. 4. Gas chromatographic separation of rat serum proteins after immunoaffinity purification (N^{ϵ}-(dichloroacetyl)-L-lysine antibody coupled to NHS-activated agarose columns), protein hydrolysis and derivatization of amino acids with 1,3-dichloro-1,1,3,3-tetrafluoroacetone. Characteristic fragments in the mass spectrum using chemical ionisation and negative ion detection of the derivative of N^{ϵ}-(dichloroacetyl)-L-lysine were monitored during the gas chromatographic separation. (A) One hundred fmol of synthetic N^{ϵ}-(dichloroacetyl)-L-lysine; (B) male rat mitochondrial proteins (37 mg) 24 h after perchloroethene exposure (40 ppm for 6 h); (C) plasma sample from a male rat (53 mg protein) exposed identically; (D) plasma sample from a human volunteer (172 mg protein) exposed identically. (B, C) Two μl out of 500 μl sample injected; (D) 2 μl out of 100 μl sample injected.

electrophore derivatization with 1,3-dichloro-1,1,3,3-tetrafluoroacetone. This procedure was applicable for the detection of the modified amino acid residue after acidic hydrolysis of N^{ϵ}-(dichloroacetyl)-L-lysine containing synthetic reference proteins. However, protein modifications in biological samples from perchloroethene exposure failed to be detected prior to specific sample preparation by enrichment of dichloroacetylated proteins. We successfully applied immunoaffinity chromatography on an immobilized anti-N^{ϵ}-(dichloroacetyl)-L-lysine antibody for the highly selective extraction of modified proteins from blood plasma of exposed rats and human volunteers.

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Table 2
Quantitation of modified proteins in biological tissues of rats and human volunteers 24 h after exposure to 40 ppm perchloroethene for 6 h (pmol N^{ϵ}-(dichloroacetyl)-L-lysine/mg protein±standard deviation; $n=4$)

	Rats male	Female	Human volunteers (male and female)
Kidney mitochondria	2.26±0.02	1.2±0.05	–
Blood plasma	0.47±0.006	0.34±0.02	<0.01

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