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The Application of Covalent Affinity Chromatography with Thiol-Disulphide Interchange for Quantitative Determination of Metallothionein-Specific Protein from Human Fluids, as Indicator of Environmental Exposure to Heavy Metals

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Abstract

The aim of this study was to present a new analytical method for the quantitative determination of metallothioneins (MT) protein in human body fluids and tissues, in order to determine the level of environmental and industrial exposition to heavy metals. For MTs isolation covalent affinity chromatography with thiol-disulphide interchange (CAC-TDI) was applied, which is a modern technique of separation of a high affinity, good repeatability and reproducibility, allowing specific isolation of the thiolproteins and metallothiolproteins. Fundamentals of indirect determination of the contents of metallothioneins protein were worked out throught estimation of the quantities of metals bound with metallothionein protein and adsorbed on covalent affinity chromatography gel as on solid-phase extraction support during a separating process.

The (CAC-TDI) gel, specially prepared, was used as a solid phase extraction support (SPE) for preconcentration of Hg-Thionein (Hg-Th), Cd-thionein (Cd-Th), Zn-thionein (Zn-Th) and Cu-thionein (Cu-Th) proteins and Hg, Cd, Zn and Cu bonded with MTs from spiked water, urine, human plasma, breast milk and tissues' homogenates.

Keywords: Covalent affinity chromatography with thiol-disulphide interchange, solid-phase extraction, environmental and industrial exposition to heavy metals, quantitative isolation and determination of metallothionein from human fluids

Introduction

An exposure to heavy metals is a big problem of environmental toxicology. The metals and their compounds are adsorbed through the air passages and alimentary canal with food and drinking water. The most of the heavy metals, especially: Hg, Co, Cd, Bi, Zn, Cu, Ag, Au, Pb, Pt, Sn, Ni, Rb, Tc are very dangerous for a human body. They disturb the economy of endogenous metals and biochemical equilibrium. They have etiological influence on hypertension, cancer decrease the ventila of the lungs and other lung-diseases. The heavy metals are the source of degeneration, reduction of pancreases efficiency and disfunction of kidneys [1-3]. The quite good indicators of the exposure to Cd and other heavy metals were measurements of its concentration in urine, blood, human breast milk, liver and kidneys. An other indicator is the contents of endogenous metals (Zn, Cu) in these tissues. The similar indicators are used at Hg exposition [4-6].

In 1956 for the first time the increased contents of aminoacids in urine after exposition to Cd was noticed. The presence of the low molecular weight proteins (MW < 40.0 kDa) was observed in urine, resulting from the damage of kidneys ducts [7]. Among these peptides the most important ones are: β_2-microglobulin, albumin, orosomu-coid, transferin, retinol binding protein (RBP), α_2-microglobulin, β_{2x}-globulin, post-γ-protein, ribonucleases, lysozyme, minialbumin, β- and γ-trace free immunoglobulin light chains (Table 1) [8, 9]. The correlation coefficient (r) between Cd concentration in blood and urine, and the concentrations of these proteins were about 0.1-0.6 (Table 2) [8-10].

The specific protein appearing in urine, plasma and human milk only after exposition to metals is metallothionein (MT). MT was discovered in 1957 by Margoshes and Val

Table 1. The low-molecular weight proteins (MW<40,000 Da) present in human blood and urine after exposition to Cd and Hg [8, 9]

No.	Protein	Metal	MW (Da)
1.	β_{2x}-Globuli	Cd	21,000
2.	Post-γ-protein (γ-trace)	Cd	10,000
3.	β-trace protein	Cd	30,000
4.	Minialbumin	Cd, Hg	10,000
5.	β_2-Microglobulin	Cd, Hg	11,800
6.	Lysozyme	Cd	14,600
7.	Ribonuclease	Cd	13-; 20-; 32,000
8.	Retinol-binding protein	Cd, Hg	21,400
9.	Ig free light chains	Cd	23,000
10.	$3S\gamma_1$-Globulin	Cd	25,000
11.	Orosomucoid	Cd	44,000

lee and characterized by Kagi and Vallee as the low molecular weight protein of about 6.5 kDa. MT is rich in -SH groups, containing about 30% of cysteine in the molecule. It is chelated with: Cd, Zn, Cu, Hg, Au, Ag, Bi, Co which are stimulators of its biosynthesis and all these metals bind to the induced protein [11-15]. The mammalian MTs binding a total 7 equivalents of the multivalent ions and have the higher stoichiometries (12 equivalents) with inivalent d(10) ions such as: Cu(I), Ag(I) and Au(I). The affinity of the metal ions for the binding sites of mammalian MTs follows the order of thiolate metal complexes: Zn(II)<Pb(II)<Cd(II)<Cu(I), Ag(I), Hg(II), Bi(III) [16]. MTs have two domains {-α}(31-61) and {-β}(1-29) about 15-20 A diameter, which contain two metallothiolate clusters of different structures and different affinity to individual metals ([A – three-metal cluster, preference Cu, Zn, Cd], [B – four-metal cluster, preference Cd, Zn, Cu]) [17-20]. In

Table 2. The correlation coefficient (r) between concentration of Cd in human blood and urine and concentration of different proteins in human fluids [8-10].

No.	Correlation	(r)
1.	log (Cd/U - log (Cd/B)	0.77
2.	log (Cd/U - log (P/U)	0.11
3.	log (Cd/U) - log (A/U)	0.14
4.	log (Cd/U) - log (β_2M/U)	0.18
5.	log (Cd/U) - log (O/U)	0.37
6.	log (Cd/B) - log (P/U)	0.27
7.	log (Cd/B) - log (A/U)	0.31
8.	log (Cd/B) - log (β_2M/U)	0.28
9.	log (Cd/B) - log (O/U)	0.61
10.	log (Cd/B) - log (β_2M/B)	0.16
11.	log (Cd/B) - log (β_2-M/U)	0.16

(U - urine, B - blood, P - total protein, A - Albumin, O - Orosomucoid, β_2M - β_2-Microglobulin)

Table 3. The correlation coefficients (r) between metallothionein concentration and concentration of Cd in human urine, blood, liver and kidney [21, 22].

No.	Correlation	(r)
1.	log (MT/U) - log (Cd/U)	0.89
2.	log (MT/U) - log (Cd/L)	0.75
3.	log (MT/U) - log (Cd/K)	0.85
4.	log (Cd/U) - log (MT/U)	0.94
5.	log (Cd/B) - log (MT/U)	0.82
6.	log (MT/U) - log (Cd/U)	0.80
7.	log (MT/U) - log (Cd/U)	0.74
8.	log (MT/U) - log (Cd/U)	0.70
9.	log (MT/U) - log (Cd/U)	0.64
10.	log (MT/U) - log (Cd/B)	0.65

(MT - Metallothionein, U - urine, B - blood, L - liver, K - kidney)

physiological states MT exist in trace amounts. Metallothionein concentration in plasma and urine was found to be about 2.4 +/- 2.2 μg MT protein per gram of creatinine [21], and about 10 μg/l in human breast milk [23, 24]. After exposition to Cd, MT concentration in urine and plasma ranged from 20 μg/g -2,500 μg/g creatinine [22, 25, 26]. The application of this technique allowed determination of a very good correlation between the contents of MT in human fluids and concentration of Cd in the blood, urine, breast milk, kidney and liver, ranging from 0.64-0.94. These correlations are very good indicators of the environmental exposition to these heavy metals (Table 3) [21, 22].

In experiments using RIA and LIA techniques the main problem is however obtaining of metallothionein antibodies, because the molecular mass of MT is too low as a hapten and also need the long period of immunization of experimental animals. The metallothionein protein before used must be polymerized, but in this process the random aggregation also occur. The immunological answer of immunological system is not homogenic and antibodies give unspecific reaction with the other proteins present in plasma and urine and analysis is not quantitative. In order to avoid all the difficulties, investigations on quantitative concentration of metallothioneins the new method was tested. MTs were carried out by covalent affinity chromatography gel as a solid phase extraction (SPE) support, specific for the thiol proteins, gives the best results than the classical physicochemical methods [27-32]. The idea of this method was based on earlier results obtained for separation of different metallothioneins by CAC-TDI method and proposed mechanism of separation [33-36].

Covalent affinity chromatography with thiol-disulphide interchange (CAC-TDI) is one of special techniques of separation of affinity chromatography based on exchange reaction between disulphide bridges (-S-S-) fixed on the insoluble support and thiol groups (-SH) of the proteins being separated [37, 38]. CAC-TDI technique was first applied for the separation of MTs from tissues by Squibb, Cousins, Ryden and Deutsch in 1977/78 [39, 40]. The yields of separation of different MTs were about 10-50% for applied protein and about 15-80% for metals bonded formerly with MTs (Table 4).

Table 4. Mean yields of separation of different metallothioneins by covalent affinity chromatography with thiol-disulphide interchange method [40-46].

No.	MT	pH	Protein recoveries		Metals recoveries		Ref.
			(mg)	(%)	(μg)	(%)	
1.	Hg-Th	8.0	0.40	25.2	292.0	78.9	41
2.	Cd-Th	4.0	0.68	24.4	36.4	17.6	42
3.	Cd-Th	8.0	0.48	19.3	32.7	17.8	42
4.	Cd-Th	9.0	0.41	29.7	22.3	21.4	44
5.	Apo-Th	4.0	1.28	50.4	-	-	43
6.	Apo-Th	8.0	1.17	46.1	-	-	43
7.	Zn-Th	4.0	0.73	29.9	62.6	48.3	45
8.	Zn-Th	8.0	0.62	25.3	36.4	28.1	45
9.	Zn-Th	9.0	0.87	35.6	38.6	29.8	45
10.	Cu-Th	4.0	0.57	8.5	31.2	53.7	46
11.	Cu-Th	5.5	61.20	10.6	84.6	49.3	40
12.	Cu-Th	7.0	0.89	15.6	27.6	42.7	46
13.	Cu-Th	8.0	1.93	28.7	22.4	38.5	46
14.	Cu-Th	9.0	2.42	36.1	16.9	29.1	46

Experimental

Preparation of Metallothioneins

The prepurified metallothioneins (Hg-thionein, Hg-Th; Cd-thionein, Cd-Th; Zn-thionein, Zn-Th and Cu-thionein, Cu-Th) and cadmium apothionein (Apo-Th) were obtained from critical tissues (Hg – kidney; Cd, Zn, Cu – liver) of the experimental female white rats after exposition to salt of heavy metals, administered subcutaneously in the form of physiological salt solutions:

$HgCl_2$ 1.0 mg of Hg/kg 3 times per week for 4 weeks;
$CdCl_2$ 1.0 mg of Cd/kg 3 times per week for 4 weeks;
$ZnSO_4$ 20.0 mg of Zn/kg 7 times per week for 2 weeks,
$CuSO_4$ 3.0 mg of Cu/kg 7 times per week for 2 weeks.

The homogenates of tissues [liver 25% (m/v) and kidney 20% (m/v)] in 1.15% KCl were prepared. Homogenates were centrifuged (3000g for 30 min) and after initial dialysis (dialysing bag Serva 20/32 and Aquacide II from Serva), the supernatants (5.0 ml) were separated by low pressure gel permeation chromatography (LPGPC) method on Sephadex G-75 (column K26/100, Pharmacia – Uppsala, 0.1 mol/l ammonium-formate buffer, pH = 8.0, ionic strength m = 0.1, 0.05% NaN_3, flow rate 20.0 ml/h, fractions of 5.0 ml). The fractions of MTs were collected and the concentrations of protein, thiol groups and metals were determined. The molecular weight and the control of quality of obtained MTs and their physicochemical parameters were measured by high-pressure gel permeation chromatography (HPGPC) (HPLC Pump 2248, autosampler Model 2157, UV/VIS detector VWM 2141, low pressure mixer, fraction collector Redi-Frac – LKB Pharmacia, control panel Chromatopic C-R5A – Shimadzu, GPC column Superdex 75HR 10/30 with gel of mean particle size 13 mm – Pharmacia, Uppsala, 0.1 mol/l ammonium-formate buffer, pH = 8.0, ionic strength μ = 0.1, flow rate 1.0 ml/min). All fractions were liophilized [41-46].

Synthesis of Gels for Covalent Affinity Chromatography

The gel for covalent affinity chromatography with thiol-disulphide interchange (CAC-TDI) Sepharose-X (where X was 5,5′-dithiobis-(2-nitrobenzoate)[Sepharose-DTNB] or 2,2′-dithiobis-(2-nitropyridine)[Sepharose-DTNP]) were prepared. The CH-Sepharose 4B gel (Pharmacia, Uppsala), DTNB (Windsor Lab.Ltd.), DTNP (BDH Ltd.) and N-cyclohexyl-N′-[2-(N-methylmorpholino)ethyl] carbodiimide-p-tolueno sulphonate (carbodiimide CMC, BDH Ltd.) were used for CAC-TDI gels synthesis. The degrees of substitution were about 0.15-0.18 mmol DTNB or DTNP/g Sepharose and were similar to these types of the commercial materials [32].

Preparation of Biological Material

Physiological human urine, plasma and human milk were collected from 4-5 people (non-smokers) in good state of health after clinical control. All samples from different people were collected and mixed for unification of their properties. The samples were protected by addition of sodium azide (NaN_3) in final concentration 0.05%. The concentration of protein, thiol groups and metals (Hg, Cd, Zn and Cu) were measured and physicochemical parameters of urine, plasma and milk were controled by low- and high-pressure GPC method (Sephadex G-75, Sephadex G-200 and Sephadex G-10, Superdex 75HR – Pharmacia, Uppsala) [27-31, 47-49].

Table 5. The correlation coefficients (r) between metallothionein concentration added to the different mediums and determined by indirect method.

No.	Correlation	(r)
1.	Hg-Th/water	0.95
2.	Hg-Th/urine	0.87
3.	Hg-Th/plasma	0.73
4.	Cd-Th/water	0.97
5.	Cd-Th/urine	0.84
6.	Cd-Th/plasma	0.81
7.	Cd-Th/human milk	0.72
8.	Cd-Th/homogenate	0.71
9.	Zn-Th/water	0.96
10.	Zn-Th/urine	0.84
11.	Zn-Th/plasma	0.72
12.	Cu-Th/water	0.96
13.	Cu-Th/urine	0.80
14.	Cu-Th/plasma	0.71

Table 6. The correlation coefficients (r) between metallothionein concentration added to the different mediums and determined by chemical methods.

No.	Sample	Correlation Coefficient (r)	
		Lowry method	Tannin method
1.	Hg-Th/water	0.89	0.92
2.	Hg-Th/urine	0.70	0.74
3.	Hg-Th/plasma	0.59	0.68
4.	Cd-Th/water	0.87	0.89
5.	Cd-Th/urine	0.64	0.69
6.	Cd-Th/plasma	0.51	0.55
7.	Cd-Th/milk	0.45	0.53
8.	Cd-Th/homogenate	0.42	0.53
9.	Zn-Th/water	0.87	0.91
10.	Zn-Th/urine	0.75	0.83
11.	Zn-Th/plasma	0.62	0.74
12.	Cu-Th/water	0.88	0.90
13.	Cu-Th/urine	0.74	0.83
14.	Cu-Th/plasma	0.57	0.72

Chromatographic Preconcentration of Metallothioneins Samples with Application of Covalent Affinity Chromatography Gel and Their Quantitation

Hg-Th, Cd-Th, Zn-Th and Cu-Th were added to 3 times distilled water, physiological human urine, plasma or human breast milk in different concentrations. The proteins were applied on Sepharose-DTNB or Sepharose-DTNP columns (10 × 130 mm, v~ 10 ml) in volume 5.0 ml and were eluted with: (a) 0.05 mol/l phosphate buffer, pH 8.0 (b) 0.04 mol/l β-mercaptoethanol in phosphate buffer. The fractions of 2.0 ml were collected at the flow rate 30.0 ml/h. The columns were regenerated by DTNB or DTNP in concentration 0.04 mol/l in phosphate buffer. The concentration of MTs were calculated with metal concentration on CAC-TDI support, determined by AAS technique and correlation coefficient between known concentration and calculated by indirect method were determined [27-31, 47-49].

The concentrations of MTs protein in samples were calculated according to the equation {1} [27-32]:

$$c = (A \times 100\% \times M_{MT}) / (M_M \times m \times R\%) \quad (1)$$

where:
(c) is calculated concentration of MTs in the sample [μg], (A) is content of metals bonded to the CAC-TDI gel [mg], (M_{MT}) is molecular weight of metallothionein protein [g/mol], (M_M) is molecular weight of the determined metals [g/mol], (m) is the coefficient determined experimentally, characterizing the content of the metals in metallothionein molecule [mol of metal/mol of protein] and (R) is the mean recovery of adsorption of the metals in the process of metallothionein separation with use of the CAC-TDI method [%]. The added amounts of MTs protein in the samples have been compared with the contents as determined with use of the indirect method based on determining of the heavy metals as: Hg, Cd, Zn or Cu contents in biological samples.

Table 7. The mean errors (%) between added amount of metallothionein protein and determined by chemical and proposed indirect method for the lowest and highest concentration of MT protein.

No.	Sample	The mean error in determination of MT content (*)					
		Concentration 5.0 μg/ml			Concentration 250.0 μg/ml		
		LM%	TM%	IM%	LM%	TM%	IM%
1.	Hg-Th/water	9.1	6.5	2.0	6.3	4.1	0.8
2.	Hg-Th/urine	10.2	7.4	2.0	6.8	4.5	0.9
3.	Hg-Th/plasma	12.5	8.1	2.1	7.8	4.5	1.3
4.	Cd-Th/water	9.8	6.3	2.8	5.1	3.8	1.2
5.	Cd-Th/urine	11.3	7.8	2.8	6.4	4.4	1.4
6.	Cd-Th/plasma	13.1	8.9	3.1	8.1	4.6	1.9
7.	Cd-Th/human milk	12.5	11.3	4.3	10.5	5.7	2.2
8.	Cd-Th/homogenate	13.3	10.9	4.2	11.3	6.2	2.1
9.	Zn-Th/water	7.8	5.6	2.1	6.2	3.9	1.2
10.	Zn-Th/urine	7.8	6.8	2.7	8.2	4.6	1.5
11.	Zn-Th/plasma	9.4	8.9	3.4	9.9	4.9	1.9
12.	Cu-Th/water	8.7	6.0	1.2	6.3	4.0	1.1
13.	Cu-Th/urine	9.3	7.7	2.9	8.7	4.5	2.0
14.	Cu-Th/plasma	11.8	8.8	3.9	11.0	6.6	2.4

(*) – The mean errors were calculated for the higher and lower concentration of MT protein added to the samples with n = 5 samples
LM – concentration determined by Lowry method
TM – concentration determined by tannin method
IM – concentration determined by indirect method based on the analysis of metals contents, adsorbed on CAC-TDI gel

Analytical Methods

In the investigations the following determinations were carried out: protein concentration [50-53], thiol groups concentration [54, 55], metals (Hg, Cd, Zn and Cu) concentration by atomic absorption spectrometry (AAS method) [41, 42, 44-46].

Results and Discussion

The mean correlation coefficients (r) between concentration of MTs added to different samples (water, urine, plasma, human milk and tissues' homogenates) and MTs concentration determined by indirect method are given in Table 5, and by Lowry and tannin method are given in Table 6. The mean errors between added amounts of metallothionein protein and determined by indirect method,

Table 8. The mean detection limits for different metallothioneins protein obtained from different mediums with preconcentration by SPE method on CAC-TDI supports.

No.	Materials	The detection limits (ng/ml)			
		Hg-Th	Cd-Th	Zn-Th	Cu-Th
1.	Water	5.470 +/- 0.027	7.670 +/- 0.067	6.915 +/- 0.063	6.734 +/- 0.087
2.	Urine	5.465 +/- 0.051	7.741 +/- 0.083	7.215 +/- 0.112	7.331 +/- 0.135
3.	Plasma	6.019 +/- 0.182	7.943 +/- 0.136	7.586 +/- 0.153	7.854 +/- 0.197
4.	Milk	–	7.987 +/- 0.169	–	–
5.	Homog.	–	8.867 +/- 0.185	–	–

Lowry and tannin method are given in Table 7. The mean detection limits for different types of metallothionein in different samples are given in Table 8.

Previous results showed that mechanism of separation of polythiolmetalloproteins is very complicated and that the separated proteins change their native structures [33-36] and the yields of protein separation were rather low. Additionally metals formerly bonded with MTs were adsorbed on the CAC-TDI gel not only via immobilized proteins, but also by direct adsorption on the supports and the yields of these reactions are very constant [33-36]. As follows from previous data (Table 4) [40-46], the direct concentration of metallothionein protein for analytic purpose is rather impossible, because of too low yield of binding the MTs protein with CAC-TDI gel, due to the occurrence of reactions, competitive to the binding ones [33-36] and very high detection limit (0.1-5.0 μg/ml) of chemical methods used for the protein quantitation [28]. The binding of 10-50% for different MTs is too low for quantitative isolation, and variations in these values are to big for its theoretical estimation. The situation is not improved even by higher efficiency of binding MT protein in case of its small concentrations in a sample. This fact was observed in synthesis in polysomal cell-free system, where the reaction of intermolecular oxidation of protein -SH groups is much less [39, 56-60]. An important fact is very high and constant content of metals (Hg, Cd, Zn and Cu) in fractions bounded to the CAC-TDI gels. Thus, it is possible to determine the contents of Hg-Th, Cd-Th, Zn-Th and Cu-Th indirectly by the estimation of Hg, Cd, Zn and Cu concentration in the fractions bounded to CAC-TDI gels [27-31, 47-49].

Urine, plasma, human milk samples and corresponding solutions of tissue MTs must be collected. After addition of MTs samples to urine, plasma or the other physiological fluids, which play a role of a model of physiological fluids, samples containing MTs, from people after exposition to heavy metals, it seems necessary to carry out pretreatment by the high- or low-pressure GPC method, in order to remove proteins heavier than MT, and also amino acids, oligopeptides and low-molecular metal compounds, mainly Hg ones (Fig. 1). This procedure is essential while investigating MTs in the human urine, plasma and milk, which contains some amount of proteins, oligopeptides and amino acids with quite high contents of -SH groups. The other important problem at investigations is carried out in a short time after exposition to heavy metals appearing in body fluids in form of low-molecular compounds [61, 62]. The preparation obtained in this way can be considered as an initial fraction. Application of CAC-TDI method at a further stage allows recovery of only about 20-30% of applied MT protein, about 30-40% of Cd, Zn or Cu and about 80% of Hg (Fig. 1). The samples, prepared in this way does not contain any other ballast proteins of 10,000 Da mass, which could contain only few -SH groups or none at all [63, 64]. This method allows obtaining of a concentrated preparations, containing Hg, Cd, Zn and Cu mobilized from mercaptide bonds in Hg-Th, Cd-Th, Zn-Th and Cu-Th. The metals can be determined by AAS method already at this stage, in the presence of MTs protein.

The given data show that the actual contents of protein as well as the ones determined by the indirect method demonstrate a very good correlation in a wide range of concentrations (Table 5). The correlation coefficients were ranged between 0.71-0.97. The lowest correlation coefficients were for plasma, homogenate and milk and higher were for urine and water, of course. In comparision the correlation coefficients (r) between present concentration and determined by chemical methods (Lowry and tannin methods) were ranging from 0.53-0.92 for tannin method and 0.42-0.89 for Lowry method, but for all physiological fluids were lower than 0.83 for tannin method and lower than 0.75 for Lowry method (Table 6). The error between the actual content of protein in the sample and the concentration as determined by the indirect method consisting in metals analysis of the metals bound with CAC-TDI does not exceed 2.2% high concentration of added MT protein (250 μg/ml) and 4.3% for low concentration of added MT protein (5 mg/ml). For the chemical method the errors ranging between 3.8-11.3% for tannin method and between 5.1-13.3% for modified Lowry method and were few times higher than for proposed indirect CAC-TDI method (Table 7). The detection limits for CAC-TDI methods are about 5.5-8.9 ng/ml for different types of MTs (Table 8). Thus, the described method may be applied in the indirect determination of metalloproteins in physiological fluids by means of evaluation of the metal contents on the CAC-TDI gel after adding of metalloprotein samples. The process of metal binding itself has a quantitative character unlike binding of thiolproteins where a complex mechanism of interaction CAC-TDI gel – separating protein causes lowering of binding efficiency [33-36]. The effectivity of adsorption depend on structure of CAC-TDI gel (length of spacer arms, their modification and type of ligand). Additionally pH of using buffers has the influence on the mechanism of adsorption reaction (cationic or anionic), and by this way on yields of the separation [32, 65-68]. Thus, the described method of indirect evaluation of protein content may be applied in the clinical and toxicological analysis. The CAC-TDI gel itself may function as a sorbent for precon-

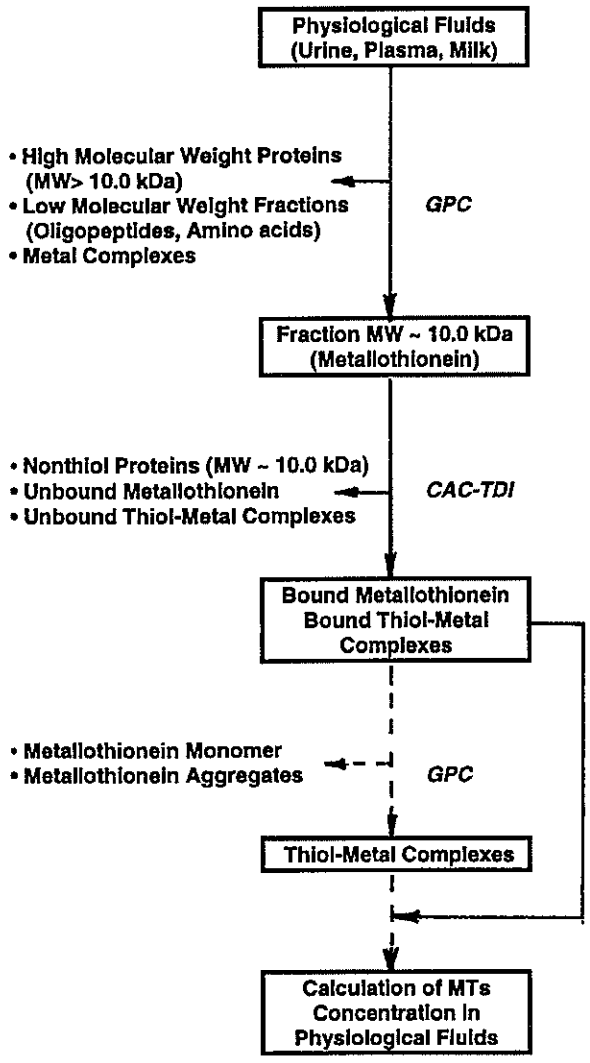


Fig. 1. The scheme of procedure of preconcentration of metallothionein and metals bound to it by covalent affinity chromatography with thiol-disulphide interchange (CAC-TDI) as gel for solid phase extraction (SPE) method.

centration of thiol- and metallothiolproteins at solid phase extraction (SPE) technique allowing to concentrate proteins. It may also function in a similar way in relation to heavy metals as: Hg, Cd, Co, Cu, Mo, Zn, Au, Ag, etc., acting both on free ions of these metals and those bound with proteins. The metals bound with the CAC-TDI gel with participation of mercaptide bonds of metal-sulphur may be easily washed from it by means of low-molecular thiolcompounds. Easiness of adsorption and desorption are among the basic factors qualifying deposits of CAC-TDI types as sorbent in the SPE method.

As the short conclusions we can emphasize that, the supports for covalent affinity chromatography with thiol-disulphide interchange (CAC-TDI) can be used as a solid phase extraction (SPE) gel for preconcentration of metallothioneins protein and metals bonded to them. The concentration of metallothioneins in human fluids can be determined by the indirect method, based on calculation of MTs concentration from content of metals bonded to the covalent affinity chromatography column and the detection limits for these methods are about 5.5-8.9 ng/ml and correlations coefficients are 0.71-0.97. Presented method can be used in toxicological analysis for the determination of exposure to heavy metals, and environmental analysis for determination the value of intoxication of the environment by heavy metals.

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