Journal of Chromatography, 228 (1982) 285—291
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1137

Note

High-performance liquid chromatographic determination of tissue metallothionein in monkeys chronically exposed to cadmium

KAZUO NOMIYAMA* and HIROKO NOMIYAMA

Department of Environmental Health, Jichi Medical School, Minamikawachi-Machi, Tochigi-Ken 329-04 (Japan)

(First received August 10th, 1981; revised manuscript received September 18th, 1981)

Metallothionein (MT) was first separated from equine renal cortex by Margoshes and Vallee in 1957 [1], and was thought to play a role in a wide range of potential homeostatic mechanisms, either in catalysis, storage, immune phenomena or detoxification [2]. The chemical structure [3] and biological functions [4] of MT have since been studied extensively.

The combination of high-performance liquid chromatography (HPLC) and atomic absorption spectrophotometry has recently enabled a quicker and better separation of MTs [5]. In the present experiments, therefore, tissue MTs from monkeys fed cadmium for a long period were subjected to HPLC analysis, and the HPLC elution profiles of the renal cortex are discussed in association with cadmium-induced nephropathy.

EXPERIMENTAL

Specimens

Rhesus monkeys were fed daily 150 g of CLEA monkey pelleted food containing cadmium chloride at dose levels of 0, 3, 10, 30 or 100 mg/kg cadmium over a period of 153 weeks. Control pelleted food (CLEA) contained 0.23 mg/kg cadmium. Biological effects of cadmium have been reported elsewhere [6]. The renal cortex and the liver specimens were subjected to HPLC analysis at the 101st week of the experiment. MTs from other organs were analysed in specimens from a monkey fed cadmium at a dose level of 30 mg/kg for 153 weeks.

Preparation of metallothioneins

One monkey of the 100 mg/kg group was subjected to tissue MT preparation

0378-4347/82/0000—0000/$02.75

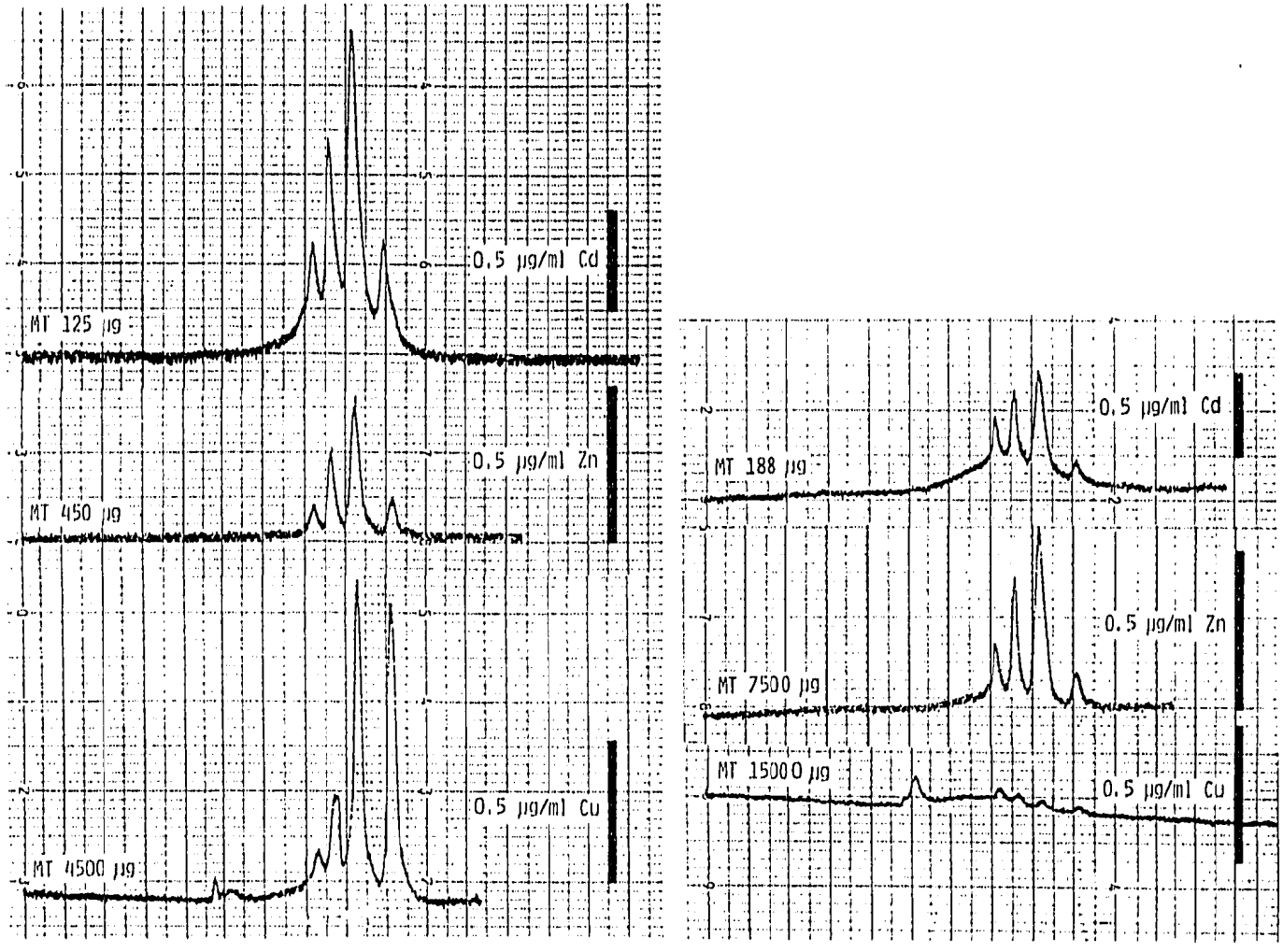

Fig. 1. HPLC elution profiles (cadmium, zinc and copper) of purified MT from the renal cortex (left) and the liver (right) of a monkey of the 100 mg/kg group at 101 weeks.

at the 101st week. Tissues were homogenized in 5 volumes of 0.25 *M* sucrose—0.02 *M* Tris—HCl buffer (pH 8.0), and the homogenate was heated at 80°C for 3 min. Then, it was centrifuged at 40,000 *g* for 60 min. The supernatant was filtered on a Sephadex G-75 column (95 cm × 5.0 cm) at a flow-rate of 60 ml/h in 0.02 *M* Tris—HCl buffer (pH 8.0). After deionization on Sephadex G-25 MT was obtained by freeze-drying the eluate.

High-performance liquid chromatography

Tissues were homogenized in 5 volumes of 0.25 *M* sucrose—0.02 *M* Tris—HCl buffer (pH 8.0), and the homogenate was centrifuged at 40,000 *g* for 60 min. Five hundred microliters of the supernatant were subjected to HPLC analysis. MT composition was analysed using Suzuki's procedure [5] with the use of a Toyo Soda high-performance liquid chromatograph Model 803 equipped with a gel permeation column (Toyo Soda TSK GEL SW 3000, 600 × 21.5 mm I.D.) and 0.05 *M* Tris—HCl buffer solution (pH 8.0) containing 0.1% sodium dodecyl sulfate at a flow-rate of 3.5 ml/min. The eluate was directly introduced into a Varian-Techtron atomic absorption spectrophotometer Model AA 1100 equipped with background corrector BC 6, and the atomic absorptions of heavy metals were continuously monitored. The chart speed was 2 mm/min.

RESULTS

Heavy metals in metallothioneins

As seen in Fig. 1, the elution profiles of purified MTs from the renal cortex and the liver of a monkey of the 100 mg/kg group at the 101st week, showed four MTs: MT 1 (retention time 44.5 min), MT 2 (40.3 min), MT 3 (37.8 min) and MT 4 (35.0 min). The molar ratios of cadmium, zinc and copper are listed in Table I. Cadmium accounted for 80—90% of the heavy metals. The copper content was higher in renal MTs than in hepatic MTs, while zinc was higher in hepatic MTs than in renal MTs. MT 1 from the renal cortex and MT 1 from the liver were the richest in copper among the four MTs.

Renal metallothioneins

As shown in Fig. 2, HPLC elution profiles of renal cortex cytosol from monkeys exposed to cadmium at various dose levels for 101 weeks indicated that cadmium bound with higher-molecular-weight (HMW) proteins as well as with MTs such as MT 1, MT 2, MT 3 and MT 4. MT dimers were also identified by reducing them to MTs by adding neat mercaptoethanol [7]. Low-molecular-weight cadmiums (LMW-Cds) were detected at longer retention times of 54, 57 and 71 min. HMW-Cd and MTs were elevated with increases in cadmium level, and the ratios of the four MTs also changed with cadmium level.

Quantitative analysis indicated that the total MTs were elevated with increases in cadmium level by the 30 mg/kg level. HMW-Cd increased very slightly. With increases in the cadmium level, MT 1 was elevated and reached a maximum at the 30 mg/kg level, and then decreased slightly. MT 2, MT 3 and MT 4 were elevated with increases in cadmium level. MT 2 was the largest fraction at the 100 mg/kg level. LMW-Cds, which had longer retention times of 54, 57 and 71 min, were detected in the 10, 30 and 100 mg/kg levels.

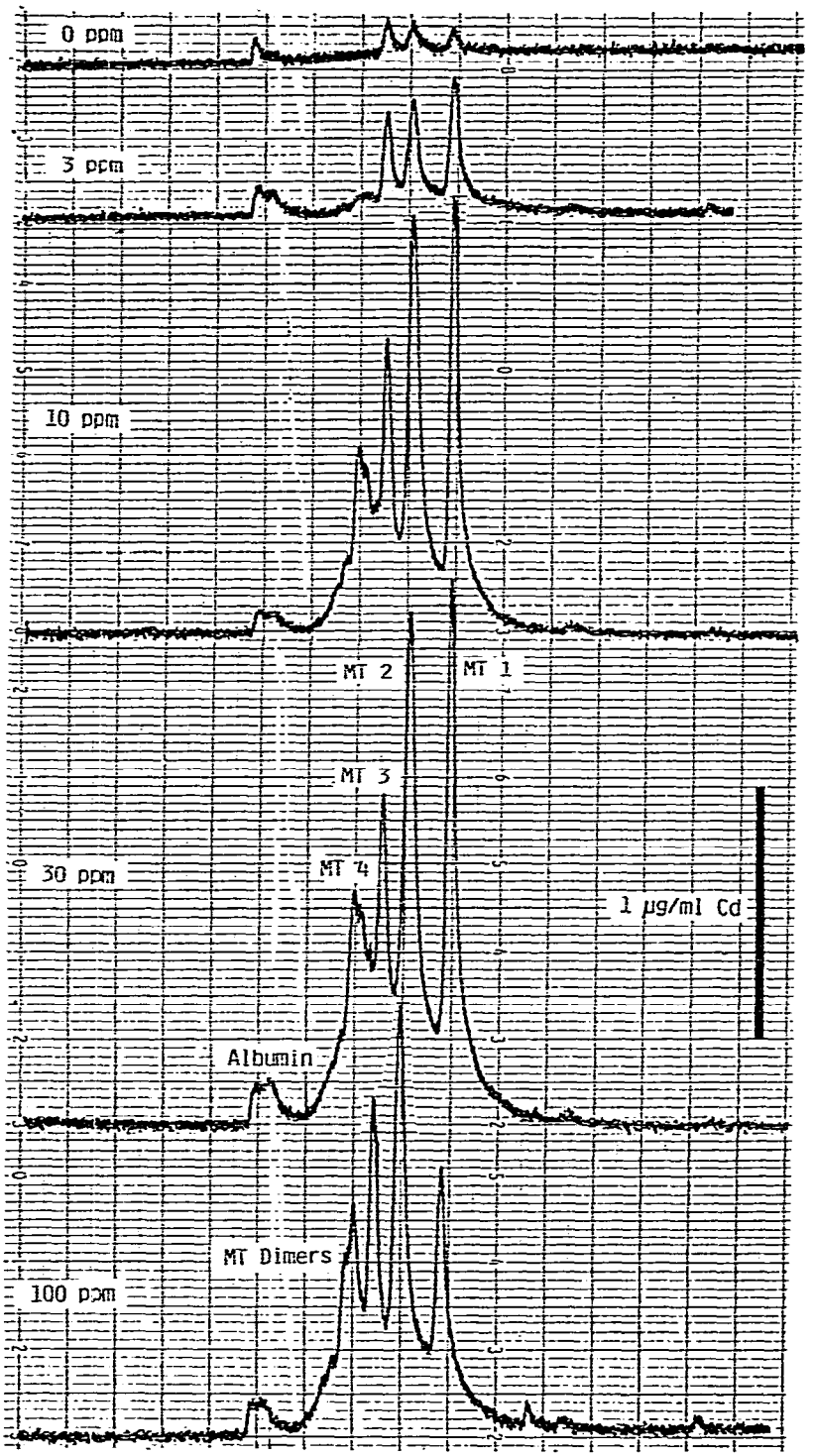

Fig. 2. HPLC elution profiles (cadmium) of MT from the renal cortex of monkeys of the 0, 3, 10, 30 and 100 mg/kg groups at 101 weeks. Reproduced from ref. 6, with permission.

TABLE I

MOLAR RATIOS OF HEAVY METALS IN MONKEY RENAL AND HEPATIC METALLOTHIONEINS

Data are expressed as percentages.

	Renal cortex			Liver		
	Cd	Zn	Cu	Cd	Zn	Cu
MT 1	84.9	8.7	6.4	80.6	19.0	0.4
MT 2	87.9	9.6	2.6	79.1	20.9	0.1
MT 3	87.4	10.7	1.7	80.9	19.0	0.2
MT 4	89.4	8.7	1.9	85.1	14.6	0.2

Hepatic metallothioneins

In the control group a scarce amount of cadmium was detected mainly as HMW-Cd and MT 3. MT 1, MT 2, MT 4 and MT dimers increased with increase in cadmium level. LMW-Cds were also detected at longer retention times of 54, 57 and 71 min in the 100 mg/kg group.

Quantitative analysis indicated the increases in HMW-Cd and MTs with cadmium level. MT 2 was the largest fraction among all the MTs, followed by MT 3. MT 1 was found to increase with cadmium level to reach a maximum at the 30 mg/kg level. MT 4 was detected at the 10 mg/kg level, and was elevated rapidly with the cadmium level. HMW-Cd and LMW-Cds were minor fractions. HMW-Cd was increased slightly with the cadmium level. LMW-Cds were detected at the 10 mg/kg level, and were elevated rapidly with the cadmium level.

Methallothioneins from other organs

Tissues from a monkey fed cadmium at a dose level of 30 mg/kg for 153 weeks were subjected to HPLC analysis. Because the detection limit of cadmium was 30 mg/l, tissues of above 10 mg/kg cadmium were subjected to the HPLC analysis as shown in Fig. 3.

The pancreas contained a very large amount of MT 2, MT 3 and MT 4, as well as LMW-Cds with longer retention times of 54 and 57 min; a scarce amount of MT 1 was also detected. MT 2, MT 3 and MT 1 were found in the stomach and jejunum; small amounts of HMW-Cd and LMW-Cd with a retention time of 57 min were detected as well. From the duodenum, small amounts of MT 1 and MT 2 were determined as well as HMW-Cd and LMW-Cd with a retention time of 52 min. Very small amounts of MT 2 and MT 3 were detected in the bladder, heart and lungs. The mandibular glands contained a scarce amount of MT 1, MT 2 and MT 3, as well as HMW-Cd and LMW-Cds with retention times of 54 and 57 min. The parotid glands contained a relatively large amount of MT 2 and MT 3 and a small amount of MT 1. MT 2, MT 3 and HMW-Cd were found in the testis. Cadmium in the muscle seemed to bind with HMW proteins, MT 2 and MT 3 as well as with MT 1.

DISCUSSION

The combination of HPLC and atomic absorption spectrophotometry [5] en-

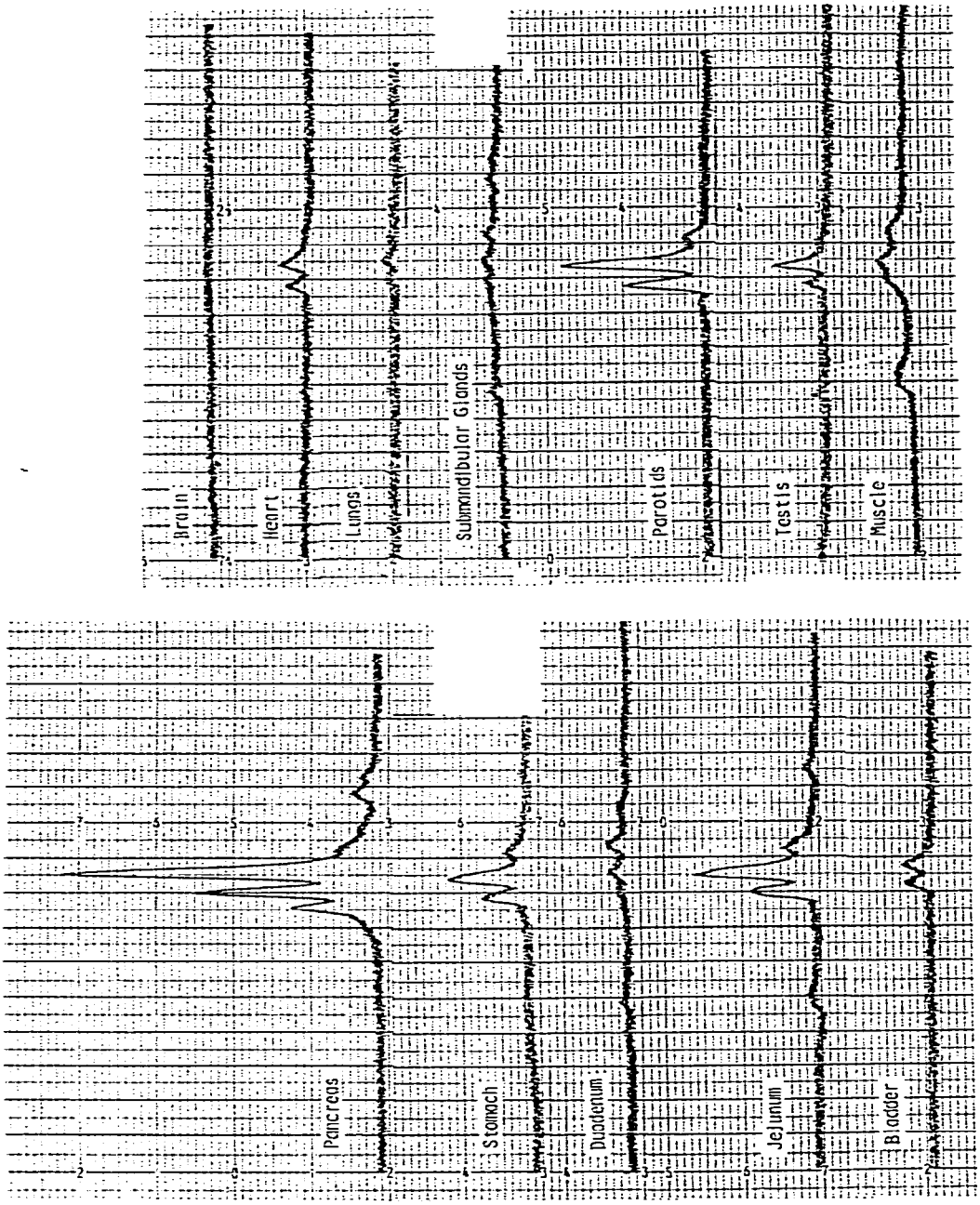

Fig. 3. HPLC elution profiles (cadmium) of MT from various organs of monkeys of the 30 mg/kg group at 153 weeks.

abled us to obtain a quicker and better separation of tissue MTs. Also, monkey renal cortex and liver were found to contain more kinds of MTs than other animal species in the present study. By ion-exchange chromatography Kimura et al. [8] separated seven MTs from African green monkey kidney cells pretreated with cadmium though not sufficiently clearly, and five MTs from the kidneys and liver of a rhesus monkey previously given fourteen subcutaneous injections of cadmium at a dose level of 3 mg/kg. Our MT 1 seemed to correspond to MTs A and B of Kimura et al. [8]. It has not been fully elucidated why the number of separated MTs are different in the two studies.

As previously reported [6], the total MTs in the renal cortex was elevated with the increase in cadmium level, except at the 100 mg/kg level, at which level renal damage was found. Because the biological half-time of MT was reported to be short [3], the MT concentration in the renal cortex of the 100 mg/kg group was expected to decrease rapidly once MT production in the renal cortex was depressed due to renal damage. MT 1 especially was much smaller at the 100 mg/kg level than at the 30 mg/kg level. MT 1 production might be in parallel with the viability of the renal cortex cells.

Cadmium is thought to be detoxified by binding with MT [3, 9]. In the present experiments almost the same amounts of LMW-Cds were found at the 10, 30 and 100 mg/kg levels, at which levels renal dysfunctions were detected at the time of experiment (after the 120th week only for the 10 mg/kg group) [6]. This fact might suggest that LMW-Cds are an etiological agent for inducing nephropathy. The hypothesis accorded well with our previous reports that non-MT Cd is responsible for inducing nephropathy [6, 10].

ACKNOWLEDGEMENTS

We express heartfelt thanks to Drs. F. Akahori and T. Masaoka of Azabu University School of Veterinary Medicine for providing us with biological specimen from monkeys fed cadmium for a long period.

This work was supported by grants from the Japan Environment Agency (1977—1981) and the Japanese Ministry of Education, Science and Culture (Grant-in-Aid for Scientific Research B-56480150, 1981).

REFERENCES

1 M. Margoshes and B.L. Vallee, J. Amer. Chem. Soc., 79 (1957) 4813.
2 J.H.R. Kägi and B.L. Vallee, J. Biol. Chem., 235 (1960) 3460.
3 J.H.R. Kägi and M. Nordberg (Editors), Metallothionein, Birkhäuser, Basel, 1979.
4 E.C. Foulkes (Editor), Biological Role of Metallothionein, Elsevier, New York, 1982.
5 K.T. Suzuki, Anal. Biochem., 102 (1980) 31.
6 K. Nomiyama, H. Nomiyama, F. Akahori and T. Masaoka, in Proc. 3rd Int. Cadmium Conf., Miami, Metal Bulletin, London, in press.
7 K.T. Suzuki and M. Yamamura, Biochem. Pharmacol., 29 (1980) 689.
8 M. Kimura, N. Otaki and T. Kakefuda, in J.H.R. Kägi and M. Nordberg (Editors), Metallothioneins, Birkhäuser, Basel, 1979, p. 187.
9 M. Webb (Editor), The Chemistry, Biochemistry and Biology of Cadmium, Elsevier, Amsterdam, 1979.
10 K. Nomiyama and H. Nomiyama, in E.C. Foulkes (Editor), Biological Role of Metallothionein, Elsevier, New York, 1982, p. 47.