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THE DISTRIBUTION AND BINDING OF ZINC IN THE HIPPOCAMPUS[1]

SHERYL M. SATO,[2] JOHN M. FRAZIER, AND ALAN M. GOLDBERG

The Johns Hopkins University, School of Hygiene and Public Health, Department of Environmental Health Sciences, Baltimore, Maryland 21205

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Abstract

Quantitative and qualitative studies suggest that zinc is concentrated in the mossy fiber boutons of the hippocampus and is believed to exist as a chelatable cytosolic pool. These studies were aimed at testing the hypothesis that a zinc-binding protein(s) or an amino acid pool in the cytosol is responsible for the sequestration of zinc. For comparison with the hippocampus, the cerebellum was chosen as a control region since it has been reported to contain lower levels of zinc both quantitatively and qualitatively. Initially, we confirmed that a quantitative difference in the levels of zinc exists between the hippocampus (12.59 ± 0.85 μg of zinc/gm wet weight tissue, $\bar{X}$ ± SD) and that this difference is reflected in cytosolic zinc levels. Using Ultrogel AcA 34 gel permeation chromatography, three major zinc-binding species were resolved. Two of these binding species appeared to account for most of the difference observed in the cytosolic levels of the two brain regions. Molecular weight criteria and differential pulse polarography behavior suggest that one of the species is a metallothionein-like protein. Based upon both molecular weight and ion exchange chromatography criteria, the other binding species may be a zinc-glutathione complex. There are no qualitative differences in the zinc-binding species localized in the cytosol of the hippocampus as compared with the cerebellum. However, the amount of binding species, in particular, the metallothionein-like protein and the putative zinc-glutathione complex, is greater in the hippocampus. These findings support the hypothesis that a cytosolic zinc-binding protein(s) may be responsible for the sequestration of zinc observed in the hippocampus.

Several histochemical studies utilizing Timm's silver sulfide stain (Timm, 1958) or dithizone (Maske, 1955; Fleischhauer and Horstmann, 1957; Frederickson et al., 1981), in addition to autoradiographical studies utilizing ^{65}Zn (von Euler, 1962; Hassler and Söremark, 1968; Dencker and Tjälve, 1979), have demonstrated a concentrated zinc pool localized in the hippocampus. The histochemical observations, demonstrating higher levels of zinc in the hippocampus, were confirmed by quantitative analysis of zinc in several mammalian species ranging from rodents to man (Hu and Friede, 1968; Harrison et al., 1968; Wong and Fritze, 1969; Fjerdingstad et al., 1974, 1977; Hock et al., 1975). More detailed electron microscopic studies utilizing histochemistry have shown zinc to be localized in the mossy fiber boutons of the hippocampus (Ibata and Otsuka, 1969; Haug, 1975). This striking and unique localization of zinc in the mossy fiber boutons of hippocampus has led many to speculate on the functional role for zinc, including a role in synaptic transmission (von Euler, 1962; Crawford and Conner, 1973; Hesse, 1979). Recent evidence suggests that the zinc pool associated with the mossy fiber boutons may be released by nerve stimulation. A study by Sloviter (1984) has demonstrated a selective loss of Timm's staining in the hippocampal mossy fiber boutons after sustained electrical stimulation of the preforant pathway, while Howell and Frederickson (1984) reported stimulation-induced release of zinc in rat hippocampal slices. The biochemical nature of the zinc pool, which is associated with the mossy fiber boutons of the hippocampus, has not been identified and will be the subject of this study. An examination of the molecular nature of the zinc pools found in the hippocampus can provide information that may enable us better to define the functional role of zinc in the mossy fiber bouton pathway. For comparison with the hippocampus, the cerebellum was

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[2] To whom correspondence should be addressed at Johns Hopkins University, School of Medicine, Department of Neuroscience, Room 914, 725 Wolfe St., Baltimore, MD 21205.

chosen as the control region because it displays little heavy metal staining and has been previously shown quantitatively to have lower levels of zinc. We tested the hypothesis that a zinc-binding protein(s) and/or an amino acid pool may be responsible for sequestering zinc in the hippocampus.

Materials and Methods

Experimental animals. Male Long-Evans hooded rats (Blue Spruce Farms, Altamont, NY), weighing 300 to 350 gm, were used in this study. Animals were housed in plastic cages with filter tops, given free access to commercial rat chow (Charles River) and tap water, and maintained on a 12-hr light-dark cycle.

Subcellular fractionation method. We used a modification of a differential centrifugation method developed by Gray and Whittaker (1962). Our interest was in maximizing the recovery of the cytosolic zinc; therefore, hypo-osmotic shock of both pellet 1 and pellet 2 was used to release any occluded cytoplasm (see Fig. 1).

Biochemical assays. To verify the subcellular fractionation method, several markers were assayed. Deoxypentose was assayed by the method of Burton (1956). Fumarase was determined by the method of Racker (1950). Lactate dehydrogenase (LDH) was assayed using a Sigma LDH assay kit. Protein was determined by the Biorad assay kit. In normalizing data in Table IV, protein was assayed by the method of Lowry et al. (1951), and DNA was extracted by the method of Munro and Fleck (1972) and assayed by the method of Burton (1956).

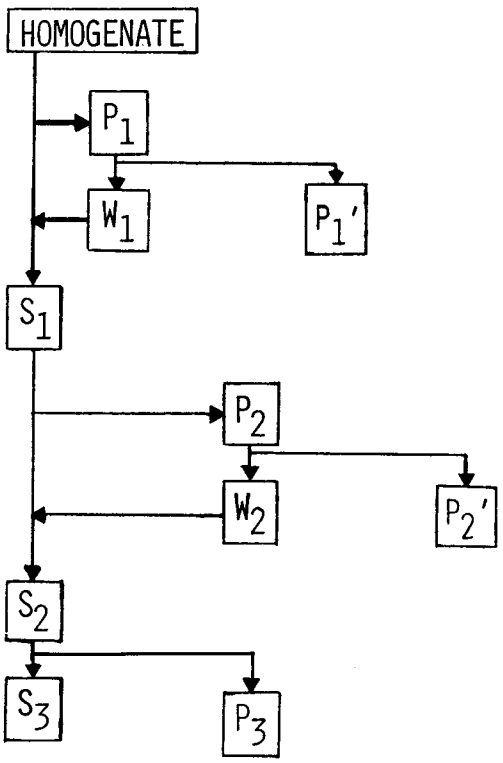

Figure 1. Method of subcellular fractionation. The hippocampi or cerebella obtained from six rats were dissected by the method of Glowinski and Iverson (1966) and homogenized (1 to 9, W/V) in a Potter Elvehjem homogenizer in 0.32 M sucrose, 50 mM Tris, pH 7.4. The homogenate was centrifuged at 900 × *g* for 10 min. Hypo-osmotic shock of pellet 1 (P_1) was accomplished with rehomogenization of the pellet in 3.0 ml of deionized, distilled water per gram of starting tissue. The wash (W_1) was combined with the first supernatant (S_1) and centrifuged at 12,500 × *g* for 20 min. Hypo-osmotic shock of pellet 2 (P_2) was accomplished with rehomogenization of the pellet in 2.0 ml of deionized, distilled water per gram of starting tissue. The second wash (W_2) was combined with supernatant 2 (S_2) and centrifuged at 100,000 × *g* for 1 hr, yielding pellet 3 (P_3) and the cytosol (S_3).

Zinc-free conditions. All glassware was soaked in 10% nitric acid for 24 hr and rinsed four times with deionized, distilled water. All solutions were eluted through a chelating resin (Chelex-100, 50 to 100 mesh, sodium form, Biorad Laboratories) to remove any zinc contamination. Column gels were washed with 2 bed volumes of 1% EDTA and 5 bed volumes of deionized water to remove zinc present in the gel. Dialysis tubing was soaked for several days in 2% EDTA to remove zinc. The need for caution in eliminating potential zinc contamination cannot be overemphasized.

Materials. Column chromatography was performed using Ultrogel ACA 34 (LKB), Sephadex G-75 and Sephadex G-25 (Pharmacia), DEAE-Sephacel (Sigma), and columns, 1.6 × 70 cm (Pharmacia). Columns were calibrated with blue dextran (Pharmacia Fine Chemicals), bovine serum albumin (Sigma), metallothionein A (see "Purification of Metallothionein"), myoglobin (Sigma), bacitracin (Sigma), and 3-OH-tyramine (Sigma). ^{109}Cd (carrier-free) was purchased from New England Nuclear. Membrane dialysis tubing (Spectrapor, M_r-3500 cut off) was purchased from Spectrum Industries.

Determination of zinc and cadmium. Subcellular fractions were wet-ashed with 5 ml of Ultrex nitric acid (Baker) and decolorized with 2 ml of 30% hydrogen peroxide (Fisher Scientific). Zinc and cadmium were analyzed using a flame atomic absorption spectrophotometer (Varian AA5) at 213.9 nm and 228.9 nm, respectively. National Bureau of Standards standard reference material 1577 bovine liver was digested and analyzed concurrently with samples for quality control.

Differential pulse polarography. The method of differential pulse polarography was described by Palecék and Pechan (1971) and was applied to metallothioneins by Olafson and Sim (1979). The polarograph utilized was a E505 Metrohm Herisau with a C26 Polarcord recorder.

Purification of metallothionein. The metallothionein standard was prepared by the method of Ohi et al. (1981) with some modifications. Briefly, these modifications were as follows: The metallothionein peak was identified by the presence of cadmium at a V_e/V_0 ratio of 1.9 to 2.0 on a Sephadex G-75 column using atomic absorption spectrophotometry. Final purification was accomplished using ion exchange chromatography (DEAE-Sephacel), and proteins were eluted with a gradient of 0.05 M to 0.2 M Tris, pH 8.6, at 23°C. Purity of the two isoproteins, metallothioneins A and B, was established using a nondenaturing 8% polyacrylamide gel electrophoresis method described by Laemmli (1970).

Results

Validation of the subcellular fractionation procedure. With the modification in the subcellular fractionation

TABLE I

The distribution of subcellular fractionation markers

LDH, fumarase, deoxypentose, and protein activity were determined as described under "Materials and Methods." The data presented are the summay of two independent assays, in triplicate, for each subcellular fractionation marker.

Component	Concentration in Homogenate	Distribution of Markers in Subcellular Fractions (Percentage of Total Recovered Material)				Recovery (Percentage of Homogenate)
		P_1	P_2	P_3	S_3	
Hippocampus						
LDH (units/gm of tissue)	64.38	23	4	2	70	86
Fumarase (units/gm of tissue)	8.65	44	48	1	7	84
Deoxypentose (mg/gm of tissue)	6.98	86	ND[a]	4	10	87
Protein (mg/gm of tissue)	1.26	40	15	7	38	104
Cerebellum						
LDH (units/gm of tissue)	54.45	23	2	2	73	88
Fumarase (units/gm of tissue)	16.21	52	40	1	7	82
Deoxypentose (mg/gm of tissue)	13.22	92	1	2	4	110
Protein (mg/gm of tissue)	1.48	48	10	6	35	104

[a] ND, not detectable.

TABLE II

The distribution of subcellular fractionation markers in hippocampus and cerebellum

The relative specific activity is obtained by dividing the specific activity (units per milligram of protein) for each fraction by the specific activity of the whole homogenate. The average of two independent determinations, in triplicate, is presented for each subcellular fractionation marker. In the overall study, less than 10% variability was apparent for each assay. Values in italics indicate the proper localization of each subcellular fractionation marker.

	Homogenate	Relative Specific Activity			
		P_1	P_2	P_3	S_3
Hippocampus					
Deoxypentose	1.0	*2.15*	ND[a]	0.54	0.24
Fumarase	1.0	0.91	*2.81*	0.10	0.14
LDH	1.0	0.48	0.25	0.30	*1.50*
Cerebellum					
Deoxypentose	1.0	*2.00*	0.10	0.04	0.10
Fumarase	1.0	0.84	*3.42*	0.10	0.18
LDH	1.0	0.40	0.12	0.26	*1.80*

[a] ND, not detectable.

TABLE III

Subcellular distribution of zinc in the hippocampus and the cerbellum

The zinc analysis was performed as described under "Materials and Methods." Data are the results of six experiments utilizing six rats per experiment, and data are expressed as $\bar{X} \pm SD$. Significance at the $p < 0.01$ level, denoted by *, was established by the Student's t test.

	Hippocampus		Cerebellum	
	Micrograms of Zinc per Gram Wet Weight Tissue	Percentage of Total	Micrograms of Zinc per Gram Wet Weight Tissue	Percentage of Total
Homogenate	12.59 ± 0.85		9.25 ± 0.59*	
P_1	4.20 ± 0.30	34	4.83 ± 0.39	50
P_2	1.92 ± 0.54	15	0.79 ± 0.12*	8
P_3	1.05 ± 0.38	9	0.84 ± 0.39	9
S_3	5.26 ± 0.86	42	3.19 ± 0.54*	33
Recovery	95 ± 8.5%		104 ± 4.9%	

TABLE IV

Zinc levels in the hippocampus and the cerebellum

The zinc levels were determined as described under "Materials and Methods." The DNA and protein determinations were made in different animals of the same sex, weight, and strain and were normalized on a per gram weight basis to compare with the zinc levels. Six independent values, in triplicate, were generated for the DNA and protein assays.

	Micrograms of Zinc per gram Wet Weight	Micrograms of Zinc per Milligram of DNA	Micrograms of Zinc per Milligram of Protein
Hippocampus	12.59	8.28	0.100
Cerebellum	9.25	1.05	0.078

procedure, three biochemical markers were assayed to assure that the distribution of expected cellular components was not altered. The distribution of deoxypentose, an indicator of nuclei; fumarase, a mitochondrial marker; LDH, a cytosolic marker, and protein is shown in Table I. In Table II, the relative specific activity is a measure depicting the enrichment of certain subcellular fractions with the appropriate subcellular marker. These data are comparable with values in the literature (Fonnum, 1968; Whittaker and Barker, 1972).

Comparison of zinc levels in the hippocampus and the cerebellum. The subcellular distribution of zinc in the two brain regions was determined, and the data are summarized in Table III. We observed a significant difference in zinc levels between the hippocampus and the cerebellum—the cerebellum being 36% lower in zinc content. Although the mitochondrial level of zinc (pellet 2) was less in the cerebellum, this difference is small when compared with the difference noted in cytosolic zinc levels between the two regions. The hippocampal cytosol contains 40% more zinc compared with the cerebellum, and this can account for the regional differences in zinc. The recovery of zinc was calculated for each experiment to control for zinc contamination, a major problem in trace element studies.

A comparison of zinc levels in the hippocampus and the cerebellum, normalized to grams wet weight, milli-

grams of DNA, and milligrams of protein, is shown in Table IV. In all cases the hippocampus has greater levels of zinc compared with the cerebellum. Most striking are the data normalized to milligrams of DNA, a measure indicative of cell number. Per milligram of DNA, the hippocampus has 8 times more zinc than the cerebellum.

Cytosolic zinc-binding species in the hippocampus and the cerebellum. After establishing regional differences in zinc levels between the hippocampus and cerebellum, we compared the distribution of cytosolic zinc in the two brain regions on Ultrogel AcA 34 (Fig. 2, *A* and *B*). Three major zinc-binding species were resolved in both brain regions which had V_e/V_0 ratios of 1.9, 2.4, and 2.7, corresponding roughly to M_r = 60,000 and 13,000 and a small molecular weight species eluting just prior to the internal volume of the column. The species will be referred to as peak 1, peak 2, and peak 3, respectively. The quantitative difference observed in cytosolic levels between the cerebellum and the hippocampus can be accounted for by the differences in amounts of zinc found in peaks 2 and 3. After exhaustive dialysis of the hippocampal cytosol, it was noted that all three binding species were retained despite the loss of the small molecular weight species having absorbance at 280 λ (see Fig. 3).

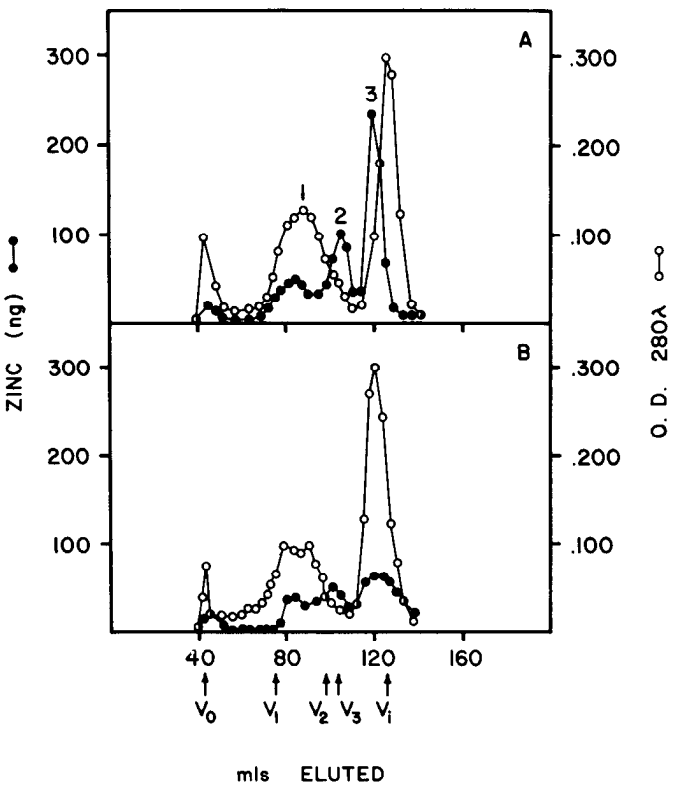

Figure 2. Separation of cytosolic zinc-binding species on Ultrogel AcA 34. Cytosols from the hippocampus (*A*) and cerebellum (*B*) were prepared and fractionated simultaneously on matched Ultrogel AcA 34 columns. Eluted fractions were monitored for zinc by atomic absorption, shown in the *solid circles*, and 280 λ shown in the *open circles*. The columns were calibrated as follows: V_0 = void volume, determined by blue dextran; V_t = total volume of the column determined by 3-OH-tyramine; V_1 = position of bovine albumin, V_e/V_0 = 1.8; V_2 = position of myoglobin, V_e/V_0 = 2.3; and V_3 = position of metallothionein A, V_e/V_0 = 2.4.

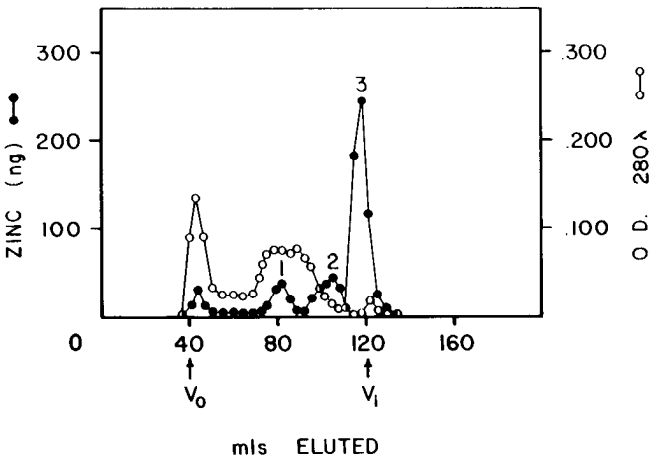

Figure 3. Separation of hippocampal cytosolic zinc-binding species after dialysis. Cytosol from the hippocampus was dialyzed against 2000 ml of 50 mM Tris, 0.9% NaCl, pH 7.4, at 4°C for 12 to 14 hr and was chromatographed on Ultrogel AcA 34.

TABLE V

Polarographic characterization of cytosolic zinc-binding species

Polarography was performed as described under "Materials and Methods." Heat treatment involved heating the samples at 80°C for 1 min. Data were obtained from four experiments; samples were assayed in duplicate and are expressed as $\bar{X} \pm SD$. Purified metallothionein A was used to establish a standard curve. A wave potential at −1.47 V was observed for metallothionein A.

	Before Heat Treatment	After Heat Treatment
	nAmps/μG of protein	
Hippocampus		
Cytosol	0.33 ± 0.16	0.37 ± 0.07
Peak 1	0.17 ± 0.05	No response
Peak 2	1.95 ± 0.60	1.77 ± 0.37
Peak 3	No response	
Cerebellum		
Cytosol	0.20 ± 0.06	0.31 ± 0.09
Peak 1	0.17 ± 0.04	No response
Peak 2	0.86 ± 0.07	1.10 ± 0.39
Peak 3	No response	

Partial characterization of cytosolic zinc-binding species. From the Ultrogel AcA 34 chromatography, it was apparent that peak 2 might be a metallothionein. Therefore, we used a method that was developed to identify metal-binding proteins rich in sulfhydryl groups, in particular, metallothioneins. Peaks 1, 2, and 3, obtained from Ultrogel AcA 34 chromatography, were further characterized using differential pulse polarography, and the data are shown in Table V. Heat treatment was also used in the characterization because metallothioneins are known to be heat-stable. Peak 1 gave a small response, which was heat-labile. Peak 3 did not respond in the polarography assay, most likely because it was too small to be detected. Peak 2 gave a response similar to that observed with rat metallothionein A. Like metallothionein A, peak 2 was heat-stable and had a maximum wave potential at −1.47 V. The zinc in metallothionein is exchangeable with cadmium, and it is this property that allowed us to characterize peak 2 further. ^{109}Cd was demonstrated to exchange with the zinc bound in peak

2. The result of the *in vitro* labeling of hippocampal cytosol with ^{109}Cd is shown in Figure 4. The ^{109}Cd-labeled peak 2 was noted to co-elute with rat metallothionein. Also under these conditions, the ^{109}Cd did not exchange with peak 3. The positions of hippocampal peak 2 compared with rat liver metallothionein A and B on DEAE-Sephacel are shown in Figure 5, *A* and *B*, respectively. Metallothionein A and B have different elution profiles compared with the hippocampal sample, suggesting that different charged species are found in the hippocampus.

To characterize peak 3 further, the small molecular weight species, Sephadex G-25 chromatography was used to get a better indication of peak 3's molecular weight. After rechromatographing peak 3 on Sephadex G-25, a peak eluting just prior to bacitracin was observed, suggesting a M_r = 1500. By virtue of its similar size, we tested the possibility of the small molecular weight species, peak 3, being identified as a zinc-glutathione complex. A zinc-glutathione complex was made and shown to co-elute with the hippocampal zinc-binding species, peak 3, on both Sephadex G-25 and DEAE-Sephacel, shown in Figures 6 and 7, respectively.

Discussion

Although histochemical and autoradiographical studies have demonstrated the presence of zinc, they have not been sufficiently sensitive to resolve the exact location or form of the zinc associated with the mossy fiber boutons. However, from the studies of Ibata and Otsuka (1969) and Haug (1975), it is believed that the zinc is not concentrated in the mitochondria or associated with membranes in the synaptic region. Therefore, we focused our efforts on investigating cytosolic zinc pools. It was not possible for us to look specifically at just mossy fiber bouton-associated zinc. Consequently, we investigated cytosolic zinc-binding species derived from the whole hippocampus. Initially, we modified the Gray and Whittaker (1962) fractionation procedure in order to maximize our recovery of cytosol. Crawford and Conner (1972) suggested that a portion of cytosolic zinc in the hippocampus was trapped within large synaptosomes, more definitively within the large mossy fiber boutons believed to be found in pellet 1. Therefore, we employed hypo-osmotic shock of pellet 1 and pellet 2 to release occluded cytoplasm. There is also the possibility that the zinc is contained inside the synaptic vesicles (Ibata and Otsuka, 1969; Haug, 1975; and Hesse, 1979). It is not known from our study whether these putative zinc-containing vesicles are hypo-osmotically labile. Therefore, we cannot determine the contribution of vesicular zinc pools to the total cytosolic pool of zinc. Our results on the subcellular distribution of zinc are similar to those reported by Crawford and Conner (1972). However, after hypotonic shock of pellets 1 and 2, we observed that more of the total zinc is localized in the cytosolic fraction.

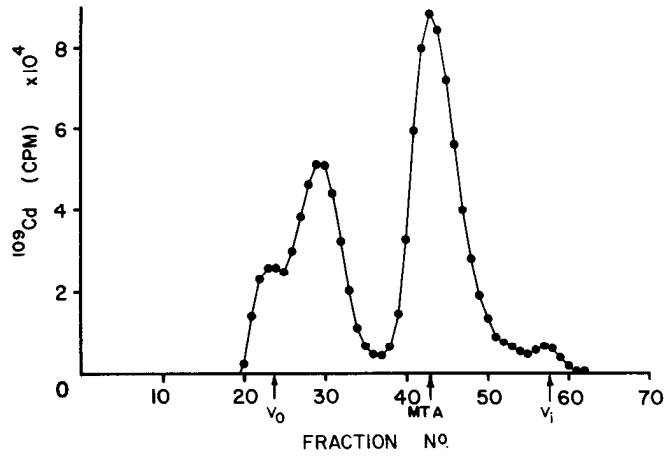

Figure 4. In vitro ^{109}Cd exchange in hippocampal cytosol. The hippocampal cytosol was equilibrated for 1 hr with 3.75 μCi (carrier-free) of ^{109}Cd. Cytosolic ^{109}Cd-labeled species were separated on Ultrogel AcA 34, and fractions were monitored for ^{109}Cd. Proteins were eluted with 0.02 M Tris, pH 8.6, at 4°C. The positions of metallothionein A (*mt A*) V_0 (blue dextran), and V_i (3-OH-tyramine) are shown by *arrows*.

We can attribute the quantitative differences observed in cytosolic levels between the hippocampus and the cerebellum to two zinc-binding species, specifically, a metallothionein-like protein and a putative zinc-glutathione complex. We can only speculate as to the relative biological importance of these two zinc-binding species in the brain. The metallothionein-like protein of the brain reported by Chen and Ganther (1975) and Oskarsson et al. (1982) may play a similar role to that attributed to liver metallothionein, that is, possibly act as a storage pool of cellular zinc important in zinc homeostasis (Webb and Cain, 1982). Glutathione has been known to form complexes with copper (Marzullo et al., 1977) and methyl-mercury (Thomas and Smith, 1979) in the brain. Glutathione also has been shown to form complexes with zinc *in vitro* (Fuhr and Rabenstein, 1973), and these authors suggest that the zinc molecule most likely reacts with the sulfhydryl groups of two glutathione molecules to form the complex. Therefore, the formation of a zinc-glutathione complex in the brain would not be unprecedented.

More information about the affinity of the glutathione for zinc is needed. It is interesting to note that this zinc-binding species did not exchange its zinc for ^{109}Cd after 1 hr of incubation. Since the study was not conducted under conditions of saturation, other zinc-binding sites present could have competed more effectively for the ^{109}Cd.

A puzzling factor is that zinc-glutathione complexes are not found in the liver where glutathione is abundant (J. M. Frazier, unpublished observation). Perhaps this observation at the present time can be explained by suggesting that brain glutathione has a different compartmentalization as compared to liver glutathione. Also, there may be higher concentrations of zinc-binding ligands with greater zinc affinity relative to glutathione in the liver.

The role for a zinc-glutathione complex in brain is unknown. However, it could behave in a fashion like the copper-glutathione complex in the brain which has been shown to modulate opiate receptors (Marzullo et al., 1977). Alternatively, the zinc-glutathione complex may be an important intracellular storage pool of zinc involved in zinc homeostasis, playing a role similar to that proposed for metallothionein.

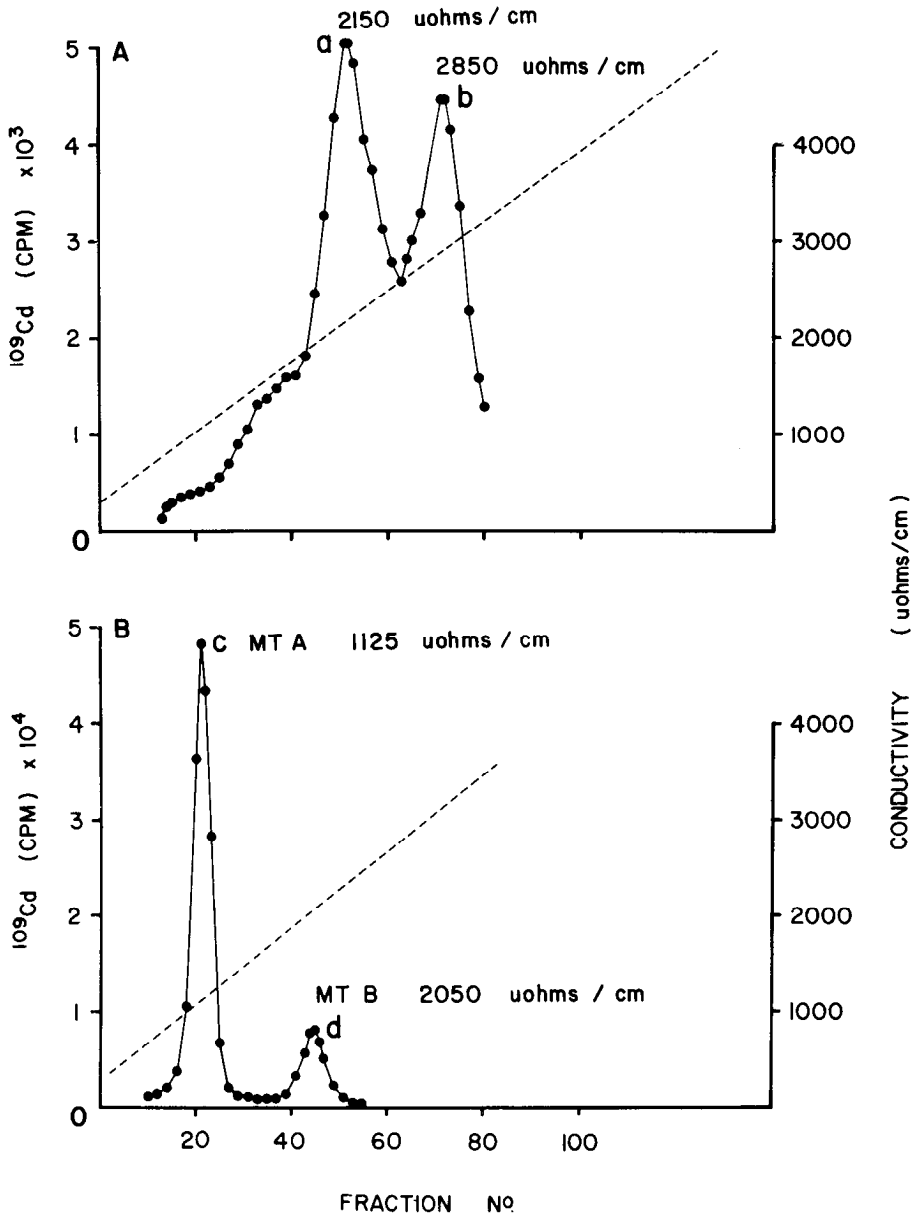

Figure 5. DEAE-Sephacel chromatography of ^{109}Cd-labeled protein. The hippocampal peak 2, obtained from Ultrogel AcA 34 chromatography (see Fig. 4), was pooled and rechromatographed on DEAE-Sephacel (*A*). Positions of rat liver metallothioneins A and B were established (*B*). Proteins were eluted with a gradient (- - -) of 0.02 M Tris to 0.25 M Tris, pH 8.6, at 23°C.

The question concerning the speciation of the zinc localized in the hippocampal mossy fibers has been addressed in other studies. In one study, glutamic acid, the neurotransmitter thought to be used in the mossy fiber boutons of the hippocampus, has been suggested as a possible zinc-binding site (Crawford and Conner, 1973). Although our data do not support glutamic acid to be a possible zinc-binding species, the formation of a glutamic acid-zinc complex cannot be entirely ruled out. Additionally, a report by Kuznetsov et al. (1973) described a protein uniquely localized in the hippocampal superior. However, the differences in the methods used in their study and ours make a comparison of proteins impossible.

In summary, we established a quantitative difference in zinc levels between the hippocampus and the cerebellum and that this difference is reflected in cytosolic zinc levels. The amounts of two cytosolic zinc-binding species can account for most of the quantitative differences noted between the two brain regions. These species were partially characterized as being a metallothionein-like protein and the other as being a zinc-glutathione complex. In conclusion, our data on cytosolic zinc-binding species support our hypothesis that a zinc-binding protein(s) may be responsible for the sequestration of zinc in the hippocampus. It will be important in future studies to determine what role these zinc-binding species play in hippocampal mossy fiber bouton function. For instance,

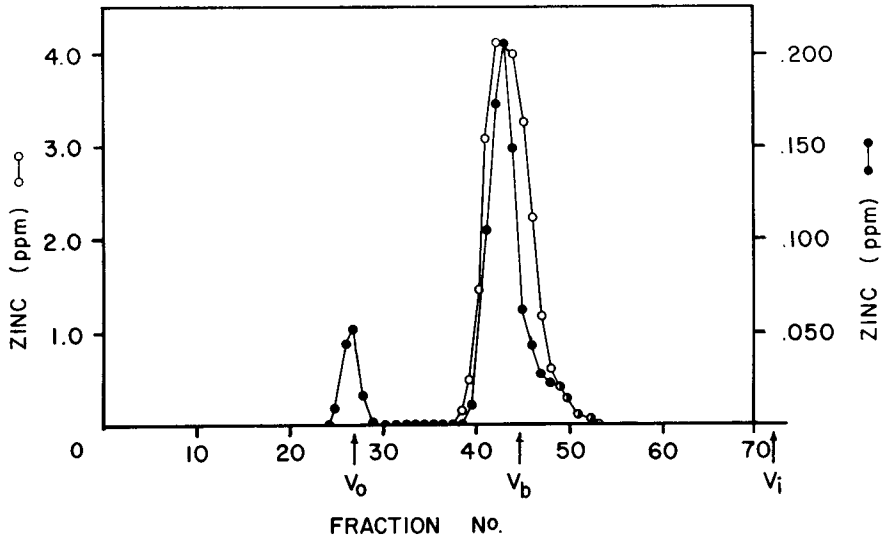

Figure 6. Co-elution of the zinc-glutathione complex and hippocampal zinc-binding species on Sephadex G-25. The small molecular weight zinc-binding species from the hippocampus was rechromatographed on Sephadex G-25, shown in the *solid circles*. A zinc-glutathione complex was prepared by mixing reduced glutathione (Sigma) and zinc oxide (Fisher Scientific) in a 6:1 molar ratio, and its elution profile is shown in the *open circles*.

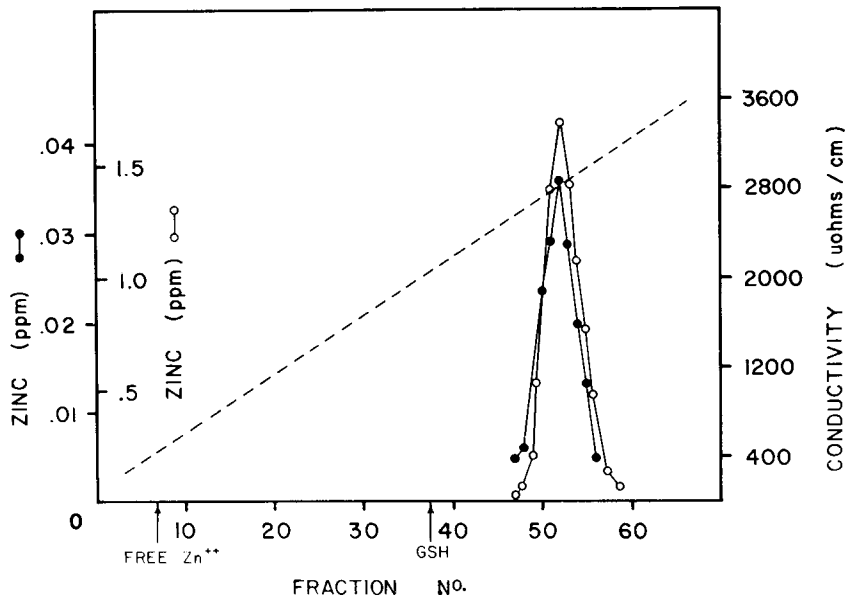

Figure 7. Co-elution of the zinc glutathione complex and hippocampal zinc-binding species on DEAE-Sephacel. The hippocampal zinc-binding species and zinc-glutathione complex obtained from Sephadex G-25 chromatography (see Fig. 6) were desalted and rechromatographed on DEAE-Sephacel as shown in the *solid* and *open circles*, respectively. A gradient, 0.01 M Tris to 0.15 M Tris, pH 8.6, was used to elute the samples (– – –). The positions of reduced glutathione (*GSH*) and free zinc (Zn^{++}) are shown by *arrows*.

what is the molecular nature of the zinc that is believed to be released by electrical stimulation from the mossy fiber boutons? In light of the recent hypothesis linking mossy fiber bouton zinc and the initiation of sympathetic sprouting (Crutcher and Davis, 1981; Stewart et al., 1982; Frederickson et al., 1984), it will also be of interest to determine whether these zinc-binding species exhibit trophic properties, thus influencing hippocampal plasticity.

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