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REACTIVITY OF METALLOTHIONEINS OF FROG *Rana ridibunda* TREATED BY COPPER AND ZINC IONS

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The metal-buffering and stress proteins metallothioneins (MTs) of frog are characterised by unusually high content of copper as for vertebrate animals and instability that was shown in our previous studies. They easily lost copper and especially zinc under unfavourable conditions. The aim of this study was to examine the reactivity of SH groups in the MTs from the liver of frog Rana ridibunda after the effect of Cu^{2+} (0.01 mg/l) and Zn^{2+} (0.1 mg/l) ions on the organism during 14 days. The α- and β-domains of MTs with molecular weights of about 4 kDa were separated by the size-exclusion chromatography on Sephadex G-50. Unlike higher vertebrates, frogs demonstrated higher reactivity of α-domain than β-domain with the Ellman's reagent (DTNB). The signs of partial oxidations in β-domain included the creation of by-products with molecular weight about 12 kDa, low reactivity of SH-groups, and typical of -S-S-bonds peculiarities of UV-spectra. The effect of both metal ions on frog provoked the elevation of SH-groups reactivity in α-domain with the appearance of by-product with molecular weight of 16 kDa and its reduction in β-domain. The incubation of MTs of control animals with 0.5 and 5.0 mM of H_2O_2 did not affect its chromatographic characteristics. In the frogs loaded by Cu^{2+} and Zn^{2+} the effect of 5.0 mM H_2O_2 on MTs provoked the release of 4 kDa product. So the α-domain is responsible for the increased release of metals from injured MTs in frogs, whereas extremely high oxidizability of β-domain makes its participation in the exchange of metals elusive and provokes the aggregation of MTs.

Key words: copper, zinc, metallothionein, oxidation, frog, Rana ridibunda.

Metallothioneins (MTs) are thermostable, highly inducible rich in SH-groups intracellular proteins with conservative cysteine number and position. They are considered to participate in the storage and detoxification of such metals as zinc (Zn), copper (Cu), and cadmium (Cd), and in the scavenging of reactive oxygen species in a wide range of living organisms [1]. The vertebrate MTs are composed of two globular domains [2]. The N-terminal right-handed β-domain binds three bivalent metal ions (usually Zn(II) or Cd(II)) and the C-terminal right-handed α-domain binds four bivalent metal ions. Each domain can also bind six monovalent ions, usually Cu(I). In heterometallic MTs, Cd and Zn are preferentially located in the α-domain, and Cu(I)-binding is more intrinsic to the β-domain [3]. It is considered that different behaviours of MTs in various species of vertebrate animals are linked to their domain structure specificity and interaction between the two domains. For example, higher Zn mobility was demonstrated rather in MTs of fish than of mouse [4].

Only a few studies have been devoted to the MTs in amphibians [5–8]. They reflected an unusually high as for vertebrate animals rate of copper in their composition, a high variability of the chromatographic forms of MTs, especially in the animals from polluted area, and a tendency to create insoluble aggregates. The weakness of the MTs ability to bind copper and especially zinc under the effect of ecologically realistic natural pollution or under the exposure to the thiocarbamate fungicide was demonstrated [7]. Therefore, the aim of this study was to clarify these peculiarities by examining the MTs reactivity in frogs loaded by the ions of Cu^{2+} or Zn^{2+}.

Materials and Methods

Sampling and laboratory setup

The individuals of frog *Rana ridibunda* were collected in July at the forestry site near the riverhead of the Seret (Ternopil region). Any sources of industrial pollution and big farms are absent in surrounding. The individuals were transported to the laboratory in the cages with native water, kept in 40 l plastic containers (8 animals per container) and fed with commercial sticks "Turtle menu" (Akvarius). Experiments were performed with permission of the Ministry of Environment Protection of Ukraine, No. 292/2007. One group was control and two other treated by 0.01 mg Cu^{2+} ($CuSO_4$)/l or 0.1 mg Zn^{2+} ($ZnSO_4$)/l, correspondingly. The used concentrations corresponded to situations of field pollution in the area [9]. Water was renewed

every two days. The remaining food was always cleaned out. Animals were anesthetized with ether and sacrificed after 14 days of exposure. The liver was dissected out. Each procedure of tissue analysis was carried out at a temperature of about 4 °C.

Chemicals

5,5′-Dithio-bis(2-nitrobenzoic acid) (DTNB), β-mercaptoethanol, EDTA, chymotrypsinogen, cytochrome *c*, insulin chain B oxidized, myoglobin, Sephadex G-50 superfine, Sephadex G-25, subtilisin and trypsin inhibitor were purchased from Sigma. All other chemicals were of analytical grade.

Metallothioneins and their separate domains isolation

MTs were obtained as thermostable proteins by size-exclusion chromatography on Sephadex G-50 [10]. Tissue samples from five individuals of the group were pooled in aliquot quality (total mass 1 g) and homogenized in ice-cold 10 mM Tris-HCl buffer, pH 8.0, containing 10 mM 2-mercaptoethanol. The obtained 5% homogenate was centrifuged at 10 000 g for 45 min at 4 °C. The supernatant was incubated under the 85 °C for 5 min and subsequently centrifuged at 10 000 g for 45 min at 4 °C. The obtained thermostable supernatant was applied to the MTs purification. The chromatography on Sephadex G-50 column (1.5 × 80 cm) was carried out in the same buffer at a flow rate 0.33 ml·min^{-1}. Fractions (5 ml) were collected and analyzed for absorbance at 280 and 254 nm. Column calibration was achieved by applying a mixture of the next standards: chymotrypsinogen (25.8 kDa), myoglobin (17.0 kDa), cytohrome *c* (12.3 kDa), trypsin inhibitor (8.4 kDa), insulin chain B oxidized (3.5 kDa). The fractions of peak with high absorbance at 254 nm were pooled (total 10 ml) for the ultraviolet (UV) absorption spectra. MTs-containing fraction was identified based upon peculiar spectral features (comparatively high density ratio D_{254}/D_{280}), thermostability, and low molecular weight [1,2,4].

The α-domain (Cd-saturated form) was prepared by using the Winge and Miklossy procedure [11]. MTs-containing fraction reacted with 4.5 M $CdCl_2$ and a slight excess of EDTA under argon (Ar) atmosphere at room temperature. The 10 mM Tris-HCl buffer, pH 8.0 was used throughout the isolation process. After 1.5 h of incubation, subtilisin was added to the solution in a 20 : 1 protein to enzyme ratio, and the mixture was incubated for 18 h under Ar atmosphere at 37 °C. A precalibrated Sephadex G-50 column (1.5 cm × 50 cm) was used to separate the domain from other smaller digestion fragments and from the EDTA complexes of metal ions. To locate the α-Cd-domain, the fractions were analysed for UV spectra at 250 nm.

The β-domain (Cu-saturated form) was prepared according to the method described by Nielson and Winge [12]. Firstly, apothionein was obtained by MT incubation at pH 3.0 followed by Sephadex G-25 chromatography in 0.05 M HCl to isolate the protein. The verification of apothionein location was performed by UV absorption at 220 nm. In order to avoid oxidation of apothionein or the Cu(I)-containing product below, 5.0 mM β-mercaptoethanol was added to the apothionein and anaerobic conditions were used. Upon neutralization of the protein solution with solid Tris, it reacted with 6 equivalents of 0.6% CuCl in 0.1 M HCl deaerated solution containing 4% NaCl. After 1.5 h, subtilisin was added to the solution in a 20 : 1 protein to enzyme ratio, and the mixture was incubated for 18 h under Ar atmosphere at 37 °C. β-domain was isolated after chromatography over Sephadex G-50.

To quantify apoMT concentrations, the UV absorbance at 220 nm and pH 2, where the metals are completely removed, was used. Under these conditions the molar absorption coefficient is 48000 $M^{-1}\cdot cm^{-1}$. To quantify MTs and their separate domains concentrations, the UV absorbance at 220 nm and pH 7.4 was used. Under these conditions the molar absorption coefficients are for hole MTs – 159000 $M^{-1}\cdot cm^{-1}$, α-Cd-domain – 78900 $M^{-1}\cdot cm^{-1}$ and β-Cu-domain – 69100 $M^{-1}\cdot cm^{-1}$ [13,14]. All MTs chemical analyses were carried out in triplicate.

Thiol reactivity of the separated domains

The thiol reactivity of the separated domains was assayed spectrophotometrically at 412 nm and 25 °C with Ellman's reagent DTNB [5.5′-dithiobis(2-nitrobenzoic acid)] over time against an equivalent amount of DTNB as reference, using the procedure described by Jiang et al. [14] with slight modifications (10 mM Tris-HCl buffer, pH 8.0) for the optimization of the reactivity of DTNB. Reactions were carried out under pseudo-first-order conditions ([DTNB] >> [SH-MT]) in 10 mM Tris-HCl buffer with 0.1 M KCl at pH 8.0. DTNB concentrations ranged from 0.25 to 6.0 mM, and domains concentrations were kept constant at 16 μM. Pseudo-first-order plots were obtained by plotting Ln $[(D_\infty - D_t)]$ vs DTNB concentration.

To determine the effects of the exposure of MTs to H_2O_2, the incubation system consisted of MTs (48 μM) in 10 mM Tris-HCI buffer pH 8.0

and H_2O_2, to a final concentration of 0.5 mM or to 5 mM [15]. After 60 min treatment the incubation system was chromatographed on Sephadex G-50 column at the same condition for MTs isolation.

Statistical analysis

MTs chemical analysis was carried out in triplicate on the joint from five animal aliquots. The UV-spectra were expressed in relative units as the ratio $(D_d - D_c)/D_c$, where D_d and D_c, density of compared samples at the same wavelength. For detection of correlation between spectra expressed in relative units, the Pearson's correlation test was performed at a 0.05 level of significance.All statistical calculations were performed on the separate data with SPSS 15.0 software, Statistica v 7.0 and Excel for Windows-2000.

Results and Discussion

Gel-filtration of a thermostable solution from the liver of frogs revealed two protein fractions (Fig. 1*A*). A low molecular weight fraction, which had an apparent molecular weight of 8 kDa, was identified as the MTs-containing fraction based upon its spectral features (comparatively high density ratio D_{254}/D_{280} 4.2 against 1.5 for the high molecular weight fraction), thermostability, and molecular weight [16]. Running this fraction on anion-exchange chromatography gave two peaks (MT-1 and MT-2) [17], similar to that of the standard MTII from rabbit liver, confirming this identification. The UV spectrum of MTs demonstrated a high level of absorption in the area of 245-255 nm (Fig. 2,*A*), indicating the presence of characteristic metals-thiolate clusters [16]. The effect of Cu^{2+} or Zn^{2+} on frogs did not provoke any changes in the elution of the thermostable proteins but caused a shift in the absorbance maximum in the UV spectra of MTs, specific for each kind of ions (absence of the correlation between spectra, $r = 0.05$, $p > 0.05$).

Incubation of Cd-enriched MTs with EDTA and subtilisin produced α-domain, whilst the preferential binding of Cu(I) to the β domain of the apothionein and the tolerance toward proteolysis of the Cu(I) bound domain permit the isolation of β-domain [12]. The eluted α- and β-domains had the molecular weights of about 4 kDa (Fig. 1,*B*; 1,*C*). In the case of β-domain, chromatography of the proteolytic sample yielded an additional peak with the molecular weight about 12 kDa. The maximum absorption of α- and β-domains in the UV spectra was about 245-255 nm and 260-270 nm, respectively, with the absence of correlation between them ($r = 0.04$, $p > 0.05$). The by-product with molecular weight 12 kDa had the peak as a broader shoulder in the middle UV (Fig. 2,*B*; 2,*C*).

In frogs exposed to Zn^{2+} and Cu^{2+}, separated domains demonstrated different ability to create by-products as compared to control frogs. The α-domain was accompanied by the product with molecular weight about 16 kDa after both kinds of exposure. On the other hand, β-domain lost the ability to create additional form, especially after the effect of Zn^{2+}. In the spectra of 16 kDa, 12 kDa by-products and β-domain after exposure, the typical of MTs hyperchromic effect in the middle of the UV spectrum was not indicated.

To study the sensibility of the thiols to oxidation, we treated MTs with the model oxidant, H_2O_2. In the case of control frogs, this treatment did not reveal any changes in the elution profile of MTs (Fig. 3). In the frogs, affected by Zn^{2+} and Cu^{2+}, the treatment of MTs with higher H_2O_2 concentration (5.0 mM) led to the disappearance of MTs with molecular weight of 8 kDa and appearance of 4 kDa- and 16 kDa-forms. The UV spectra of all forms obtained under the effect of H_2O_2 had the typical peak at the middle UV with the exception of 16 kDa form (Fig. 4). The UV-spectrum of 4 kDa product in the frogs loaded by Zn^{2+} was similar to spectrum of α-domain ($r = 0.64$, at $r(0.95, 23)=0.42$) and differed from the spectrum of β-domain ($r = - 0.59$).

In order to characterize the reactivity of the cysteinyl groups in separate domains, we studied their reactions with a disulfide (DTNB). The reaction with excess DTNB was complete within 120 s and was biphasic with some exceptions (Fig. 5, Table). For the α-domain, a fast reaction phase was exhibited first, followed by a slow reaction phase. For the β-domain and 12 kDa form, the slow step occurred first. The observed rate constants were clearly DTNB dependent (Table). The order of kinetic constants for the first phase was α-domain > 12 kDa-by-product> β-domain and for the second phase β-domain ≈ 12 kDa by-product > α-domain.

The exposure of frogs to Zn^{2+} and Cu^{2+} did not change the specificity of the first phase in the reaction of α- and β-domains or high molecular weight by-products with DTNB. However, the treatment led to changes in the rates of DTNB reactions. In the α-domain, the reactivity of thiol groups was enhanced. In contrast, the thiol groups of β-domain and 12 kDa by-product lost their reactivity and the dependence on the DTNB concentration of the second phase of the reaction. Low weight products (4 kDa) obtained after the effect of 5.0 mM of H_2O_2 on MTs of treated frogs had high reaction rate with DTNB and different dependence

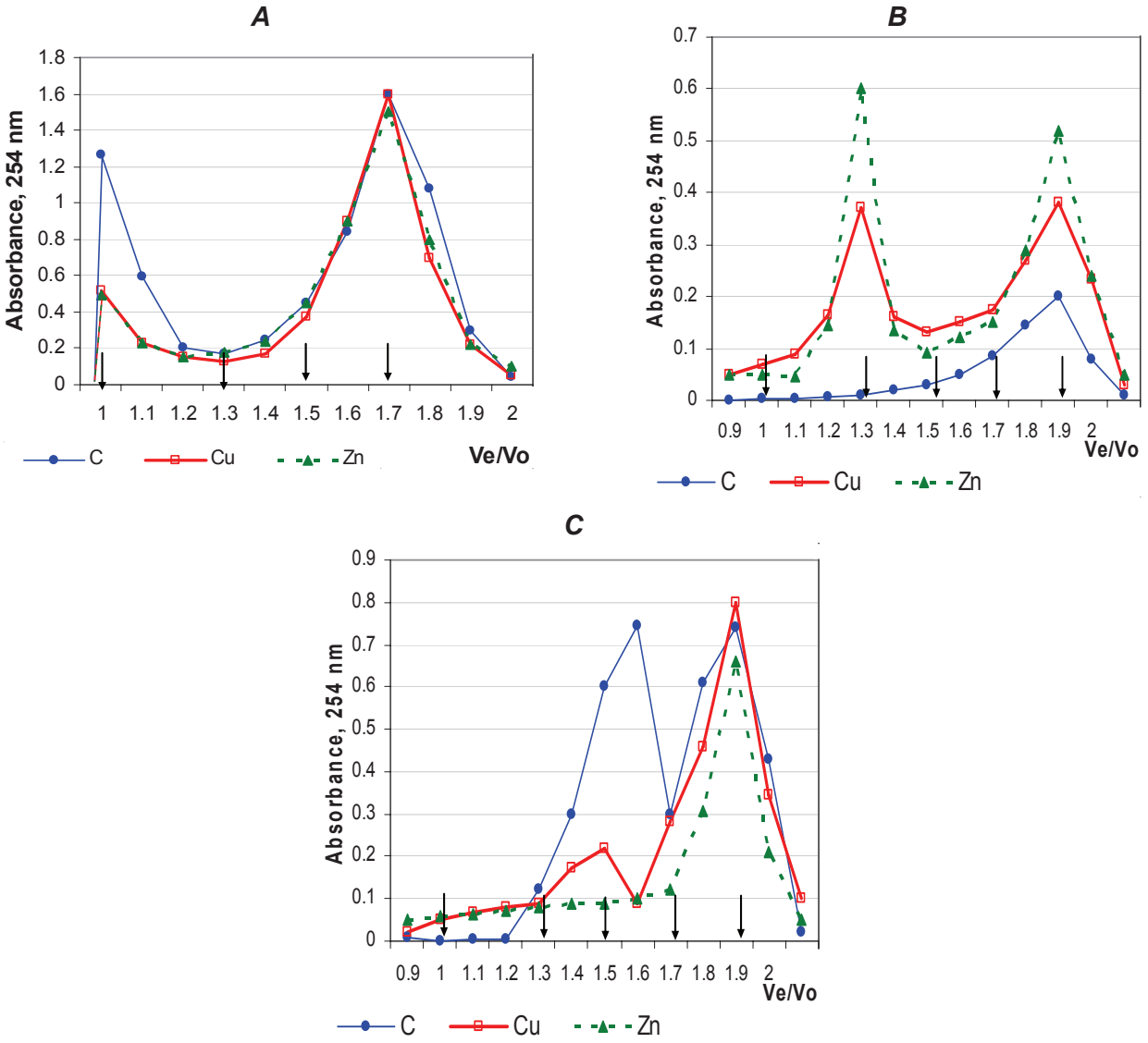

Fig. 1. The elution profiles on Sephadex G-50 of the thermostable extract (A), separated α- (B) and β- (C) domains of the metallothioneins from the liver of frogs affected by copper and zinc ions. In this and other Fig.: arrows highlight the elution volume of markers: 25.8 kDa, 17.0 kDa, 12.3 kDa, 8.4 kDa, 3.5 kDa; C, control, Cu, Zn, effect of Cu^{2+} and Zn^{2+} on the frog correspondingly; Ve, elution volume; Vo, void volume of the column

on the DTNB concentration for the frogs treated by Zn^{2+} and Cu^{2+}.

Despite the universality of the metal-buffering function of MTs in animals, its peculiarities in different organisms should be noted [4]. Our previous studies reflected that frogs exhibit copper levels in the liver, some 5 to 50 times above those found in other vertebrate species (fishes, mammalian) [9]. Consequently, MTs in frog liver bind only a little part of this amount (about 1–3%) and do not play an important role in the buffering of copper despite high rate of copper : zinc in their composition [17]. This ability to withstand a high level of unbound copper was expected to be reflected by MTs reactivity.

In our study, the intrinsic for vertebrate animals presence of both α- and β-domain in MTs was demonstrated for frog by the well recognized methods [12,18]. The successful binding of the appropriate ions in the metal-saturated domain against apothionein was confirmed by their UV-spectra in which typical Cd-MT and Cu(I)-MT maximums [19] were indicated.

MTs react with DTNB to release 5-thio-2-nitrobenzoate and metal ions. It was shown that only the simply coordinated cysteinyl groups in

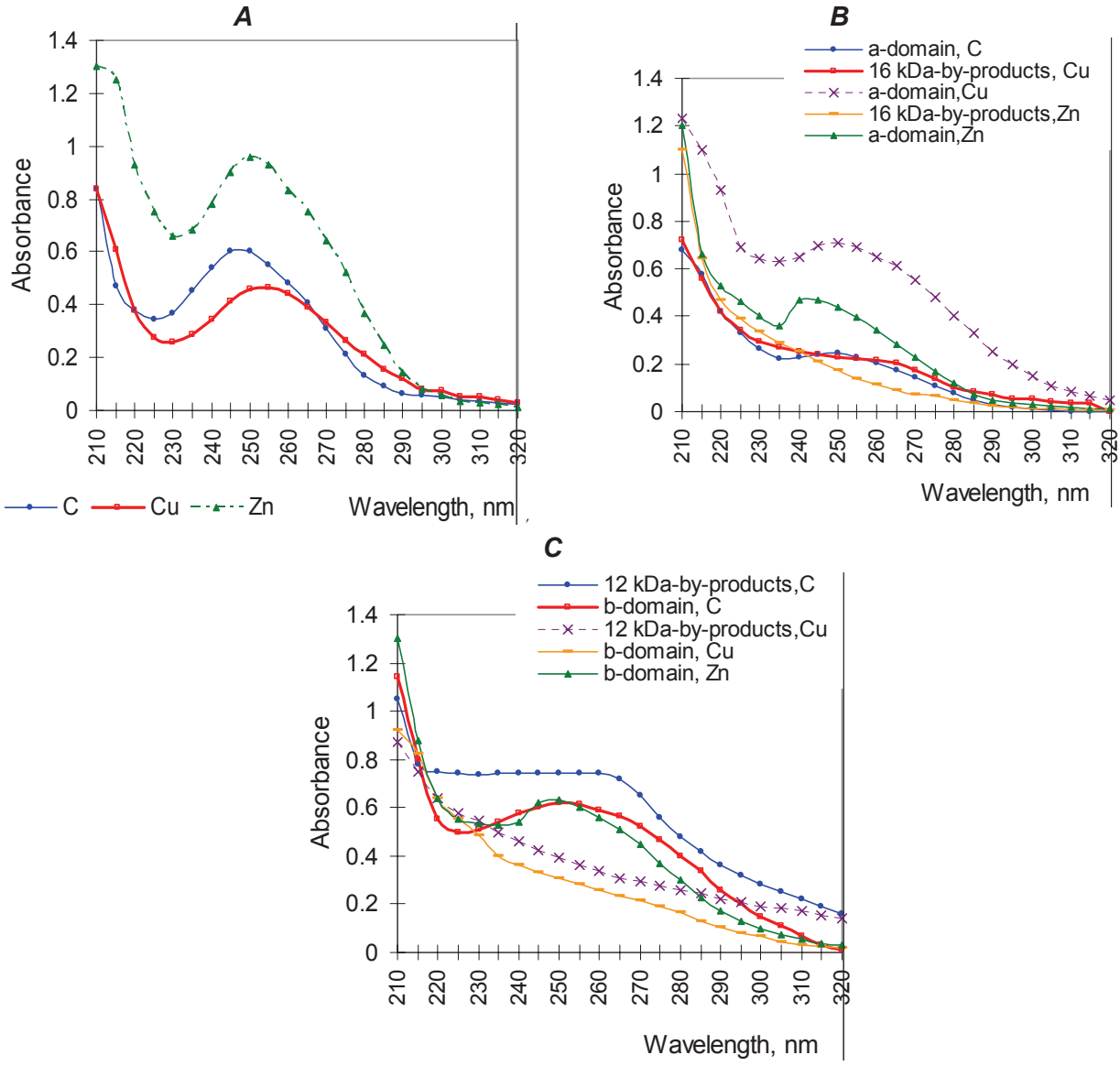

Fig. 2. UV-spectra of the metallothioneins of frog (A), their separated α-domain (B) and β-domain (C) in the liver of control animals and after the loading by Cu^{2+} and Zn^{2+}

MTs reacted fast with DTNB, whilst slow phase of the reaction corresponded to the reactions of the bridging cysteine thiolate ions [13]. Therefore, it was assumed that the tightly binding of metal ions in the thiolate cluster decreased the reactivity of SH-groups. Studies on individual β- or α-domains of mammalian MTs indicate that the Zn–thiolate cluster in the α-domain have a lower reactivity of thiolate groups than that in the β-domain due to higher stability [20]. Zangger et al. [21] have found that the exposure of MTs of rat to nitric oxide leads to a selective release of all three metals from the β-domain while leaving the four metals in the α-domain untouched. At the same time, the enhanced oxidation of Cu(I)-containing MTs even in the presence of β-mercaptoethanol and the inability of the Cu(I)-bound β-domain to react with DTNB is also known [18,22]. Therefore, in the case of MTs of frog, the likely reason for lesser reactivity of β-domain compared to α-domain is high susceptibility of its SH-groups to oxidation.

The creation of by-product with M 12 kDa also seems to be the consequence of partial oxidizing due to the spectral features of -S-S- bonds and low SH-reactivity in this form. The evidence for the oligomerization or aggregation of MTs that is a feature of partly its oxidation in *in vitro* and *in vivo* metal binding situations was reported for vertebrates [22,23]. The studies devoted to amphibian MTs indicate especially wide variability of the numbers of their chromatographic forms and the tendency of MTs to create high molecular weight

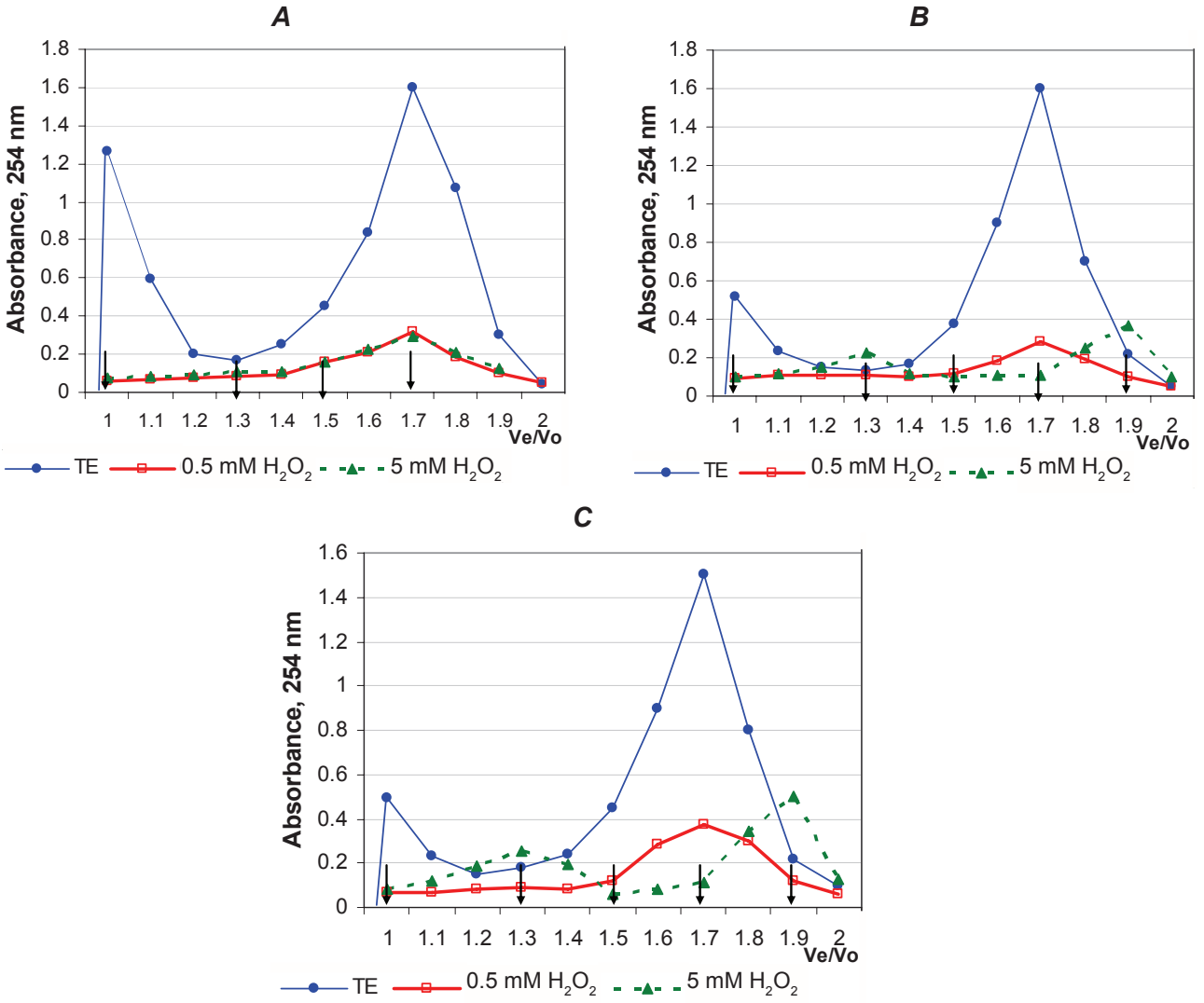

Fig. 3. The elution profiles on Sephadex G-50 of the thermostable extract and separated metallothioneins from the liver under the treatment by hydrogen peroxide in control (A) and after the effect of Cu^{2+} (B) and Zn^{2+} (C) on frogs. TE – thermostable extract

forms and insoluble aggregates [6,10,17], even if the isolation of MTs was performed with the necessary adjustments needed to avoid their oxidation [18].

In the loaded frogs the susceptibility to the oxidation of both separate domains was enhanced, preserving the comparative difference in the reactivity between two domains. However, that was revealed differently for two domains: in β-domain as a loss of the specific spectral features, SH-groups reactivity and ability to create by-products, and in α-domain the oxidative damage provoked the elevation of the SH-groups reactivity and the ability to create specific for this domain by-products. This regularity can explain why the reduction of the metal binding ability of MTs in frog that was reflected in the unfavorable conditions of being [7,17] is connected mostly to Zn^{2+} and less to Cu^{2+}. Indeed, α-domain is the main source of releasing Zn^{2+} in the injured MTs of frog and its functional state can determine the distribution of zinc between MTs and other cellular targets. On the other hand, the release of copper from the β-domain cannot change the distribution of copper within the cells of the frog liver prominently. So the dependence on the high levels of copper in the tissue and in MTs in the frog liver makes these MTs sensitive to the oxidation and unable to tightly bind metals.

The comparison of the effect of Zn^{2+} and Cu^{2+} indicates a more prominent oxidative consequence of Zn^{2+} than Cu^{2+}. The utilization of a standard oxidant, H_2O_2, revealed weakness in the linkage between domains of treated frogs by releasing products that had especially high reactivity of SH-groups and creating of 16 kDa by-product in the

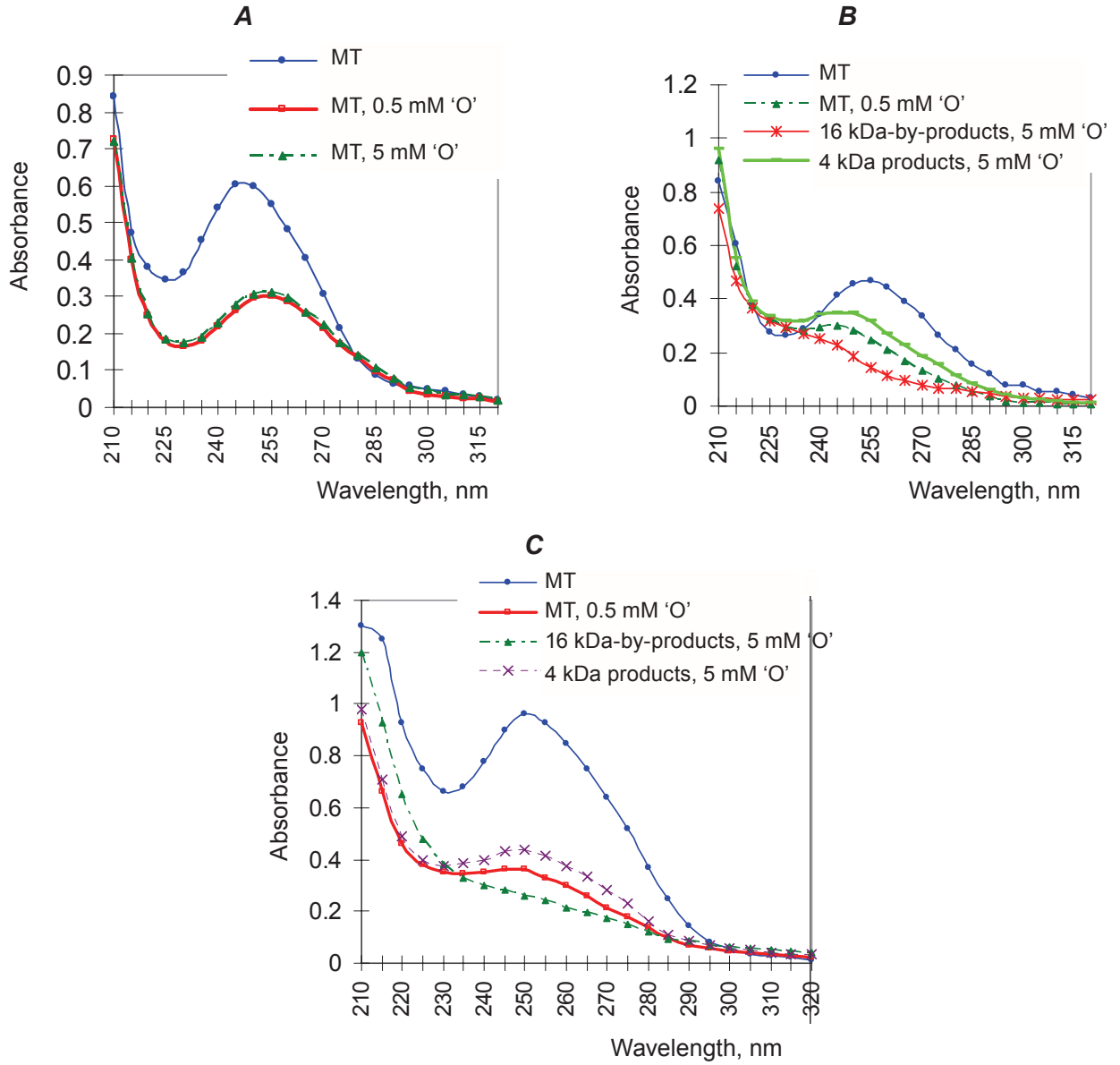

Fig. 4. UV-spectra of the metallothioneins (MT) of frog under the treatment by hydrogen peroxide in control (A) and after the effect of Cu^{2+} (B) and Zn^{2+} (C) on frogs. In Fig. 4, 5: "O", the treatment of metallothioneins by hydrogen peroxide

case of frogs treated by Zn^{2+}. This fact is confirmed by our results of the study of Zn^{2+} and Cu^{2+} effects in frogs [24]. It was shown that both effects caused the increase of the MTs content (especially Cu^{2+}) and inhibition of the Mn- and Cu,Zn-superoxide dismutase and catalase activities in the liver of frog. However, only Zn^{2+} provoked the oxidative damage: increase of the superoxide anion formation and lipid peroxidation and also the inhibition of the cholinesterase activity

Taken together, the results of determining reactivity of domains and their UV-spectra provide a strong argument in favour of the merely seeming stability of the frog MTs even under low, environmentally acceptable concentrations of pollution. The faults specific to each domain were demonstrated in the frogs treated with Cu^{2+} or, especially, Zn^{2+} ions and was better revealed under the attendant circumstances (oxidative damage, caused by H_2O_2). They seem to be crucial for interaction with other molecules, metal transfer and redox potentials in the loaded animals. The oxidative stress may significantly reinforce the injury of MTs provoked by heavy metals and especially affect the distribution of zinc in the cell, whilst extremely high oxidizability of β-domain in frog MTs and

Rates constants (K) of two-phased (I and II) reaction with DTNB for metallothioneins (MT), their separated domains (α and β) and by-products in frog treated by Cu^{2+} and Zn^{2+}, (s-1 M-1)

Metallothionein form	K(I)	K(II)
α-domain	1.3218[DTNB] + 0.6918	0.1704[DTNB] + 2.4941
α-domain, Cu^{2+}	3.3703[DTNB] + 2.4909	0.2824[DTNB] + 7.3361
α-16 kDa-by-product, Cu^{2+}	3.2693[DTNB] + 2.1922	0.2942[DTNB] + 7.012
α-domain, Zn^{2+}	3.3314[DTNB] + 2.3984	0.4987[DTNB] + 6.9762
α-16 kDa-by-product, Zn^{2+}	0.9959[DTNB] + 1.0576	1.8367[DTNB] − 1.115
β-domain	0.8176[DTNB] + 0.2335	0.7674[DTNB] + 1.0878
β-12kDa-by-product	0.6135[DTNB] + 0.7219	0.7804[DTNB] + 0.9653
β-domain, Cu^{2+}	0.3942[DTNB] + 0.0992	0.0276[DTNB] + 0.7161
β-12 kDa-by-product, Cu^{2+}	1.3659[DTNB] + 0.8905	0.2528[DTNB] + 2.9861
β-domain, Zn^{2+}	0.2122[DTNB] + 0.3021	0.043[DTNB] + 0.8919
MT, 0.5 mM “O”	0.5684[DTNB] + 0.253	0.5136[DTNB] + 0.1744
MT, 5.0 mM “O”	0.3979[DTNB] + 0.2733	0.403[DTNB] + 0.7868
MT, 0.5 mM ”O”, Zn^{2+}	4.0041[DTNB] + 0.6509	0.2639[DTNB] + 6.7258
MT, 0.5 mM “O”, Cu^{2+}	3.3078[DTNB] + 2.3043	0.2892[DTNB] + 7.1957
4 kDa product, 5.0 mM”O”, Zn^{2+}	3.1278[DTNB] + 1.3528	0.321[DTNB] + 5.9264
4 kDa product, 5.0 mM “O”, Cu^{2+}	0.9807[DTNB] + 3.6382	0.3444[DTNB] + 4.7865

Footnote. Cu^{2+}, Zn, effect of Cu^{2+} and Zn^{2+} ions on the frog correspondingly; “O”, the treatment of metallothioneins by hydrogen peroxide

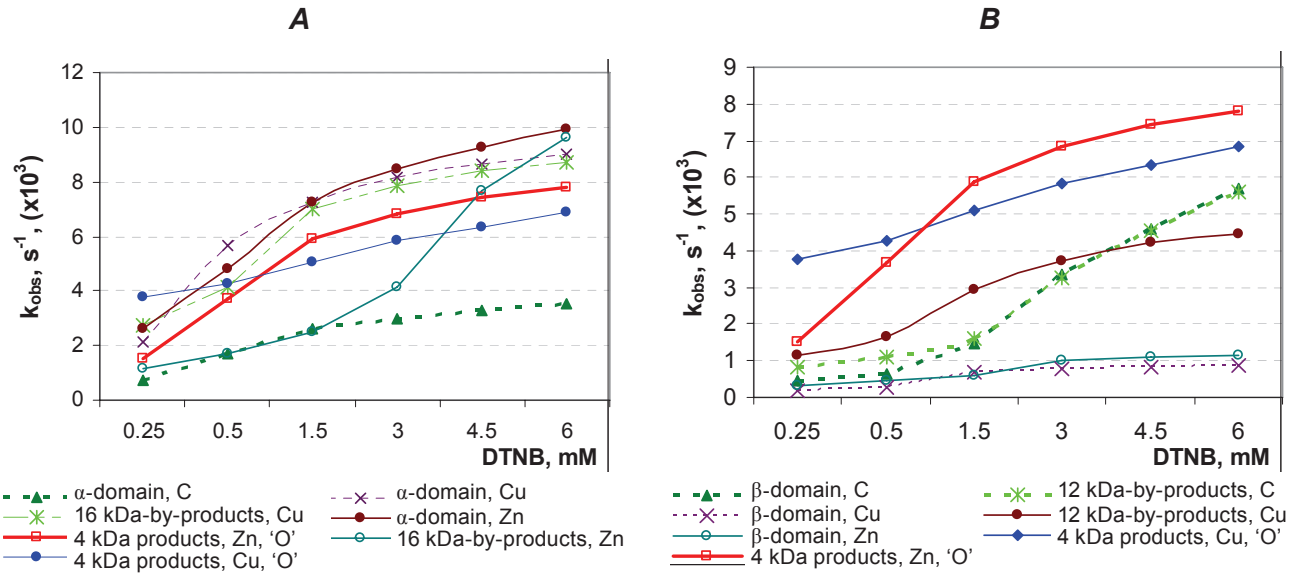

Fig. 5. The plot of absorbance Ln (D_∞ - D_t) at 412 nm vs. concentration of the DTNB for the reaction with α-domain (A), β-domain (B), their by-products and forms obtained under the effect of H_2O_2 (“O”) on MTs (in both figures). The concentration of each MTs form was 16.5 μM (in 10 mM Tris−HCl buffer, pH 8.0 with 100 mM KCl at 25 °C)

high level of copper in the cells make the participation of β-domain in the exchange of metals elusive and determine the aggregation of MTs.

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РЕАКТИВНІСТЬ МЕТАЛОТІОНЕЇНІВ ЖАБИ *Rana ridibunda* ЗА ВПЛИВУ ІОНІВ МІДІ ТА ЦИНКУ

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Металдепонуючі та стресорні білки металотіонеїни (МТ) жаби характеризуються незвичайно високим, як для хребетних тварин, вмістом міді та виявляють нестабільність, що було встановлено в наших попередніх роботах. За несприятливих умов вони легко втрачають ці іони, передусім цинку. Метою цього дослідження було вивчити реактивність SH-груп в МТ печінки жаби *Rana ridibunda* після дії Cu^{2+} (0,01 мг/л) та Zn^{2+} (0,1 мг/л) на їхній організм протягом 14 діб. Шляхом гель-розподільної хроматографії на сефадексі G-50 було виділено α- та β-домени МТ з молекулярною масою близько 4 кДа. В реакції з реактивом Еллмана (ДТНБ), на відміну від вищих хребетних, у жаб було встановлено вищу реактивність α-домену, ніж β-домену. Про часткове окислення β-домену свідчить утворення додаткового продукту з молекулярною масою близько 12 кДа, низька реактивність SH-груп та типові для S-S-зв'язків особливості УФ-спектра. Дія обох іонів металів на жаб зумовлює збільшення реактивності SH-груп в α-домені з появою додаткового продукту з *M* 16 кДа та її зменшення в β-домені. Інкубація МТ контрольних тварин з 0,5 або 5,0 мМ H_2O_2 не змінює їхні хроматографічні характеристики. За дії на жаб Cu^{2+} та Zn^{2+} інкубація МТ з 5,0 мМ H_2O_2 призводить до вивільнення продукту з *M* 4 кДа. Отже, у жаб α-домен МТ відповідає за збільшення вивільнення іонів металів з ушкодженої молекули, тоді як дуже висока окислювальна здатність β-домену робить його участь в обміні металів непомітною та зумовлює агрегацію МТ.

Ключові слова: мідь, цинк, металотіонеїн, окислення, жаба, *Rana ridibunda*.

РЕАКТИВНОСТЬ МЕТАЛЛОТИОНЕИНОВ ЛЯГУШКИ *Rana ridibunda* ПОД ВОЗДЕЙСТВИЕМ ИОНОВ МЕДИ И ЦИНКА

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Металл-депонирующие и стрессорные белки металлотионеины (МТ) лягушки характеризуются необычно высоким, как для позвоночных животных, содержанием меди и проявляют нестабильность, что было установлено в наших предыдущих работах. В неблагоприятных условиях они легко теряют ионы меди и, прежде всего, цинка. Целью данного исследования было изучить реактивность SH-групп в МТ печени лягушки *Rana ridibunda* после воздействия Cu^{2+} (0,01 мг/л) и Zn^{2+} (0,1 мг/л) на их организм на протяжении 14 суток. В результате гель-распределительной хроматографии на сефадексе G-50 было выделено α- и β-домены МТ с молекулярной массой около 4 кДа. В реакции с реактивом Еллмана (ДТНБ), в отличие от высших позвоночных, у лягушек была установлена более высокая реактивность α-домена, чем β-домена. О частичном окислении β-домена свидетельствует образование дополнительного продукта с молекулярной массой около 12 кДа, низкая реактивность SH-групп и типичные для S-S-связей особенности УФ-спектра. Действие обоих ионов металлов на лягушек вызывает увеличение реактивности SH-групп в α-домене с появлением дополнительного продукта с *M* 16 кДа и ее уменьшение в β-домене. Инкубация МТ контрольных животных с 0,5 или 5,0 мМ H_2O_2 не изменяет их хроматографические характеристики. При действии на лягушек ионов Cu^{2+} и Zn^{2+} инкубация МТ с 5,0 мМ H_2O_2 приводит к высвобождению продукта с *M* 4 кДа. Таким образом, у лягушек α-домен МТ отвечает за увеличение высвобождения ионов металлов из поврежденной молекулы, тогда как очень высокая окислительная способность β-домена делает его участие в обмене металлов несущественным и обусловливает агрегацию МТ.

Ключевые слова: медь, цинк, металлотионеин, окисление, лягушка, *Rana ridibunda*.

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