PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2021, **55**(3), p. 248–254

Biology

THE ROLE OF THIOL GROUPS IN THE EXPRESSION OF THE ACTIVITY OF ARGINASE I AND II ISOENZYMES

M. K. ISKANDARYAN [*], E. Kh. BARSEGHYAN [**]

Chair of Biochemistry, Microbiology and Biotechnology, YSU, Armenia

There are some aspects of the proteins «self-organization» that are not well studied, understanding the relationship between conformational folding linked with the formation of a disulfide bond is important and challenging from both biophysical and biochemical perspectives. In the studies, an attempt was made to elucidate the role of thiol groups in maintaining of native conformation and activity of arginase I and II of various organ origins. It was identified that depending on the stage of enzyme reactivation and the stability of the conformational state of the formed oligomers, para-chloromercuribenzoate affects the «self-organized» oligomers in different ways.

https://doi.org/10.46991/PYSU:B/2021.55.3.248

***Keywords*:** reversible inactivation, self-organization, arginase I, arginase II, para-chloromercuribenzoate (PCMB), chimeric oligomers.

Introduction. The conformational state of a molecule is essential for functional properties of mediator proteins. Minor changes of conformation can occur as a result of changes in the internal environment, such as temperature, hydrogen ions or chemical compounds, leading to complete or partial inactivation of proteins, including enzymes, contributing to a number of pathological conditions [1]. From this point of view, the study of changes in the structure of isofunctional proteins in the process of reversible inactivation (inactivation and reactivation or "self-organization") is of great interest. "Self-organization" or the formation of the spatial structure of globular proteins is a complex, multi-stage and directed process involving a variety of molecular chaperones [2].

The experiment was based on the study of changes in the oligomer structure of rabbit arginase I and II in order to clarify the specific aspects of "self-organization" of mammalian arginase, the role of Mn^{2+} and Ni^{2+} cations in the process of reversible inactivation of arginase isoforms, to investigate the importance of thiol (–SH) groups for the activity of inactivated and reactivated arginase I and II. Our research group at the Chair of Biochemistry, Microbiology and Biotechnology of YSU in recent years was identified that hepatic (liver) arginase I "self-organized" oligomers in the presence of Ni^{2+} cation are 1.3–1.8 times more active than oligomers reactivated in the presence of Mn^{2+} cation. It has also been shown that the physiological stimulant

[*] E-mail: meri.iskandaryan98@mail.ru
[**] E-mail: e.barseghyan@ysu.am

of the enzyme Mn^{2+} cation inhibits the catalytic activity of "self-organized" Ni^{2+} oligomers by about 20% [3].

Additionally, it is known fact that arginase I and arginase II perform the same catalytic function, but differ in tissue distribution, cell localization, metabolic function, physicochemical-kinetic properties, and immunological cross-reactivity.

The studies have attempted to elucidate the role of –SH groups in maintaining the native conformation and activity of the enzyme, because according to the literature, in contrast to mammalian liver arginase the microorganisms, plants, birds and mice renal arginase has –SH groups associated with enzyme activity. However, rabbit liver arginase is completely inactivated by a reagent that breaks down disulfide bridges when it does not affect horse liver arginase [4].

Disulfide bonds play an important role in the determination of protein structures, as they are the most stable, conservative part of the proteome. Disulfide bonds or bridges (SdS) are usually formed by the oxidation of thiol groups, particularly in biologically active systems [3]. Proper disulfide bridge formation is important to maintain the physiological state of the organism or to prevent the occurrence of a pathological condition. Compounds containing disulfide bridges may participate in disulfide exchange reactions ("interchange") with thiol groups that occur over a wide range of pH and buffer conditions, including physiological conditions [5].

The method of chemical modification was used to study the active structure of the enzyme and to identify the functional groups that ensure its activity. By studying the interaction of specific chemical reagents (ex. para-chloromercuribenzoate) with a protein, it is possible to regulate enzyme activity, as some targeted drugs have been developed [4].

Materials and Methods.

The Experimental Object is a Rabbit (*Oryctolagus cuniculus domesticus)* with the mass of 2000–2600 *g*. The animals were kept a temperature of 18–23°C, a correlation 12/12 *h* night/morning with a standard diet. Experimental procedures with animals and feeding were carried out in accordance with the provisions of the European Directive on the Protection of Animals Used for Scientific Purposes (Directive 2010/63/EU, European Parliament and Council of the European Union, 2010), and according to the rules of laboratory practice of the Russian Federation (State standard GOST 53434–2009).

Animals' liver, kidney and brain were used for experiments. Separated organs were carefully dried on filter paper, weighed and homogenized with a Potter-Elvehjem glass homogenizer at +4°C ("Potter-Elvehjem Glass", Germany). 10% homogeneities of the test tissue were prepared using distilled water. The homogeneity was centrifuged at 20,000 × g for 30 *min* at 0–4°C. The extract served as a source of enzyme. Arginase activity was determined by the Ratner method with some modifications. The enzyme hydrolysed L-arginine to L-ornithine and urea. The amount of the final product, urea, was determined by the Archibald method with modifications by Moore and Kaufman. The optical density of the final product (urea) was determined by spectrophotometric method (GENESYS 10S UV-Vis, "Thermo scientific", USA) at a light length of 478 *nm*.

Partial Purification of Enzyme. The partial purification of the enzyme was carried out by gel-filtration method. 2 *mL* of homogenate was added to the tower

filled with Sephadex G-200 (“Pharmacia”, Uppsala, Sweden) (1.5 *cm* × 75 *cm*). The equilibrium elution volume was 4 *mL*, the rate – 25 *mL*/*h*. After fractionation, protein absorption was measured at 280 *nm* and the arginase activity in each fraction was determined by the Ratner method at 478 *nm* (GENESYS 10S UV-VIS) [6].

***Experimental Design*.** Acid inactivation of the enzyme was carried out by by exposure to 0.05 *M* glycine-HCl buffer (pH 4.0) at room temperature for 18 *h*. Arginase was reactivated with 0.05 *M* glycine-NaOH buffer (pH 9.5) at +20°C (72 *h*), in the presence of divalent cations Mn^{2+} (physiological stimulant) and Ni^{2+} (25 *μmol* in 1 *mL* of test tube). Metal chlorides were used in the experiment. 10% enzymatic partially purified extracts of arginase I (liver) and arginase II (kidney and brain) served as the object of study. The activity of the native enzyme was taken as 100%.

Results and Discussion. In this study, para-chloromercuribenzoate (PCMB) was used as a thiol reagent. It binds to free thiol groups in the protein to form mercaptides, contributing to a decrease in enzyme activity. The mentioned thiol reagent may also bind to reactive groups of enzymes: histidine, lysine and methionine. Depending on the experimental conditions and organ origin PCMB can affect the activity of the enzyme in different ways [4].

The main research facts were observed using three experimental models, namely reversible inactivation of hepatic arginase I, renal arginase II and brain arginase II, in the presence of bivalent cations. At the first stage of the experiment, native arginase I (liver) and II (kidney, brain) were exposed to 10 *μmol*/*mL* PCMB for 60 *min* (Fig. 1). The concentration of PCMB was selected according to the literature data, where the corresponding concentration of the thiol reagent were used for the various objects and for different enzymes [7].

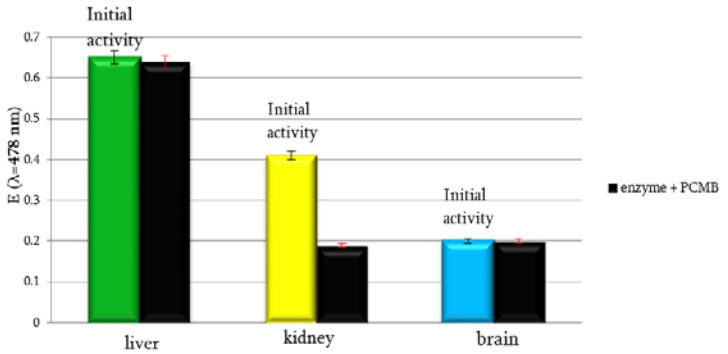

Fig. 1. Effect of PCMB (10 *μmol*/*mL*) on the activity of arginase I (liver) and II (kidney, brain) (n = 5, $M \pm m$, p <0.05).

As shown in Fig. 1, PCMB had practically no effect on arginase I and the brain-derived arginase II, which according to the literature indicates the absence of reactive –SH groups responsible for enzyme’s activity. However, PCMB suppresses the activity of renal arginase II (kidney) by 50%, which probably indicates the presence of –SH groups responsible for the activity.

The Anfinsen principle, according to which the sequence of a protein uniquely determines its structure, is based on experiments on oxidative refolding of a protein

with disulphide bonds [8]. In the case of acid inactivation, disulphide bonds are cleaved first, but upon restoration of environmental conditions, "self-organization" of the protein and partial or complete reduction of disulfide bonds occurs. Therefore, it was studied to what extent disulfide bridges associated with the enzyme activity of oligomers are formed in presence of divalent cations [9].

In the next figures (Fig. 2–4) are shown the effect of PCMB (10 *μmol/mL*) on the activity of "self-organized" chimeric oligomers ("Ni^{2+} oligomer", "Mn^{2+} oligomer") of various organ origin.

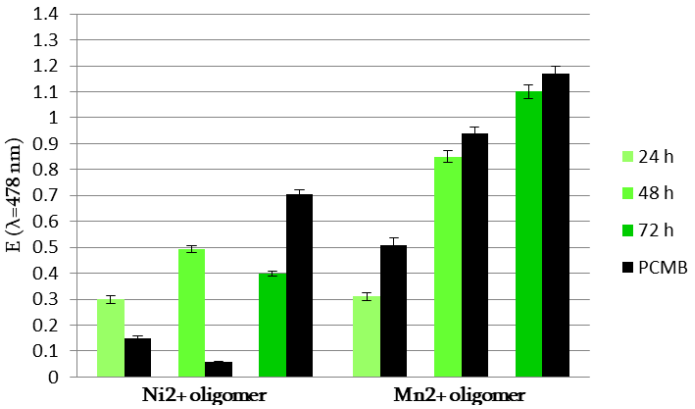

Fig. 2. Effect of PCMB (10 *μmol/mL*) on the activity of Hepatic arginase I "self-organized" oligomers in the presence of Ni^{2+} and Mn^{2+} cations (reactivation (pH 9.5), $n = 5$, $M \pm m$, $p < 0.05$).

As shown in Fig. 2, in the case of reversible inactivation of liver arginase I, PCMB had an inhibitory effect of 50% and 88% on Ni^{2+} oligomers reactivated for 24 *h* and 48 *h*, and a stimulating effect of 76.47% on oligomer reactivated for 72 *h*. This probably indicates the repair of cleaved disulfide bridges as a result of reversible inactivation, and the stimulus effect may be due to a conformational change in the oligomers, resulting in the PCMB interaction with the active group of His residues in the active site of the enzyme (Fig. 2). According to the literature, His and Asp residues responsible for enzyme activity are located near the Mn^{2+} binuclear cluster.

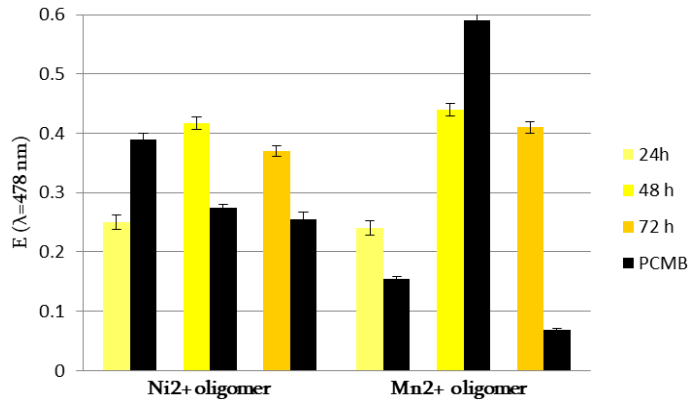

Fig. 3. Effect of PCMB (10 *μmol/mL*) on the activity of Renal arginase II "self-organized" oligomers in the presence of Ni^{2+} and Mn^{2+} cations (reactivation (pH 9.5), $n = 5$, $M \pm m$, $p < 0.05$).

PCMB have a stimulating effect of 64.52%, 10.6% and 6.36% on Mn^{2+} oligomers reactivated for 24, 48 and 72 *h*. Based on the data obtained, it can be concluded that the stabilization of the conformational state contributed to the stabilization of the picture of the PCMB stimulating effect (Fig. 2).

Moreover, on renal arginase II, PCMB had a 56% stimulatory effect on 24 *h* reactivated Ni^{2+} oligomers, but it had an inhibitory effect of 34.3% and 31.08% on oligomers reactivated for 48 *h* and 72 *h* (Fig.3).

On Mn^{2+} oligomers of renal arginase II, after reactivation for 24 *h* and 72 *h*, the PCMB had an inhibitory effect by 35.4% and 83.4%, and stimulating effect of 34.1% on oligomers reactivated for 48 *h* (Fig. 3).

In the case of reversible inactivation of brain arginase II, PCMB had no effect on 24 *h* reactivated Ni^{2+} oligomers, while exerting an inhibitory effect of 92.3% and 85.7% on oligomers reactivated for 48 *h* and 72 *h*, respectively. However, PCMB had no effect on Mn^{2+} oligomers after 24 and 72 *h* of reactivation, but had an inhibitory effect of 92.3% on oligomers reactivated for 48 *h* (Fig. 4). According to some authors, it is possible that not all cleaved disulfide bridges are restored as a result of reversible inactivation, despite the fact that "self-organized" Ni^{2+} oligomers exhibit significantly higher catalytic activity.

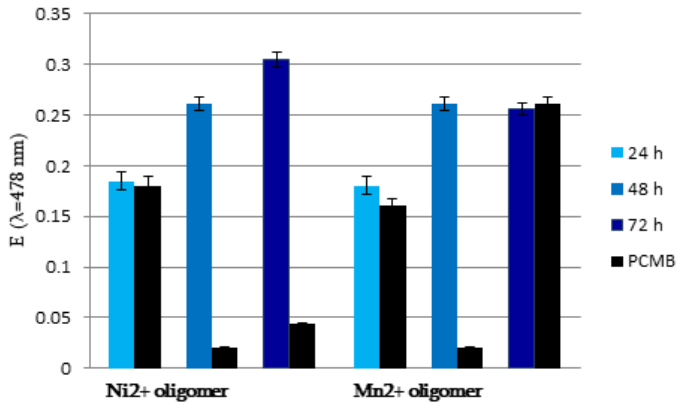

Fig. 4. Effect of PCMB (10 *μmol/mL*) on the activity of Brain arginase II "self-organized" oligomers in the presence of Ni^{2+} and Mn^{2+} cations (reactivation (pH 9.5), $n = 5$, $M \pm m$, $p < 0.05$).

Conclusion. In two of the three models considered, "self-organized" Ni^{2+} oligomers exhibited higher activity than "self-organized" Mn^{2+} oligomers; however, according to the registered PCMB impact on "self-organized" Mn^{2+} oligomers, the conformation was close to that of native arginase I and II.

Received 16.09.2021
Reviewed 24.11.2021
Accepted 03.12.2021

REFERENCES

1. Acharya N., Mishra, P., Jha S.K. Evidence for Dry Molten Globule-Like Domains in the pH-Induced Equilibrium Folding Intermediate of a Multidomain Protein. *J. Phys. Chem. Lett*. **7** (2016), 173–179. https://doi.org/10.1021/acs.jpclett.5b02545
2. Bychkova V.E., Semisotnov G.V., et al. The Molten Globule Concept: 45 Years Later. *Biochemistry (Moscow)* **83** (2018), S33–S47. https://doi.org/10.1134/S0006297918140043
3. Iskandaryan M.K., Barseghyan E.Kh. Study of Protein Mediators “Self-formation” Processes in Rabbit Liver Arginase as Example. *Biol J. Armenia* **1–2** (2020), 121–125.
4. Khachatryan N.S. Influence of p-Chloromercuribenzoate on the Arginase Isoenzimes of Rat Organs in the Presence of N^G-Hydroxiarginine. *Proc. YSU B: Chem. Biol. Sci*. **45** (2011), 40–45. https://doi.org/10.46991/PYSU:B/2011.45.3.040
5. Jha S.K., Udgaonkar J.B. Direct Evidence for a Dry Molten Globule Intermediate During the Unfolding of a Small Protein. *PNAS* **106** (2009), 12289–12294. https://doi.org/10.1073/pnas.0905744106
6. Shamirian A.S. Characteristics of Rana Ridibunda Frog Liver Arginase Inactivation by EDTA and Further Reactivation Processes. *Electron. J. Nat. Sci.* **21** (2013), 26–29.
7. Sandeep K., Kayastha A.M. Soybean (*Glycine max*) Urease: Significance of Sulfhydryl Groups in Urea Catalysis. *Plant Physiol. Biochem.* **48** (2010), 746–750. https://doi.org/10.1016/j.plaphy.2010.05.007
8. Finkelstein A.V., Badretdin A.J., et al. There and Back Again: Two Views on the Protein Folding Puzzle. *Phys. Life Rev.* **21** (2017), 56–71. https://doi.org/10.1016/j.plrev.2017.01.025
9. Qina M., Wanga W., Thirumalai D. Protein Folding Guides Disulfide Bond Formation. *PNAS* **112** (2015), 11241–11246. https://doi.org/10.1073/pnas.1503909112

Մ. Կ. ԻՍԿԱՆԴԱՐՅԱՆ, Է. Խ. ԲԱՐՍԵՂՅԱՆ

ԹԻՈԼԱՅԻՆ ԽՄԲԵՐԻ ԴԵՐՆ ԱՐԳԻՆԱԶ I-Ի ԵՎ II-Ի ԻՉՈՖԵՐՄԵՆՏՆԵՐԻ ԱԿՏԻՎՈՒԹՅԱՆ ԴՐՍԵՎՈՐՄԱՆ ՀԱՐՑՈՒՄ

Գոյություն ունեն սպիտակուցների «ինքնահավաքման» որոշ ոլորտներ, որոնք դեռևս լավ ուսումնասիրված չեն: Կոնֆորմացիոն ֆոլդինգը կապված դիսուլֆիդային կապերի ձևավորման հետ կարևոր և դժվար խնդիր է ինչպես կենսաֆիզիկական, այնպես էլ կենսաքիմիական տեսանկյուններից: Հետազոտությունների ընթացքում փորձ է կատարվել պարզաբանել թիոլային խմբերի դերը տարբեր օրգանական ծագում ունեցող արգինազի I և II նատիվ կոնֆորմացիայի և ակտիվության պահպանման գործընթացում: Ցույց է տրվել, որ կախված ֆերմենտի ռեակտիվացման փուլից և ձևավորված օլիգոմերի կոնֆորմացիոն վիճակի կայունությունից պարա-քլորմերկուրիբենզուատը տարբեր կերպ է ազդում «ինքնահավաքված» օլիգոմերների վրա:

М. К. ИСКАНДАРЯН, Э. Х. БАРСЕГЯН

РОЛЬ ТИОЛОВЫХ ГРУПП В ВЫРАЖЕНИИ АКТИВНОСТИ ИЗОФЕРМЕНТОВ АРГИНАЗЫ I И II

Понимание взаимосвязи между конформационным фолдингом, связанным с образованием дисульфидной связи, является важным и сложным как с биофизической, так и с биохимической точки зрения, и некоторые аспекты «самоорганизации» белков недостаточно изучены. В рамках исследования была предпринята попытка выяснить роль тиоловых групп в поддержании нативной конформации и активности аргиназ I и II различного органного происхождения. Показано, что в зависимости от стадии реактивации фермента и стабильности конформационного состояния образованных олигомеров парахлормеркурибензоат по-разному влияет на «самоорганизованные» олигомеры.