

The influence of aluminum ions on actomyosin superprecipitation and myosin ATPase activity in cardiac muscle

K.I. Bogutska^{1*}, Yu.I. Prylutskyi¹, Yu.P. Sklyarov²

¹ Taras Shevchenko National University of Kyiv
64 Volodymyrska Str., Kyiv, 01601, Ukraine

² National O. Bogomolets Medical University
13 T. Shevchenko Avenue, Kyiv, 01601, Ukraine

Summary. The inhibition of actomyosin superprecipitation reaction and myosin ATPase activity by aluminum ions in cardiac muscle have been found to depend on the concentration of aluminum ions, which causes the development of pathological processes in organism.

Key words: cardiac muscle, actomyosin, myosin, ATPase, superprecipitation.

Introduction. It is of general knowledge that aluminum ions produce toxic action on the living organisms as they are introduced and accumulated by the organs and tissues, which is accompanied by the disorder of metabolic processes and development of various kinds of pathologies. Aluminum is usually introduced in the body as polyphenol complexes from vegetables, fresh water, food, various cosmetics, pharmaceutical products (sorbents, antacidic drugs, protectors and anesthetics) and vaccines [1–6]. The hybrid materials Al-C-O used in traumatic and orthopedic surgery also contribute into aluminum content in the body since they contain the aluminum matrix with uniformly distributed Al₄C₃ and Al₂O₃ nanoparticles. However, it has been supposed that the material high purity as well as aluminum oxide and carbide inertness to bone and muscle tissues makes the material applicable for external and internal osteosynthesis [7]. In recent years the problem of small radiation doses affecting the human organism with simultaneous intoxication by aluminum has become

very important in some regions of Ukraine [8]. At present much attention is paid to investigating the action of aluminum phosphide on humans and animals which is widely used to produce pesticides, as well as insecticides and rodenticides (zoocides) that can cause poisoning [9, 10]. The study of molecular mechanism by which aluminum affects human body's different systems is no less important since it harms not only the central nervous system causing different forms of aluminosis, but also osteal, contractile and cardiovascular systems [1–4, 11, 12]. In particular, cardiac rhythm disturbance caused by accumulation of aluminum in the cardiac muscle is one of the pathologies associated with the increased content of aluminum in the organism. As for the role played by aluminum ions in the mechanisms of muscle contraction an assumption can be made that they influence not only neuromuscular transition but also have direct effect on the contractile apparatus.

Given the above, the purpose of this work was to investigate the aluminum ions influence on the actomyosin superprecipitation (SPP) and myosin ATPase activity in cardiac muscle.

Materials and Methods. The method used to extract myosin from a bull's cardiac muscle was

* Corresponding author.

Tel.: +38044-5213325

E-mail address: biophys@univ.kiev.ua

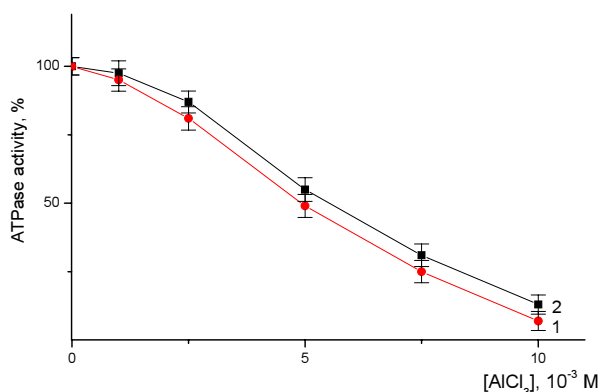


Fig. 1. The impact of aluminum ions on ATPase activity of cardiac muscle myosin: 1 — at low (0.05 M KCl) and 2 — at high (0.5 M KCl) ion strength of the medium with 5 mM Ca^{2+} .

the somewhat modified Margosyan method [13]. Myosin extraction was carried out for an hour by the solution containing 0.2 M KCl, 0.15 M Tris-HCl, pH 8.0, 1 mM EDTA, 5 mM MgCl_2 , 0.2 mM PMSF, 1 mM NaN_3 , 3.5 mM ATP. Actomyosin was precipitated by adding 10 volumes of cold distilled water to the solution previously diluted by 0,1 n vinegar acid to pH~6.2. The protein was separated by centrifuging at 3000 g for 15 min. After dissolving the protein in 40 mM $\text{Na}_4\text{P}_2\text{O}_7$, pH 7.5, 1 mM DTT, 1 mM NaN_3 , which was followed by dialysis, it was centrifuged for an hour at 100000 g. The supernatant was deposited onto the diethylaminoethanol (DEAE)-Sephacrose (4x45 cm) that was balanced by the same buffer. The elution from the column was made by the linear gradient [0÷0.5 M] NaCl. The general volume of the gradient was 900 ml. Myosin from the column was in the concentration range of NaCl from 0.07 to 0.14 M. The fractions of this peak were separated and subjected to dialysis against buffer of 0.05 M KCl, 5 mM imidazole, pH 6.2, 0.5 mM DTT, 1 mM NaN_3 . The resulting myosin precipitation, was extracted by centrifuging and dissolved in the buffer of 0.5 M KCl, 5 mM Tris-HCl, pH 7.5, 1 mM NaN_3 , 0.5 mM DTT. The activity of myosin ATPase was measured at 37 °C in the media of the total volume 1,8 ml containing the following components: 20 mM imidazole (pH 7.5), 5 mM CaCl_2 , 2.5 mM MgCl_2 , 1 mM EDTA, 0.5 mM DTT, 1 mM ATP, 0.5 or 0.05 M KCl, 0.14 mg/ml of protein. The amount of inorganic phosphate obtained in the result of ATP hydrolysis was estimated by Fis-

ke-Subarrov method [14]. The activity of myosin ATPase was expressed in $\mu\text{mole P}_i/\text{min}$ per 1 mg of protein.

The kinetics of SPP actomyosin was registered on spectrophotometer «Spekord M-40» (Germany) by the change of actomyosin optical density at the wavelength of 450 nm and the temperature of 25 °C for 20 min. The mixture for the reaction of SPP actomyosin contained 20 mM Tris-HCl (pH 7.5), 0.15 M KCl, 1 mM MgCl_2 , 0.1 mM CaCl_2 . The actomyosin concentration in the total volume of 2 ml sample was 0.1 mg/ml. SPP was initiated by adding ATP into the medium. The speed of SPP reaction was estimated by the value of time $t_{1/2}$ during which the optical density of actomyosin increased to the value of $D/2$, where D is the optical density of actomyosin after SPP reaction is completed.

In order to study the influence of aluminum ions on ATPase activity of myosin and SPP actomyosin these cations (in the form of aluminum chloride solution) were added in appropriate concentrations to the incubation medium.

The reagents that have been used are those produced by home and foreign producers with the high level of purity. Water solutions of the reagents as well as incubation media were prepared from bidistilled and deionized water.

Results and Discussion. The research involved the solution of three problems: (1) to study ATPase activity of cardiac muscle myosin in the presence of aluminum ions. The investigations were performed in the concentration range of AlCl_3 from 0,1 to 10 mM at the presence of 5 mM CaCl_2 . ATPase activity of cardiac muscle myosin showed a dose depending decrease at these concentrations of aluminum ions (Fig. 1, curves 1 and 2). At the concentration of aluminum ions 5 mM half maximum deceleration of ATPase myosin activity in cardiac muscle was observed.

The next problem was (2) to study the SPP reaction at the presence of aluminum ions. It should be noted that actomyosin SPP reaction enables to investigate of certain properties of muscle contractile protein complex under the influence of various factors, in particular metal ions [15, 16]. The analysis of formation and dissociation of precipitate during SPP actomyosin reproduces the actual process of muscle contraction almost completely: with available magne-

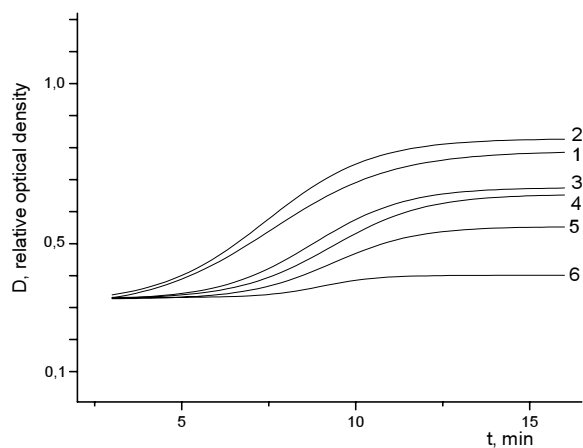


Fig. 2. The kinetic curves of cardiac muscle actomyosin SPP reaction at different concentrations of AlCl_3 (mM): 1 – control; 2 – 0.01; 3 – 0.05; 4 – 0.1; 5 – 0.5; 6 – 1.

sium ions and ATP the calcium ions cause the muscle contraction while after removing calcium ions with the help of EGTA and at the presence of ATP the muscle is relaxed. Ions of Ca^{2+} and Mg^{2+} are known to have opposite action on SPP: calcium ions accelerate whereas magnesium ions decelerate this process. The model study of actomyosin structural stability under the action of different factors is also of interest since it causes the changes in intramolecular interactions and produces effect on SPP. These investigations are aimed to reveal the forces responsible for the stabilization of the unique myosin molecule structure as well as the changes that can occur during muscle contraction.

Fig. 2 presents the kinetic curves of actomyosin SPP reaction in the cardiac muscle at different concentrations of aluminum ions.

In particular, when adding ATP to the reaction mixture (curve 1) SPP reaction demonstrates gradual increase in actomyosin optical density followed by a plateau and further stabilization. The value of $t_{1/2}$ in this case is about 5 min. The addition of 0.01 mM AlCl_3 (curve 2) does not result in noticeable change in speed and degree of SPP during adding ATP to actomyosin (curve 1). With the increase of aluminum ions concentration from 0.05 to 0.5 mM (curves 3–5), the behavior of SPP reaction changes: SPP actomyosin curves display a refractory phase (it is a delayed phase of a slight increase in optical density of actomyosin without noticeable precipitation structures), which further transforms into a

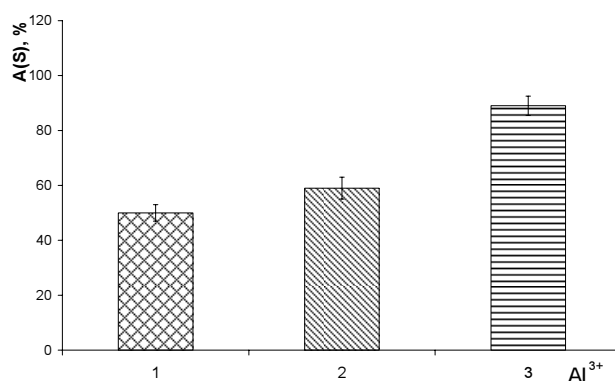


Fig. 3. SPP (S) (1) of actomyosin, ATPase activity (A) of actomyosin (2) and of myosin (3) in cardiac muscle at the presence of aluminum ions (Al^{3+} , 1 mM). The values of these parameters are assumed to be 100 % at the presence of 1 mM Mg^{2+} (for ATPase activity and actomyosin SPP) or of Ca^{2+} (for ATPase activity of myosin) ($M \pm m$, $n=7$).

phase of fast increase in optical density and then finishes with a plateau on SPP reaction curves. At the concentration of aluminum ions 1 mM the SPP reaction is inhibited (curve 6).

Our next task was (3) to compare the results obtained from the research in the action of aluminum ions on actomyosin SPP reaction and ATPase activity of actomyosin and myosin in the cardiac muscle (Fig. 3).

At the concentration of aluminum ions 1 mM in contractile proteins of cardiac muscle the actomyosin SPP reaction as well as myosin ATPase reaction are inhibited. The same is observed for different (in terms of functioning) ATPases in the other tissues [17, 18]. Since ATP hydrolysis is produced by myosin one may expect that the structural reconstruction taking place during the formation of myosin intermediates should also occur during SPP actomyosin. The stable analogues of intermediate states of myosin in ATPase reaction are used for investigating the myosin subfragment 1 (S1 structure), one of which is S1 complex with ADP and AlF_4^- [19]. The dissociation of this complex is analogous to the dissociation of key intermediate in the process of ATPase reaction. S1 myosin complexes with $\text{MgADP} \cdot \text{AlCl}_4$ also imitates different structural states of myosin head [20]. Besides, aluminum ions can influence certain characteristics of actin filaments [21]. There are data about the interaction between aluminum ions and regulatory proteins, in particular about their

Table 1
The physical characteristics of metals

Metal	Al	Mg	Ca
Atomic radius, nm	0.143	0.160	0.197
Ion radius, nm	0.053 (4)* 0.062 (5) 0.067 (6)	0.071 (4) 0.080 (5) 0.086 (6) 0.103 (8)	0.114 (6) 0.126 (8) 0.137 (10) 0.148 (12)

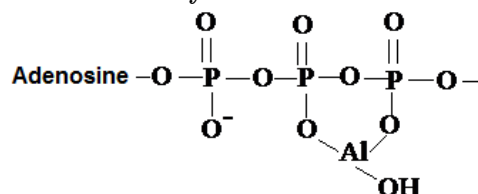
*Note: the figure given in the parenthesis is the coordination number.

influence on spectral properties of Ca²⁺-binding subunit of troponin — troponin C [22]. The troponin C binding with aluminum induces the increase of its fluorescence and makes the hydrophobic site of this protein accessible to binding with fluorescent probes. Aluminum ions influence also Ca²⁺ and/or Mg²⁺-induced changes in troponin C.

The ions of Eu³⁺ like Ca²⁺ and Mg²⁺ ions are known not to interact with the purine fraction of ATP in the solution but to bind with three-phosphate cluster (with β- and γ-phosphates of ATP), with Eu³⁺ ion being bound much stronger to ATP than Mg²⁺ (the difference between their binding constants is greater by over an order of magnitude) [19]. Unfortunately, so far there have been no available data concerning aluminum

ions binding to ATP. However, the comparison of ion radii values for Al, Mg and Ca given in Table 1 shows that the above values for Al and Mg are very close while for Ca they differ considerably.

Thus, there seems to be enough evidence to conclude that aluminum is also capable of forming complexes with β- and γ-phosphate groups of ATP. Basing on this assumption, one can propose the most likely its structure:



Conclusion. The detailed analysis of actomyosin SPP reaction and ATPase activity of myosin in cardiac muscle in the presence of aluminum ions acting as an external factor has shown that aluminum ions in certain concentrations can inhibit SPP actomyosin and myosin ATPase reactions in the cardiac muscle. The obtained results provide a deeper insight into the mechanisms of muscle contractile proteins functioning in the presence of aluminum ions in the muscle and are a basis for correction of norm/pathology states.

Надійшла в редакцію 11.12.2011 р.

Вплив іонів алюмінію на суперпреципітацію актоміозину та АТФазну активність міозину серцевого м'яза

К.І. Богуцька¹, Ю.І. Прилуцький¹, Ю.П. Склярів²

¹ Київський національний університет імені Тараса Шевченка
вул. Володимирська, 64, Київ, 01601, Україна

² Національний медичний університет імені О.О. Богомольця
бульв. Т. Шевченка, 13, Київ, 01601, Україна

Резюме. Виявлено концентраційно-залежне інгібування реакції суперпреципітації актоміозину й АТФазної активності міозину серцевого м'яза іонами алюмінію, які здатні викликати розвиток патологічних процесів в організмі.

Ключові слова: серцевий м'яз, актоміозин, міозин, АТФаза, суперпреципітація.

References

1. Peto M.V. Aluminium and iron in humans: bioaccumulation, pathology and removal // *Rejuvenation Res.* — 2010. — Vol. 13, N 5. — P. 589-598.
2. Avtzin A.P., Zchavoronkov A.A., Rish M.A., Strochkova L.S. Microelementoses in humans: etiology, classification, organ pathology. — M.: Medicine, 1991. — 496 p. (in Russian)
3. Skalniy A.V., Rudakov I.A. Bioelements in medicine. — M.: Onix 21 Century, 2004. — 272 p. (in Russian)
4. Nayak P. Aluminium: impacts and disease // *Environ. Res.* — 2002. — Vol. 89, N 2. — P. 101-115.
5. Clapp T., Siebert P., Chen D., Jones Braun L. Vaccines with aluminum-containing adjuvants: optimizing vaccine efficacy and thermal stability // *J. Pharm. Sci.* — 2011. — Vol. 100, N 2. — P. 388-401.
6. Tomljenovic L., Shaw C.A. Aluminum vaccine adjuvants: are they safe? // *Curr. Med. Chem.* — 2011. — Vol. 18, N 17. — P. 2630-2637.
7. Shalunov E.P., Matrosov A.L., Shvedov M.A.

Granulated aluminum nanocomposition materials in extreme conditions of operation // II Russ. Conf. Nanomatter «NANO 2007», 2007. — 406 p. (*in Russian*)

8. Rudenko S.S., Ozerova I.O., Ribitzka M.M., Voloshchuk K.O. The influence of aluminum intoxication and γ -irradiation on the state of body's antioxidant system and the possibility of its managing // Ukr. Biokhim. Zh. — 1998. — Vol. 70, N 2. — P. 84-88. (*in Russian*)

9. Gurjar M., Baronia A.K., Azim A., Sharma K. Managing aluminum phosphide poisonings // J. Emerg. Trauma Shock. — 2011. — Vol. 4, N 3. — P. 378-384.

10. Proudfoot A. Aluminium and zinc phosphide poisoning // Clin. Toxicol. — 2009. — Vol. 47, N 2. — P. 89-100.

11. Miroshnichenko M.S., Minchenko P.G., Shuba M.F. Aluminium impact on actomyosin precipitation // DAN of Ukr. — 1993. — N 6. — P. 78-81. (*in Russian*)

12. Bogutska K.I., Danilova V.M., Minchenko P.G., Sheremet L.P. The influence of aluminium ions on actomyosin precipitation in cardiac and skeleton muscles // Biopolymers and cell. — 2003. — Vol. 19, N 4. — P. 362-366. (*in Russian*)

13. Margossian S.S. Reversible dissociation of dog cardiac myosin regulatory light chain and its influence on ATP hydrolysis // J. Biol. Chem. — 1985. — Vol. 260, N 25. — P. 13747-13754.

14. Severin S.E., Solov'eva G.A. Biochemical practices. — Leningrad: LGU, 1989. — 509 p. (*in Russian*)

15. Tickunov B.A. The character of actin-myosin interaction in two stages of superprecipitation reaction

// Biofizika. — 1991. — Vol. 36, N 2. — P. 261-265. (*in Russian*)

16. Tickunov B.A. The impact of magnesium ions on the two-stage kinetics of superprecipitation and ATPase of natural actomyosin // Biokhimia. — 1990. — Vol. 55, N 5. — P. 822-828. (*in Russian*)

17. Slinchenko N.M. Effect of fluoroaluminate on the ATP-hydrolysing and Ca^{2+} -transporting activity of the purified Ca^{2+} , Mg^{2+} -ATPase of myometrium cell plasma membranes // Ukr. Biokhim. Zh. — 2003. — Vol. 75, N 5. — P. 95-98. (*in Russian*)

18. Sushma N.J., Rao K.J. Total ATPases activity in different tissues of albino mice exposed to aluminium acetate // J. Environ. Biol. — 2007. — Vol. 28, Sup. 2. — P. 483-494.

19. Ponomarev M.A., Timofeev V.P., Levitsky D.I. The difference between ADP-beryllium fluoride and ADP-aluminium fluoride complexes of the spin-labelled myosin subfragment 1 // FEBS Lett. — 1995. — Vol. 371, N 3. — P. 261-263.

20. Levitzky D.I., Litvinov I.S., Poglazov B.F. The study of the binding of cations with subfragment 1 of myosin by means of fluorescent Eu^{3+} probe // Biokhimia. — 1982. — Vol. 47, N 9. — P. 1504-1511. (*in Russian*)

21. Arnoys E.J., Schindler M. Aluminium modifies the viscosity of filamentous actin solutions as measured by optical displacement microviscometry // Anal. Biochem. — 2000. — Vol. 277, N 1. — P. 1-10.

22. Phan B.C., Reisler E. Aluminium fluoride interactions with troponin C // Biophys. J. — 1993. — Vol. 65, N 6. — P. 2511-2516.