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INFLUENCE OF SHORT-TERM HEATING ON THE ARRANGEMENT OF SARCOPLASMIC RETICULUM Ca-ATPase

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LÜHIAJALISE KUUMUTAMISE MÕJU Ca-ATPaasile SARKOPLASMAATILISES RETIIKULUMIS. Erika GEIMONEN, Aleksander RUBTSOV, Marina BATRUKOVA

ВЛИЯНИЕ КРАТКОВРЕМЕННОЙ ТЕРМООБРАБОТКИ НА ОЛИГОМЕРНОЕ СОСТОЯНИЕ Ca-АТФазы САРКОПЛАЗМАТИЧЕСКОГО РЕТИКУЛУМА. Эрика ГЕЙМОНЕН, Александр РУБЦОВ, Марина БАТРУКОВА

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It is known that short-term heating of sarcoplasmic reticulum (SR) vesicles at 42—45 °C leads to a sharp decrease of Ca-pump efficiency without any effect on the Ca-ATPase hydrolytic activity [1]. The decrease of the pump efficiency is connected with the release of accumulated Ca^{2+} from the vesicles as a result of a strong increase of the membrane permeability for this ion [2]. Analysis of the phenomenon called thermo-uncoupling may be useful for understanding the possible pathways of Ca^{2+} release from SR. The appearance of such new pathways for Ca^{2+} release from SR can be relevant to the regulation of Ca^{2+} exchange in muscle tissue under normal and pathological conditions.

The formation of Ca-permeable channels between Ca-ATPase molecules at enzyme clusterization is one of the possibilities of the Ca^{2+} release from SR [3]. It has been shown earlier that the Ca-pump thermo-uncoupling is connected with the decrease of the SR lipid bilayer microviscosity that can induce protein oligomerization [2, 4]. As shown elsewhere, thermotreatment of SR vesicles decreases the membrane surface potential that seems to reflect clusterization of the membrane proteins [5]. However, all approaches used previously have given only indirect information about the Ca-ATPase protein arrangement in the SR membranes.

Bifunctional chemical cross-linking of target groups of the neighbouring protein molecules can be used for a more direct investigation of the protein arrangement in biological membranes. We have used this approach in studying the Ca-ATPase oligomeric state in control and thermotreated SR preparations. Cupric *o*-phenanthroline [6] and 1,5-difluoro-2,4-dinitrobenzene (DFDNB) [7] were used in our study. These compounds cross-link the proteins located in a close proximity: cupric *o*-phenanthroline induces the formation of S-S-bridges, and DFDNB interacts with the SH-groups the distance between which is about 5 Å.

Electrophoresis of the control and thermotreated SR preparations in the presence of SDS revealed distinct differences between them (Fig. 1). In the thermotreated SR preparations some new protein bands with molecular weights 140, 240, 280, and 310 kDa appeared, and a significant amount of the protein did not enter the gel. The appearance of the 140 kDa protein band seems to be connected with intramolecular cross-linking of the Ca-ATPase protein: As it is known from literature [8], intramolecular cross-linking of Ca-ATPase protein changes its electrophoretic mobility.

The protein bands with molecular weights 240, 280, and 310 kDa in Fig. 1 probably correspond to dimers and trimers of Ca-ATPase. The differences in their electrophoretic mobility seem to be connected with the different number of intramolecular bonds in individual Ca-ATPase molecules forming these di- and trimers. The Ca-ATPase oligomers with the higher molecular weights did not enter the gel.

A study of the nature of the intra- and intermolecular bonds forming after thermotreatment showed that SDS treatment (2 min at 100 °C, 15 min at 60 °C, or 60 min at 37 °C) had no effect on the mobility of the new protein bands, neither did the addition of β-mercaptoethanol (5 mM) or dithiotreitol (5 mM).

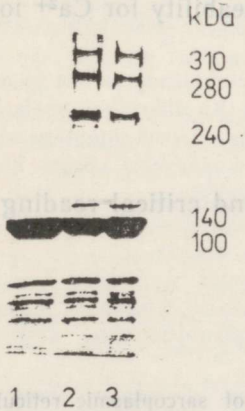

Fig. 1. SDS-gel electrophoresis patterns of sarcoplasmic reticulum vesicles. *1* — control vesicles, *2* — thermotreated vesicles, *3* — control vesicles incubated 2 min with cupric *o*-phenanthroline.

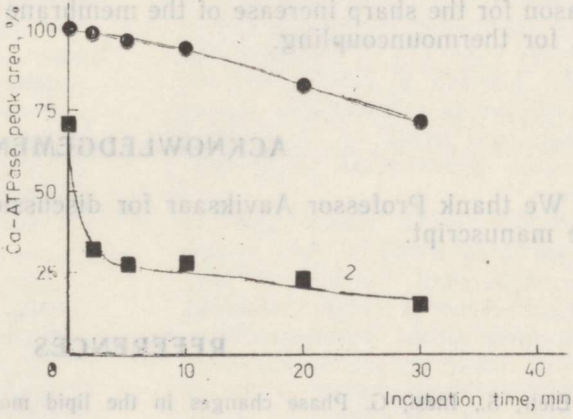

Fig. 2. Rate of cross-linking of Ca-ATPase with cupric *o*-phenanthroline by SDS-gel electrophoresis. *1* — control vesicles, *2* — thermotreated vesicles. The standard assay for cross-linking contained 100 mM MOPS (pH 7.2), 1 mM $CaCl_2$, 0.1 mM $CuSO_4$, 2 mg/ml protein of sarcoplasmic reticulum, 0.3 mM *o*-phenanthroline at 25 °C. The reaction was stopped at different incubation intervals by 1 mM EDTA, 16 mM *N*-ethylmaleimide, and 10 mg/ml SDS-Na. Electrophoresis was carried out according to Laemmli [9].

Addition of β-mercaptoethanol and dithiotreitol in the above-mentioned concentrations into the medium before thermotreatment resulted in a nonsignificant decrease of the intensity of the high-molecular weight protein bands. However, incubation of the thermotreated preparations in the presence of 50 mM β-mercaptoethanol at 100 °C decreased significantly the amount of Ca-ATPase oligomers (data not shown). Therefore, we conclude that thermotreatment resulted in covalent cross-linking of the Ca-ATPase protein; the bonds formed seem to be S-S-bridges.

Fig. 1 shows that incubation of the control SR vesicles with the cross-linking agent cupric *o*-phenanthroline results in the appearance of high-molecular weight protein bands which are practically identical in mobility to those in the thermotreated preparations. Comparison of the cross-linking process in the control and thermotreated preparations showed a significant difference in the kinetics of cross-linking. Thus, in the thermotreated SR vesicles the Ca-ATPase protein is already partially cross-linked before the addition of cross-linking agents. After 30 min incubation with cupric *o*-phenanthroline the amount of Ca-ATPase monomer decreased by 85% (Fig. 2) and after 90 min incubation with DFDNB, by 90% (data not shown). Under the same conditions, the amount of Ca-ATPase monomer in the control SR vesicles decreased by 30% after 30 min incubation with cupric *o*-phenanthroline (Fig. 2) and by 30% after 90 min incubation with DFDNB (data not shown).

Analysis of the minor protein contents after electrophoresis showed that thermotreatment did not change their amount. Therefore, these low-molecular weight proteins obviously did not participate in the formation of the high-molecular weight oligomers consisting only of Ca-ATPase molecules.

From the obtained data we can make the conclusion that thermotreatment of SR vesicles induces a strong oligomerization of the Ca-ATPase protein and part of the Ca-ATPase molecules acquire inter- and intramolecular covalent bonds. We suggest that clusterization of the Ca-ATPase protein in SR membranes on thermotreatment is the main reason for the sharp increase of the membrane permeability for Ca^{2+} ions, i.e. for thermouncoupling.

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REFERENCES

1. Eletr, S., Inesi, G. Phase changes in the lipid moieties of sarcoplasmic reticulum membranes induced by temperature and protein conformational changes. — Biochim. Biophys. Acta, 1972, **290**, 178—185.
2. Геймонен Э. Р., Рубцов А. М., Болдырев А. А. Механизм температурного разобщения Са-насоса саркоплазматического ретикулума: изменение функциональных свойств и структурного состояния молекулы фермента. — Биохимия, in press.
3. Moller, J. V., Andersen, J. P., Le Maire, M. The sarcoplasmic reticulum Ca-ATPase. — Mol. Cell. Biochem., 1982, **42**, 83—107.
4. Болдырев А. А., Куин П. Дж., Лущак В. И. Механизм термоиндуцированного функционального разобщения Са-насоса саркоплазматического ретикулума скелетных мышц. — Биохимия, 1986, **51**, 1, 150—159.

5. Geimonen, E., Rubtsov, A. Study of membrane surface potential of sarcoplasmic reticulum vesicles using pH-sensitive dye and paramagnetic probe. — Proc. Estonian Acad. Sci. Chem., 1993, **42**, 3, 153—157.
6. Murphy, A. J. Cross-linking of the sarcoplasmic reticulum ATPase protein. — Biochem. Biophys. Res. Commun., 1976, **70**, 160—166.
7. Shaltier, S., Tauber-Finkelstein, M. Introduction of an intramolecular crosslink at the active site of glyceraldehyde 3-phosphate dehydrogenase. — Biochem. Biophys. Res. Commun., 1971, **44**, 482—490.
8. Ross, D. C., McIntosh, D. B. Intramolecular cross-linking of domains at the active site links A1 and B subfragments of the Ca-ATPase of sarcoplasmic reticulum. — J. Biol. Chem., 1987, **262**, 2042—2049.
9. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. — Nature, 1970, **227**, 680—685.