Chaperone Activity of α-Crystallin in Relation to the Sarcoplasmic Reticulum Ca^{2+} Pump of Skeletal Muscles in Stress and Adaptation

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α-Crystallin, an endogenous low-molecular-weight protein with chaperone activity, exerted protective effects on membrane systems of Ca^{2+} transport into the sarcoplasmic reticulum of skeletal muscles. Protective action of α-crystallin depended on the body state. This effect was not observed in the control and after adaptation to stress, while after stress, especially against the background of adaptation, α-crystallin increased the rate of Ca^{2+} transport into the sarcoplasmic reticulum and thermal resistance of Ca^{2+} pump. The mechanisms of α-crystallin activation during stress are discussed.

Key Words: *Ca^{2+} transport; α-crystallin; adaptation; stress*

Realization of protective effects of α-crystallin during stress-induced protein damages is well studied.

α-Crystallin constituting about 30% of all soluble proteins of the eye lens [14] displays a considerable chaperone activity in relation to other crystallins and prevents eye lens opacity induced by free-radical, thermal, and other damages [9,13,21]. α-Crystallin is present practically in all body tissues. The heart and skeletal muscles contain the highest levels of α-crystallin (5% of soluble cytoplasmic proteins) [14]. The range of protein structures, whose thermal denaturation is prevented or inhibited by α-crystallin, is extending. Therefore, this nonspecific protector has the general biological importance [8,11,12].

Protective effects of α-crystallin are realized only in relation to proteins with damaged structures formed after thermal injuries, pH decrease, oxidative modification, and effects of other factors [5,12,18]. It can be assumed that chaperone activity of α-crystallin depends on the degree of protein structural damages. Here we compared the effects of α-crystallin on thermal resistance of sarcoplasmic reticulum Ca^{2+} pump of skeletal muscles in various body states (control, stress, adaptation, and stress against the background of adaptation).

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-220 g. The animals were divided into 4 groups. Group 1 rats served as the control. Group 2 rats were exposed to long-term immobilization stress (fixation of four limbs in the supine position for 3 h). Group 3 rats were adapted to short-term stress (15 min on day 1, 30 min on day 2, 45 min on day 3, and then 60 min per day over 3 weeks). Group 4 rats were immobilized for 3 h after adaptation to short-term stress. The animals were decapitated 2 h after stress or 24 h after adaptation. The thigh muscles were removed, washed with ice-cold physiological saline, and frozen in liquid nitrogen.

For isolation of sarcoplasmic reticulum (SR) membranes, skeletal muscles were minced in an Ultra-Turrax knife homogenizer for 30 sec at 12,000 rpm.

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The medium contained 100 mM KCl and 10 mM histidine (pH 7.5, 1:4 tissue:medium ratio). The homogenate was centrifuged at 105,000*g* for 20 min, the precipitate was removed, and the supernatant was filtered through a 4-layer gauze and centrifuged at 105,000*g* for 60 min. The precipitate was suspended in 0.6 M KCl with 5 mM histidine (pH 7.0) using a Teflon-glass homogenizer. The membrane suspension was then centrifuged at 105,000*g* for 60 min, the supernatant was removed, and the precipitate was suspended in a medium containing 20% glycerol, 5 mM imidazole (pH 7.0), and 50 mM KCl. All procedures were performed at 3-4°C. SR preparations were stored in liquid nitrogen.

The rate of Ca^{2+} transport was determined by the rate of Ca^{2+} uptake by SR vesicles (Ca^{2+} content in the incubation medium was recorded on line) in an Orion EA 940 ionomer (Orion Res.) equipped with a Ca^{2+} selective electrode (model 93-20) as described previously [15]. The rate of Ca^{2+} transport in SR was measured in 7 ml incubation medium containing 100 mM KCl, 15 mM potassium oxalate, 20 mM HEPES (pH 7.1), 4 mM $MgCl_2$, and 5 mM NaN_3 at 37°C. The reaction was initiated by the addition of ATP and Ca^{2+} at final concentrations of 4 mM and 10 μM, respectively, into the medium containing 40 μl SR preparation.

α-Crystallin from bovine eye lens was used. Its effects were analyzed by the addition of 0.4 mg α-crystallin to the suspension containing 80 μg SR protein before thermal inactivation. In control samples, α-crystallin was replaced with BSA for evaluation of nonspecific protein effects.

Conformational stability of the Ca^{2+} pump molecule was evaluated by analyzing its resistance to thermal denaturation [2,3]. After 2-min preincubation, thermal denaturation was initiated: SR preparations were incubated in the absence or presence of α-crystallin in temperature-controlled cells at 41°C and constant mixing for 5-20 min. The results were analyzed by Excel software.

RESULTS

In the control we observed intense thermal inactivation of skeletal muscle SR Ca^{2+} pump leading to 70% loss of its activity over 20 min (Fig. 1, *a*). Stress decreased the initial rate of Ca^{2+} transport in SR by 1.5 times (Fig. 2, *a*) and sharply increased the rate of thermal inactivation of the enzyme: over 10-min thermal inactivation, its activity in control and stressed animals decreased by 25% and 60%, respectively. Adaptation to stress had no effect on the rate of Ca^{2+} transport in SR, but considerably improved thermal resistance of the enzyme, especially during long-term thermal inactivation (by 2.3 times). Immobilization after adaptation to stress had no effect on the rate of Ca^{2+} transport and thermal resistance of the enzyme, and these parameters did not differ from the control (Fig. 2, *b*).

Dose-effect calibration was performed before analyzing the effect of α-crystallin on membrane systems of Ca^{2+} transport to SR in control, stressed, and adapted animals. The maximum effect of α-crystallin was observed within a narrow concentration range (4.4-5.6 mg/mg protein) at a 5:1 α-crystallin:protein ratio and did not change after increasing this ratio to 7-10:1 (Fig. 3). This agrees with previously reported optimum α-crystallin/protein ratio, at which the protective effect on soluble and cytoskeletal proteins was observed [8,14].

In the control, α-crystallin had no stimulatory effects on the initial rate of Ca^{2+} transport in SR and thermal resistance of Ca^{2+} pump (Fig. 1, *a*). Only after 10-min thermal exposure (41°C), the rate of Ca^{2+} transport in the presence of α-crystallin increased by 27%. Adaptation to stress considerably improved thermal resistance of Ca^{2+} pump, which was probably responsible for the absence of α-crystallin-induced activation of Ca^{2+} transport in adapted animals. Indeed, the rate and thermal resistance of the enzyme did not change after various thermal exposures in the presence of α-crystallin (Fig. 1, *b*).

By contrast, the effects of α-crystallin in acute stress were different (Fig. 2). α-Crystallin prevented stress-induced inhibition of Ca^{2+} pump, whose parameters returned to the control. However, thermal resistance of the enzyme remained low. α-Crystallin also

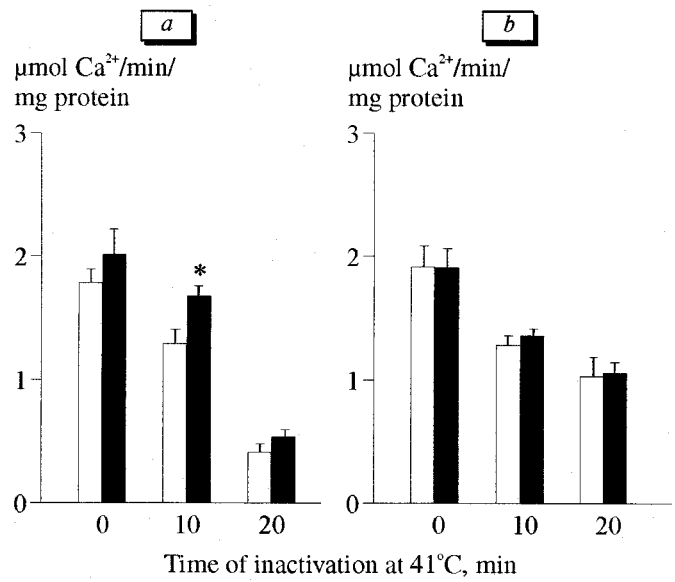

Fig. 1. Effects of α-crystallin (dark bars) on the rate and thermal resistance of the sarcoplasmic reticulum Ca^{2+} pump of skeletal muscles in the control (*a*) and after adaptation to stress (*b*). *$p<0.05$ compared with preparations without α-crystallin (light bars).

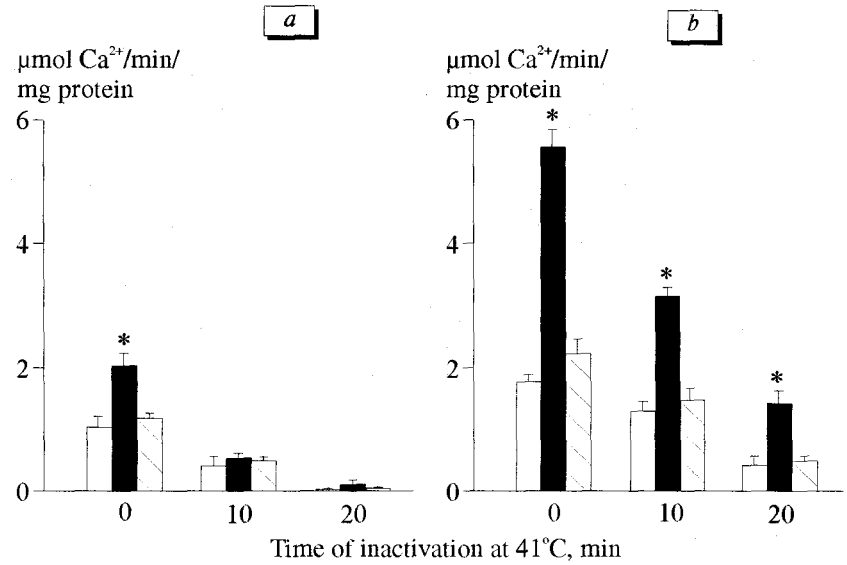

Fig. 2. Effects of α-crystallin (dark bars) and BSA (shaded bars) on the rate of Ca^{2+} transport and thermal resistance of the sarcoplasmic reticulum Ca^{2+} pump in skeletal muscles during acute stress in control animals (*a*) and in stress against the background of adaptation (*b*). *$p<0.05$ compared with preparations without α-crystalline (light bars).

displayed considerable activating effects during stress against the background of adaptation (Fig. 2, *b*). The maximum protective effect of α-crystallin was observed in acute stress after long-term adaptation. The initial rate of Ca^{2+} transport in SR increased more than 3-fold. This was not associated with nonspecific action of protein, because BSA displayed no such effects.

In this group, thermal resistance of the enzyme did not decrease during Ca^{2+} pump activation in the presence of α-crystallin. Such activation was not observed under the effect of α-crystallin on skeletal muscle SR after single acute stress. However, a 1.5-3-fold increase in the SR Ca^{2+} pump rate accompanied by a sharp decrease in its resistance to various damaging factors, in particular high Ca^{2+} content, was observed at the initial stages (1-4 sessions) of adaptation to physical exercises (swimming). Similar changes were observed in the heart: physical exercise increased the rate of Ca^{2+} transport in SR by 27% and led to an 8-10-fold decrease in thermal resistance of the enzyme [1]. Thus, activation of the SR Ca^{2+} pump can be accompanied by a considerable decrease in its resistance to damaging factors and, therefore, its lifetime [6]. That is why, the preserved thermal resistance of SR Ca^{2+} pump in skeletal muscles despite manifold increase in the enzyme activity induced by α-crystallin is of considerable importance. Hence, at any time of thermal inactivation, the rate of Ca^{2+} transport in SR after stress against the background of adaptation in the presence of α-crystallin 2.3-3-fold surpassed the rate in the absence of the protein.

After stress against the background of adaptation and without α-crystalline, the rate of Ca^{2+} transport in SR and thermal resistance of the enzyme remained at the control level. However, α-crystalline considerably activated Ca^{2+}-transporting systems during stress in adapted animals (by contrast to its effects in controls). The data suggest that in adapted animals, acute stress induces accumulation of α-crystallin activators including low-molecular-weigh proteins of the hsp family (for example, hsp25 and hsp27) [10,16]. These proteins promote α-crystallin phosphorylation or transformation to the membrane-bound form and, therefore, its inactivation. Moreover, SH groups are probably involved in potentiation of α-crystallin protective ef-

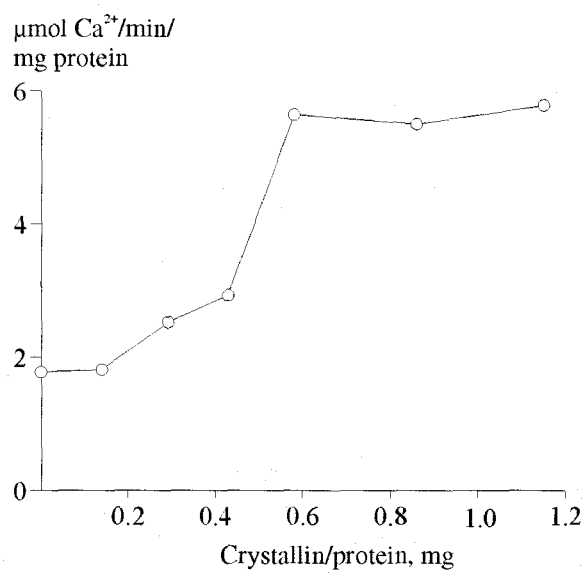

Fig. 3. Activation of sarcoplasmic reticulum Ca^{2+} pump in skeletal muscles in the presence of various doses of α-crystallin.

fects [17] during acute and chronic adaptive procedures [4,7,19,20].

Thus, α-crystallin displays the highest chaperone activity in relation to the SR Ca^{2+}-transporting system of skeletal muscles during acute stress accompanied by structural damages in this enzyme complex. Therefore, α-crystallin can be used for promoting adaptation during combined action of this thermal stabilizer and various adaptive factors.

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