Biochemical and Biophysical Research Communications 438 (2013) 218–223

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Differential effects of mutant SOD1 on protein structure of skeletal muscle and spinal cord of familial amyotrophic lateral sclerosis: Role of chaperone network

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ARTICLE INFO

Article history:
Received 12 July 2013
Available online 23 July 2013

Keywords:
Heat shock proteins
Familial ALS
BisANS
Protein conformation
Mutant SOD1
Surface hydrophobicity

ABSTRACT

Protein misfolding is considered to be a potential contributing factor for motor neuron and muscle loss in diseases like Amyotrophic lateral sclerosis (ALS). Several independent studies have demonstrated using over-expressed mutated Cu/Zn-superoxide dismutase (mSOD1) transgenic mouse models which mimic familial ALS (f-ALS), that both muscle and motor neurons undergo degeneration during disease progression. However, it is unknown whether protein conformation of skeletal muscle and spinal cord is equally or differentially affected by mSOD1-induced toxicity. It is also unclear whether heat shock proteins (Hsp′s) differentially modulate skeletal muscle and spinal cord protein structure during ALS disease progression. We report three intriguing observations utilizing the f-ALS mouse model and cell-free *in vitro* system; (i) muscle proteins are equally sensitive to misfolding as spinal cord proteins despite the presence of low level of soluble and absence of insoluble G93A protein aggregate, unlike in spinal cord, (ii) Hsp′s levels are lower in muscle compared to spinal cord at any stage of the disease, and (iii) G93ASOD1 enzyme-induced toxicity selectively affects muscle protein conformation over spinal cord proteins. Together, these findings strongly suggest that differential chaperone levels between skeletal muscle and spinal cord may be a critical determinant for G93A-induced protein misfolding in ALS.

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1. Introduction

Protein misfolding is believed to be the key element in the pathology of most neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) that is characterized by progressive loss of motor neurons followed by muscle weakness, paralysis and death [1]. The etiology of ALS is currently unknown; however, approximately 20% of familial ALS (f-ALS) cases have been linked to mutations in the antioxidant gene, copper–zinc superoxide dismutase (SOD1) [2] and transgenic mice overexpressing the mutant SOD1 (mSOD1) closely mimic ALS pathology [3]. It is unclear whether mSOD1 mediated toxicity is confined solely to motor neurons or capable of affecting other tissues (e.g., skeletal muscle) prior to the onset and during disease progression. With regards to muscle cells, it has been reported that muscle dysfunction and neuromuscular junction (NMJ) degeneration occur long before the onset of symptoms and motor neuron death [4,5]. Two independent studies have claimed that skeletal muscle is the primary target of mSOD1-mediated toxicity as muscle-specific G93A overexpression induces neurodegeneration and muscle atrophy [6,7]. However a recent study found that muscle-specific overexpression of the transcriptional coactivator, PGC-1α delays muscle atrophy and improves muscle endurance, but does not extend lifespan of G93A mice [8]. In addition, there are contradictory reports with regard to the role of motor neurons in ALS pathogenesis; it has been shown that overexpression of two mSOD1 proteins (G93A and G85R) selectively in motor neurons fail to produce any detectable sign of pathology or disease [9], whereas, another study found that motor neuron restricted expression of mSOD1 was sufficient to cause ALS pathology [10]. A recent study from Cleveland's labora-

Abbreviations: BisANS, 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid dipotassium salt; f-ALS, familial Amyotrophic lateral sclerosis; Hsp, heat shock protein.

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0006-291X/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.bbrc.2013.07.060

tory [11] raises an open question on the classical dogma about the pathogenic link between mSOD1 aggregation and mitochondrial dysfunction. The study showed that reduction of mSOD1 aggregates and motor neuron cell death by enhancing mitochondrial calcium homeostasis does not extend survival, prevent muscle denervation or motor axon degeneration in f-ALS mice. All of these data thus suggest that both motor neurons and skeletal muscle are likely be the targets of mSOD1-induced toxicity and may synergestically, not individually, contribute to ALS pathology.

Despite the fact that protein misfolding plays a causative role in initiation and progression of ALS [12,13], less study has been done on changes in protein conformation. We have previously shown that certain skeletal muscle proteins are commonly affected in two mouse models of f-ALS (G93A and H46RH48Q), both structurally and functionally [14]. Our recent study showed that skeletal muscle proteins are misfolded prior to the loss of muscle mass and disease onset in G93A mice which was not dependent on aggregation of G93A protein [15]. However, it is not known if mSOD1-induced toxicity equally or differentially affects conformation of skeletal muscle and spinal cord proteins during disease progression. It is also unclear whether the status of chaperone proteins differ between skeletal muscle and spinal cord tissues during ALS disease progression. In this study, we have addressed these questions and made two intriguing observations: (i) muscle proteins are equally sensitive to misfolding as spinal cord proteins despite the presence of low soluble and no insoluble G93A aggregates, when compared to G93ASOD1 enzyme level in the soluble and insoluble form of aggregates in spinal cord at any stage of the disease, and (ii) skeletal muscles from G93A mice express lower levels of heat shock proteins (Hsp′s), when compared to spinal cords from G93A mice. We further demonstrate that muscle proteins of wild-type mice are more vulnerable to misfolding compared to spinal cord proteins when challenged with the purified recombinant G93ASOD1 enzyme *in vitro*. The difference in chaperone levels between skeletal muscle and spinal cord can explain the vulnerability of skeletal muscle proteins to misfolding over spinal cord proteins during ALS disease progression and may likely be a critical determining factor for G93A-induced protein misfolding in ALS pathogenesis.

2. Materials and methods

2.1. Animals

Male B6SJLTg (SOD1-G93A) Gur1/J mice backcrossed to C57Bl/6 J and C57Bl/6 J wild-type (WT) mice were sacrificed at three different time points (50–65 days, pre-symptomatic; 95–100 days, onset and 130–135 days, post-symptomatic). All procedures for handling animals in this study were reviewed and approved by the IACUC (Institutional Animal Care and Use Committee) of University of Texas Health Science Center at San Antonio and the IACUC at the Audie L. Murphy Memorial Veterans Hospital.

2.2. Change in protein surface hydrophobicity

The changes in protein surface hydrophobicity were determined by the method described earlier [14,15]. In brief, gastrocnemius muscle and spinal cord tissues from G93A mice (pre- to post-symptomatic disease stages) and age-matched WT mice were homogenized in 50 mM Tris, pH 7.4 containing 1 mM $MgSO_4$ and protease inhibitor and centrifuged at 100,000×*g* for 1 h at 4 °C to obtain the cytosolic fraction. To study the effects of recombinant G93ASOD1 enzyme on protein conformation, equal amount of cytosolic proteins (100 μg) containing 1 mM ATP was treated with WTSOD1 (Sigma–Aldrich) and G93ASOD1 (a kind gift from Dr. John Hart, Department of Biochemistry, University of Texas Health Science Center at San Antonio) at 0, 2, 5 and 10 μM concentrations for 1 h at 37 °C. Thereafter, equal amount of proteins (1 mg/ml) were photo-labeled by 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid, dipotassium salt (BisANS, 0.1 mM) under UV light. BisANS-labeled proteins (10 μg) were then subject to 12% SDS–PAGE. After electrophoresis, BisANS fluorescence was captured under 365 nm UV light with an AlphaImage™ 3400. Gels were stained in Coomassie blue or SYPRO Ruby overnight to normalize for protein loading.

2.3. Western blot for heat shock proteins and G93A SOD1 enzyme

Gastrocnemius muscle and spinal cord tissues from G93A mice (pre- to post-symptomatic disease stages) and age-matched WT mice were homogenized in 20 mM phosphate buffer, pH 7.4 containing 0.5 mM $MgCl_2$, and 1 mM EDTA and protease inhibitors and centrifuged at 100,000×*g* for 1 h at 4 °C to obtain the cytosolic fraction. Equal amount of cytosolic proteins (60 μg) were subjected to 12% SDS–PAGE followed by western blot using primary antibodies against Hsp′s (40, 60, 70 and 90; Cell Signaling, Danvers, MA), human-SOD1 and ß-Actin (Abcam, Cambridge, MA). The pellets were dissolved in 20 mM potassium phosphate containing 2% SDS, 0.5% IGEPAL CA-630, and 0.5% sodium deoxycholate and 2 μg of protein was used for western blot against human-SOD1 and tubulin (Abcam, Cambridge, MA).

3. Results

3.1. Spinal cord and skeletal muscle of G93A mice show distinct pattern of soluble and detergent-soluble G93ASOD1 enzyme

G93ASOD1 enzyme-mediated toxicity is considered to be the basis for motor neuron loss and muscle degeneration in the G93A mouse model [15,16], we first investigated the partition of the G93ASOD1 enzyme between soluble and insoluble fractions of skeletal muscle and spinal cord at various disease stages. Fig. 1 clearly demonstrates that distribution of G93ASOD1 enzyme in skeletal muscle and spinal cord tissues are quite different; first, there are no G93ASOD1 aggregates detected in skeletal muscle prior to the onset and during disease progression (data not shown) as shown in our recent study [15], while significantly higher level of G93ASOD1 enzyme aggregates are detected in spinal cord even at the pre-symptomatic stage (50–65 days) of the disease; second, significantly lower level (∼80- to 100-fold) of soluble G93ASOD1 is detected in skeletal muscle compared to spinal cord at any stage of the disease.

3.2. G93A mice skeletal muscle and spinal cord proteins are equally sensitive to misfolding

Since we observed a significant difference in the distribution of G93ASOD1 enzyme in skeletal muscle and spinal cord at different stages of disease, we investigated whether G93A-induced toxicity equally or differentially affects global protein conformation of skeletal muscle and spinal cord proteins at different stages of the disease using the BisANS photo-labeling assay [14]. Fig. 2 clearly indicates that both skeletal muscle and spinal cord proteins of G93A mice undergo similar pattern of changes in protein conformation; there is significant increase (∼25–50%) in exposure of hydrophobic domain of proteins at the pre-onset stage of the disease (50 days) which collapses (∼25%) with the progression of disease (from 95 to 130 days), in both spinal cord and skeletal muscle tissues ($p = 0.05$). Based on our findings in Figs. 1 and 2, there ap-

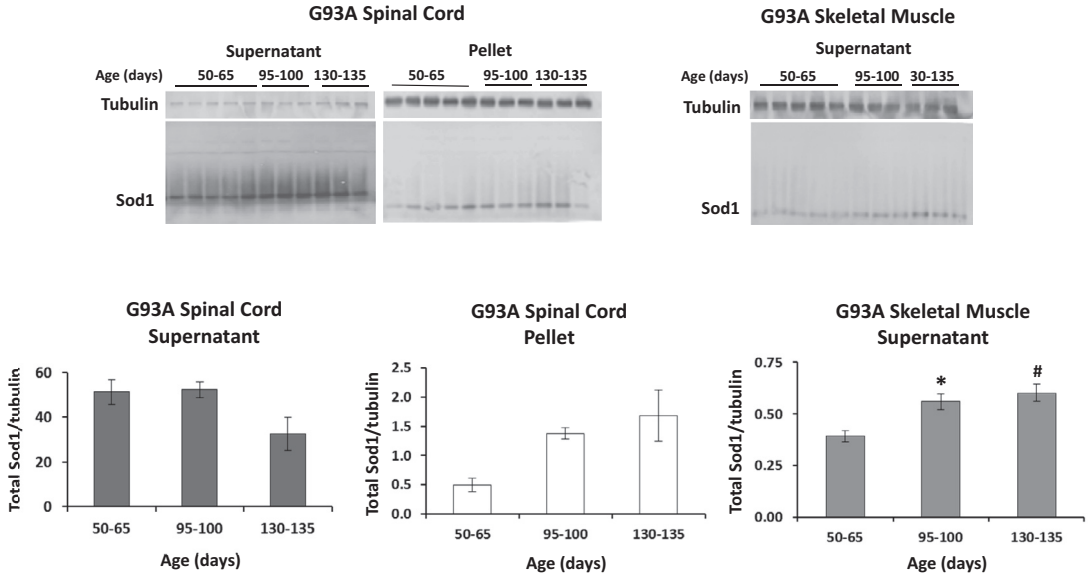

Fig. 1. SOD1 aggregation in spinal cord and skeletal muscle supernatant and insoluble pellet from pre-onset to symptomatic stages of ALS disease in G93A mice. Equal amounts of protein (2 μg) from supernatant and insoluble pellet from spinal cord and skeletal muscles were subjected to SDS–PAGE. The levels of SOD1 protein were determined by western blot using anti-human SOD1 antibody ($n = 3–5$, $^{*}p < 0.05$, $^{\#}p < 0.01$ versus 50–65 days by two-tailed t-test). Results are expressed as mean ± SEM.

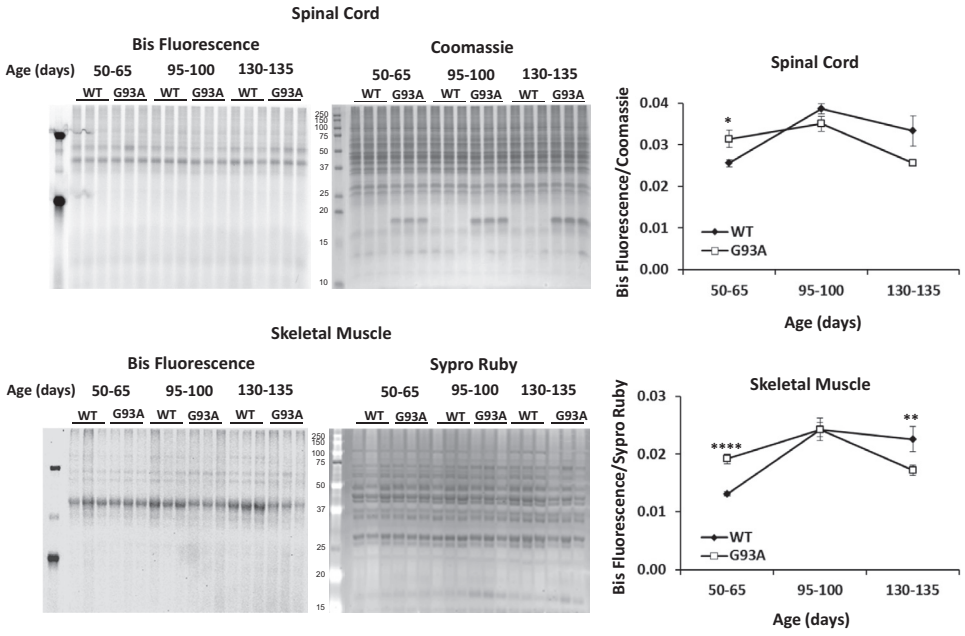

Fig. 2. Global surface hydrophobicity changes in spinal cord and skeletal muscle from pre-onset to symptomatic stages of ALS disease in G93A mice. Cytosolic skeletal muscle and spinal cord proteins from pre-onset to symptomatic stages of ALS in age-matched wild-type (WT) and G93A mice were photolabeled with BisANS. Equal amounts of BisANS-labeled protein were subjected to SDS–PAGE for visualization of BisANS labeling followed by SYPRO Ruby staining. Results were analyzed by two-tailed t-test ($^{*}p < 0.05$, $^{**}p < 0.01$ and $^{****}p < 0.0001$) and expressed as mean ± SEM ($n = 3$).

pears to be a disconnect between G93A protein level and its impact on protein conformation.

3.3. Heat shock proteins are significantly elevated in spinal cords compared to skeletal muscle at any disease stage in G93A mice

The data above led us to speculate whether the status of chaperone proteins can explain the disconnect between G93ASOD1-induced toxicity and alteration of protein conformation as Hsp′s play a major role in preventing proteins from misfolding [17]. Therefore, we next accessed the levels of Hsp′s at various stages of the disease in spinal cord and skeletal muscle. The data in Fig. 3 demonstrates that spinal cords from both WT and G93A mice have significantly higher level of Hsp′s (∼4- to 10-fold) compared to skeletal muscles and remains consistently higher during disease progression in G93A mice, although all the Hsp′s were found to decrease in spinal cord tissues in both WT and G93A over the time course of the study.

3.4. Spinal cord proteins are more resistant to G93ASOD1 mediated misfolding compared to skeletal muscle proteins in vitro

As G93ASOD1 enzyme-induced toxic effect on global protein conformation was found to be similar in skeletal muscle and spinal

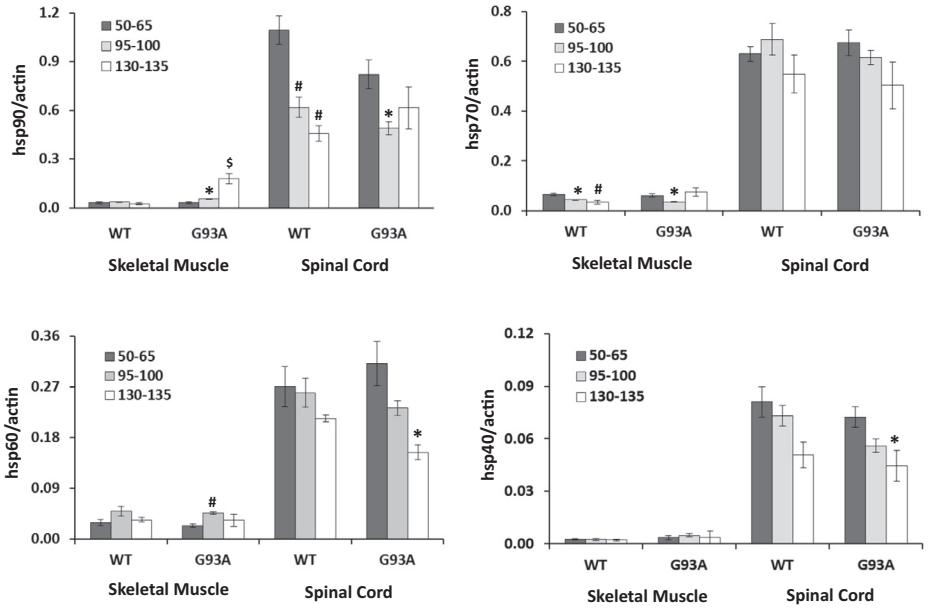

Fig. 3. Heat shock protein expression in spinal cord and skeletal muscle from pre-onset to symptomatic stages of ALS disease in G93A mice. Cytosolic skeletal muscle and spinal cord proteins from pre-onset to symptomatic stages of ALS in age-matched wild type (WT) and G93A mice were subject to SDS–PAGE. Western blot was performed using primary antibodies against Hsp40, 60, 70 and 90. Results were analyzed by two-tailed *t*-test ($^{*}p < 0.05$, $^{\#}p < 0.01$ and $^{\$}p < 0.0001$ versus 50–65 days for each tissue and genotype) and expressed as mean ± SEM ($n = 3–6$).

cord proteins despite of presence of dramatic difference in G93ASOD1 enzyme level between these tissues, we performed an *in vitro* experiment using equal amount of purified G93ASOD1 enzyme to determine if G93ASOD1 enzyme has equal or differential effects on global protein conformation of spinal cord and muscle proteins. We used WTSOD1 enzyme as control to study specific conformational changes contributed by the mutant enzyme. We first examined the structural state of G93ASOD1 and WTSOD1 enzymes in their native state using BisANS labeling. Fig. 4A shows that WTSOD1 and G93ASOD1 are structurally distinct in their native states; G93ASOD1 has more exposure of hydrophobic pockets compared to WTSOD1 in the native state, as heat-induced denaturation abolishes the hydrophobic pockets. Utilizing this unique structural characteristic of the G93ASOD1 enzyme, we next incubated both skeletal muscle and spinal cord cytosolic proteins with G93ASOD1 and WTSOD1 enzymes. The data in Fig. 4B shows that the kinetics of changes in exposure of hydrophobic domain in response to increasing concentrations of G93ASOD1 enzyme is significantly different between spinal cord and skeletal muscle when compared to their respective WTSOD1 controls; muscle proteins were significantly affected by G93ASOD1 enzyme, while spinal cord proteins were comparatively resistant.

4. Discussion

In this study, we provide the first compelling evidence that spinal cord proteins from f-ALS mice are less susceptible to misfolding compared to muscle proteins despite a ~100-fold higher presence of soluble G93ASOD1 and ~3-fold increase in G93ASOD1 aggregates. We propose that this dichotomy may be linked, in part, to higher expression of Hsp′s in spinal cord versus skeletal muscle of G93A mice. The dramatic difference in chaperone proteins between spinal cord and skeletal muscle can further explain their resistance to G93ASOD1 mediated misfolding *in vitro*.

Several studies in the last decade have attempted to understand if muscle or neuron-specific overexpression of mSOD1 protein is sufficient to cause ALS pathogenesis with contradictory results [6,9,10]. The study by Dobrowolny et al. reported that muscle-specific overexpression of mSOD1 (G93A) was sufficient to induce muscle atrophy [6]. Wong et al. further demonstrated that mice with skeletal muscle-specific expression of human SOD1 leads to muscle degeneration and impacts the survival of spinal cord motorneurons [7]. These findings suggest a retrograde mechanism, i.e., degeneration of spinal cord motor neurons may occur as a consequence of the loss of muscle cells and degeneration of NMJ. Moreover, a study reported that lack of muscle-derived growth factor (which protects motor neuron death) may have a role in NMJ degeneration in ALS [18]. Together, these studies make a strong argument for skeletal muscle as an important target for mSOD1 mediated toxicity. However, muscle-specific over-expression of PGC-1α in a f-ALS mouse model significantly reduced muscle atrophy but failed to affect the onset of symptoms and lifespan [8]. There are also contradictory reports with regards to the role of neuronal mSOD1 in ALS pathogenesis. Mice with neuron-specific overexpression of mSOD1 enzyme failed to produce any detectable sign of ALS pathology [9]. However, another study reported that motor neuron-restricted expression of mSOD1 is sufficient to cause ALS pathogenesis [7]. These seemingly contradictory results suggest that both motor neurons and skeletal muscle may be targets for mSOD1 toxicity, and individually or synergestically may be causative in ALS pathogenesis. However, there are two important questions that remain unanswered: first, does mSOD1-induced toxicity equally or differentially affect the structure of skeletal muscle and motor neuron proteins during ALS disease progression? Second, does the skeletal muscle and motor neuron cellular environment, namely the chaperone proteins/Hsp′s, differentially modulate mSOD1-induced protein misfolding during disease progression?

Skeletal muscle degeneration is an early event and precedes the onset of symptoms and loss of motor neurons in the G93A mouse model of f-ALS [11,15]. Our results found striking differences in the expression of G93ASOD1 enzyme between spinal cord and skeletal muscle soluble fractions over the course of the disease. At any stage of the disease, the G93A expression was at least ~80-fold higher in spinal cord compared to skeletal muscle. Moreover, there

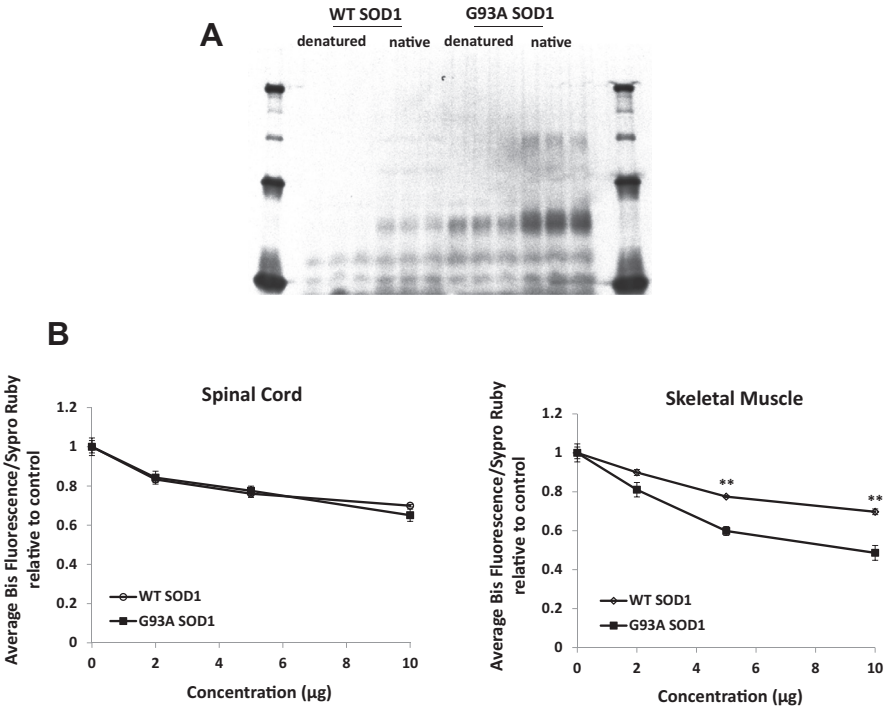

Fig. 4. *In vitro* study of the effect of mutant G93ASOD1 enzyme on global protein surface hydrophobicity in wild-type spinal cord and skeletal muscle. (A) BisANS fluorescence of WTSOD1 and G93ASOD1 proteins (0.75 µg) under native (no stress) and denaturing (100 °C for 30 min) conditions. (B) WT cytosolic spinal cord and skeletal muscle proteins containing 1 M ATP were incubated for 1 h with either WTSOD1 or G93ASOD1 at 37 °C. Thereafter, proteins were photolabeled with BisANS and subjected to SDS–PAGE for visualization of BisANS labeling followed by SYPRO Ruby staining. Data is expressed as average BisANS fluorescence/SYPRO Ruby, relative to control. Results were analyzed by two-tailed *t*-test (**$p < 0.01$) and expressed as mean ± SEM ($n = 3$).

was no evidence for G93ASOD1 aggregates in skeletal muscle unlike in spinal cord as shown in our earlier study [15]. Recently, Onesto et al. [19] also made a similar observation; they did not find any aggregates of G93ASOD1 enzyme in G93A overexpressing muscle cells and detected significant accumulation of G93A protein as aggregates in G93A overexpressing neuronal cells. Onesto et al. further reported that proteasome function is significantly higher in muscle cells compared to neuronal cells overexpressing G93A [19]. We made a similar finding in spinal cord and skeletal muscle tissues over the course of the disease (data not shown). This observation can explain the differential aggregation status of G93A enzyme in skeletal muscle versus spinal cord tissue of G93A mice. Nevertheless, all of these findings would imply that spinal cord proteins may be more vulnerable to G93ASOD1 mediated toxicity compared to muscle proteins. Contrary to our expectations, we found that spinal cord and skeletal muscle proteins were almost identical in their sensitivity to misfolding during the course of the disease. This led us to conclude that the expression of G93A protein alone, in spinal cord versus skeletal muscle was not sufficient to explain the disconnection with f-ALS pathology. Therefore, we predicted that the cellular environment (i.e., the molecular chaperones) might differ considerably between skeletal muscle and spinal cord tissues and possibly explain this disconnect. We found that at any time during the course of the disease, the expression of Hsp′s was ~4- to 10-fold higher in spinal cord versus skeletal muscle in both WT and G93A mice. It was interesting to note that the level of Hsp′s, in general, declined during disease progression in spinal cords of G93A mice, despite being considerably higher than skeletal muscle, which could potentially explain partial protein misfolding and the death of motor neurons during the post-symptomatic stage of the disease.

The differences in the expression of Hsp′s between spinal cord and skeletal muscle tissues may also possibly explain why spinal cord proteins from WT mice, subjected to *in vitro* challenge with G93ASOD1 enzyme were more resistant to misfolding, compared to skeletal muscle proteins. We postulate that chaperone response is one of the key components that distinguish the spinal cord environment from the skeletal muscle environment and can possibly explain the resistance of spinal cord proteins to misfolding despite the high presence of soluble and insoluble mSOD1 aggregates. It has been shown that upregulation of heat shock factor 1 mediated activation of multiple Hsp′s is protective in a primary cell culture model of ALS [20]. Moreover, motor neurons from mice overexpressing Hsp70 and transfected with mSOD1 have increased survival in culture [21]. Interestingly, G93A mice overexpressing Hsp70 or Hsp27 do not exhibit extension in lifespan [22,23]. However, recent studies have shown that intraperitoneal injection with recombinant human Hsp70 and a heat shock response co-inducer, arimoclomol, can extend the lifespan of G93A mice [24–26] and improve some aspects of f-ALS pathology such as denervation and fiber type switching [27], which clearly demonstrates the importance of chaperone response in the progression of f-ALS.

In conclusion, we propose that significantly lower steady-state level of molecular chaperones can explain the vulnerability of skeletal muscle proteins to misfolding, compared to spinal cord proteins and can possibly explain why muscle atrophy occurs long before the loss of motor neurons in the G93A mouse model of f-ALS.

Acknowledgments

This work was supported by VA-Merit Grant (to A.R.C.). We like to thank Dr. Holly Van Remmen for providing wild-type and G93A mouse tissues and to Dr. John Hart for providing purified recombinant G93ASOD1 enzyme.

References

[1] L.P. Rowland, Looking for the cause of amyotrophic lateral sclerosis, N. Engl. J. Med. 311 (1984) 979–981.

[2] D.R. Rosen, T. Siddique, D. Patterson, D.A. Figlewicz, P. Sapp, A. Hentati, D. Donaldson, J. Goto, J.P. O'Regan, H.X. Deng, et al., Mutations in Cu/Zn-superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis, Nature 362 (1993) 59–62.

[3] M.E. Gurney, H. Pu, A.Y. Chiu, M.C. Dal Canto, C.Y. Polchow, D.D. Alexander, J. Caliendo, A. Hentati, Y.W. Kwon, H.X. Deng, et al., Motor neuron degeneration in mice that express a human Cu/Zn-superoxide dismutase mutation, Science 264 (1994) 1772–1775.

[4] L.R. Fischer, D.G. Culver, P. Tennant, A.A. Davis, M. Wang, A. Castellano-Sanchez, J. Khan, M.A. Polak, J.D. Glass, Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man, Exp. Neurol. 185 (2004) 232–240.

[5] D. Frey, C. Schneider, L. Xu, J. Borg, W. Spooren, P. Caroni, Early and selective loss of neuromuscular synapse subtypes with low sprouting competence in motoneuron diseases, J. Neurosci. 20 (2000) 2534–2542.

[6] G. Dobrowolny, M. Aucello, E. Rizzuto, S. Beccafico, C. Mammucari, S. Boncompagni, S. Belia, F. Wannenes, C. Nicoletti, Z. Del Prete, N. Rosenthal, M. Molinaro, F. Protasi, G. Fano, M. Sandri, A. Musaro, Skeletal muscle is a primary target of SOD1G93A-mediated toxicity, Cell Metab. 8 (2008) 425–436.

[7] M. Wong, L.J. Martin, Skeletal muscle-restricted expression of human SOD1 causes motor neuron degeneration in transgenic mice, Hum. Mol. Genet. 19 (2010) 2284–2302.

[8] S. Da Cruz, P.A. Parone, V.S. Lopes, C. Lillo, M. McAlonis-Downes, S.K. Lee, A.P. Vetto, S. Petrosyan, M. Marsala, A.N. Murphy, D.S. Williams, B.M. Spiegelman, D.W. Cleveland, Elevated PGC-1alpha activity sustains mitochondrial biogenesis and muscle function without extending survival in a mouse model of inherited ALS, Cell Metab. 15 (2012) 778–786.

[9] M.M. Lino, C. Schneider, P. Caroni, Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease, J. Neurosci. 22 (2002) 4825–4832.

[10] L. Wang, K. Sharma, H.X. Deng, T. Siddique, G. Grisotti, E. Liu, R.P. Roos, Restricted expression of mutant SOD1 in spinal motor neurons and interneurons induces motor neuron pathology, Neurobiol. Dis. 29 (2008) 400–408.

[11] P.A. Parone, S. Da Cruz, J.S. Han, M. McAlonis-Downes, A.P. Vetto, S.K. Lee, E. Tseng, D.W. Cleveland, Enhancing mitochondrial calcium buffering capacity reduces aggregation of misfolded SOD1 and motor neuron cell death without extending survival in mouse models of inherited amyotrophic lateral sclerosis, J. Neurosci. 33 (2013) 4657–4671.

[12] M. Prudencio, D.R. Borchelt, Superoxide dismutase 1 encoding mutations linked to ALS adopts a spectrum of misfolded states, Mol. Neurodegener. 6 (2011) 77.

[13] H.N. Liu, S. Tjostheim, K. Dasilva, D. Taylor, B. Zhao, R. Rakhit, M. Brown, A. Chakrabartty, J. McLaurin, J. Robertson, Targeting of monomer/misfolded SOD1 as a therapeutic strategy for amyotrophic lateral sclerosis, J. Neurosci. 32 (2012) 8791–8799.

[14] A. Pierce, H. Mirzaei, F. Muller, E. De Waal, A.B. Taylor, S. Leonard, H. Van Remmen, F. Regnier, A. Richardson, A. Chaudhuri, GAPDH is conformationally and functionally altered in association with oxidative stress in mouse models of amyotrophic lateral sclerosis, J. Mol. Biol. 382 (2008) 1195–1210.

[15] R. Wei, A. Bhattacharya, N. Chintalaramulu, A.L. Jernigan, Y. Liu, H. Van Remmen, A.R. Chaudhuri, Protein misfolding, mitochondrial dysfunction and muscle loss are not directly dependent on soluble and aggregation state of mSOD1 protein in skeletal muscle of ALS, Biochem. Biophys. Res. Commun. 417 (2012) 1275–1279.

[16] D. Jaarsma, E.D. Haasdijk, J.A. Grashorn, R. Hawkins, W. van Duijn, H.W. Verspaget, J. London, J.C. Holstege, Human Cu/Zn-superoxide dismutase (SOD1) overexpression in mice causes mitochondrial vacuolization, axonal degeneration, and premature motoneuron death and accelerates motoneuron disease in mice expressing a familial amyotrophic lateral sclerosis mutant SOD1, Neurobiol. Dis. 7 (2000) 623–643.

[17] R.I. Morimoto, The heat shock response: systems biology of proteotoxic stress in aging and disease, Cold Spring Harb. Symp. Quant. Biol. 76 (2011) 91–99.

[18] W. Li, D. Brakefield, Y. Pan, D. Hunter, T.M. Myckatyn, A. Parsadanian, Muscle-derived but not centrally derived transgene GDNF is neuroprotective in G93ASOD1 mouse model of ALS, Exp. Neurol. 203 (2007) 457–471.

[19] E. Onesto, P. Rusmini, V. Crippa, N. Ferri, A. Zito, M. Galbiati, A. Poletti, Muscle cells and motoneurons differentially remove mutant SOD1 causing familial amyotrophic lateral sclerosis, J. Neurochem. 118 (2011) 266–280.

[20] Z. Batulan, D.M. Taylor, R.J. Aarons, S. Minotti, M.M. Doroudchi, J. Nalbantoglu, H.D. Durham, Induction of multiple heat shock proteins and neuroprotection in a primary culture model of familial amyotrophic lateral sclerosis, Neurobiol. Dis. 24 (2006) 213–225.

[21] W. Bruening, J. Roy, B. Giasson, D.A. Figlewicz, W.E. Mushynski, H.D. Durham, Up-regulation of protein chaperones preserves viability of cells expressing toxic Cu/Zn-superoxide dismutase mutants associated with amyotrophic lateral sclerosis, J. Neurochem. 72 (1999) 693–699.

[22] J. Krishnan, R. Lemmens, W. Robberecht, L. Van Den Bosch, Role of heat shock response and Hsp27 in mutant SOD1-dependent cell death, Exp. Neurol. 200 (2006) 301–310.

[23] J. Liu, L.A. Shinobu, C.M. Ward, D. Young, D.W. Cleveland, Elevation of the Hsp70 chaperone does not effect toxicity in mouse models of familial amyotrophic lateral sclerosis, J. Neurochem. 93 (2005) 875–882.

[24] D.J. Gifondorwa, M.B. Robinson, C.D. Hayes, A.R. Taylor, D.M. Prevette, R.W. Oppenheim, J. Caress, C.E. Milligan, Exogenous delivery of heat shock protein 70 increases lifespan in a mouse model of amyotrophic lateral sclerosis, J. Neurosci. 27 (2007) 13173–13180.

[25] B. Kalmar, S. Novoselov, A. Gray, M.E. Cheetham, B. Margulis, L. Greensmith, Late stage treatment with arimoclomol delays disease progression and prevents protein aggregation in the SOD1 mouse model of ALS, J. Neurochem. 107 (2008) 339–350.

[26] D. Kieran, B. Kalmar, J.R. Dick, J. Riddoch-Contreras, G. Burnstock, L. Greensmith, Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice, Nat. Med. 10 (2004) 402–405.

[27] B. Kalmar, E. Edet-Amana, L. Greensmith, Treatment with a coinducer of the heat shock response delays muscle denervation in the SOD1-G93A mouse model of amyotrophic lateral sclerosis, Amyotroph. Lateral. Scler. 13 (2012) 378–392.