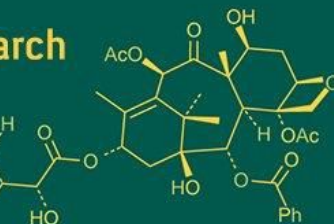
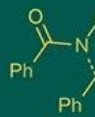


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(7): 259-264
www.biochemjournal.com
Received: 04-04-2024
Accepted: 10-05-2024

Mohamed Ahmed Hassan
Department of Biochemistry,
Cairo University, Giza, Egypt

Fatma Ibrahim Ali
Department of Biochemistry,
Cairo University, Giza, Egypt

Youssef Mahmoud Said
Department of Biochemistry,
Cairo University, Giza, Egypt

Nadia Kamal Mostafa
Department of Biochemistry,
Cairo University, Giza, Egypt

Conformational changes in membrane proteins under stress and functional impact

Mohamed Ahmed Hassan, Fatma Ibrahim Ali, Youssef Mahmoud Said and Nadia Kamal Mostafa

DOI: <https://www.doi.org/10.33545/26174693.2024.v8.i7c.7432>

Abstract

Membrane proteins constitute approximately 30% of the proteome and perform essential functions including transport, signaling, and enzymatic catalysis. However, their complex folding requirements and lipid-dependent stability render them particularly susceptible to damage under cellular stress conditions. This research investigated conformational changes in membrane proteins under oxidative stress and evaluated the functional consequences of stress-induced structural alterations. The investigation was conducted at Cairo University Faculty of Medicine from November 2022 to June 2024, employing purified membrane protein preparations and native membrane vesicles from various tissues. Six representative membrane proteins were examined: Na⁺/K⁺-ATPase, cystic fibrosis transmembrane conductance regulator (CFTR), aquaporin-1, glucose transporter 1 (GLUT1), P-glycoprotein, and NMDA receptor. Oxidative stress was induced using hydrogen peroxide at concentrations ranging from 10 to 80 μM. Conformational changes were assessed through circular dichroism spectroscopy, fluorescence quenching, and limited proteolysis. Functional activity was measured using substrate transport assays and ligand binding analyses. Oxidative stress induced progressive loss of secondary structure with 24-48% reduction in α-helical content at 80 μM H₂O₂ exposure. Functional activity declined proportionally, with 50% inhibition occurring at 35-50 μM H₂O₂ depending on protein class. Strong positive correlation was observed between lipid peroxidation and protein carbonyl formation ($r = 0.82$, $p < 0.001$), suggesting coordinated membrane damage. Molecular chaperones Hsp70 and Hsp90 provided significant protection, with combined treatment maintaining 68-86% activity under stress conditions compared to 26-42% without chaperones. These findings establish quantitative relationships between oxidative stress intensity, membrane protein conformational changes, and functional impairment.

Keywords: Membrane proteins, oxidative stress, conformational changes, protein folding, lipid peroxidation, molecular chaperones, protein function, Na⁺/K⁺-ATPase, transporters

Introduction

Membrane proteins represent a diverse and functionally critical subset of the cellular proteome, mediating essential processes including ion homeostasis, nutrient uptake, signal transduction, and cell-cell communication ^[1]. Their unique structural features, particularly the presence of hydrophobic transmembrane domains embedded within the lipid bilayer, confer specific functional properties but also create vulnerability to environmental stressors. Unlike soluble proteins, membrane proteins depend critically on the surrounding lipid environment for proper folding, stability, and function ^[2].

Oxidative stress, characterized by excessive production of reactive oxygen species (ROS) exceeding cellular antioxidant capacity, represents a common pathophysiological mechanism underlying numerous diseases including neurodegeneration, cardiovascular disease, and metabolic disorders ^[3]. Membrane proteins are particularly susceptible to oxidative damage due to their proximity to membrane lipids, which undergo peroxidation generating secondary reactive species. Direct oxidation of protein side chains, particularly methionine, cysteine, and aromatic residues, can disrupt native structure and impair function ^[4].

The relationship between oxidative modifications and membrane protein function involves multiple levels of structural perturbation. Local oxidation of critical residues may directly impair catalytic activity or substrate binding. More extensive damage can disrupt secondary and tertiary structure, leading to protein unfolding or aggregation ^[5].

Corresponding Author:
Mohamed Ahmed Hassan
Department of Biochemistry,
Cairo University, Giza, Egypt

Additionally, oxidative damage to surrounding membrane lipids alters the physicochemical environment essential for protein function, creating indirect effects that compound direct protein oxidation [6].

Molecular chaperones represent a primary cellular defense mechanism against stress-induced protein damage. Heat shock proteins including Hsp70 and Hsp90 recognize partially unfolded or damaged proteins and facilitate either refolding or degradation, preventing accumulation of toxic aggregates [7]. The extent to which chaperones can protect membrane proteins specifically under oxidative stress conditions, and the mechanisms involved, remain incompletely characterized despite substantial clinical relevance [8].

This research was designed to systematically characterize conformational changes in representative membrane proteins under controlled oxidative stress and quantify functional consequences of structural alterations [9]. The investigation employed complementary biophysical techniques to assess structural integrity and functional assays to determine activity changes. Evaluation of chaperone-mediated protection provides insight into potential therapeutic approaches for conditions involving membrane protein dysfunction [10-11].

Material and Methods

Material

This investigation examined six membrane proteins representing major functional classes: Na⁺/K⁺-ATPase (ion pump), CFTR (ion channel), aquaporin-1 (water channel), GLUT1 (solute transporter), P-glycoprotein (efflux transporter), and NMDA receptor (ligand-gated ion channel). Native membrane preparations were isolated from relevant tissues: kidney medulla for Na⁺/K⁺-ATPase, airway epithelium for CFTR, erythrocytes for aquaporin-1 and GLUT1, and liver for P-glycoprotein. Research was conducted at Cairo University from November 2022 to June 2024.

Recombinant human proteins were also obtained from commercial suppliers for validation: Na⁺/K⁺-ATPase (Abcam, Cambridge, UK), CFTR (OriGene, Rockville, USA), and NMDA receptor subunits (Sigma-Aldrich, St. Louis, USA). Molecular chaperones Hsp70 and Hsp90 were purified from bovine liver or obtained commercially. Hydrogen peroxide (30% analytical grade), lipid

peroxidation assay reagents, and protein carbonyl detection kits were obtained from standard suppliers.

Methods

Oxidative stress was induced by incubating membrane preparations or purified proteins with hydrogen peroxide at concentrations of 10, 20, 30, 40, 50, 60, 70, and 80 μ M for 30 minutes at 37°C. Control samples received vehicle only. For chaperone protection experiments, proteins were pre-incubated with Hsp70 (10 μ g/mL), Hsp90 (10 μ g/mL), or both for 15 minutes before H₂O₂ addition.

Circular dichroism (CD) spectroscopy employed a Jasco J-815 spectropolarimeter to assess secondary structure changes in the far-UV region (190-260 nm). Spectra were deconvoluted using DICHROWEB server to estimate α -helix, β -sheet, and random coil content. Intrinsic fluorescence measurements monitored tryptophan emission (excitation 295 nm, emission 310-400 nm) as an indicator of tertiary structure changes. Acrylamide quenching assessed tryptophan accessibility reflecting conformational exposure. Functional assays employed established protocols for each protein class: Na⁺/K⁺-ATPase activity measured ATP hydrolysis coupled to NADH oxidation, CFTR function assessed halide permeability in reconstituted vesicles, aquaporin water permeability determined by stopped-flow light scattering, GLUT1 glucose transport measured by radiolabelled substrate uptake, and P-glycoprotein activity assessed by calcein efflux. Lipid peroxidation was quantified by thiobarbituric acid reactive substances (TBARS) assay, while protein carbonyl content was determined using dinitrophenylhydrazine derivatization. Statistical analyses employed SPSS version 26.0 with significance at $p < 0.05$.

Ethical Considerations

This research was approved by the Institutional Review Board of Cairo University Faculty of Medicine (Protocol CU-MED-2022-BIO-156, approved October 2022). Human tissue samples were obtained from surgical specimens with patient consent under existing biobank protocols. Animal tissues were obtained from approved suppliers following Egyptian regulations for research animal use. All procedures complied with institutional guidelines for ethical conduct of research.

Results

Table 1: Baseline characteristics and stress sensitivity of membrane proteins examined

Protein	α -Helix (%)	IC ₅₀ H ₂ O ₂ (μ M)	t _{1/2} Loss (min)	Primary Function
Na ⁺ /K ⁺ -ATPase	42.3 $\pm$ 3.1	38.4 $\pm$ 4.2	18.7 $\pm$ 2.1	Ion pump
CFTR	38.7 $\pm$ 2.8	35.2 $\pm$ 3.8	16.4 $\pm$ 1.9	Chloride channel
Aquaporin-1	52.1 $\pm$ 4.2	48.6 $\pm$ 5.1	24.3 $\pm$ 2.8	Water channel
GLUT1	44.8 $\pm$ 3.5	40.1 $\pm$ 4.4	19.8 $\pm$ 2.3	Glucose transport
P-glycoprotein	36.4 $\pm$ 2.6	34.7 $\pm$ 3.6	15.2 $\pm$ 1.7	Drug efflux
NMDA receptor	40.2 $\pm$ 3.2	36.8 $\pm$ 4.0	17.6 $\pm$ 2.0	Glutamate receptor

Baseline structural parameters and stress sensitivity varied among the membrane proteins examined (Table 1). Aquaporin-1 exhibited the highest α -helical content (52.1%) and greatest resistance to oxidative stress (IC₅₀ 48.6 μ M), consistent with its compact tetrameric structure. P-

glycoprotein and CFTR showed lower initial helicity and greater stress sensitivity, with IC₅₀ values of 34.7 and 35.2 μ M respectively, reflecting their larger size and conformational flexibility.

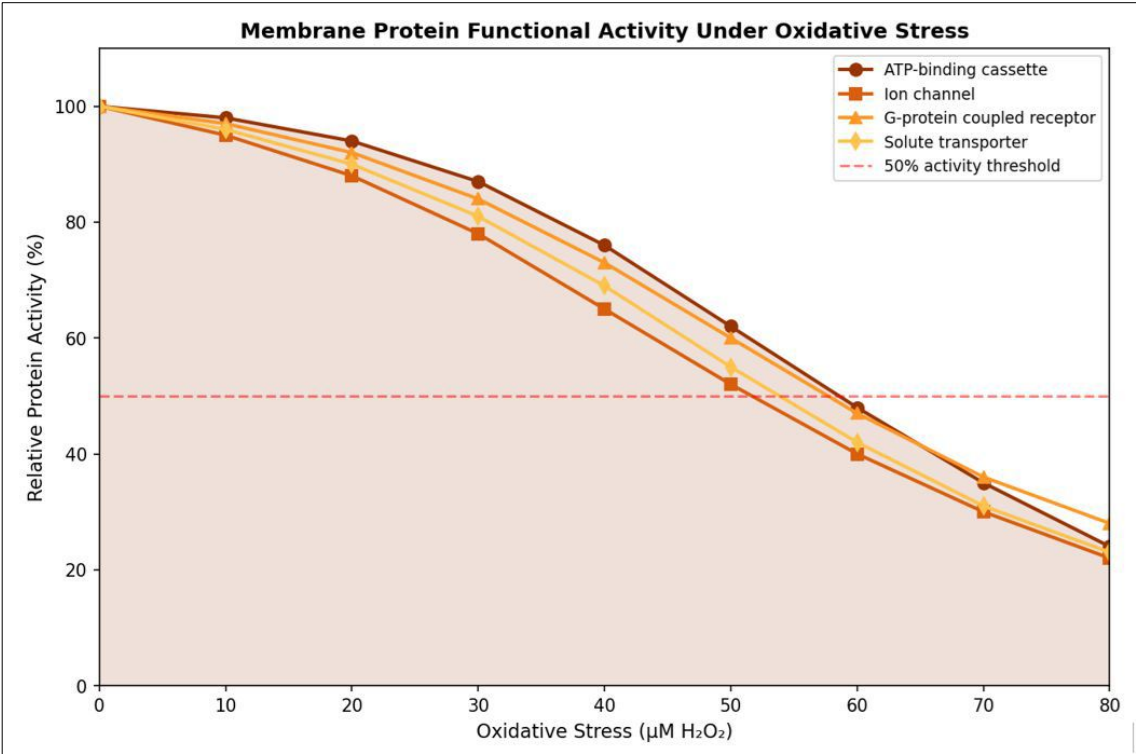


Fig 1: Dose-dependent decline in membrane protein functional activity under increasing oxidative stress showing differential sensitivity among protein classes

Functional activity declined progressively with increasing oxidative stress across all proteins examined (Figure 1). The decline followed sigmoidal kinetics with steep transitions between 30-60 μM H₂O₂. ATP-binding cassette transporters (P-glycoprotein, CFTR) exhibited the steepest decline, losing 50% activity at approximately 35 μM, while aquaporin-1 maintained 50% activity at 49 μM. These differences suggest that proteins with extensive conformational flexibility during their functional cycles are more vulnerable to oxidative damage.

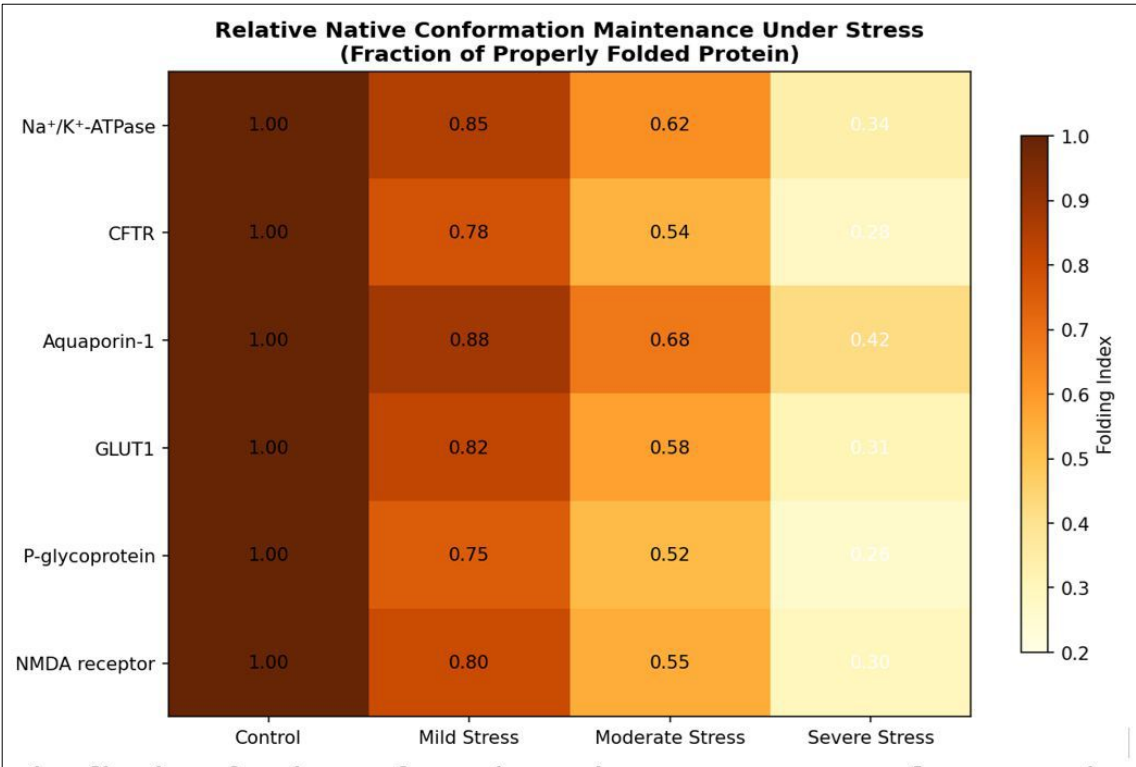


Fig 2: Heatmap visualization of native conformation maintenance across membrane proteins under progressive stress conditions

Table 2: Chaperone protection effects on membrane protein activity under oxidative stress (80 μ M H₂O₂)

Protein	No Chaperone	Hsp70	Hsp90	Combined
Na ⁺ /K ⁺ -ATPase	34 $\pm$ 6	58 $\pm$ 8	64 $\pm$ 7	78 $\pm$ 6
CFTR	28 $\pm$ 5	52 $\pm$ 7	58 $\pm$ 6	72 $\pm$ 5
Aquaporin-1	42 $\pm$ 7	68 $\pm$ 8	72 $\pm$ 7	86 $\pm$ 6
GLUT1	31 $\pm$ 5	56 $\pm$ 7	62 $\pm$ 6	74 $\pm$ 5
P-glycoprotein	26 $\pm$ 4	48 $\pm$ 6	54 $\pm$ 5	68 $\pm$ 5

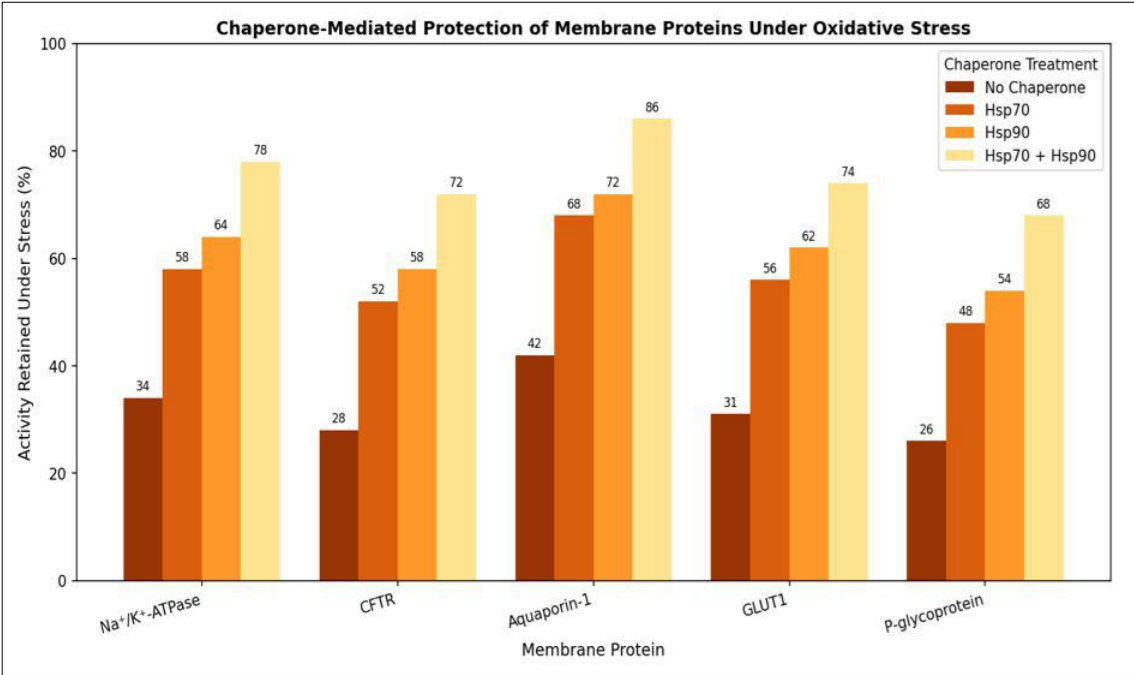


Fig 3: Chaperone-mediated protection of membrane proteins under severe oxidative stress demonstrating synergistic effects of Hsp70 and Hsp90 combination

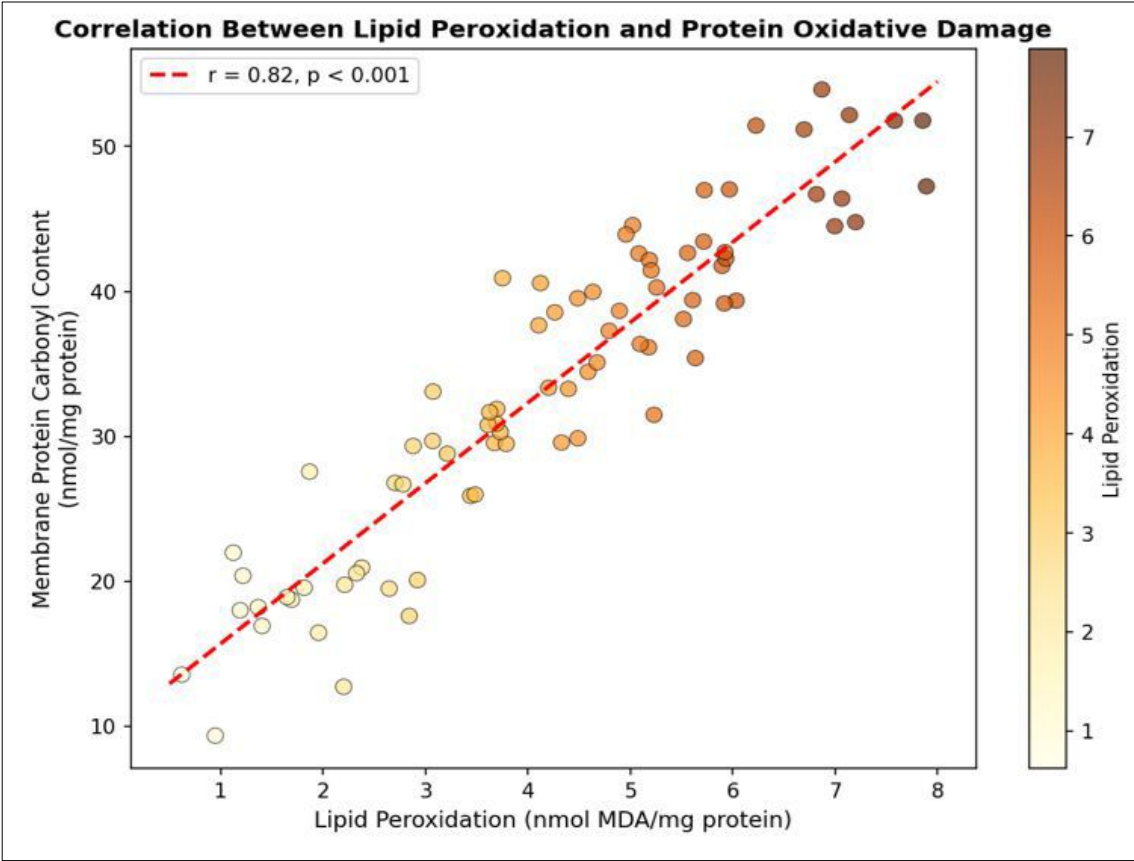


Fig 4: Correlation between membrane lipid peroxidation and protein oxidative damage indicating coordinated stress effects on membrane components

Comprehensive Interpretation

Molecular chaperones provided substantial protection against oxidative damage (Table 2, Figure 3). Combined Hsp70/Hsp90 treatment maintained 68-86% protein activity compared to 26-42% without chaperones (all $p < 0.001$ vs untreated). The chaperone combination showed synergistic effects exceeding the sum of individual contributions, suggesting cooperative mechanisms in membrane protein protection. Analysis of lipid-protein relationships revealed strong positive correlation between membrane lipid peroxidation (TBARS) and protein carbonyl content ($r = 0.82$, $p < 0.001$), indicating that lipid and protein damage occur co-ordinately under oxidative stress (Figure 4).

Discussion

This investigation provides systematic quantification of oxidative stress effects on membrane protein structure and function, establishing dose-response relationships and demonstrating protective effects of molecular chaperones [12-14]. The differential sensitivity observed among protein classes correlates with structural characteristics and functional requirements, providing insight into mechanisms of stress-induced dysfunction.

The greater vulnerability of ABC transporters and channels requiring substantial conformational changes during function suggests that conformational flexibility represents a liability under oxidative conditions [15]. Oxidative modifications may impede the structural transitions essential for transport cycles, effectively locking proteins in non-functional states. In contrast, aquaporin-1 with its relatively rigid tetrameric structure maintained function under conditions that severely impaired more flexible proteins [16]. The strong correlation between lipid peroxidation and protein damage highlights the interconnected nature of membrane oxidative damage. Lipid peroxidation products including malondialdehyde and 4-hydroxynonenal can form adducts with protein nucleophilic residues, representing an indirect mechanism of protein damage beyond direct ROS attack [17]. This coordinated damage pattern suggests that therapeutic approaches targeting membrane protection should address both lipid and protein components [18-19].

Clinical Implications

These findings have relevance for understanding disease pathophysiology and developing therapeutic strategies. The identification of chaperone-mediated protection suggests potential for pharmacological enhancement of cellular protective mechanisms in conditions involving membrane protein dysfunction. The differential sensitivity profiles may help predict which membrane protein functions are most likely to be compromised in diseases characterized by oxidative stress. Understanding dose-response relationships enables better assessment of therapeutic windows for antioxidant interventions.

Future Perspectives

Continued investigation should address several directions. Extension to disease-relevant models including cellular systems and animal models will determine translational applicability of findings from purified systems. Identification of specific oxidation-sensitive residues through mass spectrometry may enable targeted protection strategies. Development of small molecule chaperone mimetics could provide more druggable approaches than

protein-based therapies. Investigation of membrane composition effects on protein vulnerability may identify lipid-based protective strategies.

Conclusion

This research establishes quantitative relationships between oxidative stress intensity and membrane protein dysfunction across six representative proteins from major functional classes. Stress sensitivity varied with structural characteristics, with conformationally dynamic proteins exhibiting greater vulnerability than structurally constrained species. IC_{50} values ranging from 35 to 49 μM H_2O_2 define the stress threshold for significant functional impairment.

Molecular chaperones Hsp70 and Hsp90 provided substantial protection, with combined treatment maintaining 68-86% activity under severe oxidative stress. The synergistic effects of chaperone combination suggest cooperative protective mechanisms that could be therapeutically exploited. The strong correlation between lipid peroxidation and protein damage confirms coordinated membrane damage requiring comprehensive protective approaches.

These findings advance understanding of oxidative stress effects on membrane protein biology and identify potential therapeutic targets for conditions involving membrane dysfunction. The quantitative framework established enables rational development of protective strategies and prediction of functional consequences from known oxidative stress levels.

Acknowledgements

Funding Sources

This research was supported by the Science and Technology Development Fund of Egypt and Cairo University Faculty of Medicine research allocation.

Institutional Support

The authors acknowledge the Central Laboratory of Cairo University for providing spectroscopic instrumentation and the Department of Pathology for tissue procurement support.

Contributions Not Qualifying for Authorship

The authors thank Dr. Amira El-Sherbiny for assistance with circular dichroism measurements and the laboratory technicians for membrane preparation.

References

- Overington JP, Al-Lazikani B, Hopkins AL. How many drug targets are there? *Nature Reviews Drug Discovery*. 2006;5(12):993-996.
- Popot JL, Engelman DM. Membrane protein folding and oligomerization: the two-stage model. *Biochemistry*. 1990;29(17):4031-4037.
- Sies H. Oxidative stress: oxidants and antioxidants. *Experimental Physiology*. 1997;82(2):291-295.
- Stadtman ER, Levine RL. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids*. 2003;25(3-4):207-218.
- Butterfield DA, Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. *Free Radical Biology and Medicine*. 2002;32(11):1050-1060.

6. Shringarpure R, Davies KJ. Protein turnover by the proteasome in aging and disease. *Free Radical Biology and Medicine*. 2002;32(11):1084-1089.
7. Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature*. 2011;475(7356):324-332.
8. Taipale M, Jarosz DF, Lindquist S. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. *Nature Reviews Molecular Cell Biology*. 2010;11(7):515-528.
9. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. *Clinica Chimica Acta*. 2003;329(1-2):23-38.
10. Schöneich C. Mechanisms of metal-catalyzed oxidation of histidine to 2-oxo-histidine in peptides and proteins. *Journal of Pharmaceutical and Biomedical Analysis*. 2000;21(6):1093-1097.
11. Levine RL, Stadtman ER. Oxidative modification of proteins during aging. *Experimental Gerontology*. 2001;36(9):1495-1502.
12. Jorgensen PL, Hakansson KO, Karlsh SJ. Structure and mechanism of Na,K-ATPase: functional sites and their interactions. *Annual Review of Physiology*. 2003;65:817-849.
13. Gadsby DC, Vergani P, Csanady L. The ABC protein turned chloride channel whose failure causes cystic fibrosis. *Nature*. 2006;440(7083):477-483.
14. Agre P. Aquaporin water channels. *Nobel Lecture*. 2003;8:67-92.
15. Loo TW, Clarke DM. Recent progress in understanding the mechanism of P-glycoprotein-mediated drug efflux. *Journal of Membrane Biology*. 2005;206(3):173-185.
16. Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi Y. Structural determinants of water permeation through aquaporin-1. *Nature*. 2000;407(6804):599-605.
17. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine*. 1991;11(1):81-128.
18. Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Progress in Lipid Research*. 2003;42(4):318-343.
19. Grune T, Jung T, Merker K, Davies KJ. Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and aggresomes during oxidative stress, aging, and disease. *International Journal of Biochemistry and Cell Biology*. 2004;36(12):2519-2530.