Effect of hyperglycemic condition on observed surface area to volume ratio of human cancerous cells

Mohammad Hassan Naseri[1,4], Saeed Hesami Tackallou[2*], Mostafa Rezaei Tavirani[3]

1 Faculty of Medicine, Alborz University of Medical Sciences, Karaj, Iran.
[2] Islamic Azad University, Garmsar Branch, Garmsar, Iran.
[3] Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Baqiyate Allah University of Medical Sciences, Tehran, Iran

*Corresponding Author: email address: tackallou@gmail.com (S. Hessami Tackallou)

ABSTRACT

The human blood basal glucose level is a completely controlled range. Information on the relationship between culture glucose concentration and changes in the cell's surface area, volume and surface area to volume (S/V) ratio are lacking. Cancerous epithelial-like cell lines SW480, SW742 and T-47D were cultured in mediums nourished with 4.7 mM per liter of glucose as the control group and three other groups with glucose concentrations of 9.4, 14.1 and 18.8 mM at 37 °C for 48hr. Digital images of cells were analyzed using the *ImageJ* software. Observed changes in surface area, volume, and surface area to volume (S/V) ratio were significantly ($P<0.05$) different between the control group and the X4 group (18.8 mM glucose) in the three cell lines tested. Cultured cells responded delicately but sharply to glucose elevation. The goal of this research is to show the dictating of changes via pathologic conditions in cellular levels that could be a good answer to changing the body metabolic parameters. Besides the S/V ratio could be studied as a variant parameter in other metabolic challenges.

Keywords: hyperglycemia; surface area to volume ratio; cell morphology; fasting blood sugar; glucose

INTRODUCTION

Morphological parameters, such as the surface area of all eukaryotic cells are dependent upon cell adhesion to a suitable substratum, cell flattening [36], cytoskeletal microfilaments [12], such as actin [16], and temperature [13,30]. The effect of temperature changes has been studied on many biomolecular and cellular functions and characteristics, such as cell morphology [1-7]. Many structural transitions of proteins [8], such as the sodium-potassium pump [2], cytoskeleton [7], tropomyosin [14], transmembrane proteins [26] and many other structural, carrier or enzymatic proteins [15-18,22,23,29,35,37,38] are affected by physical changes of the medium.

Cytoskeleton is always in a dynamic state. It can play an important role in glucose regulation [9], mainly through the following three strategies. First, by guiding the intracellular transport of insulin-containing vesicles, and regulating the release of insulin. Second, by regulating the distribution of insulin receptor substrate, GLUT4 translocation, and internalization of insulin receptor, and third by directing the intracellular distribution of glucose metabolism-related enzymes including glycogen synthase and many glycolysis enzymes [17]. The addition of D-glucose or D-galactose to a culture medium increased the survival of heated eukaryotic cells. Heat protection by galactose appeared rapidly (within 1 hr), while glucose was a less effective heat protector [10].

Cell volume, obviously, correlates with cell morphology and cell surface area. The ratio between surface area and volume of the cells has an enormous impact on their biology [13]. An increased surface area to volume ratio (S/V) could mean an increased exposure to the surrounding environment, which may be regarded as an objective fact for cellular normality [30]. Cells can get around having a low S/V ratio by being long and thin (nerve cells) or convoluted (microvilli) [13]. Decreased surface area can also lead to biological problems [30]. More contact with the environment through the surface of a cell or an organ (relative to its volume) is a good sign for the health of cells. High surface area to volume ratios present problems, such as temperature control in unfavorable environments [30].

The range of human blood basal glucose concentration is 3.6-5.8 mM per lit. This glucose content is mostly expressed by term fasting blood sugar (FBS), that is equal to 4.7 mM per lit. So, upper glucose levels can be known as a trigger for cellular and molecular perturbation of structure and function [8] . So, from the stand point of physiology, it could be logical to speculate that normal glucose concentration should fit best with normal cell morphology and structural properties [8].

MATERIALS AND METHODS

Cell culture

Cancerous epithelial-like cell lines SW480 (colon cancer), SW742 (colonorectal cancer) and T-47D (breast cancer) were purchased from the National Cell Bank of Islamic Republic of Iran, and were grown in RPMI 1640 (Sigma, USA), supplemented with 10% FBS (Sigma, USA), non-essential amino acids, 5 mM L-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin.

Cell Treatment

All three cell lines were cultured for 48 hr in four different concentrations of glucose: 4.7 mM per liter as the control group (X1 group), 9.4 mM per liter (X2 group), 14.1 mM per liter (X3 group), and 18.8 mM per liter (X4 group). SW480, SW742 and T-47D cells were cultured at 37 $^{\circ}$C for 48hr. Glucose-treatments were triplicated, and the results of pooled data for each cell line were reported.

Evaluation of Observed Cell Surface

Digital images of control and treated cells were obtained using an stereomicroscope equipped with the digital camera NIKON S500, and analyzed using the ImageJ software (1.42l, Wayne Rasband, National Institutes of Health, USA), a public domain Java-based image processing program.

Cell Volume Evaluation

Control and treated cell suspensions were obtained by repeated gentle pipetting with 0.06% trypsin/0.02% EDTA (pH 7.3). The radius of suspended cells, which had spherical dimensions, were measured using the ImageJ software. The volume was calculated mathematically.

Statistics

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 13.0 (Chicago, IL, USA). Data were presented as Mean ± SD. The comparisons of the variables in the three groups were performed with Kruskal-Wallis test, while the comparisons for two groups were performed with Mann-Whitney U test. Differences were considered as statistically significant at $P<0.05$.

RESULTS

Effect of glucose concentration stress on cell volume and cell surface

Figure 1 shows the mean observed surface area changes ± standard deviation (mm^2) of three cell-linesm, cultured in 4.7(X1), 9.4 (X2), 14.1 (X3) and 18.8 (X4) mM per liter. Kruskal-Wallis test shows that observed surface area is significantly different in four examined concentrations in the three cell-lines tested ($P<0.05$). 2×2 comparisons were performed with Mann-Whitney U test. Surface area changes were not significant in T47D cells between X2 and X3 ($P=0.581$). Changes in 2×2 comparisons between other cell lines' observed surface area were significant ($P<0.05$). In cell lines SW480 and SW742, the observed surface area in X4 was significantly ($P=0.000$) smaller than that of X1, X2 and X3. T47D and SW742 cell lines' observed surface area in X1 were significantly ($P=0.000$) greater than those of X2, X3 and X4. Figure 2 shows volume changes ± standard deviation (mm^3) in three cell lines cultured in 4.7(X1), 9.4 (X2), 14.1 (X3) and 18.8 (X4) mM per liter.

Kruskal-Wallis test shows that volume changes are significantly different in 4.7(X1), 9.4 (X2), 14.1 (X3) and 18.8 (X4) mM per liter ($P<0.05$). 2×2 comparisons were performed with Mann-Whitney U test. Volume changes were not significant in T47D cells between X1 and X2 ($P=0.471$), in SW480 cells between X1 and X2 ($P=0.392$), in SW742 cells between X1 and X2 ($P=0.603$) and between X2 and X3 ($P=0.665$). But changes in 2×2 comparisons between other cell lines' volume were significant ($P<0.05$). The volume measured for all three cell lines in X1 and X2 were significantly smaller than their volume in X4 concentration ($P=0.000$).

Figure 3 shows changes in "surface area" to "volume" ratio (S/V ratio, mm^{-1}) ± standard deviation in the three cell-lines.

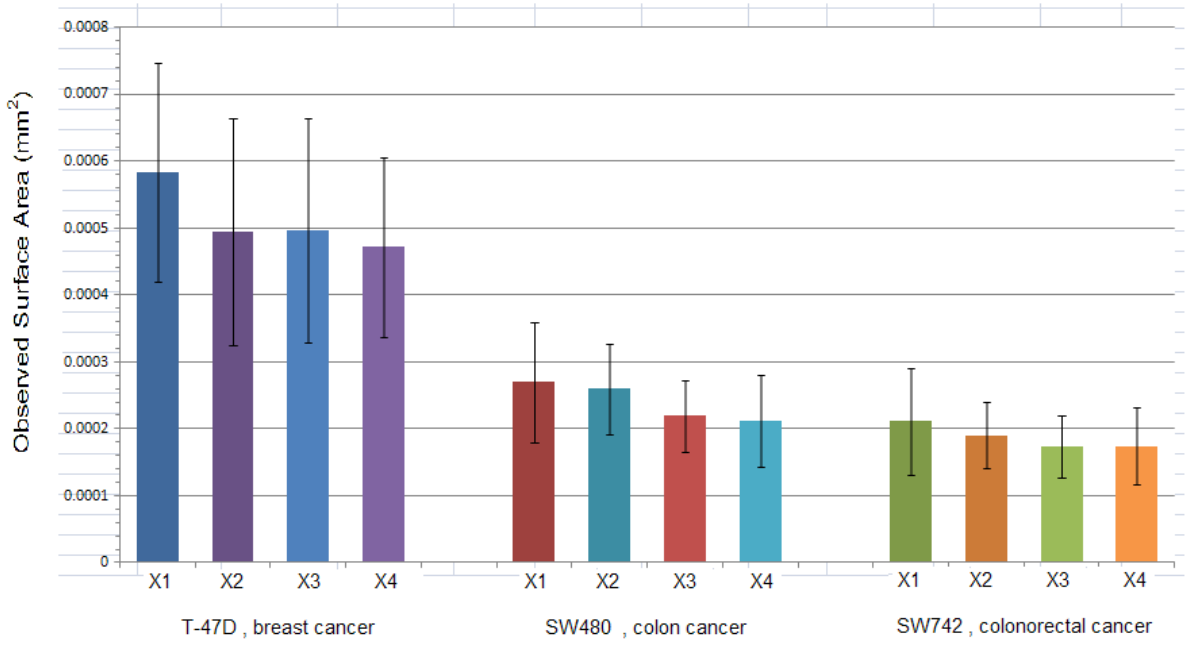

Figure 1. Mean observed surface area changes ± SD (mm^2) of the three cell-lines cultured in 4.7(X1), 9.4 (X2), 14.1 (X3) and 18.8 (X4) mM per liter for 48hr.

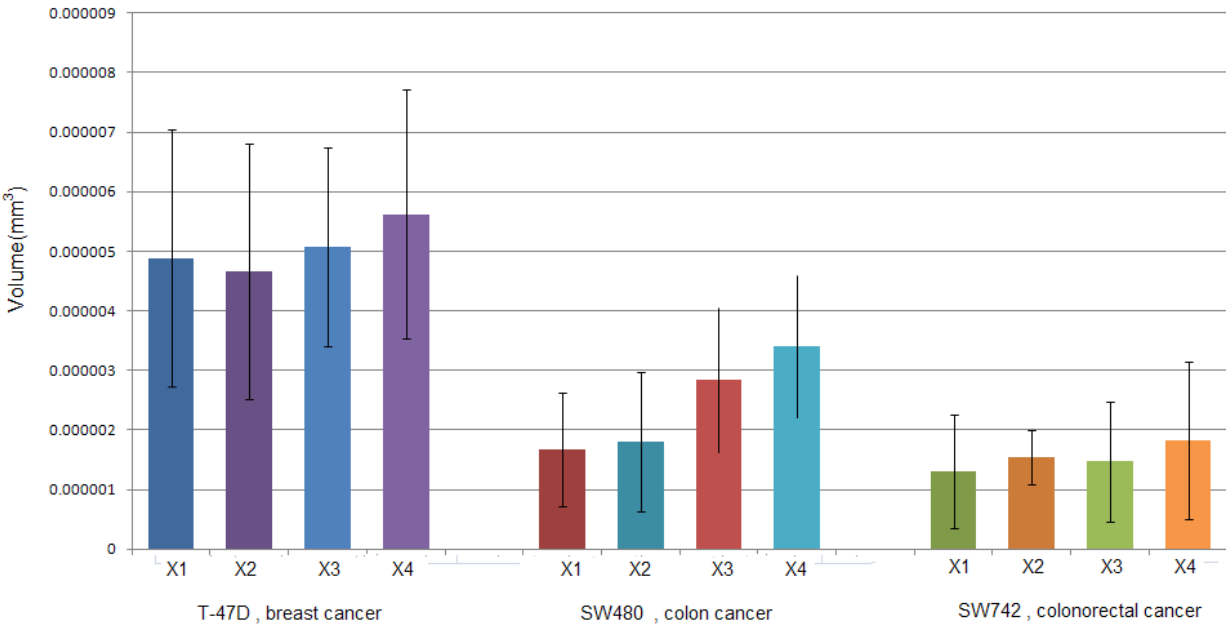

Figure 2. Mean volume changes ± SD (mm3) of three cell-lines cultured in 4.7(X1), 9.4 (X2), 14.1 (X3) and 18.8 (X4) mM per liter for 48hr.

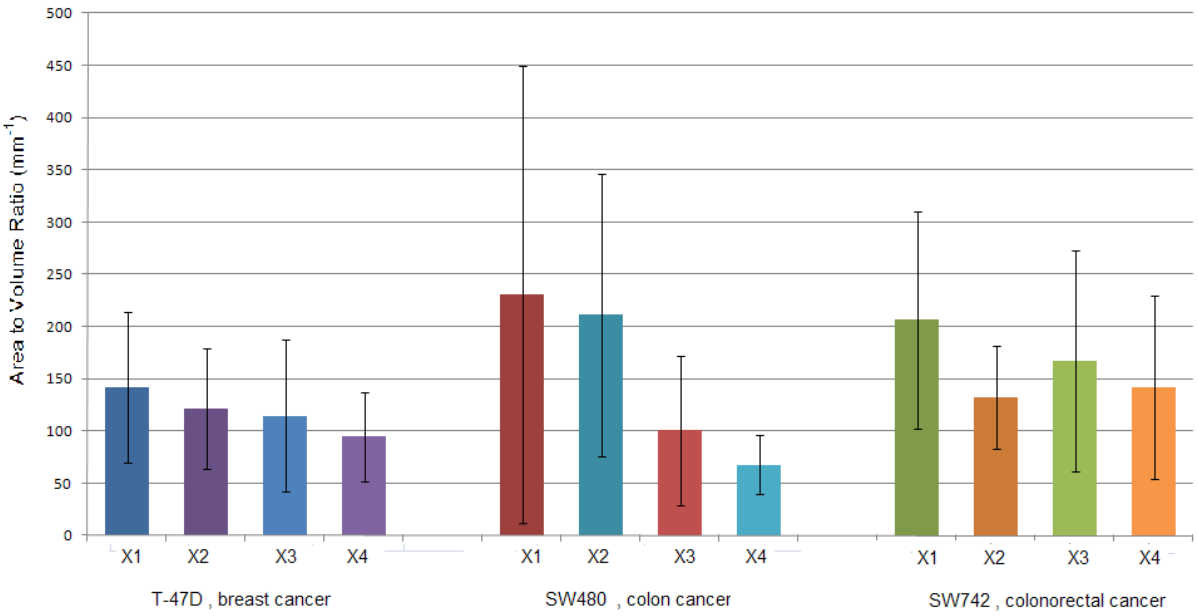

Figure 3. Mean observed surface area to volume ratio, S/V ratio changes ± SD (mm^{-1}) in three cell-lines cultured in 4.7(X1), 9.4 (X2), 14.1 (X3) and 18.8 (X4) mM per liter for 48hr.

Kruskal-Wallis test shows that S/V ratio changes are significantly different in 4.7(X1), 9.4 (X2), 14.1 (X3) and 18.8 (X4) mM per liter ($P<0.05$). 2×2 comparisons for S/V ratio changes were performed with Mann-Whitney U test. Changes in 2×2 comparisons between all cell lines' S/V ratio between X1 and X4 were significant. Measured S/V ratio in X4 for all three cell lines were significantly smaller than their volume in X1 concentration ($P<0.05$).

DISCUSSION

The physiologic levels of glucose concentration in human blood is between 3.6-5.8 mM per lit. This glucose content is expressed by term fasting blood sugar (FBS), that is equal to 4.7 mM per lit [8]. A raised blood glucose level can increase metabolic rate, and makes the immune response more efficient [27]. Cytoskeleton is the main controller of cell morphology, and is always in a dynamic state. It can play an important role in glucose regulation, and glucose can affect it [9]. The addition of D-glucose to the culture medium increased survival of heated eukaryotic cells. Heat protection by glucose is less effective than by galactose, but the importance of glucose as the main blood sugar is not disregardable [10]. Weak noncovalent bonding forces govern functioning and structural cohesion of cells. Direct measurements of these forces through mechanical means has recently become an important tool in the study of biological molecules.[6]

In this study, surface area to volume ratio was selected, as it is the simplest and most obvious biophysically measurable cell parameter against environment-related physical changes. Since the values of observed cell surface area (with direct relation) and cell volume (with reverse relation) control the value of surface to volume ratio, it is preferred to investigate cell surface area and volume, independently, before analyzing the S/V ratio.

As it is depicted in figure 1, the comparisons of surface area for two groups were performed with Mann-Whitney U test. Surface area changes were not significant in T47D cells between X2 and X3 ($P=0.581$). Changes in 2×2 comparisons between other cell lines' observed surface area were significant ($P<0.05$). In cell lines SW480 and SW742, the observed surface area in X4 was significantly smaller than those of X1, X2 and X3 ($P=0.000$). T47D and SW742 cell lines' observed surface area in X1 were significantly greater than their observed surface area in X2, X3 and X4 ($P=0.000$). These nonhomogenous alternations of observed surface area compel us to not introduce it as a key parameter in changing S/A ratio. The decrease of cell surface area in X4 (with four-

fold glucose concentration) could be due to the irregularity of cell surface and morphology controlling systems, such as the cytoskeleton components. Perhaps these systems are more sensitive to glucose concentration, because they show more response higher glucose levels.

As it is clarified in figure 2, volume of all three cell-lines is different between the tested glucose levels. Volume changes were not significant in T47D cells between X1 and X2 ($P=0.471$), in SW480 cells between X1 and X2 ($P=0.392$), in SW742 cells between X1 and X2 ($P=0.603$), and between X2 and X3 ($P=0.665$). But changes in 2×2 comparisons between other cell lines' volume were significant ($P<0.05$). The volume measured for all three cell lines in X1 and X2 were significantly smaller than their volume in X4 concentration ($P=0.000$).

After analyzing the observed cell surface area and volume, the cell surface area to volume ratio (S/V ratio) was investigated. As depicted in figure 3, the surface area to volume ratio of cell lines were compared in different glucose concentrations. Because of a reverse relationship between volume and S/V ratio, it was expected the S/V ratio to decrease at higher glucose concentrations, in comparison with the physiologic glucose levels. In addition, the decrease in S/V ratio was more significant for X4 groups ($P=0.000$), as they were exposed to the highest glucose concentration. The regular order of cell lines' apparent decrease in S/V ratio by an elevating glucose concentration can easily be perceived. The other point is that the standard deviation of all three cell lines is much greater in the X1 group (the physiologic concentration) than in the other concentrations. This great freedom of values in physiologic blood sugar shows cell's health, wealthy and normality. On the other hand, cells exposed to glucose shock showed a decrease in the S/V ratio by changing their morphology to a spherical shape, since the S/V ratio of a sphere is the least among all geometrical three-dimensional shapes. Therefore, deviation from this exact shape could be less possible, and so the standard deviation of all three cells is much smaller at these two non-physiologic sugar concentrations.

Finally, by taking a cross look at all cellular findings, it may be safe to assume that human body, both at the cellular level and even as a whole, respond delicately but sharply to glucose elevations. The goal of this research was to show that the dictating of changes via pathologic conditions in cellular levels could be a good answer to changing the body metabolic parameters. Besides, the S/V ratio could be studied as a variant parameter due to other metabolic conditions and can be calibrated for detecting the state of cell health.

ACKNOWLEDGEMENT

This research resulted from PhD thesis of Saeed Hesami Tackallou.

REFERENCES

1. D.A.Bates, G.C.Le, J.H.Bates, A.Loutfi, and W.J.Mackillop, Effects of thermal adaptation at 40 degrees C on membrane viscosity and the sodium-potassium pump in Chinese hamster ovary cells, Cancer Res. 45 (1985) 4895.
2. D.A.Bates and W.J.Mackillop, The effect of hyperthermia on the sodium-potassium pump in Chinese hamster ovary cells, Radiat. Res. 103 (1985) 441.
3. C.Bhattacharjee, S.Saha, A.Biswas, M.Kundu, L.Ghosh, and K.P.Das, Structural changes of beta-lactoglobulin during thermal unfolding and refolding--an FT-IR and circular dichroism study, Protein J. 24 (2005) 27.
4. M.Broom, Physiology of fever, Paediatr. Nurs. 19 (2007) 40.
5. K.Charalambous, A.O.O'Reilly, P.Bullough, and B.A.Wallace, Thermal and chemical unfolding and refolding of a eukaryotic sodium channel, Biochim. Biophys. Acta (2009).
6. M.Cieplak, T.X.Hoang, and M.O.Robbins, Thermal folding and mechanical unfolding pathways of protein secondary structures, Proteins 49 (2002) 104.
7. W.T.Coakley, Hyperthermia effects on the cytoskeleton and on cell morphology, Symp. Soc. Exp. Biol. 41 (1987) 187.
8.M. E. Daly "Acute effects on insulin sensitivity and diurnal metabolic profiles of a high-sucrose compared with a high starch diet, Am J Clin Nutr 67 (1998) 1186–1196.

9. N.Gao and T.Y.Zhao, [Cytoskeleton and insulin related signal transduction pathways], Sheng Li Ke. Xue. Jin. Zhan. 39 (2008) 124.
10. K.J.Henle, T.P.Monson, A.J.Moss, and W.A.Nagle, Protection against thermal cell death in Chinese hamster ovary cells by glucose, galactose, or mannose, Cancer Res. 44 (1984) 5499.
11. K.Huus, S.Havelund, H.B.Olsen, W.M.van de, and S.Frokjaer, Thermal dissociation and unfolding of insulin, Biochemistry 44 (2005) 11171.
12. M.Iwig, E.Czeslick, A.Muller, M.Gruner, M.Spindler, and D.Glaesser, Growth regulation by cell shape alteration and organization of the cytoskeleton, Eur. J. Cell Biol. 67 (1995) 145.
13. Knut Schmidt-Nielsen, *Scaling: Why Is Animal Size So Important*?, Cambridge University Press, *New York, NY*., 1984.
14. E.Kremneva, O.Nikolaeva, R.Maytum, A.M.Arutyunyan, S.Y.Kleimenov, M.A.Geeves, and D.I.Levitsky, Thermal unfolding of smooth muscle and nonmuscle tropomyosin alpha-homodimers with alternatively spliced exons, FEBS J. 273 (2006) 588.
15. D.Lecavalier and W.J.Mackillop, The effect of hyperthermia on glucose transport in normal and thermal-tolerant Chinese hamster ovary cells, Cancer Lett. 29 (1985) 223.
16. D.I.Levitsky, A.V.Pivovarova, V.V.Mikhailova, and O.P.Nikolaeva, Thermal unfolding and aggregation of actin, FEBS J. 275 (2008) 4280.
17.Z.Liu, Y.W.Zhang, Y.S.Chang, and F.D.Fang, The role of cytoskeleton in glucose regulation, Biochemistry (Mosc.) 71 (2006) 476.
18. V.V.Mikhailova, B.I.Kurganov, A.V.Pivovarova, and D.I.Levitsky, Dissociative mechanism of F-actin thermal denaturation, Biochemistry (Mosc.) 71 (2006) 1261.
19. Z.Nikolovski, V.Buzon, M.Ribo, M.Moussaoui, M.Vilanova, C.M.Cuchillo, J.Cladera, and M.V.Nogues, Thermal unfolding of eosinophil cationic protein/ribonuclease 3: a nonreversible process, Protein Sci. 15 (2006) 2816.
20. H.G.Park, S.I.Han, S.Y.Oh, and H.S.Kang, Cellular responses to mild heat stress, Cell Mol. Life Sci. 62 (2005) 10.
21. A.V. Pivovarova, V.V. Mikhailova, I.S. Chernik, N.A. Chebotareva, D.I. Levitsky, and N.B. Gusev, Effects of small heat shock proteins on the thermal denaturation and aggregation of F-actin, Biochem. Biophys. Res. Commun. 331 (2005) 1548.
22. E.M.Puchner, G.Franzen, M.Gautel, and H.E.Gaub, Comparing proteins by their unfolding pattern, Biophys. J. 95 (2008) 426.
23. X.L.Qi, C.Holt, D.McNulty, D.T.Clarke, S.Brownlow, and G.R.Jones, Effect of temperature on the secondary structure of beta-lactoglobulin at pH 6.7, as determined by CD and IR spectroscopy: a test of the molten globule hypothesis, Biochem. J. 324 (Pt 1) (1997) 341.
24. A.Razvi and J.M.Scholtz, Lessons in stability from thermophilic proteins, Protein Sci. 15 (2006) 1569.
25. P. Relkin, Thermal unfolding of beta-lactoglobulin, alpha-lactalbumin, and bovine serum albumin. A thermodynamic approach, Crit Rev. Food Sci. Nutr. 36 (1996) 565.
26. R.Renthal, An unfolding story of helical transmembrane proteins, Biochemistry 45 (2006) 14559.
27. J.L.Roti Roti, Heat-induced cell death and radiosensitization: molecular mechanisms, Natl. Cancer Inst. Monogr 61 (1982) 3.
28. J.L.Roti Roti, Cellular responses to hyperthermia (40-46 degrees C): cell killing and molecular events, Int. J. Hyperthermia 24 (2008) 3.
29. F.Spinozzi, P.Mariani, F.Rustichelli, H.Amenitsch, F.Bennardini, G.M.Mura, A.Coi, and M.L.Ganadu, Temperature dependence of chaperone-like activity and oligomeric state of alphaB-crystallin, Biochim. Biophys. Acta 1764 (2006) 677.
30. steven vogel, Life's Devices, the Physical World of Animals and Plants, *Princeton University Press*, Princeton, 1988.
31. A.Stirpe, L.Sportelli, and R.Guzzi, A comparative investigation of the thermal unfolding of pseudoazurin in the Cu(II)-holo and apo form, Biopolymers 83 (2006) 487.
32. W.W.Streicher and G.I.Makhatadze, Unfolding thermodynamics of Trp-cage, a 20 residue miniprotein, studied by differential scanning calorimetry and circular dichroism spectroscopy, Biochemistry 46 (2007) 2876.
33. M.R.Tavirani, S.H.Takallu, S.H.Moghaddamnia, S.Kalantari, B.Ranjbar, S.Z.Moosavi-Nejad, S.A.Marashi, M.R.Rahmati, and F.Malekzad, Study of Sensitivity and Ability of Adenosine Deaminase in Response to Pre-Unfolding and Especially

Pathological Temperatures Via Changing the Enzyme Structure and Activity, 2008, pp.219-224.
34. M.R.Tavirani, S.H.Moghaddamnia, B.Ranjbar, M.Amani, and S.A.Marashi, Conformational study of human serum albumin in pre-denaturation temperatures by differential scanning calorimetry, circular dichroism and UV spectroscopy, J. Biochem. Mol. Biol. 39 (2006) 530.
35. R.L.Van Laack and J.L.Lane, Denaturation of myofibrillar proteins from chickens as affected by pH, temperature, and adenosine triphosphate concentration, Poult. Sci. 79 (2000) 105.
36. E.B.Voura, M.Sandig, V.I.Kalnins, and C.Siu, Cell shape changes and cytoskeleton reorganization during transendothelial migration of human melanoma cells, Cell Tissue Res. 293 (1998) 375.
37. Y.B.Yan, Q.Wang, H.W.He, and H.M.Zhou, Protein thermal aggregation involves distinct regions: sequential events in the heat-induced unfolding and aggregation of hemoglobin, Biophys. J. 86 (2004) 1682.
38. H.G.Zhang, K.Mehta, P.Cohen, and C.Guha, Hyperthermia on immune regulation: a temperature's story, Cancer Lett. 271 (2008) 191.