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PREDICTING METAL - BINDING PROTEINS BASED ON PROTEIN STABILITY

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ABSTRACT

Understanding the temperatures at which proteins become inactive is very important with the view of preventing some diseases that come along with it. A typical example that this can cause is protein aggregation which can lead to some disorders especially in the brain. Therefore there is the need to study the temperatures at which these proteins denature. This study is thus presented is in this paper.

The metal-binding proteins used were categorized into five main groups. The denaturation temperatures of the selected metal-binding proteins were harvested from the various scientific literatures, their sequences as well as their amino acid compositions and secondary structures were collected respectively from Web resources. Thus the total number of metal-binding proteins harvested was seventy (70). Using Microsoft Excel, a statistical analysis was carried out to compare between the selected metal-binding proteins to see if there is genuine interpretation to corroborate those of the scientific literatures.

The analysis carried out as well as the scientific literatures that were reviewed, testifies that proteins that have metal ions in them, be it as cofactors or as chelating agents are more thermally stable than their counterparts - Non-metal binding proteins.

Proteins are essential in the functionality of the body. Therefore, there is need to investigate their compositions and structures in order to understand these macromolecules. Thus this study would

help in the field of drug design in pharmaceutical sector by understanding the temperatures which proteins would be inactive. This would be helpful to prevent protein aggregation and neuron disorders mostly in the brain.

Keywords: Metal-binding, protein, denaturation temperature

1. Introduction

Proteins are big biological macromolecules made up of approximately twenty-amino acids. The primary structure, that is, the sequence of amino acids making up the backbone of the protein is held together by peptide bonds. These peptide bonds match up to a class of organic compounds called amides and are generally stable under typical biological conditions [3].

Secondary and tertiary structures represent the folding of the protein onto itself as a result of the nature of the side chains of the constituent amino acids. These structures can be disrupted quite easily, especially during routine handling. Denatured proteins means a disruption of tertiary structure which is generally useless, thus the goal of any process involving the use of active proteins must include understanding of protein stability and biological functions. [3]

Protein stability can be interpreted in different ways by different people. A physical biochemist and a biotechnologist may each mean something different when they talk about protein stability.

The physical biochemist would refer to protein stability in terms of the thermodynamic stability of a protein that unfolds and refolds rapidly, reversibly and jointly. In the case of biotechnologist, he is more concerned with the practical utility of the definition: Is the protein stable enough to function under harsh conditions of temperature or solvent? Whilst the answers to this question lie in thermodynamic stability; it may also lie in reversibility or irreversibly or slowly unfolding proteins, in kinetic stability.

The prediction of metal-binding proteins via various manual experimental methods has been beset with series of problems. The major problems are time wasting and cost of carrying out the experiments. Some of the experiments take days to years before a conclusion can be reached. There is therefore the need to explore the use of computer applications to speed up the prediction as well as save energy and most importantly complement the manual experimental results.

Research has shown that amino acid cysteine and histidine participate more in binding several metals [15]. This can be used as a discriminative feature incorporated in the computer system used

for predicting metal binding proteins.

This paper investigates the prediction of metal-binding proteins based on protein stability (temperature), of which if found to be involved in prediction of metal-binding proteins, will serve as a discriminative feature that can be incorporated in computer application to aid prediction accuracy. This will also imply that not only amino acid composition can be used as a feature but also thermostability can as well be used in predicting metal-binding proteins from non-metal binding proteins.

2. Methodology

In this study, the quantitative research method was used, having some variables with values which are analyze using statistical procedures so as to determine whether the predictive generalizations of the theory hold true. The computer application used for the statistical analysis is Microsoft Office Excel 2003.

The statistical analysis was carried out on one comparison to see if there is a genuine interpretation to corroborate those of the scientific literatures. The comparison is: "***Comparison between the selected metal-binding proteins".***

The classes of metal binding proteins used in the analysis were copper-binding proteins, zinc-binding proteins, iron- binding proteins, calcium-binding proteins and other binding proteins. Different features were extracted for each proteins namely (i) denaturation temperature (ii) amino-acid sequence (iii) amino-acid composition (iv) secondary structure (alpha-helix, extended strand and random coil). These features were critically analyzed to see which one would be suitable as a discriminative feature that can be incorporated in computer programs to aid prediction accuracy.

The total number of metal-binding proteins harvested was seventy (70). The breakdown in terms of numbers is as follows:

a. Copper Binding Proteins-------12 proteins
b. Zinc Binding Proteins----------11 proteins
c. Iron Binding Proteins----------13 proteins
d. Calcium Binding Proteins------21 proteins
e. Other Metal Proteins----------13 proteins

Table 2.1 presents the metal binding proteins used in the analysis. Also Tables 2.2a, b, c, d, e, present the list of all the selected metal-binding proteins against their denaturation temperature while Figure 2.1 presents the metal-binding proteins against the overall averages of their denaturation temperature. The analysis of the metal-binding proteins against their denaturation temperature is done in order to establish if there is a solid correlation with that in the existing scientific literatures such as [1],[8],[11],[16],[17].

Table 2.1 List of Metal Binding Proteins

Copper binding Proteins	Zinc Binding Proteins	Iron binding Proteins	Calcium binding proteins	Other Metal-binding Proteins
Cupredoxin	Aminoacylase	Ferredoxin	alpha-amylase	Concanavalin A
Nitrate reductase	Insulin	Rubredoxin	alpha-amylase	Phosphotriesterase
Amine Oxidase	Azurin	Bovine Lactoferrin	calbindin	Calbindin
	yeast alcohol	Human growth Hormone(replaced	**Human Growth	Human growth
Plastocyanin	dehydrogenase	with iron)-	Hormone	Hormone
		Iron-sulfur cluster		
Azurin	Phosphotriesterase	protein	alpha- haemolysin	Creatine Kinase
	Protease 2A(human			
Pseudoazurin	rhinovirus 2)	Apotransferin	alpha-lactalbumin	Tubulin
	Aspartate	Apo- recombinant	Psychrophilic	
Amicyanin	transcarbamoylase	wild-type myoglobin	elastase(ACE)	Apoptosis
	superoxide		Psychrophilic	
Lacasse	dismutase	Transferrin	elastase(ACE)	Beta lactoglobulin
superoxide		Glutamate	Alpha-Parvalbumin-,	Glutamine
dismutase	Carboxypeptidase A	dehydrogenase	Beta-parvalbumin	Synthetase
***Bovine				Spinach Phosystem
Serum albumin	Nucleotidase	Adrenodoxin	aldose reductase	II (OEC16)
Copper/quinon	Human Sonic	Hemopexin	Concanavalin A	

Copper/quinon			
e containing	Human Sonic		
amine oxidases	hedgehog	Hemopexin	Concanavalin A
Amyloid Fiber		Frataxin	Calprotectin
			Photosystem II PBSO
		Myoglobin	extrinsic protein
			Troponin C
			Cytosolic
			Phospholipase A2
			Subtilisin BPN
			Human Complement
			Protein
			Membrane Calcium
			Pump
			Horse plasma gelsolin
			Soybean glycinin
			Bovine trypsinogen

Table 2.2 Metal - Binding proteins against Denaturation Temperature

a. Copper-binding proteins

Copper binding Proteins	**Denaturation Temperature (oC) – 72.22**
Cupredoxin	71

b. Zinc-binding Proteins

Zinc Binding Proteins	**Denaturation Temperature (oC) – 58.56**
Aminoacylase	65
Insulin	37
Azurin	64.8 (replaced with zinc)
yeast alcohol dehydrogenase	50
Phosphotriesterase	75
Protease 2A(human rhinovirus 2)	52.5
Aspartate transcarbamoylase	60.9
superoxide dismutase	72
Carboxypeptidase A	50
Nucleotidase	65-70 (presence of DTT),55-60(absence of zinc atoms by NTA)
Human Sonic hedgehog	59 (wild -type),52-54 (H140A and D147A mutants)

c. Iron-binding Proteins

Iron binding Proteins	**Denaturation Temperature (oC) – 74.64**

Apo- recombinant wild-type myoglobin	60.4
Transferrin	>60
Glutamate dehydrogenase	113
Adrenodoxin	70
Hemopexin	64.63
Frataxin	45
Myoglobin	71.8 (mutant), 81.3 (wild-type)
Bovine milk lactoferrin	72-85

d. Calcium-binding Proteins

Calcium binding proteins	**Denaturation Temperature (oC) – 77.13**
alpha-amylase	80-90 (calcium added as catalyst)
alpha-amylase	83 (calcium added) , 48 (calcium removed)
Calbindin	89->95 (calcium added) , 58 (apo state)
**Human Growth Hormone	27 (replaced by calcium)
alpha- haemolysin	100
alpha-lactalbumin	70
Psychrophilic elastase(ACE)	50.21 (absence of calcium)
Psychrophilic elastase(ACE)	55.71 (calcium added)

Alpha-Parvalbumin-, Beta-parvalbumin	45.8 (for alpha- parvalbumin), 53.6 (beta- parvalbumin)
aldose reductase	55
Concanavalin A	87.2- 90.8
Calprotectin	62.1- 88.2
Photosystem II PBSO extrinsic protein	50-60
Troponin C	75 (apo form), 80 (holo form)
Cytosolic Phospholipase A2	64.9 (calcium added), 46 (absence of calcium)
Subtilisin BPN	75 (PH 8.0), 56 (Presence of excess EDTA)
Human Complement Protein	60 (PH 6.6), 81.4 (PH 8.0)
Membrane Calcium Pump	33-35
Horse plasma gelsolin	42
Soybean glycinin	90.98
Bovine trypsinogen	68.3

e. Other Metal-binding Proteins

Other Metal- binding Proteins	**Denaturation Temperature (oC) - 65.00**
Concanavalin A	82.5-85.6 (added nickel), 85.2- 85.9 (added cobalt)
Phosphotriesterase	58 (repalced with cobalt cation)
Calbindin	75 (magnesium added), 58 (apo form)

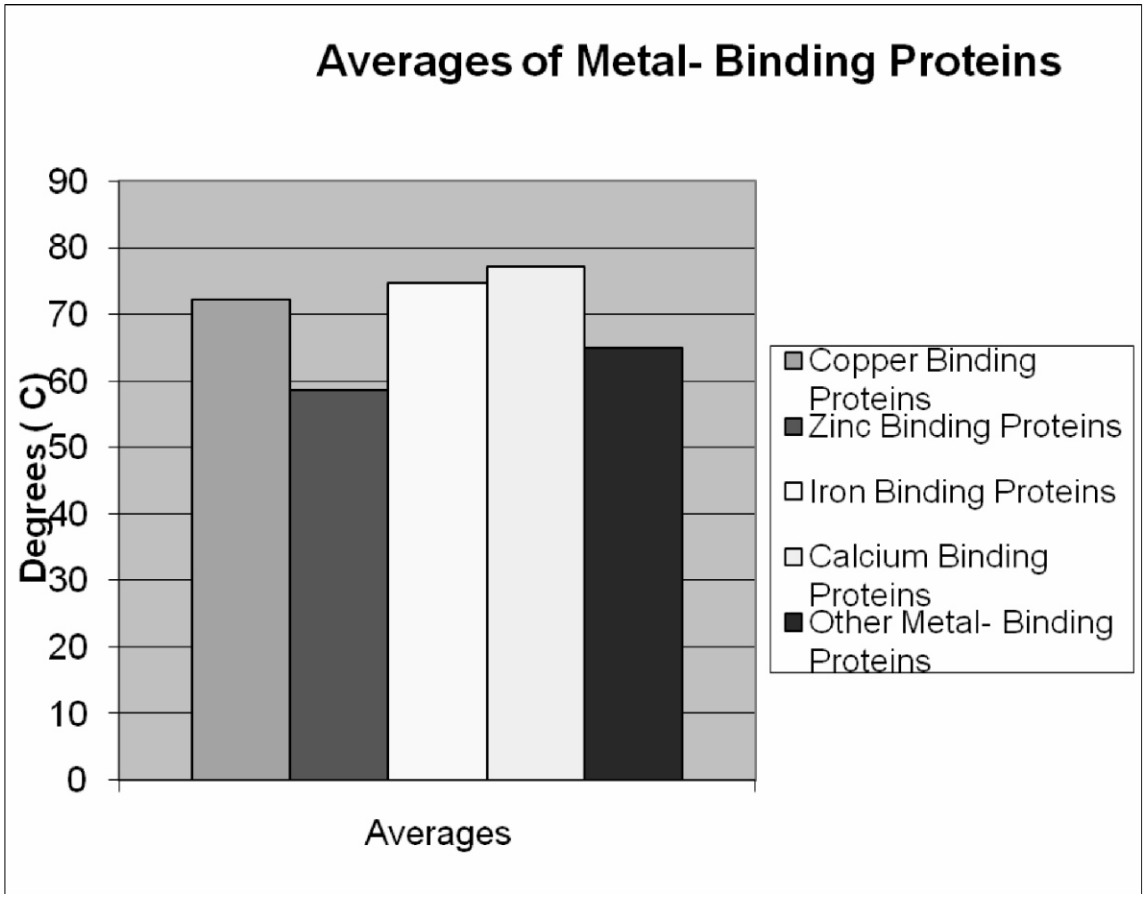


Figure 2.1Overall averages of metal-binding proteins denaturation temperature.

The analysis was done based on the following premises:

i. Denaturation temperature against Amino acid composition of each metal- binding proteins in a particular group. That is each protein under copper binding proteins. A sample is presented in Figure 2.4

ii. Denaturation temperature against Amino acid composition of all the selected metal binding proteins.

iii. Denaturation temperature against secondary structure of each metal binding protein in a particular group. Samples are presented in Figures 2.5a, b, c, d, e, f.

iv. Denaturation temperature against secondary structure of all the selected metal binding proteins. A sample is presented in Figure 2.6

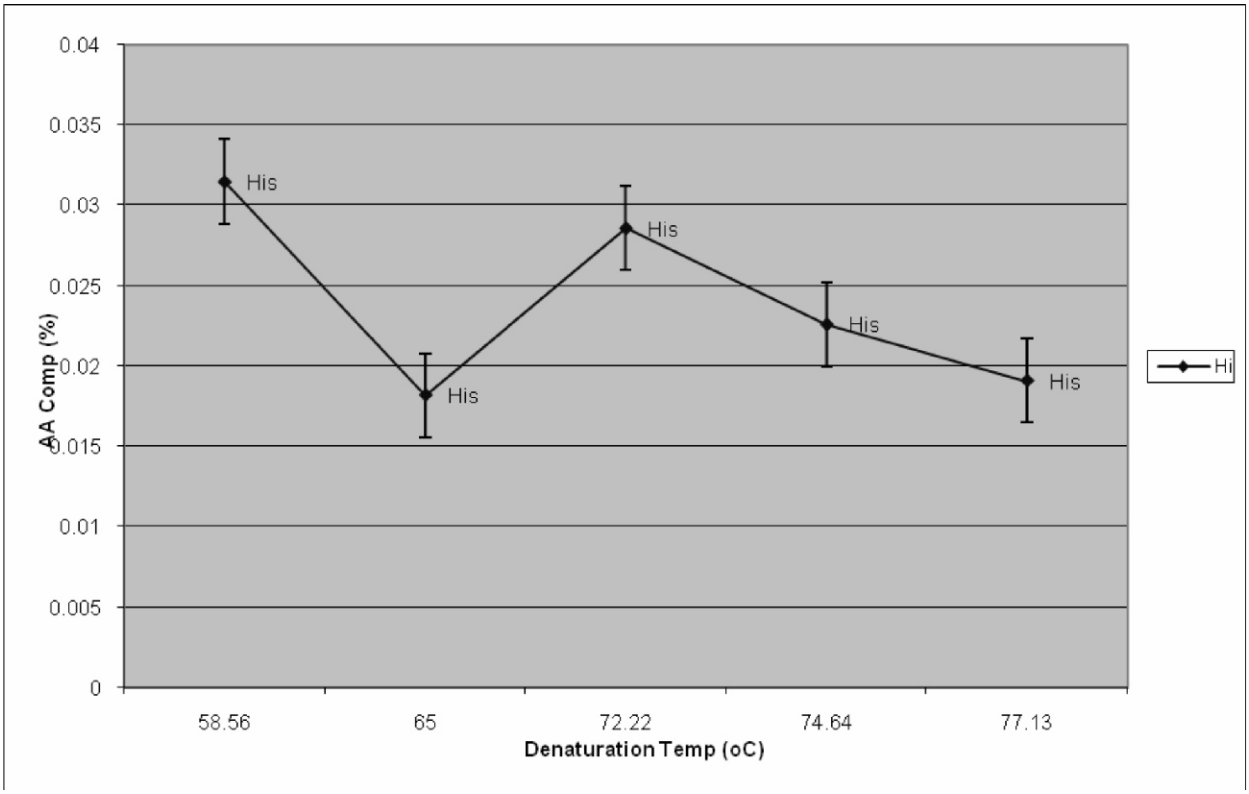


Figure 2.4Denaturation temperature against Amino Acid composition

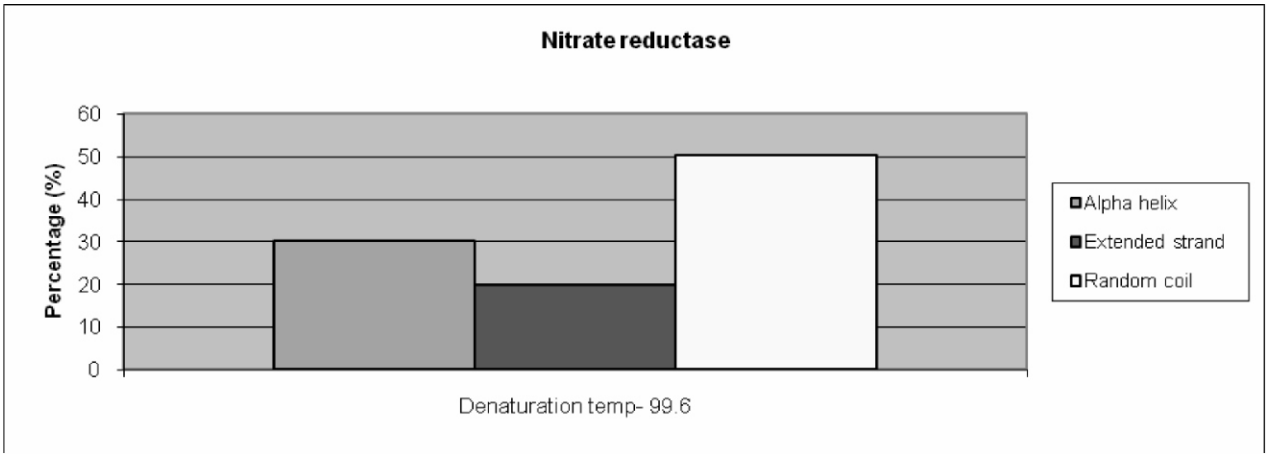


Figure 2.5a

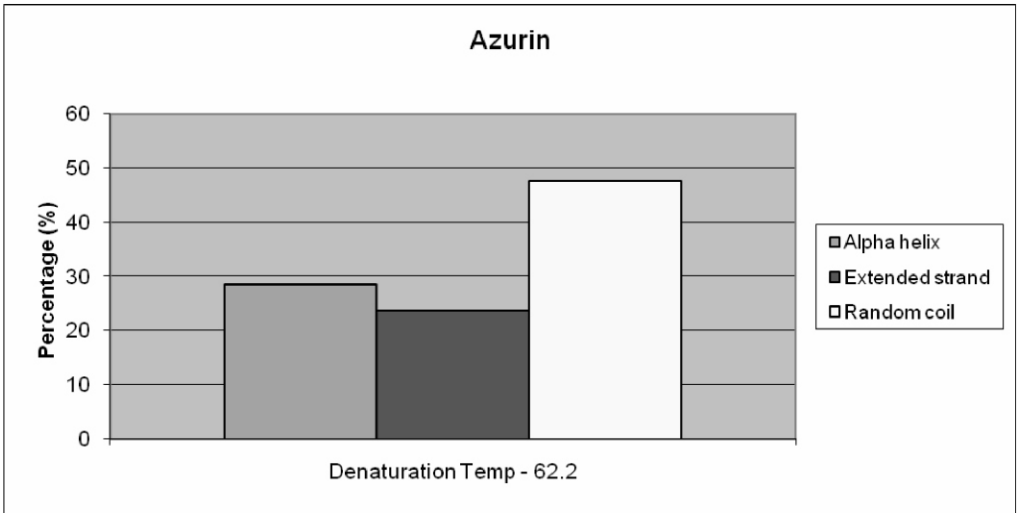
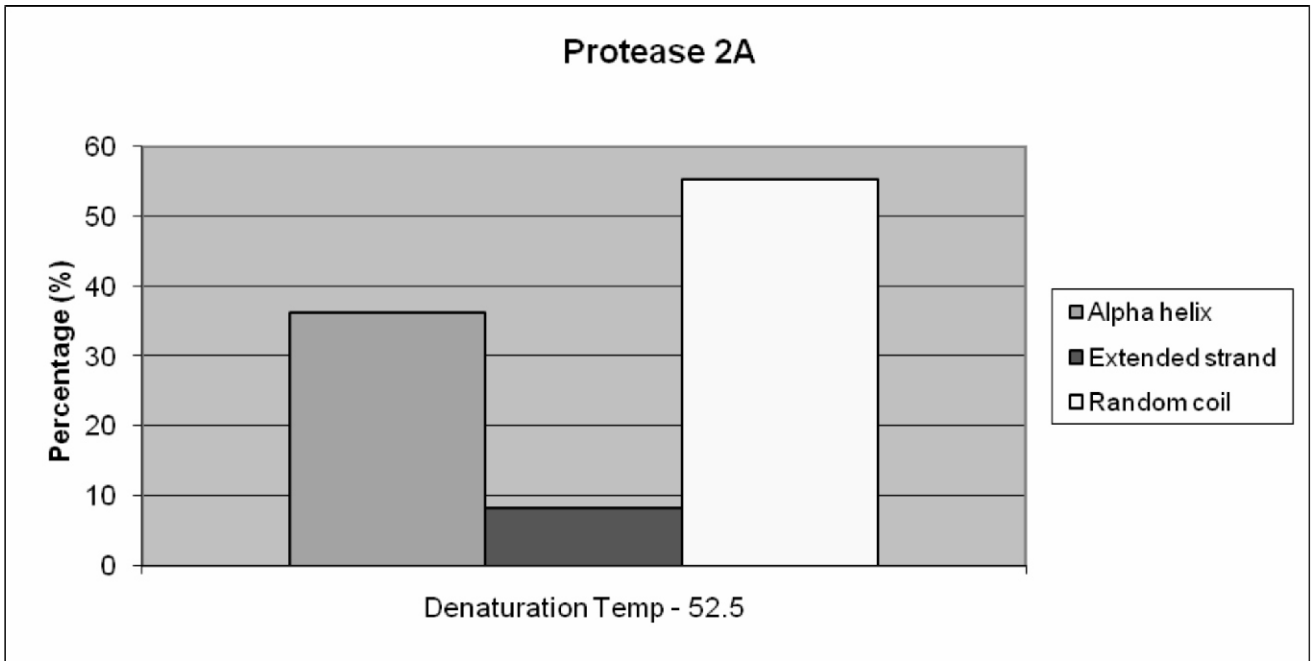


Figure 2.5b



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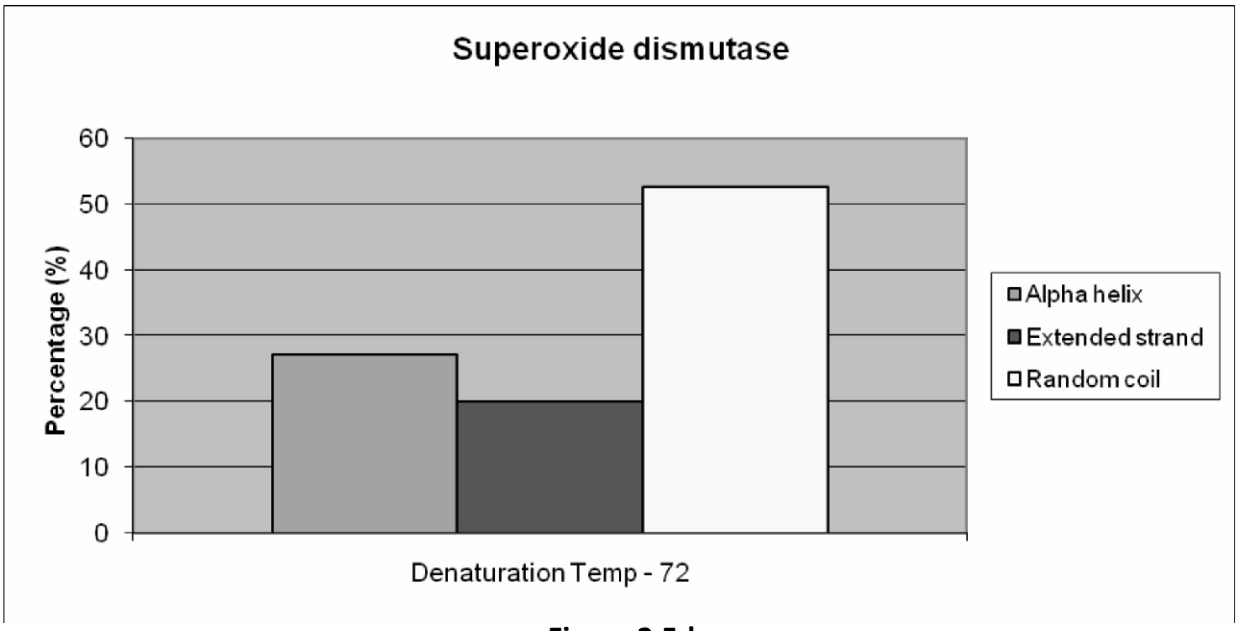

Figure 2.5d

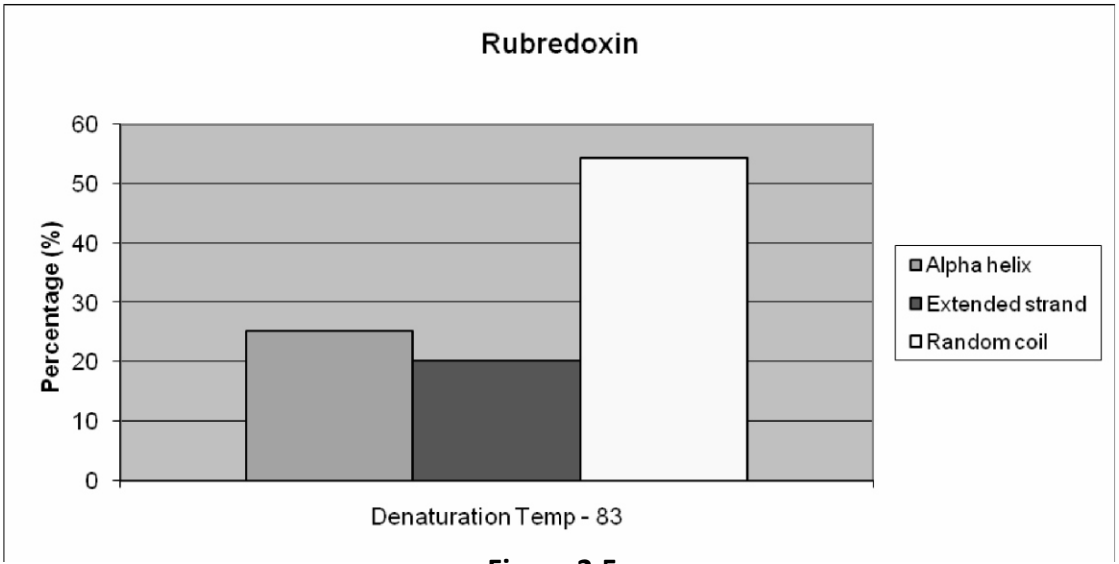

Figure 2.5e

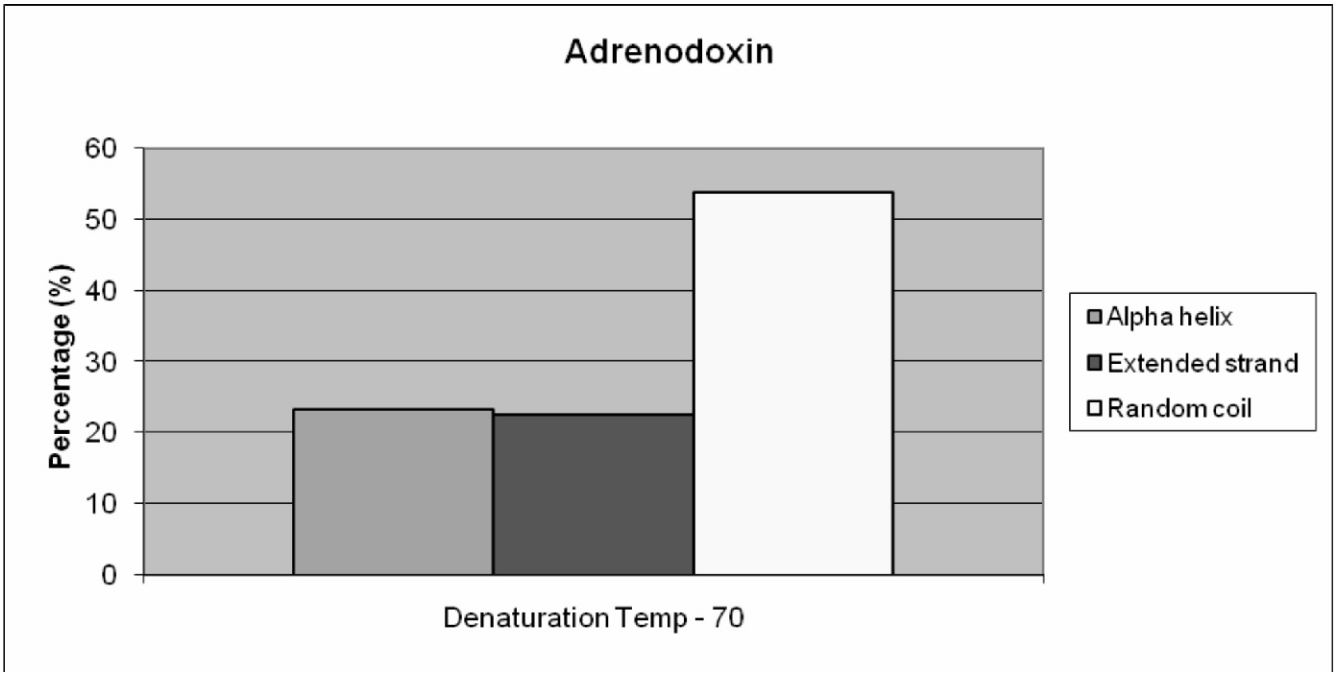

Figure 2.5f

Figure 2.5Denaturation temperature against secondary structure of each metal-binding protein

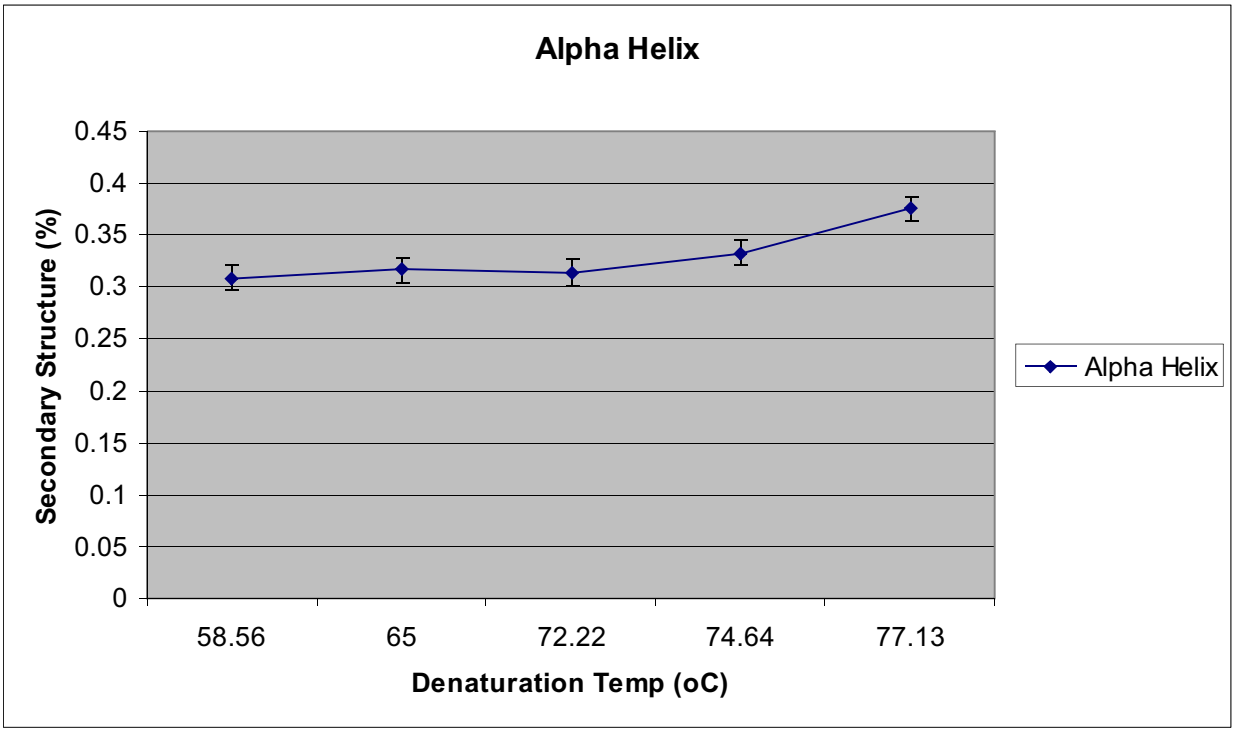

Figure 2.6Denaturation temperature against secondary structure o

f all the selected metal binding proteins

The averages of the metal-binding proteins were calculated at the individual level as well as collective level.

3. RESULTS AND DISCUSSION

The data analyzed was mainly metal-binding proteins and the results focused on which of the metal-binding proteins were more stable based on references drawn from existing works such as [1],[8],[11],[16],[17], where experimental methods were carried out.

From the analyses above, **Calcium binding proteins** seemed to be the most stable proteins as compared to the other metal-binding proteins. Its average denaturation temperature was calculated to be 77.13 degrees. Which meant an average copper binding protein would denatured at above 70 degrees. It is important to also investigate what actually constituted to the prolonged denaturation temperatures of metal- binding proteins. One of the reasons why some metal-binding proteins are stable is because some amino acids play some important role in stabilizing the protein's tertiary structure and make the protein more resistant to denature. For instance, amino acid **Cysteine** (Cys). Its sulfur atom binds easily to heavy metal ions. Therefore, disulphide bridges are usually common in such proteins. A typical example is Insulin. This explains why some proteins basically metal-binding proteins would be more stable than non-metal binding proteins because of the interaction of some amino acids that interact with the metal ions. Also, for thermophilic and hyperthermophilic organisms, the preferred amino acids are Glu, Lys, Tyr, Ile. This preference is being noticed in the graphs plotted of which Figure 3.1 is a sample. In the case of amino acid **Glu**, as the percentage of the amino acid increases, the more stable the protein is.

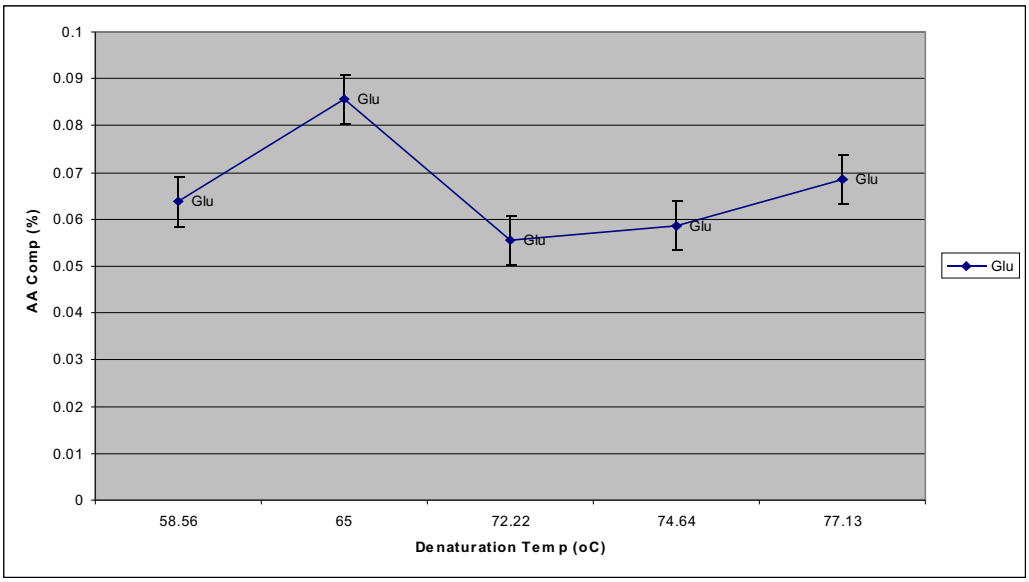

Figure 3.1AA against Averages of Metal binding proteins

The same trend was been repeated in the remaining preferred amino acids except in amino acid **Tyr**. This fact is supported in [18]

Based on secondary structure, it was observed that most calcium binding proteins increase protein thermal stability by increasing the **alpha-helix content** as well as decreasing the beta and random coil structures respectively. This was buttressed in [13]. Increased in alpha-helix contents of the secondary structure of proteins meant that there would also be an increase in the **hydrogen bonds** in the proteins and this kind of interaction increases the stability of the protein.

Again, the effect of metal substitution was looked into to have a clearer picture of what takes place. In [9] ,various metals were investigated which consisted of Co2+, Ni2+ and Cd2+ substituted for the Mn2+ ion in the SI site of Con A. It was observed by the X-ray crystallographic studies that different metal ions substituted into the SI binding site may affect the thermodynamics of binding at the carbohydrate- binding site. That any differences in the structure between the Con A may also be revealed in differences in their thermal stabilities as it was evident in the difference between derivatives of Con A where the metal ions have been removed. It was concluded that there is little difference in the thermal stabilities of ConA, CoConA and NiConA.

Still on metal substitution, from the experimental technique carried out in [7] using CD spectrum, the spectrum decreases with an increase in temperature and revealed a decrease in the level of secondary structure and other possible implications of metal ion binding to the Hx- heme complex. It was observed that the ability of hemopexin to bind metal ion meant this protein participates in the transport of metal ions in blood or in the exchange of metal ions between proteins. But because the Hx- heme complex should not remain in plasma for long prior to its removal by the liver, there is need for possibilities that the binding of metal ions such as Cu2+ and Zn2+ would promote a change in ligation and further chance that these or other metal ions may participate in the dissociation of the Hx-heme complex.

In [10], it was also found out that there was an increase in the alpha-helix content and a slight decrease in both beta and random coil structures result due to the increasing concentration of magnesium ion with respect to the protein. Overall, calcium binding proteins have been proved to be the most stable metal-binding proteins. This was buttressed in [17], in the paper, a maximum of six calcium ions were bound per calprotectin molecule. The enthalpy of denaturation was also boosted by Ca2+ the result testified that the protein shows distinct conformational changes and raised thermal stability. This was concluded to be important for its function.

4. CONCLUSION

The main objective of this paper is to investigate the prediction of metal-binding proteins based on protein stability (temperature). Protein stability can be used to predict metal-binding proteins as it has been seen that calcium binding proteins tend to be more stable than any other metal binding proteins. This is so because metal ions which bound to proteins make the proteins more stable as well as make them to be able to perform their responsibilities in the various parts of the body. Understanding the temperatures at which proteins become inactive is very important so as to prevent some diseases that it comes along with. A typical example that this can cause is protein aggregation. This can lead to some disorders especially in the brain. So, it is extremely paramount that we understand the denaturation temperatures of various proteins.

From the analysis carried out as well as the scientific literatures/journals that were reviewed, all testify that proteins that have metal ions in them be it as cofactors or as a chelating agents are more thermally stable than their counterparts- Non- metal binding proteins.

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