Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.9, 2014

Analysis of Oxygen Affinity of the Major Hemoglobin Component HbA from *Aquila chrysaetos* (Golden Eagle)

Abid Ali[1], Tayyabur Rehman[2], Roshan Ali[2]*
1. Department of Zoology, Islamia College University, Peshawar 25120, Pakistan
2. Institute of Basic Medical Sciences, Khyber Medical University, Peshawar 25000, Pakistan

The work was done partly at Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, and partly in Islamia College University, Peshawar, Pakistan

Abstract
Aquila chrysaetos(golden eagle), a high altitude bird is usually found perched on poles or cliff edges. Its hemoglobin is adapted to high altitude hypoxic condition. To understand the mechanism of respiration in *A.chrysaetos*we need to understand the three dimensional structure of its hemoglobin. During our contemplation, a three dimensional model of hemoglobinfrom *A.chrysaetos* was predicted though homology modeling using Turkey (*Meleagrisgallopavo*)'s HbA as template. MODELLER was used to create three dimensional patterns.Stereochemistry of the models was evaluated by PROCHECK and ProSA. This study is about the analysis of effects of inter-subunit contacts on oxygen affinity of the hemoglobin. The effect of the following pairs of inter-subunit contacts on the oxygen affinity of the hemoglobin have been studied *i.e.* α99 and β101, α34 and β125, α38 and β99 and β97, α119and β55, α35 and β128, and α103 and β112. The absence of bonds between these pair like other hypoxic condition dwellers might be the main cause of its living under low oxygen tension.
Keywords: High altitude, hypoxic, Inter-subunit Contacts, R-state, birds

INTRODUCTION

Hemoglobin (Hb)'s structural and functional correlation in animals other than human has been is extensively studied (Perutz, 1983, Weber *et al.*, 2004). Previous studies regarding vertebrates hemoglobin functions explain the metabolic needs and confirms that the structure and function is responsible for environmental constraints. The organisms demand for the CO2 and O2 is full filled by Hb(Stepuro and Zinchuk, 2006). The variation in the structure and thus in function of this molecule indicates the evolution in the animal kingdom for adaptation (Hourdez and Weber, 2005). The obvious variability in Hb molecule in terms of O2 affinity in response to change in surrounding has been reported long before (Riggs, 1964). Among vertebrates, birds with exception of flightless birds have acquired the ability of surviving in hypoxic condition such as flying at high altitude during migration or in deep sea diving (Weber, 1995).

Golden Eagle is a high altitude bird, and has been reported from Northern Hemisphere, comprised of Asia continent south to Himalayas and China. These birds are also found in North America, which ranges from northern Alaska south to Lower California and central Mexico (Dawson, 1923). The population of Golden eagles inhibiting at remote areas can be affected by human exploration, and hunting causing disturbance near the nest (Kaufman, 1996, Dawson, 1923). As golden eagle inhabit in high altitude, its survival at high altitude is made possible by some of the substitution in the interaction in Alpha and Beta chain residues in Hb, which enable it to survive under hypoxic condition at high altitude.

MATERIALS AND METHODS

The sequences of αA and β chains of *A.chrysaetos*'sHb(Oberthur *et al.*, 1983) were retrieved from SwissProt Database (Boeckmann *et al.*, 2003). To perform similarity searches and template selection BLAST (Altschul *et al.*, 1997, Altschul *et al.*, 2005) was used. Turkey oxy-hemoglobin A [PDB: 2QMB](Charles *et al.*, To be Published) was selected as the template because of its highest homology with the target sequence. The αA chain of *A.chrysaetos*'sHbA shows 87 % identity with αA chain of turkey's HbA and the β chain shows 95 % similarity with the β chain of turkey's HbA. The 3D structure coordinates of turkey's HbA were obtained from Brookhaven Protein Databank (PDB) (Berman *et al.*, 2000). Alignment was performed with CLUSTAL-X (Larkin *et al.*, 2007).

MODELLER 9v9 (Sali and Blundell, 1993) was used to build the homology models using turkey's HbA as template. Stereochemistry of the models was evaluated by PROCHECK (Laskowski *et al.*, 1993). The energy graphs were calculated with the help of ProSA(Sippl, 1993). The best model was selected on the basis of PROCHECK and ProSA results.

To analyze the inter subunit contacts LigPlot(Wallace *et al.*, 1995)was used. All protein structures and models were visualized and analyzed using the DS Visualizer® (v. 2, Accelrys Software Inc).

RESULTS AND DISCUSSION

A homology model of the *A.chrysaetos*'sHb has been calculated using coordinates of the structure of turkey oxy-hemoglobin. The model has general all alpha topology with no beta strands just like all other hemoglobin having eleven alpha helices in Alpha chain and thirteen helices in Beta chain as illustrated in figure 1.

Analysis of the model of *A.chrysaetos* shows that 93.2% residues were in core region, 6.6% were found in allowed region, and 0.2% in generously allowed region while no residue was in the disallowed region as evaluated by PROCHECK. Energy plots of both the chains were below zero just like corresponding template chains' energy plots calculated using ProSA. The energy values of both the chains are quite similar to the corresponding chains of the template.

Inter-subunit Contacts and Their Effect on Oxygen Affinity

The functional characteristics of hemoglobin are because of the inter-subunit contacts i.e. α1β1 and α1β2 as well as its interaction with effectors molecules like Cl, CO2 and organic phosphates (Perutz, 1989). In Tufted duck's HbA, the formation of the salt bridge between α99Arg and β101Glu stabilize the R (Relaxed) state that helps in increasing the oxygen affinity of the hemoglobin(Hourdez and Weber, 2005). In golden eagle hemoglobin α99 is replaced with Lys. These two residues are close enough to interact with each other and hence making a bond that may increase the oxygen affinity of golden eagle's Hb, as represented in figure 2.

Most birds, including Bar Headed goose, possess Thr at position αA34 (α1β1 contact site). The interaction between αA34 and β125 results into a hydrogen bond. It stabilizes the T structure and hence lowering the oxygen affinity (Lutfullah *et al.*, 2005). This hydrogen bond is lost in Tufted duck's HbA because of the substitution of Thr with Ile at α34 position (Hourdez and Weber, 2005). This loss of hydrogen bond stabilizes the R structure and hence increasing the oxygen affinity of Tufted duck's HbA. Pheasant's HbA possesses Ile at α34, which cannot make a hydrogen bond with β125Glu thus having high oxygen affinity (ALI *et al.*, 2011). The golden eagle's HbA has Thr at αA34 position but it did not show any interaction with the β125 in R state which stabilized the R state and thus may be a possible cause of high oxygen affinity as represented in figure 3.

Anseriformes and some other species have Gln at position α38 (Huber *et al.*, 1988), which is responsible for stable oxy structure of hemoglobin (Perutz, 1990) by making two hydrogen bonds with β99 and β97. These bonds are also reported in Tufted duck's HbA and thus are the possible agents of its high oxygen affinity (Hourdez and Weber, 2005). In case of Golden eagleα38Gln is substituted with Pro, and beta residues are replaced by β97His and β99Asp. The distance between α38Pro and β97His residues was shorter than that of α38Pro and β99Asp as a result, unlike the Tuftedduk'sHbA, hydrogen bond was found between α38Pro and β97His. The bond was absent between α38Pro and β99Asp. The presence of this bond in R state ensures the high oxygen affinity. Figure 4 shows the interaction between these residues.

In Human HbA β55Met is involved in van der Waals interactions with α119Pro, however this type of interaction is not possible in Bar Headed goose and Andean goose because of the mutation in this pair of residues i.e. β55Leu and α119Ala in Bar Headed goose, and β55Ser and α119Pro in Andean goose(Hiebl *et al.*, 1987, Oberthur *et al.*, 1982). Due to the absence of this contact in Bar Headed goose and Andean goose HbA, T structure becomes unstable increasing the oxygen affinity (Hourdez and Weber, 2005). In pheasant, the substitution of Leu at position β55 fails to show van der Waals interactions with α119Pro due to larger distance and results into increased oxygen affinity (ALI *et al.*, 2011). In *golden eagle*'s HbAR-state, β55Leu is mutated to β55 Ile. Due to larger distance between these two residues no van der Waals interactions are observed, which is a possible inclination to lower oxygen affinity, as represented in figure 5.

Some additional contacts which effect the hemoglobin adaptation of *Golden eagle*

Some interactions between α and β subunits results into stabilization of the T-state as a result more quantity of oxygen needs for relaxation of tense state (Bettati *et al.*, 1998). It has been made clear in previous studies that more inter-subunit contacts result in lower oxygen affinity in the hemoglobin molecules (Liu *et al.*, 2001). Some additional contacts have been reported in previous studies between the *G. carbonaria*Hb results low O2 affinity relative to chicken and human Hb(Lutfullah *et al.*, 2008). A substitution in human Hb is reported at α35 where Ser is present, same position in *G. carbonaria*Hb is occupied by Val (Lutfullah *et al.*, 2008). In golden eagle Hb's alpha chain, Thr is present at α35 position and do not form hydrogen bond with β128Leu, as illustrated in figure 6, which may possibly increase oxygen affinity of golden eagle's Hb. Similarly, at β112, Ile is present in *G. carbonaria*HbD β112 is substituted by Ile and differentiate from human having Cys at same position form H-bond with α103His (Lutfullah *et al.*, 2008). Just like *G.carbonaria*HbD and unlike human Hb Ile is present at β112 but α103His is substituted by α103Ser, due to larger distance they are unable to form bond, figure 7 shows the distance between these residues. This absence of bond may have increased the oxygen affinity in golden eagle's HbA.

Heterotrophic effects

The Hb O2-binding affinity depend on allosteric effectors interactions which may be H^+ ions, organic phosphates, Cl^- and CO2. They bind strongly to deoxyHb, mainly at sites of N- and C-termini, so stabilized T-state and hence low O2 affinity by salt bridges formation (Bettati *et al.*, 1983, Perutz, 1970). At normal PH, the human Hb binds

to protons at α1Val, α122His,β2His, β82Lys, β143His, and β146His(Ho and Russu, 1987, Kilmartin *et al.*, 1978, Lukin and Ho, 2004, Perutz *et al.*, 1969). Cl^- ions binds to α1Val and α131Ser and β1Val and β82Lys (Riggs, 1988). While the CO2 combines with the N-terminal NH3+ residues of deoxyHb and change the O2 affinity through delocalized electrostatic effects (Arnone, 1974, Perutz, 1983, Perutz *et al.*, 1994). The positive charges of β-chains of deoxyHb is partially neutralized by Chloride ions and stabilizes the deoxy state. Similar to other vertebrates the above residues are conserved in golden eagle except β143His which is substituted by β143Arg, which may be the possible reason for the increased oxygen affinity and ability of surviving in lower oxygen pressure.

The joint effects of hypoxia and cold in high-altitude habitats compel to intense physiological challenges on endothermic animals. At high altitude the low oxygen pressure decreases the oxygen distribution arriving at the cells of respiring tissues (Cheviron and Brumfield, 2012). As golden eagle is a high altitude bird, it overcome these entire problems by the unique interactions in the Hb inter subunits contacts. It is concluded from the above study that the HbA of golden eagle has been adapted to hypoxic condition.

References:

ALI, R., LUTFULLAH, G., KHAN, Z. and AHMAD, A., 2011. Homology Modeling of Ring Necked Pheasant (Phasianus colchicus, Galliformes) Major Hemoglobin Component (HbA). *J. Chem. Soc. Pak.*, 33: 539.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, 25: 3389-3402.

Altschul, S.F., Wootton, J.C., Gertz, E.M., Agarwala, R., Morgulis, A., Schaffer, A.A. and Yu, Y.K., 2005. Protein database searches using compositionally adjusted substitution matrices. *FEBS J.*, 272: 5101-5109.

Arnone, A., 1974. Mechanism of action of hemoglobin. *Annu. Rev. Med.*, 25: 123-130.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N. and Bourne, P.E., 2000. The Protein Data Bank. *Nucleic Acids Res.*, 28: 235-242.

Bettati, S., Mozzarelli, A. and Perutz, M.F., 1983. Allosteric mechanism of hemoglobin: rupture of salt bridges raises oxy-gen affinity of the T-structure. *J. Mol. Biol.*, 281: 581-585.

Bettati, S., Mozzarelli, A. and Perutz, M.F., 1998. Allosteric mechanism of haemoglobin: rupture of salt-bridges raises the oxygen affinity of the T-structure. *J Mol Biol*, 281: 581-585.

Boeckmann, B., Bairoch, A., Apweiler, R., Blatter, M.C., Estreicher, A., Gasteiger, E., Martin, M.J., Michoud, K., O'Donovan, C., Phan, I., Pilbout, S. and Schneider, M., 2003. The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. *Nucleic Acids Res.*, 31: 365-370.

Charles, P., Sundaresan, S. and Ponnuswamy, M.N., To be Published. X-ray crystal structure analysis of hemoglobin from turkey(meleagris gallopavo) at 2.80 Angstrom resolution.

Cheviron, Z.A. and Brumfield, R.T., 2012. Genomic insights into adaptation to high-altitude environments. *Heredity*, 108: 354-361.

Hiebl, I., Braunitzer, G. and Schneeganss, D., 1987. The primary structures of the major and minor hemoglobin-components of adult Andean goose (Chloephaga melanoptera, Anatidae): the mutation Leu----Ser in position 55 of the beta-chains. *Biol. Chem. Hoppe-Seyler.*, 368: 1559-1569.

Ho, C. and Russu, I.M., 1987. How much do we know about the Bohr effect of hemoglobin? *Biochemistry*, 26: 6229-6305.

Hourdez, S. and Weber, R.E., 2005. Molecular and functional adaptations in deep-sea hemoglobins. *J. Inorg. Biochem.*, 99: 130-141.

Huber, K., Braunitzer, G., Schneeganss, D., Kosters, J. and Grimm, F., 1988. The primary structure of the hemoglobin of the Cormorant (Phalacrocorax carbo, Pelecaniformes). *Biol. Chem. Hoppe-Seyler*, 369: 1251-1258.

Kilmartin, J.V., Imai, K., Jones, R.T., Faruqui, A.R., Fogg, J. and Baldwin, J.M., 1978. Role of Bohr group salt bridges in cooperativity in hemoglobin. *Biochim. Biophys. Acta.*, 534: 15-25.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23: 2947-2948.

Laskowski, R.A., MacArthur, M.W., Moss, D.S. and Thornton, J.M., 1993. PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.*, 26: 283-291.

Liu, X.Z., Li, S.L., Jing, H., Liang, Y.H., Hua, Z.Q. and Lu, G.Y., 2001. Avian haemoglobins and structural basis of high affinity for oxygen: structure of bar-headed goose aquomet haemoglobin. *Acta. Crystallogr. D. Biol. Crystallogr.*, 57: 775-783.

Lukin, J.A. and Ho, C., 2004. The structure--function relationship of hemoglobin in solution at atomic resolution. *Chem. Rev.*, 104: 1219-1230.

Lutfullah, G., Ali, S.A. and Abbasi, A., 2005. Molecular mechanism of high altitude respiration: primary structure of a minor hemoglobin component from Tufted duck (Aythya fuligula, Anseriformes). *Biochem. Biophys. Res. Commun.*, 326: 123-130.

Lutfullah, G., Khalil, H.S., Amin, F. and Azhar, N., 2008. Low oxygen affinity in reptilian hemoglobin D: prediction of residue interactions in Geochelone carbonaria HbD by homology modeling. *Protein J.*, 27: 141-150.

Oberthur, W., Braunitzer, G., Grimm, F. and Kosters, J., 1983. [Hemoglobin of the golden eagle (Aquila chrysaetos, Accipitriformes): amino acid sequence of the alpha A- and beta chains of the principal component]. *Hoppe-Seyler's Zeitschrift fur. physiologische Chemie.*, 364: 851-858.

Oberthur, W., Braunitzer, G. and Wurdinger, I., 1982. [Hemoglobins, XLVII. Hemoglobins of the bar-headed goose (Anser indicus): primary structure and physiology of respiration, systematic and evolution]. *Hoppe-Seylers. Z. Physiol. Chem.*, 363: 581-590.

Perutz, M.F., 1970. Stereochemistry of cooperative effects in haemoglobin. *Nature*, 228: 726-739.

Perutz, M.F., 1983. Species adaptation in a protein molecule. *Mol. Biol. Evol.*, 1: 1-28.

Perutz, M.F., 1989. Mechanisms of cooperativity and allosteric regulation in proteins. *Q. Rev. Biophys.*, 22: 139-237.

Perutz, M.F., 1990. Mechanisms regulating the reactions of human hemoglobin with oxygen and carbon monoxide. *Annu. Rev. Physiol.*, 52: 1-25.

Perutz, M.F., Muirhead, H., Mazzarella, L., Crowther, R.A., Greer, J. and Kilmartin, J.V., 1969. Identification of residues responsible for the alkaline Bohr effect in haemoglobin. *Nature*, 222: 1240-1243.

Perutz, M.F., Shih, D.T. and Williamson, D., 1994. The chloride effect in human haemoglobin. A new kind of allosteric mechanism. *J. Mol. Biol.*, 239: 555-560.

Riggs, A., 1964. The Relation between Structure and Function in Hemoglobins. *Can. J. Biochem. Physiol.*, 42: 763-775.

Riggs, A.F., 1988. The Bohr effect. *Annu. Rev. Physiol.*, 50: 181-204.

Sali, A. and Blundell, T.L., 1993. Comparative protein modelling by satisfaction of spatial restraints. *J. Mol. Biol.*, 234: 779-815.

Sippl, M.J., 1993. Recognition of errors in three-dimensional structures of proteins. *Proteins*, 17: 355-362.

Stepuro, T.L. and Zinchuk, V.V., 2006. Nitric oxide effect on the hemoglobin-oxygen affinity. *J. Physiol. Pharmacol.*, 57: 29-38.

Wallace, A.C., Laskowski, R.A. and Thornton, J.M., 1995. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng.*, 8: 127-134.

Weber, R.E., Voelter, W., Fago, A., Echner, H., Campanella, E. and Low, P.S., 2004. Modulation of red cell glycolysis: interactions between vertebrate hemoglobins and cytoplasmic domains of band 3 red cell membrane proteins.Am. J. Physio. Regul.Integ. Comp. phys., 287: R454-464.

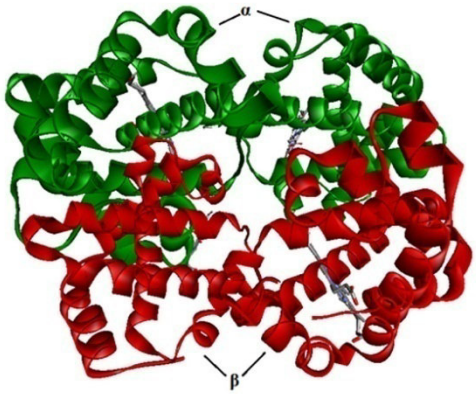

Figure 1: Schematic representation of the predicted homology model of *Aquila chrysaetos*'sHbA. Hem is represented in ball and stick representation whereas globin chains are shown as flat ribbons (Alpha chains: green and Beta chains: yellow)

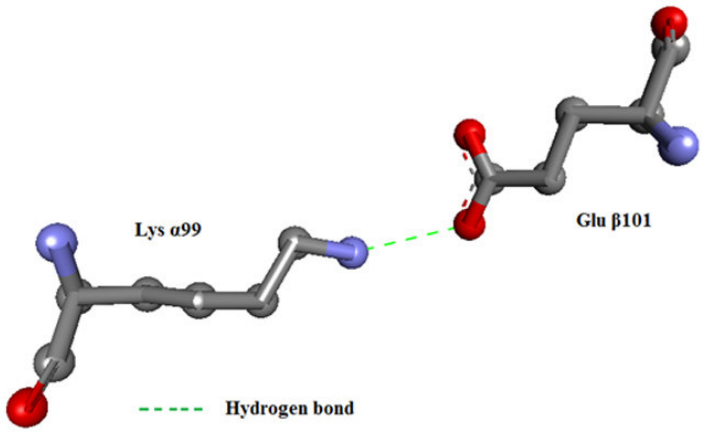

Figure 2: The hydrogen bond between α99Lys and β101Glu. The residues have been represented with ball and stick model and the hydrogen bond is shown by dotted line.

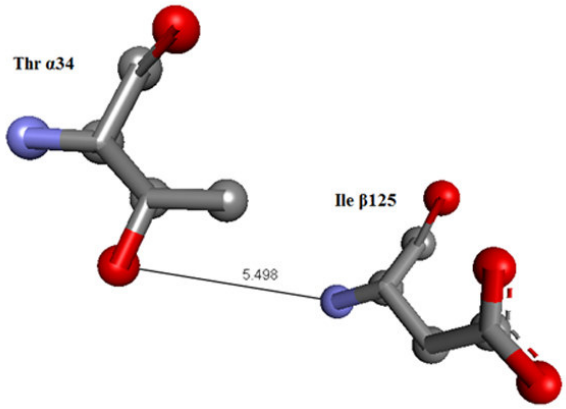

Figure 3: The distance between α34Thr and β125Ile. The residues have been represented with ball and stick model and the distance is shown with a line.

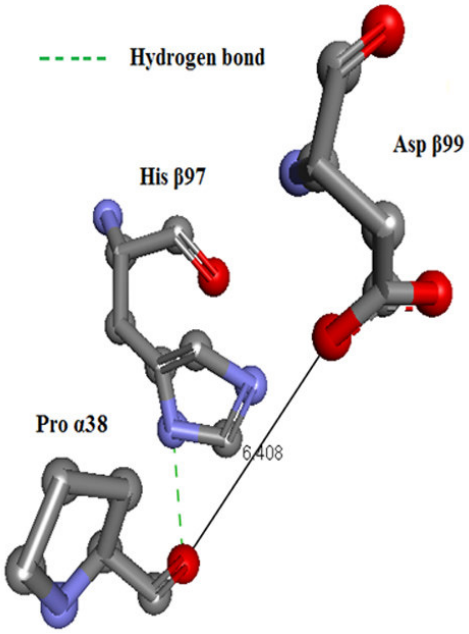

Figure 4: The distance between αPro38, β1Asp99 is shown by a line and the hydrogen bond between α1Pro38, β1His97 is represented by dotted line.

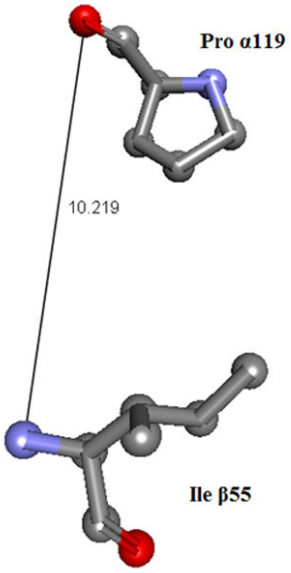

Figure 5: The representation of the distance between αPro119 and β1Ile55.

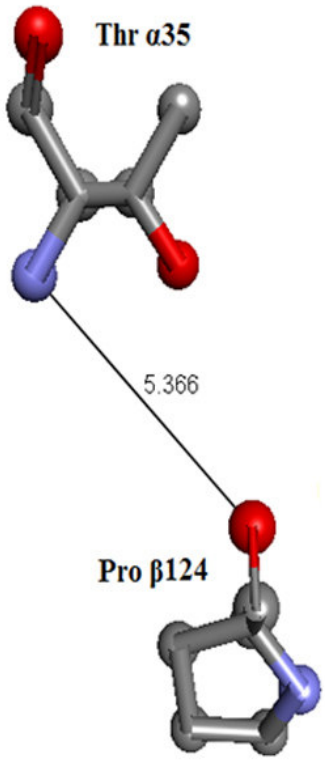

Figure 6: The distance between α35Thr and β124Pro. The residues have been represented with ball and stick model and the distance is shown with a line.

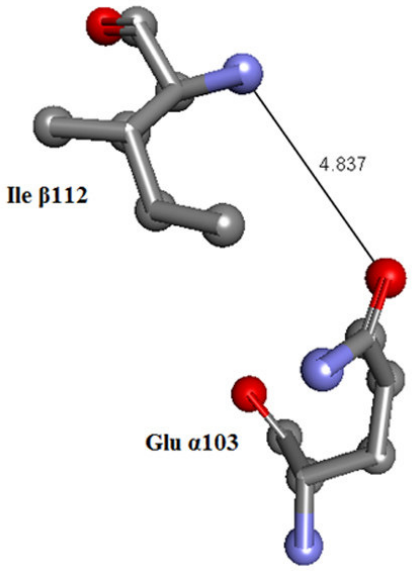

Figure 7: The residues α103Glu and β112Ile have been represented with ball and stick model and the distances have been shown with lines.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Recent conferences: http://www.iiste.org/conference/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar