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Interaction Between Low Molecular Weight Chitosan and Some Types of Drugs and Insulin

KHALDOUN AL-SOU'OD

Department of Chemistry, Faculty of Science, Al albayt University, P.O. Box 130040, Mafraq 25113, Jordan

Corresponding author: E-mail: khaldoun@aabu.edu.jo

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The complexation between ibuprofen, declofenac and insulin with the low molecular weight chitosan was studied using molecular modeling method. Molecular mechanics calculations were used to have insight into interaction stoichiometry between chitosan and ibuprofen. The results of molecular mechanics showed that the complexation of ibuprofen with low molecular weight chitosan involves ionic interaction between the ammonium group of low molecular weight chitosan and the carboxylate anion of ibuprofen. Chitosan was built in two different forms. The long one practically can be obtained from diluted solutions while the short one are from concentrated solutions. The modeling show that the chitosan-ibuprofen complex is best prepared from diluted solutions of the chitosan polymer. Human insulin is also build using a hyperchem software. It is a rather small protein, with a molecular weight of about 6000 Daltons. It is composed of two chains (A and B) held together by disulphide bonds. The interaction between human insulin and low molecular weight chitosan was studied using molecular mechanics calculations.

Key Words: Chitosan, Ibuprofen, Declofenac, Insulin, Molecular mechanics.

INTRODUCTION

Chitosan (CS) is a polysaccharide, similar in structure to cellulose. Both are made by linear β-(1→4)-linked monosaccharides (Fig. 1). However, an important difference to cellulose is that chitosan is composed of 2-amino-2-deoxy-β-D-glucan combined with glycosidic linkages[1,2]. The primary amine groups render special properties that make chitosan useful in pharmaceutical applications. The chitosan macromolecule has unique properties as a consequence of the presence of both amino and hydroxyl groups in their structure (Fig. 1). The ionic interactions of chitosan and drugs or proteins *via* the amino group of chitosan and the carboxyl group of a drug or a protein has been reported to be a key factor of particle formation. Based on several publications, the binding or interaction of oppositely charged molecules of chitosan was demonstrated by IR spectra, X-ray photoelectron spectroscopy and viscosimetry. To our best of knowledge, there is no theoretical effort dealing with this issue.

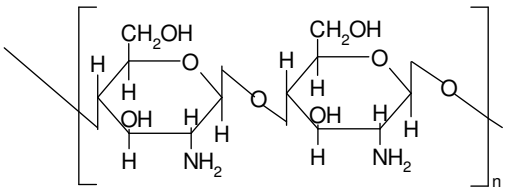


Fig. 1. Chemical structure of chitosan

The interaction between the amino group of chitosan and carboxyl group of indomethacin was mentioned because it was the reason for complexation[3,4]. Especially, the interaction of chitosans and peptides or proteins is often reported as showing a carrier and stabilizer effect[3-7].

Alonso and co-workers[7] prepared insulin loaded chitosan nanoparticles base on ionic interaction between both molecules. The loading capacity was up to 55 %. These chitosan nanoparticles released insulin in active form and enhanced nasal absorption in rabbits.

The carrier and stabilizer effect is mostly explained by an electrostatical binding of the loaded drug molecule to the cationic chitosan polymer. Due to the fact that polymers with high molecular weights (70-150 kD) showed an immunogenic side effect after parenteral application the commercially produced chitosans cannot be used for parenteral formulations[8,9].

In order to use the advantageous properties of chitosans as excipients for proteins in the parenteral pathway, short chain chitosans were produced from fungus by a biotechnological method[10,11].

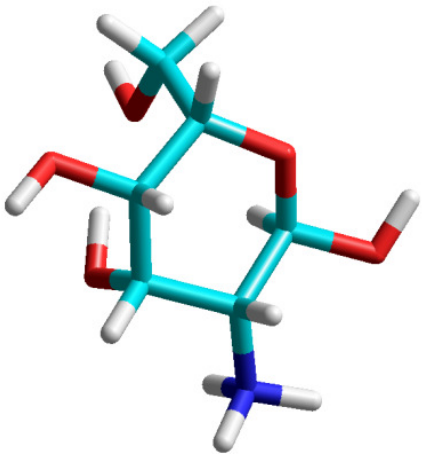
It could be shown that the properties of the short chain chitosans (MW below 5 kD, deacetylation degree above 95 %) changed completely *e.g.*, they were soluble even in alkaline solutions. This was a disappointing result of that reseach, these chitosans showed neither a binding effect to negatively charged neither molecules nor a stabilizing effect to peptides[12].

In this context, electrostatic interactions between opposite charge of drugs (insulin and benzoic acid) and chitosan was studied by ^{1}H NMR, FTIR and isothermal titration calorimetry (ITC). It was found that, no ionic interaction between the carboxyl group of benzoic acid and the amino group of high molecular weight chitosan could be detected. However, we are interested in low molecular weight chitosan and based on our preliminary studies which show that there is a strong ionic interaction between these drugs and proteins with chitosan, a theoretical investigation is needed to tackle this problem.

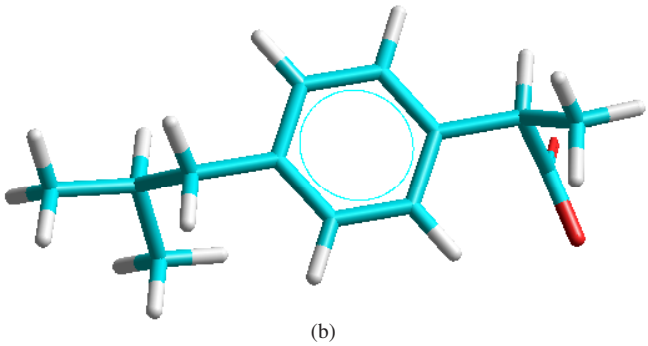
The insulin molecule has served as a model for multitudes of studies on the fundamental structure and properties of proteins. It was the first protein to have its amino acid sequence sequenced, in 1955 by Fred Sanger[13], earning him a Nobel Prize in 1958. It was also the first peptide hormone, circulating in minute amounts, to be measured by radioimmunoassay[14], earning Yalow a Novel Prize in 1977. The pathway behind the biosynthesis of insulin in pancreatic β-cells, specifically as a proinsulin precursor, was determined by Don Steiner in 1967[15]. The three-dimensional structure of insulin was ultimately solved by Dorothy Crowfoot Hodgkin and colleagues in 1969, using X-ray crystallographic methods[16]. It was also the first protein to be synthesized in microorganisms by recombinant DNA technology in the late 1970s. This supported the design of insulin analogues in order to optimize the molecule"s pharmacodynamic profile for therapeutic purposes. As a result, recombinant insulin has replaced purified insulin for therapeutic purposes. Here, we briefly discuss the structural characteristics and structure-function relationships of insulin.

EXPERIMENTAL

Computations in vacuum were performed with Hyperchem® (release 8.05), using Amber Force field implemented in Hyperchem. Partial atomic charges were obtained by performing AM1 semi-empirical calculations. Energy minimizations were obtained using the conjugate gradient algorithm (0.01 kcal/mol Å) gradient. D-glucoseamine (chitosan monomer), ibuprofen salt, declofenac salt (Fig. 2) and insulin were also built up from natural bond angles, as defined in the Hyperchem software. The structures were then minimized with the Amber force field and further optimized at the HF-*ab initio* level with the 3-21G* basis set (Fig. 2).



(a)



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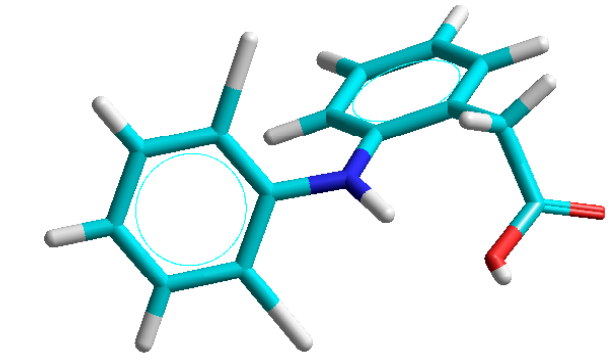


(c)

Fig. 2. Structure of (a) D-glucoseamine (b) ibuprofen salt and (c) declofenac salt

RESULTS AND DISCUSSION

Polymer build up: The resulting chitosan monomer (Fig. 2a) is defined in the HyperChem workspace. A set of named selections has been made to define the HEAD (name is "a") and TAIL (name is "b") for building the polymer (Fig. 3). An additional set of named selections is made to define the atoms involved in the torsion angle that will be used to put the monomers together. The HEAD is assumed to be connected to x (name is "x") which in turn is connected to x' (name is "x'"). The TAIL is assumed to be connected to y (name is "y") which in turn is connected to y' (name is "y'"). The torsional angle connecting monomers are then the x'-x-y-y' angle, with retention of the internal structure of the monomer. The torsion angle used to put the monomers together may be specified to have a random value rather than the constant value described above.

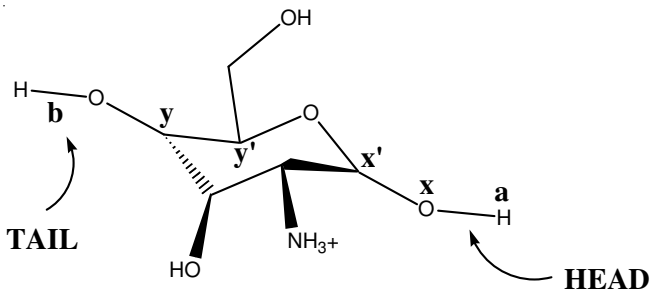


Fig. 3. Selected atoms in chitosan monomer

Low molecular weight chitosan polymers is build up by connecting 108 chitosan monomer (D-glucoseamine) through β-(1-4) site. The molecular weight of the resulting polymer is about 17 kDa.

The chitosan polymer was constructed with a suitable length from the monomer unit (Fig. 2a). Two different structures of chitosan polymer with the same number of units (108 units) were built. The first (Chit I) is longer (its length *ca.* 300 Å) and the other is shorter (Chit II) (its length *ca.* 111 Å) as shown in Figs. 4 and 5, respectively.

It's worth noting that, the ammonium groups (blue tubes) in CHIT II located inside the channel (Fig. 4), in contrast to CHIT I, which is located outside the channel.

Drug-chitosan complexes formation: The complexation between ibuprofen and low molecular weight chitosan was studied using ^{1}H NMR technique[17]. The results showed that complexation of ibuprofen with low molecular weight chitosan involve ionic interaction between the ammonium group of low molecular weight chitosan and the carboxylate anion of ibuprofen. The ratio of the integral area of H_2-atoms in chitosan to the integral area of, Ar-CH_2, in ibuprofen, $R_{ibu/chit}$, in low concentration of chitosan was found to increase from 0.29 up to 8.33, as a result of changing the weight per cent of ibuprofen from 9.1 to 60 %[17].

In present model, ibuprofen molecule (salt form) was placed manually close to (in front) ammonium groups in chitosan polymer and allowed to optimize leading to Ibup…Chitosan complex, which is further optimized by using Amber force field (0.1 gradient).

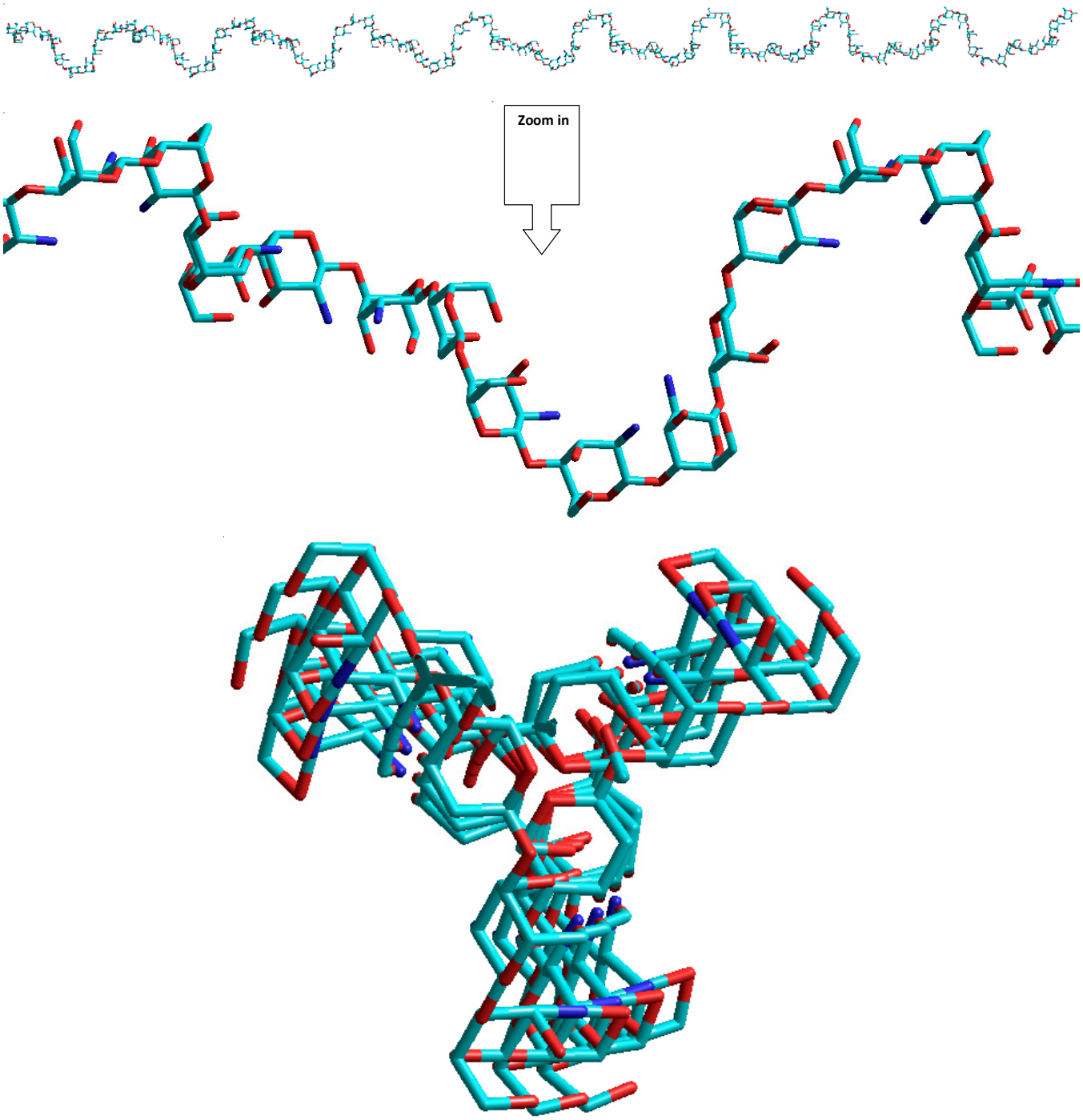


Fig. 4. Side and top view of longer chitosan (CHIT I)

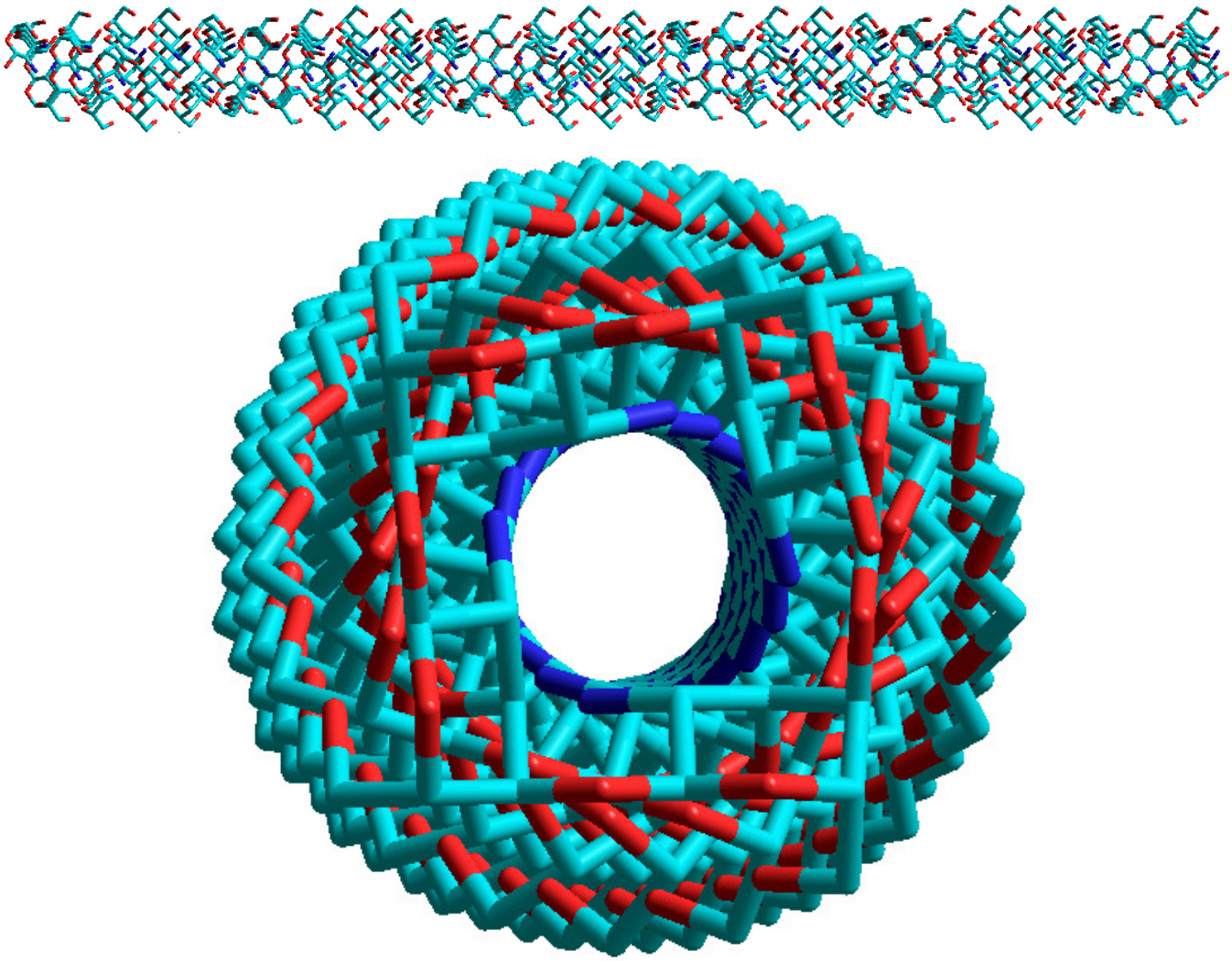
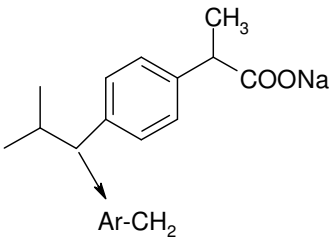


Fig. 5. Side and top view of shorter chitosan (CHIT II)

It is expected that the chitosan polymer (at low concentration) is more extended as compared to the one at high concentration and therefore it is more available to interact with Ibuprofen salt or with any other molecule. This was examined by taking two polymers (both have the same number of building units but different length; Chit I and Chit II). The results of molecular mechanics calculation show that the Chit I can accommodate ibuprofen up to 50% (2 glucoseamine with 1 ibuprofen molecule) (Fig. 4), while Chit II (shorter) interacts to a much less extent with drug which might be attributed to steric hindrance and the difficulty access to interaction between carboxylate groups of ibuprofen molecules and ammonium groups in glucoseamine, since as seen in Fig. 4 these groups located inside channels (Fig. 5). In many practical studies H-2 proton of chitosan was chosen because it is peak doesn't interfered with other peaks in the spectrum. It is noteworthy that the resulting CHIT I complex has the H-2 (Fig. 3) atoms located inside the channel in contrast to Ar-CH_2 of ibuprofen (located at the periphery of the polymer. Therefore, it is expected that H-2 atoms in low molecular weight chitosan (inside channel) are difficult to detect by ^{1}H NMR, therefore, $R_{ibu/chit}$ at low concentrations have abnormal values. In our model, the maximum value of $R_{ibu/chit}$ is 0.5, but $R_{ibu/chit}$ value in practical study reaches a very high value (8.33).



The same procedure was applied to declofenac salt, similar results were obtained, and we can say that, low molecular weight chitosan has same orientation, in spite of the type of drug that can interact with it.

Insulin build up: Insulin's empirical formula is: $C_{254}H_{377}N_{65}O_{75}S_6$ and it has a molecular weight of 5734 (Wikipedia, 2005). Insulin is built from 51 amino acids and is one of the smallest proteins in the body. It is structured with two polypeptide chains linked by two disulphide bonds, connecting the amino acid cysteine to cysteine. There is also a third disulphide bond that connects these same amino acids

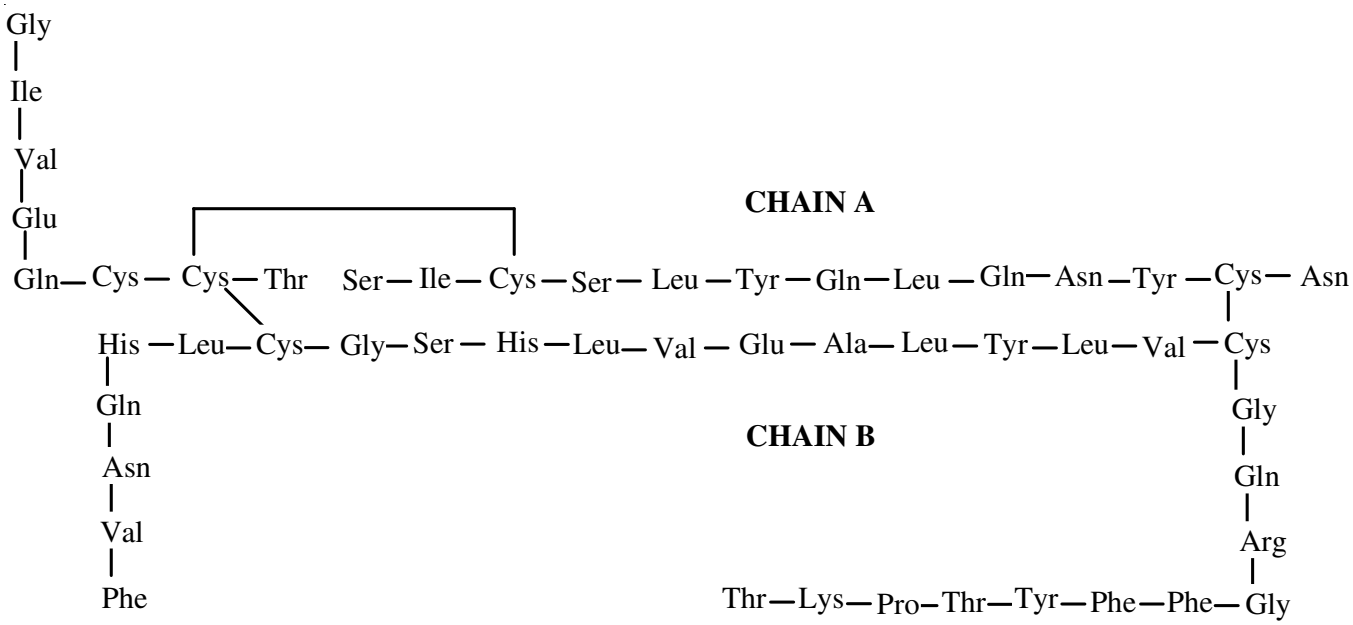


Fig. 6. Human insulin: The amino acid diagram of human insulin, showing the A and B chains and 3 disulphide bonds

within chain A. chain A consists of 21 amino acids and chain B contains 30 amino acids (Fig. 6).

Secondary structure of insulin (simple 3D form): Some of the joined amino acid residues coil to form short sections of alpha helix, due to hydrogen bonds between >N-H and >C=O groups (projecting from peptide bonds of amino acids 3 or 4 residues further along), which stabilizes the structure. Other amino acids give a turn to the amino acid chains so the overall structure is fairly compact.

The A chain, which is fairly compact, contains 2 sections of alpha helix (A2 Ile - A8 Thr and A13 Leu - A19 Tyr). In between these sections is a fairly flat ribbon which enables these helices to lie alongside one another and also brings the side chains of A2 Ile and A19 Tyr into van der Waals contact. The B chain appears to wrap around the A chain. It consists of a larger section of alpha-helix (B9 Ser-B19Cys) and the smaller glycine residues at 20 and 23 allow it to fold into V shape. This brings the C terminal residues B24 Phe and B26 Tyr into van der Waals contact with B15 Leu and B11 Leu of the alpha-helix (Fig. 7). The resulting structure of insulin was optimized using amber force field (0.10 kcal/mol Å gradient).

To explore the nature and strength of the interactions between chitosan and insulin, insulin was allowed to approach the chitosan polymer in different orientations. To obtain the energy of the most stable complex between insulin and low molecular weight chitosan, the binding energy for each complex was calculated. The binding energy was calculated using the following equation:

$$E_{binding} = E_{complex} - (E_{insulin} + E_{chitosan})$$

The binding energy of the most stable complex (Fig. 8) was found to be -38.0 kcal/mol.

Conclusion

Molecular modeling is a very useful method to give us an accurate idea about the nature of interactions that may be resulted between drugs and low molecular weight chitosan.

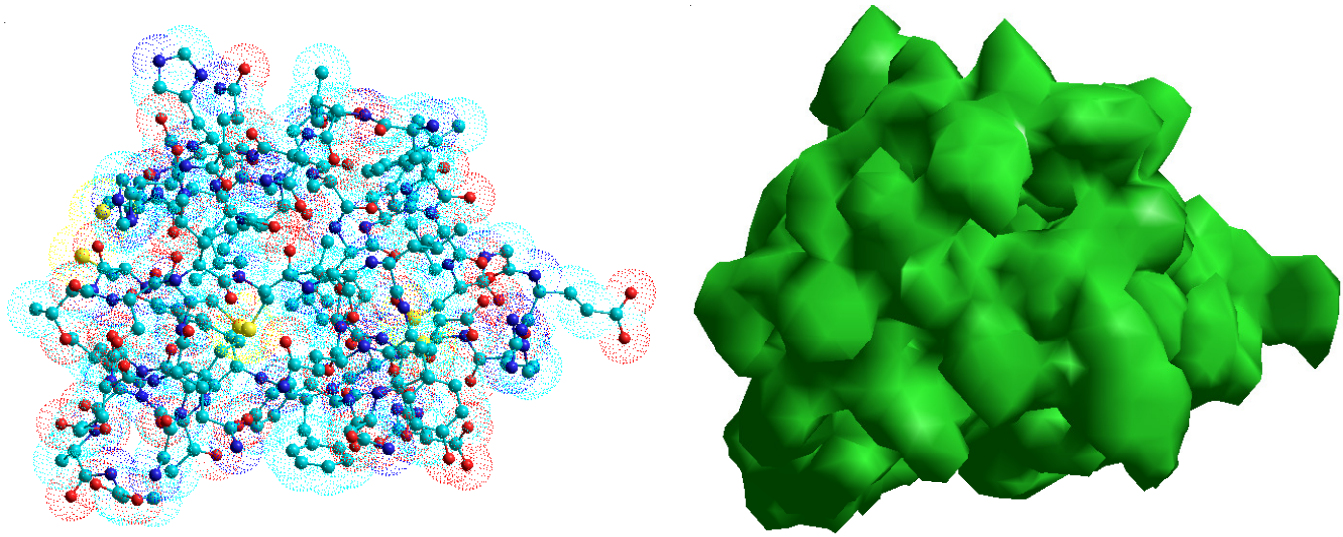


Fig. 7. Structure of human insulin

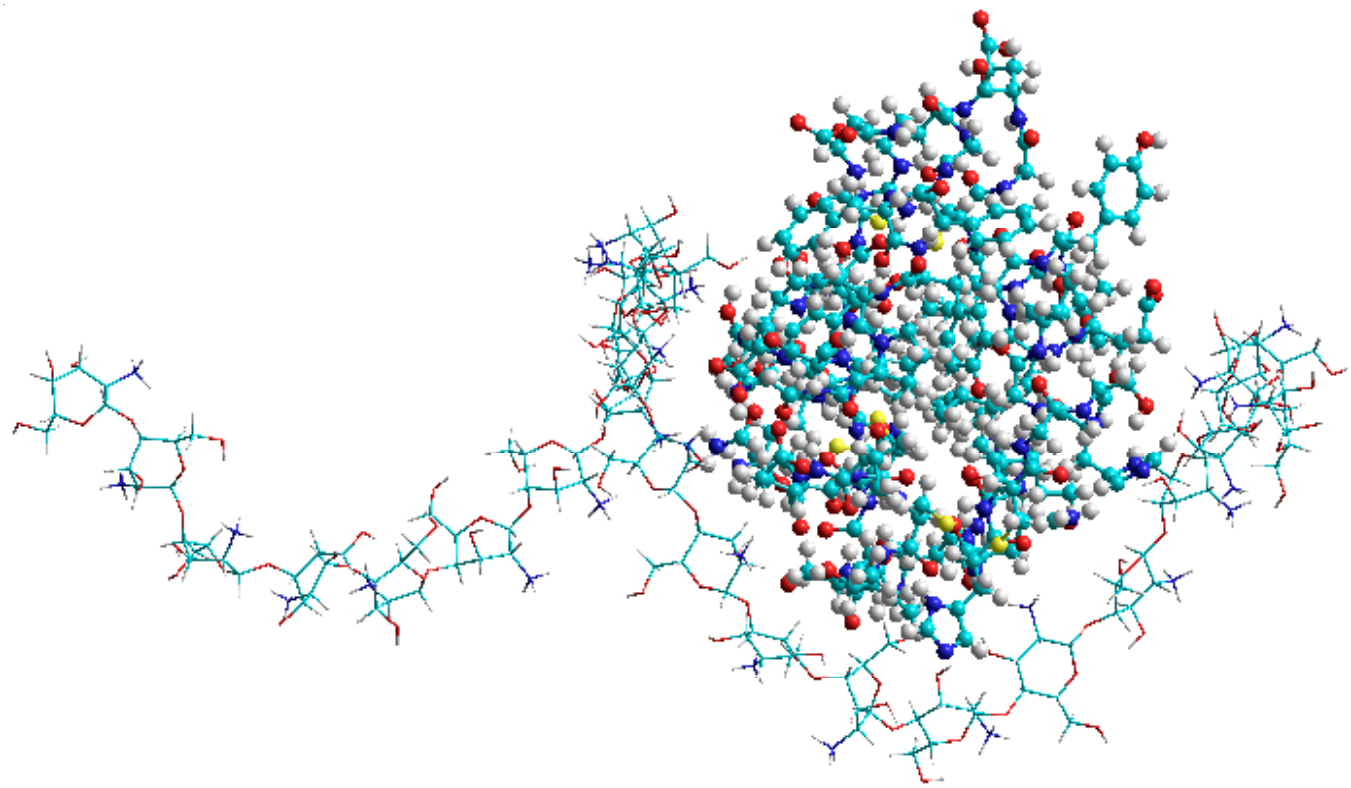


Fig. 8. Best conformation of the chitosan/insulin complex

The complexation of low molecular weight chitosan with ibuprofen or declofenac salts involves ionic interaction between the ammonium groups of low molecular weight chitosan and the carboxylate anion of the drug. It is more efficient to prepare drug-low molecular weight chitosan complexes using diluted solutions of the polymer. The latter observation leads to the assumption that low molecular weight chitosan assumes an accessible extended conformation in dilute solutions or that dilute solutions of low molecular weight chitosan might be less aggregated, both of which will lead to efficient drug-low molecular weight chitosan interactions. These results were confirmed using MM calculations by building low molecular weight chitosan with the same molecular weight but different lengths (Chit 1 and Chit. 2). We can use a hyperchem software to build human insulin and to study the nature of interactions between it and low molecular weight chitosan.

Recommendations: This project needs more study and more effort to understand the nature of interactions between low molecular weight chitosan and the human insulin. These theoretical calculations will help the researchers to prepare a new formula of insulin to be enabling the patients to take it by mouth instead of the syringe. The study of such interactions needs to build a full workstation.

Also, we hope to extend our research area by performing molecular dynamic (MD) simulations to understand the role of other human proteins, RNA and DNA in human body. The simulations will give us a full view about the structures of the complexes between proteins and RNA and with DNA their stabilities.

REFERENCES

1. R. Hejazi and M. Amiji, *J. Control. Rel.*, **89**, 151 (2003).
2. J.W. Nah and M.K. Jang, *J. Polym. Sci. A Polym. Chem.*, **40**, 3796 (2002).
3. P. Calvo, C. Remunan-Lopez, J.L. Vila-Jato and M.J. Alonso, *Pharm. Res*., **14**, 1431 (1997).
4. K. Gong, J.A. Darr and I.U. Rehman, *Int. J. Pharm*., **315**, 93 (2006).
5. A. Vila, A. Sanchez, K. Janes, I. Behrens, T. Kissel, J.L. Vila Jato and M.J. Alonso, *Eur. J. Pharm. Biopharm*., **57**, 123 (2004).
6. R.F. Urrusuno, P. Calvo, C.R. Lopez, J.L. Vila Jato and M.J. Alonso, *Pharm. Res*., **16**, 1576 (1999).
7. P. Calvo, C. Remunan-Ropez, J.L. Vila-Jato and M.J. Alonso, *J. Appl. Polym. Sci*., **63**, 125 (1997).
8. Y. Tanaka, S. Tanioka, M. Tanaka, T. Tanigawa, Y. Kitamura, S. Minami, Y. Okamoto, M. Miyashita and M. Nanno, *Biomaterials*, **18**, 591 (1997).
9. S.C.W. Richardson, H.V.J. Kolbe and R. Duncan, *Int. J. Pharm*., **178**, 231 (1999).
10. A. Niederhofer and B.W. Mueller, *Eur. J. Pharm. Sci*., **57**, 101 (2004).
11. P. Pochanavanich and W. Suntornsuk, *Lett. Appl. Microbiol*., **35**, 17 (2002).
12. A. Niederhofer, Ph.D. Thesis, Biotechnologische Herstellung von Niedermolekularem Chitosan aus Mucor Spezies als Pharmazeutischer Hilfsstoff, Christain-Albrechts-University, Kiel, Germany (2003).
13. F. Sanger, *Ann. Rev. Biochem.*, **57**, 1 (1988).
14. S.A. Berson and R.S. Yallow, *Nauchni Tr Vissh Med. Inst. Sofiia*, **191**, 1392 (1961).
15. D.F. Steiner and D.E. James, Cellular and Molecular Biology of the Beta Cell, Diabetologia, 35, Suppl 2, pp. S41-8 (1992).
16. M.J. Adams, T. Blundell, E. Dodson, G. Dodson, M. Vijayan, E. Baker, M. Harding, B. Rimmer and S. Sheat, *Nature*, **224**, 491 (1969).
17. A.M. Qandil, A.A. Obaidat, M. A. Ali, B.M. Al-Taani, B.M. Tashtoush, N.D. Al-Jbour, M.M. Al-Remawi, K.A. Al-Sou'od and A.A. Badwan, *J. Sol. Chem.*, **38**, 695 (2009).