Insulin as a model to teach three-dimensional structure of proteins

O hormônio insulina como um modelo para ensinar a estrutura tridimensional das proteínas

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Abstract

Proteins are the most ubiquitous macromolecules found in the living cells and have several physiological functions. Therefore, it is fundamental to build a solid knowledge about the proteins three-dimensional structure to better understand the living state. The hierarchical structure of proteins is usually studied in the undergraduate discipline of Biochemistry. Here we described pedagogical interventions designed to increase the pre-service teacher chemistry students’ knowledge about protein structure. The activities were made using alternative and cheap materials to encourage the application of these simple methodologies by the future teachers in the secondary school. From the primary structure of insulin chains, students had to construct a three-dimensional structure of insulin. After the activities, the students highlighted an improvement of their previous knowledge about proteins structure. The construction of a three-dimensional model together with other activities seems to be an efficient way to promote the learning about the structure of proteins to undergraduate students. The methodology used was inexpensive and simple and it can be used both in the university and in the high-school.

Keywords: Biochemistry education; undergraduate students; protein models.

Resumo

As proteínas são macromoléculas amplamente encontradas nas células e possuem inúmeras funções fisiológicas. Consequentemente, é fundamental construir um conhecimento sólido sobre estrutura tridimensional das proteínas. As estruturas proteicas são geralmente estudadas durante a graduação na disciplina de Bioquímica. Neste trabalho descrevemos intervenções pedagógicas planejadas para aumentar o conhecimento de estudantes de Química licenciatura sobre a estrutura de proteínas. As atividades foram realizadas utilizando materiais baratos para encorajar a sua implementação no ensino médio pelos futuros professores. A partir da estrutura primária da insulina, os estudantes construíram a estrutura tridimensional dessa proteína. Após as atividades, os estudantes destacaram uma melhora nos seus conhecimentos prévios sobre a estrutura das proteínas. A construção de um modelo tridimensional juntamente com outras atividades parece ser uma maneira eficiente de promover o aprendizado sobre a estrutura de proteínas aos estudantes de graduação. A metodologia utilizada foi simples e de baixo custo e pode ser utilizada tanto no nível universitário como no nível do ensino médio.

Palavras-chave: Educação em Bioquímica; estudantes de graduação; modelos de proteínas.
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| Record activity performed |
|---------------------------|
| **Title** | Insulin as a model to teach three-dimensional structure of proteins |
| **Target audience** | Pre-service chemistry Teacher Undergraduate students |
| **Related disciplines** | chemistry, Biochemistry; Physiology |
| **Educational objectives** | To improve the understanding of undergraduate students about protein structures using a concrete and virtual 3D model |
| **Justification of use** | Concrete 3D models building can improve the perception of the students about the complex 3D protein structure than textbooks. |
| **Worked contents** | Structure of proteins |
| **Estimated duration** | 8-12 h |
| **Materials used** | 10 mm styrofoam balls; 1 mm wire; 4 mm wire; styrofoam glue; Colorful pens (black, blue, red and yellow); Pliers. |
1 Introduction

The Biochemistry course is normally obligatory to undergraduate students who focus in biological, chemistry and health sciences [1, 2]. The discipline emphasizes the chemistry of cellular components and how simple building blocks are synthesized and assembled into complex macromolecules that will form the living cells [3]. To understand the cellular metabolism and structure is fundamental to know its macromolecular components, i.e., lipids, carbohydrates, nucleic acids, and proteins.

Proteins perform a variety of biochemical functions, for instance, transport, catalysis, structural, hormonal, among others. Proteins are built from the monomers called amino acids. The amino acids have a basic structural feature which includes the α-Carbon bounded to a Hydrogen, a carboxyl group, an amine group and to a lateral chain (R). The chemical nature of the R group will confer specific physicochemical properties to the amino acid as (1) Nonpolar, aliphatic; (2) Aromatic; (3) Polar, uncharged; (4) Positively charged; (5) Negatively charged. In proteins, the amino acids form polymers by a double bond-character ligation, namely, the peptide bond (Figure 1) [3, 4].

![general structure](image1.png)

**Figure 1.** Representation of the general structure of an amino acid and of the peptide bond. The distance among the atoms (Angstroms) was based in Pauling et al. [5].

In view of the ubiquitous role of proteins in cell physiology, it is of fundamental importance that students build a solid knowledge and understanding of the proteins and their spatial conformation. The study of the protein structures, namely (1) primary, (2) secondary, (3) tertiary and (4) quaternary, is extensive and involves a lot of reading and listening hours to the teacher what can become exhaustive to both students and instructors. Moreover, it is difficult to effectively understand the three-dimensional structure...
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of macromolecules by attending only to theoretical classes. Consequently, it is important to adapt the teaching strategies to be more effective [6]. Spadaro [7] observed an increase in students’ attendance and grades after the inclusion of practical activities in his biochemical theoretical classes. In this context, several papers have been published with a variety of pedagogical alternatives to improve Biochemistry learning, for instance, games, conceptual mapping, videos/movies, molecular models, among others [8-16]. The use of softwares to build and edit 3D model of proteins can also be instrumental to teach protein structure. Some examples of valuable tools are Jmol [17], Pymol [18], Rasmol [19], Kinemage [20], Avogadro [21], Discovery Studio [22], and others.

Similarly, Herman et al. [23] highlighted the importance of physical models for teaching protein structure, because they facilitate the better visualization of the abstract information taught to students in theoretical classes. There are some studies in the literature proposing models to explain the protein structure by building concrete models at different levels of organization (i.e., primary, secondary and tertiary structure) [24-26]. However, the majority of the models are based on a fictitious protein and this may be less motivating than the study of a familiar protein in cell physiology.

Although virtual 3D structure can give good ideas about the spatial distribution of atoms in macromolecules [17-22], the use of "concrete models" are also important pedagogical tools [23, 27-28] and, historically, their use was instrumental in unraveling the secondary structure of proteins (for a brief historical account see the picture of Pauling and Corey with their models of alfa-helix at [29]). Thus, in our opinion, the combination of concrete models with 3D virtual software should be more satisfactory to teach protein structure than their use in isolation.

In this context, the study of the insulin can be instrumental. The hormone is popularly associated with diabetes and glucose blood control, though the insulin metabolic role is much more complex [30, 31]. Insulin is a small protein containing 51 amino acid residues, with a molecular weight of approximately 6000 Da [31]. The deciphering of its primary structure was a hallmark in the history of biochemical sciences [32]. The insulin structure has two chains, the A (21 amino acids) and B (30 amino acids) chains, which are bound to each other by 3 disulfide bridges [30] (Figure 2). In humans, the chains are encoded by the gene located on the short arm of chromosome 11 and are synthesized as the pre-proinsulin, which is latterly processed to the active hormone [33].
Thus, to stimulate the students’ motivation and to facilitate the comprehension of the proteins spatial conformation from its primary to the tertiary structures we proposed and implemented a set of activities to construct a simple protein model, most specifically the insulin protein, to students of chemistry undergraduate course.

2 Materials and Methods

The study was applied to 46 chemistry undergraduate students who are enrolled in the Biochemistry courses from the Federal University of Santa Maria. However, the majority of results presented were from the application made with students enrolled in the discipline at the second semester of 2016, because the models obtained were more similar to the structure present in the literature [3, 36-39]. The activities were applied during biochemical theoretical classes to 17 chemistry students (in three different semesters) and biochemical practical classes to 29 chemistry students (in two different semesters). Here the entire activity was divided into four classes, with 2 hours each (Figure 3), as described below:
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First Class: The students received a printed copy of the molecular formulae of the 20 amino acids that are the building blocks of the proteins (Figure 4). They were asked to individually create a chemical classification to the 20 amino acids and after finishing the activity, all students exposed their classification criteria and discussed to create a common and logical chemical amino acids classification. Although not included here, we commented about the existence of the 21st amino acid (i.e., selenocysteine), which is analogs to serine and cysteine and found only a few number of selenoproteins in animals and others, but not all, living organisms. For instance, fungi and higher plants do not have selenoproteins [40].

![Molecular formulae of the 20 amino acids.](image)

After the development of a concise and common-sense classification of the amino acids, the printed human insulin primary structure (Figure 5) and 4 questions (Table 1)
were given to the students. It was requested then that the students identified and classified the amino acids of insulin using the classification criteria created by the group. The answers to the 4 questions were discussed near the end of the activity.

Figure 5. Primary structure of the active human insulin.

Table 1. Questions presented to the students together with the primary structure of the human insulin.

| Questions                                                                 |
|---------------------------------------------------------------------------|
| How many amino acids residues the active insulin has?                     |
| How many polypeptides are part of the active insulin?                     |
| The treatment of the insulin with dithiothreitol (DTT, a strong reducing agent) could alter the insulin tertiary conformation? If yes, will DTT alter the insulin activity? |

Second Class: The students were invited to construct a model of the insulin protein. For this, they were divided into two groups: one to build the A chain and the other the B chain.

The following material was given to the groups:
- 10 mm styrofoam balls;
- 1 mm wire;
- 4 mm wire;
- Styrofoam glue;
- Colorful pens (black, blue, red and yellow);
- Pliers.

After the material distribution, the structural and spatial aspects of the peptide bond was given to the students (see Figure 1). They are then asked to create a scale to construct their model in the wire using the Styrofoam balls as the atoms.

The student painted the styrofoam balls in accordance with the following description:
- White = Carbon atoms (approximately 250);
- Black = Hydrogen atoms (approximately 50);
- Blue = Nitrogen atoms (approximately 70);
- Red = Oxygen atoms (approximately 70);
- Yellow = Sulfur atoms (approximately 10).
The lateral chains of the insulin amino acids using styrofoam balls were constructed (Figure 6).

In the majority of classes described above, the students did not use styrofoam balls. The majority of them has used planar lateral groups drawn in a paper and covered with transparent adhesive tape to make the structure firm. Some students assembled the aromatic group of the phenylalanyl residue with wire covered with a plastic straw (Figure 7A). One group in 2015, made some spatial distribution of the lateral groups by using paper and match stick to represent the Hydrogen atoms (Figure 7B).
Another group made very simple lateral groups with adhesive tapes and classified the lateral groups only in general terms (Figure 8). However, it was not so easy to establish all the interaction clearly between the lateral groups in the secondary and tertiary structures; though it was possible to have a good idea about the tri-dimensional structure and the distribution of the lateral groups in the space. Here the results obtained with the styrofoam balls (though more time consuming to construct) were better than planar lateral groups made of paper.

Third Class: For the third class, the backbone of the protein was built. Based on the information about the sequence of the amino acids residues (primary structure), the students added the Carbon from the carbonyl group, the Carbon alpha and the Nitrogen from the amine group, corresponding to each residue, using a 4 mm wire. In this step, the peptide bond was created (Figure 9). After that, all the respective Oxygen (from the peptide bond) were added to the backbone structure. As exposed above, in the two previous classes, the atoms of the backbone of the peptides were made with permanent markers of different colors and the C=O and NH groups were made by hand using paper or adhesive tapes and glued to the wire. In the structure constructed here, the insertion of the atoms of the backbone was also time consuming, but the structure obtained gave a better indication on how the atoms are distributed in the backbone of a protein than in the previous semester.
Fourth Class: In the fourth class, the groups added the lateral chain of the residues and modulated the protein to create the secondary structure (alpha helix). The Hydrogen bonds and disulfide bridges were also built. Then the students endeavored and succeeded in joining the chain A and B with the disulfide bonds. In the other studies, the groups used either crepe tape (Figure 8) or thin wire (data not shown) to do the Hydrogen bond between the −NH- and −C=O in the peptide bonds apart 3,6 residues [3]. To help the student to build the protein, a 3D insulin structure (see Figure 2) was obtained from the Protein Data Bank (https://www.rcsb.org/pdb) [34] with the code 2HIU [35] and visualized in the software Discovery Studio Visualizer. In this stage, it was possible to visualize several conformations of the insulin molecule, determined by NMR (Nuclear Magnetic Resonance) [35], demonstrating to the student some basic aspects of the insulin dynamic in solution. Here, we asked the students to carefully observe the patterns of intermolecular interactions between the amino acids side chains. The students were also asked to see these interactions in the wire model.

Approximately two months after the end of the semester, an online questionnaire was sent to the students. The questionnaire had the questions presented in Table 2.
Table 2. Online questionnaire sent to the students.

| Questions                                                                 |
|---------------------------------------------------------------------------|
| 1- How much those classes contributed to your learning?                    |
| 2- Which are the positive points of those classes?                        |
| 3- Which are the negative points of those classes?                        |
| 4- Taking into consideration the relationship between the primary structure |
|    and the tertiary structure of proteins, how much those contributed      |
|    to your learning?                                                      |
| 5- In relation to the protein folding, how much those classes contributed  |
|    to your learning?                                                      |
| 6- How much those classes contributed to your comprehension about          |
|    intramolecular interaction in protein?                                 |

3 Results and Discussion

Before commenting the results obtained, we have to consider two points about the participants of the activities. First, the students who participated in this study were seniors of the chemistry undergraduate course, so we assumed that they have a good knowledge about chemical interactions. Second, the Biochemistry disciplines (theoretical and practical) where the activities were implemented was the third discipline dealing with Biochemistry. Consequently, we assume that students had a good comprehension of basic principles governing the chemistry of proteins.

The first activity was about amino acids and their chemical classification. In this activity, the students were required to propose their particular classification of amino acids based on their own knowledge of chemistry. Following this, individual classifications were discussed by the whole group and after that, a general classification was agreed upon. The main criteria of grouping were based on the polarity of the lateral chains of the amino acids and, therefore, amino acids were classified in polar and nonpolar. Here we emphasize that students did the classification using only their previous own chemical knowledge without any intervention of the instructors in the first moment. This task was easily performed by the students.

However, the discussion about the polarity of the lateral group of glycine, methionine, and tryptophan was stimulated by the instructors, because normally methionine and glycine were classified in different groups by students. Tryptophan was included to stimulate the reasoning about the influence of the size of the lateral groups in protein structure. As discussed above, this classification is of extreme importance for the study of protein structures, once the tertiary structure of proteins is built due to the intermolecular interactions of amino acids lateral chains [3]. Although the chemistry students have performed the task with relative easiness, we question whether this will occur in courses from the biomedical area.

The insulin 3D model construction started by asking the students to construct the
lateral chains of amino acids (see Figure 6). As all the atoms are represented by identical styrofoam balls, atoms of different chemical elements were represented by different colors. To facilitate the labeling of the atoms, Carbon atoms was agreed to be white, and Oxygen atoms red, Nitrogen blue and Sulfur yellow.

Only the Hydrogen atoms that participate in Hydrogen interactions in the peptide bond were represented in the structure as the black styrofoam balls. During the insulin model construction, students analyzed the primary structure of human insulin, creating a scale for the bonds distances between atoms of the amino acids. The distances were estimated based on the distances presented in Figure 1 and in the original paper of Pauling and Corey [4]. In this way, atoms (represented by the styrofoam balls) were distributed and glued to the wire (see Figure 9).

In all the applications of the proposed classes, the half the students built either the A or B chain. In last application, two groups of two students were formed, so each group was responsible for the construction of one of the two insulin chains. Then students tried to orientate the interactions between the chains, guided by the disulfide bridges (Figure 10). Similar activities were also applied in the previous semesters, but the complexity and quality of the insulin made in the second semester of 2016 were somewhat superior to the previous ones.

Figure 10. Finalized insulin model.
During the discussion of the questions presented in Table 1, more than 90% of students answered correctly the number of amino acids residues present in the active insulin. Besides, the ones that were wrong, after discussion, comprehended their misconceptions. In relation to the question number 2, 100% of students answered that the active insulin has two polypeptide chains. Finally, most of the students comprehended the effect of DTT on the insulin chains (reduction the S-S, disulfide bonds) and that insulin reduction by DTT will cause the loss of protein function.

It was clear for us, based on the responses obtained in online questionnaire (see Table 2), that the construction of the insulin model helped the students to improve their knowledge about the spatial distribution of the atoms and lateral groups in proteins. Before performing the activities, the students' knowledge was apparently mechanical and no detailed spatial thinking was evident. After the activities, more than 80% of the students indicated an improvement of their knowledge about the structure of the proteins. A good portion of the students had concerns about the time required to construct the models, but almost all them identified the activity as important to think about the structure of the proteins in concrete grounds. This result is in accordance with the finding of Herman et al. [17] that the physical models are very useful in introducing the basic concepts of protein structure to students. In addition, Jittivadhana et al. [41] observed that the use of physical models was better to understand 3D features of proteins than the textbooks illustrations or computer graphic representations.

At the end of the classes, 80% of the students reported that the methodology contributed to the learning of proteins structure, folding and intermolecular interactions among amino acids lateral chains. The students also reported the positive and negative sides of this model building approach. As positive sides, some of the students highlighted that the construction of a protein model promoted a better understanding of the topics via the observation of a protein in a 3D model, that was visualized, before, only in 2D figures of textbooks. In contrast, the time spent to build the proteins and the refuse of some colleagues to collaborate were pointed out by some students. We have also asked the students how to make this methodology more environmentally friendly, students complained that the materials used are recyclable, forgetting the use of styrofoam and adhesive tape as potential pollutants of the environment. Finally, in relation to the students’ opinion about this methodology, students reported that it was overall productive to improve their knowledge, to motivate them and take them off their comfort zone where they are passive receptors of knowledge.
Another aspect that we should mention is that we live in a virtual age and several tools are available to teach 3D structures of molecules, including complex protein molecules [17-22]. However, it is important to remember that our knowledge about the three-dimensional structure of the informational macromolecules was built based on concrete models deduced from x-ray diffraction of different proteins. For instance, the first models of hemoglobin (constructed by Max Perutz using wood) and myoglobin (constructed by John Kendrew using wire) [42] and the alpha-helix structure of protein proposed by Pauling and Corey (using different materials) [5]. In this context, we realize that it is primordial that students construct their knowledge using their own concrete models.

4 Conclusion

The results and observations made here indicated that the construction of concrete models of protein structure can be an efficient alternative to teach proteins structure to undergraduate students. The construction of insulin with simple materials instigated the reconstruction of student's knowledge both from group discussion-approach and from the concrete building of insulin three-dimensional structure. This can be a way to promote an increase of the Pedagogical Content Knowledge (PCK) [43] of pre-service teachers, both in chemistry and Biology, making this proposal accessible for implementation in basic education. As observed by Júnior and Souza [44], the use of materials as models that represent real structures in the educational context emphasizes the participation and experimentation of the student on the construction of their own knowledge.

On the other hand, a negative point has to be considered about the materials used to the construction of this model. The styrofoam is a material rarely recycled, as well as the reuse of the balls is limited because they are painted, perforated and glued to the wire. Therefore, it is important to consider the use a material that are less aggressive to the environment. In this way, the activities will encourage the development of attitudes that are positive toward the environment. As discussed above, the use of paper has been also effective, but usually adhesive tape had to be used and it is not recyclable as well. At the moment, we are testing new materials, but we have not yet found a definitive solution. Another important aspect that happened to us, during the process of answering the questions raised by the reviewers of this paper, was the necessity of discussing in detail with pre-service teachers the importance played by the use of concrete models in the development of the field of chemistry of proteins. The discussion will permit address important historical and humanitarian aspects that we have neglected previously.
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