In silico study of Bacillus spB201 amylase through protein sequence inspection

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Background/aim
Bioinformatics is the automatic processing of biological information, and it is a helpful tool to study the structure-function relationship of various proteins. Here, it was used in the *in silico* study of an AmyB201 from *Bacillus* strain, named AmyB201 to describe its origin and properties, which could help us to improve the performance of this enzyme through mutagenesis.

Materials and methods
The protein sequence of AmyB201 was purchased from NCBI data Base. The Signal P and the Protparam were used to determine the properties of AmyB201. In addition, programs like Swiss-model, Phyre2 and PyMOL were used to generate and manipulate the AmyB201 models.

Results
Comparaison study of Amy B201using Blast program showed an identity of 94% and 91% with amylases from *Bacillus* spUS586 and *Bacillus* spUS572, respectively. Subsequently, the analysis of the sequence by the Signal P. program revealed the presence of a signal peptide, which confirms the extracellular nature of the enzyme. Furthermore, the examination of the AmyB201 sequence by Espript showed that it has the same secondary structure with amylases from *Bacillus* spUS586 and *Bacillus* spUS572, with the exception of a few differences that could explain the specificity of each enzyme. In addition, the inspection of the 3D models showed the presence of three typical domains of amylases, namely the domains: A, B, C. Using these structures, we have been able to explain some properties of AmyB201.

Conclusion
This study was able to describe the origin of some properties of AmyB201, and could help us to improve the performance of this enzyme through mutagenesis.

Keywords:
AmyB201, *in silico* analysis, modeling, protein sequence

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Introduction

Amylases are glycosyl hydrolases (GH) that hydrolyze α-1,4 and/or α-1,6 glycosidic linkages within a linear chain of polysaccharides to generate maltose, maltotriose and branched maltodextrins. They have a variety of specific substrates [1], including amylose, amylopectin, cyclodextrins, glycogen, and dextrins, which contribute to the immense diversity of these kinds of enzymes.

According to the CAZY database, amylases are assembled with different types of glycosyl hydrolases in the GH-13 family [2]. This family is characterized by the presence of 4 conserved regions, named I; II; III and IV, which contain the catalytic triad Asp, Glu, Asp, corresponding to the residues Asp206, Glu230 and Asp297 of the Taka AmyB201 [3]. Subsequently, Janecek S in 1992, 1994 and 2002 [4–6] proposed three additional conserved sequence regions based on extensive analyses of a large number of α-amylase and related enzyme sequences. These regions contain amino acids that are essential for the stability of the topology of the enzyme [7]. On the other hand, AmyB201 has a three-dimensional structure that allows the enzyme to bind to the substrate and to promote the cleavage of glycosidic bonds through the action of highly specific catalytic groups [8]. Amylases have been shown to be composed essentially of three domains: A, B, and C. To this conserved structure, other domains such as the N, D and E domains are often added for some classes of AmyB201 [9]. As an example, maltogenic amylases have the domain N, while CGTases have the D and E domains.

Amylases can be produced by several organisms such as plants, animals and microbes [10]. However, due to efficient improvements in growth rates and cell production scales, the production of amylases by bacterial strains is usually preferred over other sources. In addition, bacterial amylases are valuable enzymes with broad applications in clinical practice and industry [2,10]. The identification and characterization of bacterial amylases can be performed by experimental methods such as purification, enzyme activity assay, and others [11]. While these methods are of widespread interest, they have recently given way to *in silico* methods, which use bioinformatics to study proteins in general and enzymes in particular. In addition, the advances in the development of computational tools over the last two decades, coordinated with growing genomic resources, has provided new opportunities to study proteins [12]. In fact, bioinformatics can provide sequence analysis, structure prediction and reconstruction, prediction of protein properties and functions, prediction of biomolecule interaction and systems biology [13]. As a result, it remains a powerful tool in the in-depth study of enzymes such as amylases.

In a previous work, an extracellular amylase from a *Bacillus* spB201 strain, named AmyB201, was characterized. This amylase has an optimum temperature of 60°C and retains more than 70% of its activity after 30 min incubation at 60°C. its optimum pH is of about 5. The SDS PAGE and the zymogram showed an active band of about 70 kDa. The proteomic analysis by mass spectrometry of this band showed 100% identity with *Bacillus* spKR8104 amylase.

In this work, we report an *in silico* study of the AmyB201. A detailed analysis of the protein structures and their influence on the physicochemical properties of this protein is also mentioned.

Materials and methods

Materials

Data analysis software; Signal P and Protparam were used to determine the properties of AmyB201. In addition, programs Swiss-model, Phyre2 and PyMOL were used to generate and manipulate the amylase models.

Study design

The data of AmyB201 obtained from BLAST was compared *in silico* with the sequence and structure of amylase from Bacillus spUS586 and Bacillus spUS572.

Ethical consideration

The present study was conducted *in silico,* which does not need approval, according to the principles expressed in the declaration of Helsinki.

Methods

Nucleic and protein sequences alignment

The BLAST program [14] was used to align and compare protein sequences.

Basic biochemical analysis of proteins

The ProtParam program was used for proteins physicochemical properties prediction [15], while the SignalP program was used to predict the cellular location of the enzyme [16].

Protein secondary structure prediction

The protein secondary structure prediction was performed using the PsiPred program [17] and the alignment with secondary structure overlay was edited using the ESPript program [18].

Structure modeling

The 3D structural model of amylase was generated using Swiss model and Phyre 2 program [19,20] and the crystal structure of *Bacillus* spKR8104 (PDB accession code 3DCO). The PyMol molecular Graphics System (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.) was used to visualize the constructed model structure and to render illustrative graphical figures.

Results

Protein sequence acquisition and BLAST comparison

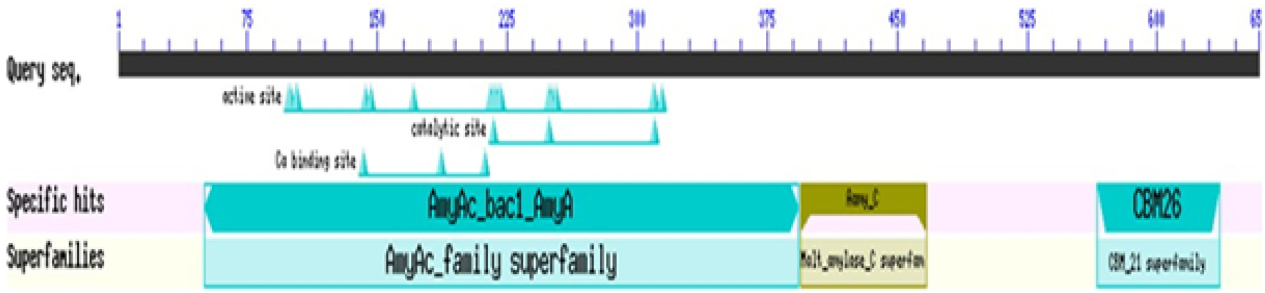
The results of the proteomic study carried out on AmyB201 (data no show) showed 100% identity with *Bacillus* spKR8104 amylase. The 3D structure of this protein was determined (PDB accession code 3DC0), and the enzyme was characterized. Furthermore, the wild-type enzyme exhibited similar physicochemical properties to AmyB201, notably their Ca independence [21]. Consequently, the protein sequence of *Bacillus* sp. KR 8104 amylase and its available 3D structure were used to perform the in *silico* study of AmyB201. The sequence of AmyB201 consists of ~659 amino acids. BlastP analysis revealed that this sequence shares 100%, 94%, and 91% identity with amylases from *Bacillus* spKR8104, *Bacillus subtilis* spUS586 [22], and *Bacillus subtilis* spUS572 [23], respectively.

Primary protein structure

Conserved domains analysis

An analysis using the NCBI CD search was conducted to identify conserved domains and important functional sites in a protein sequence [24]. The result obtained (Fig. 1) shows that AmyB201 belongs to the large GH-13 family of α-amylases, as

Figure 1



Graphical summary of NCBI CD search of Amy B201 protein. Query sequence: the Amy B201 sequence, specific hits: specific domains, bleu triangle showed the active site, the catalytic site and the Ca binding site.

it contains the characteristic catalytic triad of α-amylases: D217, E249 and D310 [25]. In addition to these catalytic amino acids, the program also identified those forming the active site. Furthermore, the analysis revealed the presence of a large domain extending from position 50–393, representing the alpha-amylase catalytic domain found in all bacterial α-amylases. Other domains were identified include a starch-binding domain (positions 565–636) and an AmyC domain (positions 394–467).

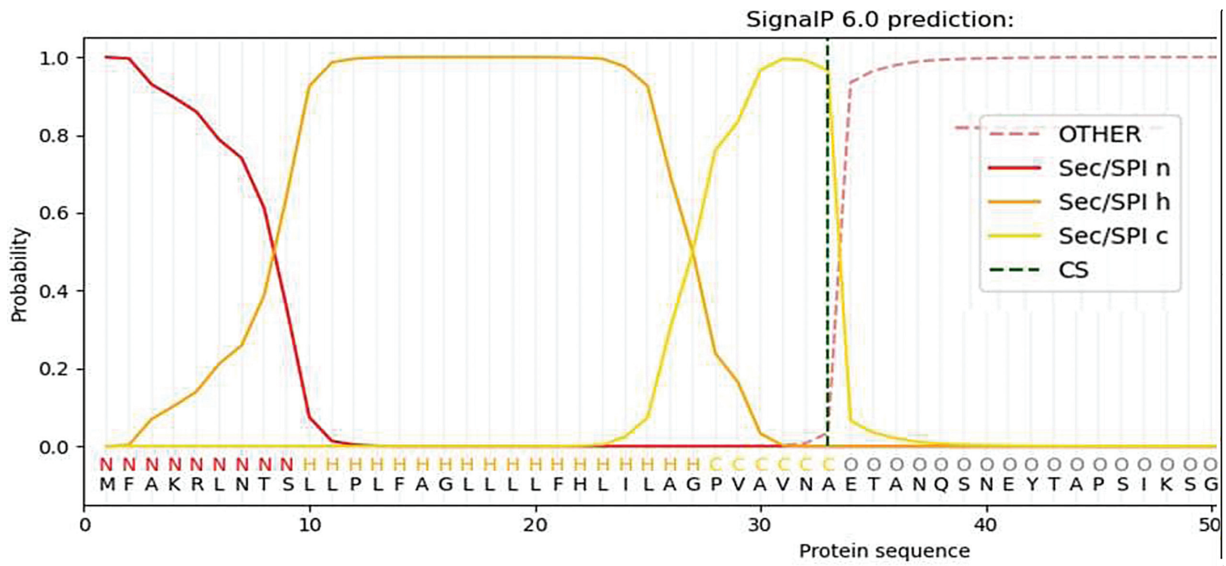
Signal peptide prediction

The signal peptide is a short sequence of around 13–50 amino acids, typically located at the N-terminus of proteins destined for secretion or integration into cell membranes. Once the protein is delivered to the correct cellular compartment, the signal peptide is removed by specialized signal peptidases (SPases) acting at the cleavage site [26]. Signal peptides generally lack sequence similarity; however, they all possess a tripartite structure [27] composed of:

(1) A positively charged amino-terminal N-domain
(2) An H domain, following the N-domain, formed by a stretch of hydrophobic residues. This domain adopts an alpha-helix conformation in the membrane.
(3) The C domain is located after the H domain and contains the SPase cleavage site, which removes the signal peptide from the mature part of the secreted protein.

Its cleavage site is located between positions 33 and 34, resulting in a mature protein of 626 amino acids (Fig. 2). This result confirms the extracellular nature of AmyB201. The inspection of the amino acid composition of this signal peptide shows that its N-

Figure 2



Signal peptide prediction of AmyB201. The N-terminal region (n) is in red. Hydrophobic region (h) is orange. The C-terminal region (c) is yellow. The cleavage site (cs) is in green.

Table 1 Number and percentage composition of certain amino acids of AmyB201

Amino acids	Number (percentage), *n* (%)
Alanine	53 (8.5)
Proline	21 (3.4)
Glycine	56 (8.9)
Cysteine	1 (0.2)
Positive charged amino acids	48 (7.7)
Negative charged amino acids	70 (11.18)

domain contains only two positive amino acids, Lys and Arg at position 4 and 5, respectively. In contrast, several SP's amylases contain 3 positive amino acids [22,23]. This result may explain the late secretion of amyB201 (after 6 h of culture) in comparison with other amylases of the same genus [22,23].

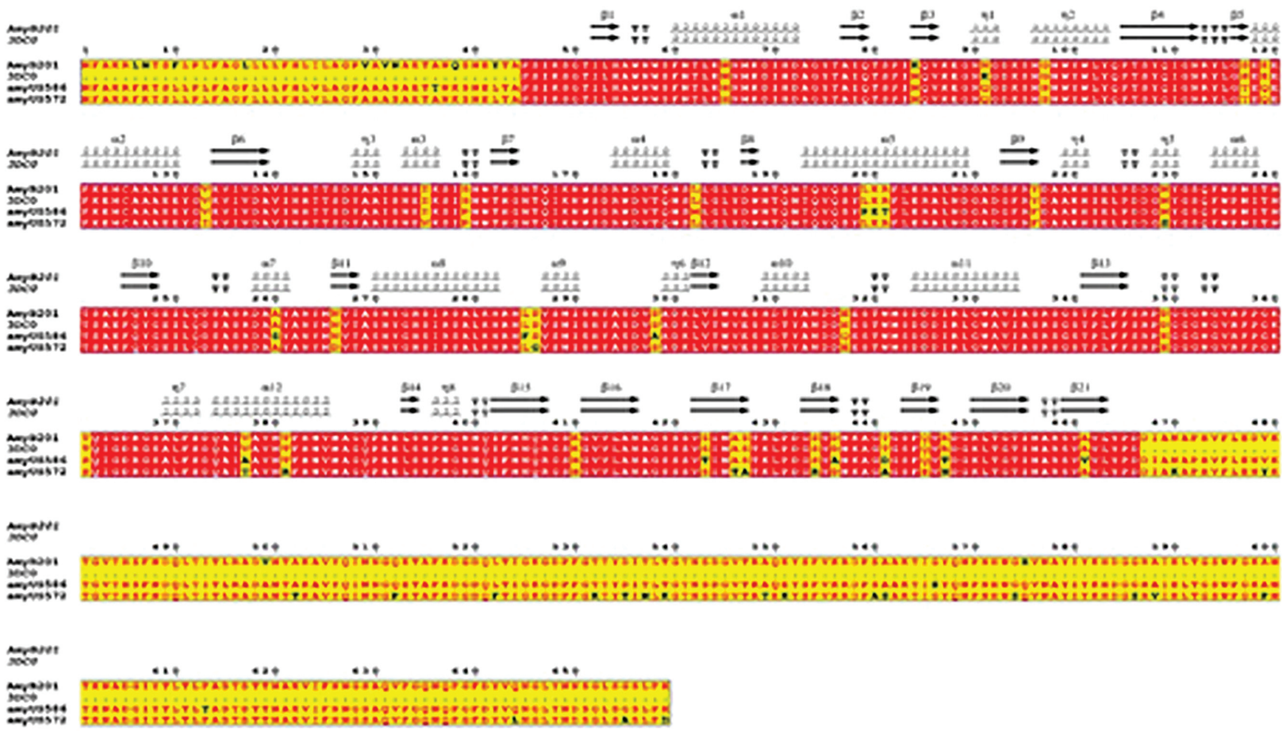
Physicochemical parameters prediction

The *in silico* analysis of various physicochemical parameters of the mature AmyB201 (without SP) was conducted using the ProtParam program on the Expasy website. This analysis revealed that the protein has a molecular weight of 68.5 KDa and a pHi of ~5.07. The amino acid composition was also determined in terms of number and percentage (Table 1).

Prediction of secondary structure

The secondary structure of proteins consists of local interactions between amino acid residues, mediated or not by hydrogen bonds. The most common secondary structures are α-helices and β-sheets [28]. In addition to these two structure types, loops can also be found, which are more irregular and thus more difficult to describe [29]. To predict the secondary structure of AmyB201, we utilized two different programs: 'PSIPRED' and 'Espript3.' PSIPRED is based on statistical computation, while Espript3 relies on known structural similarities. For our model, we selected the AmyB201 from *Bacillus* sp KR 8104 (PDB: 3DCO), which shares 100% identity with AmyB201. Additionally, we aligned the AmyB201 sequence with those of *Bacillus* spUS586 and *Bacillus* spUS572 amylases, as they have the same number of amino acids but different characteristics compared to our target protein. Using the Espript3 program, we predicted the secondary structure only up to residue 466 (Fig. 3). This limitation is due to the reference protein (3DCO) consisting of only 466 amino acids. In order to predict the secondary structure of the entirely sequence, we had used the PSIPRED program. The result indicated an identical secondary structure for the first 466 amino acids, demonstrating the program's efficiency (Fig. 4). Additionally, we successfully

Figure 3



Espript Analysis of AmyB201 sequence. 3DC0: AmyB201 of *Bacillus* spKR8401; amyUS586: AmyB201 of *Bacillus* spUS586; amyUS572: AmyB201 of *Bacillus* spUS572. The secondary structure assignment corresponds to the AmyB201 of *Bacillus* sp KR8401. Residues invariable among sequences are typed in white on a red background; residues conserved within each group are displayed as red letters on a yellow background.

Figure 4

Protein secondary structure prediction of AmyB201 using PsiPred Programm.

predicted the secondary structure of the missing segment (from position 467 to 659).

Hence, the peptide backbone of amyB201 is composed of 8 α-helices associated with 25 β-sheets. The loops are dispersed throughout the peptide chain. The high number of β sheets compared with α-helices is due to the richness of the C domain by β sheets.

Prediction of tertiary structure

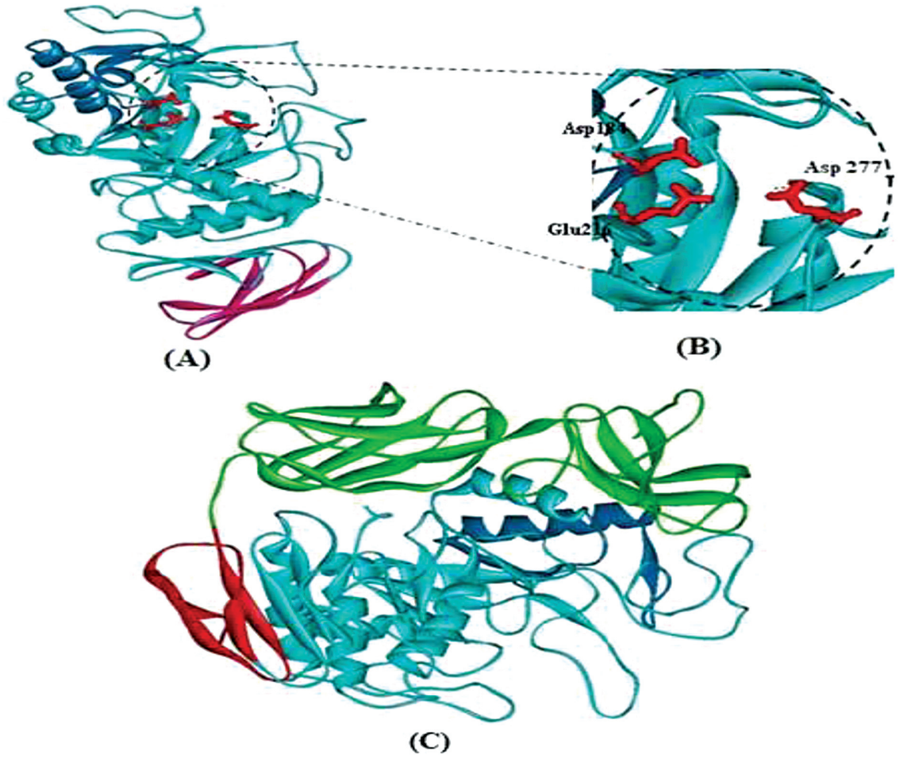
The tertiary structure of a protein represents its spatial folding [29]. Understanding the physicochemical properties of a protein and comparing them to similar proteins necessitates determining its three-dimensional structure. However, techniques for resolving protein 3D structures are often inaccessible, expensive, and time-consuming. Consequently, molecular modeling provides a valuable alternative for structural studies. This approach relies on the degree of identity between the protein of interest and its structural counterpart found in databases [30]. In addition, the prediction of 3D structures is a keystone of modern molecular biology. In fact, it can highlight features contributing to a deeper understanding of protein function and to engineer enzymes with improved properties, such as increased stability and enhanced catalytic activity.

Using the Swiss-model program, we generated a model of the three-dimensional structure of AmyB201 based on the *Bacillus* sp KR 8104 AmyB201 (PDB: 3DCO). The resulting model perfectly aligns with the structure of the reference protein with a Ramachandran Favoured of 99.28%. Furthermore, the generated model has good overall structural coherence, as indicated by the QMEANDisCo of 0.92, which is a good sign for the quality of the structure. This finding is expected since the two proteins share 100% identity. Therefore, the generated model shows the three typical domains of amylases: A, B, C (Fig. 5a): the A domain has a typical structure $(\alpha,\beta)_8$ and consists of residues 1-141 and 193-388. This domain contains the catalytic triad (Fig. 5b). The domain B is represented by a helix and a large loop. It is inserted between the third β-sheet (β3) and the third helix (α3) of domain A. This domain is made up of 50 amino acids (142–192). The domain C on the C-terminal side of the protein, which is formed by residues 389–466 and has a 'Greek key' structure. However, the model did not include the terminal part of the protein, extending from residue 467 to 659. This omission is because this segment is not present in the structure of the reference AmyB201. To generate a model including the missing part, we used another modeling program called 'Phyre2.' The new model reveals that the additional domain encompasses domain B and a significant portion of domain A (Fig. 5c). This configuration is similar to the arrangement of domains D and E found in CGTase [31].

A detailed examination of the AmyB201 model showed that Glutamate E119 is located on the protein surface at helix 3 of the A domain. This residue forms a salt bridge with Lys123, which stabilizes the helix (Fig. 6a, b). In the other two amylases, this salt bridge is replaced by a hydrogen bond since they have a glutamine residue at position 119 (Fig. 6c).

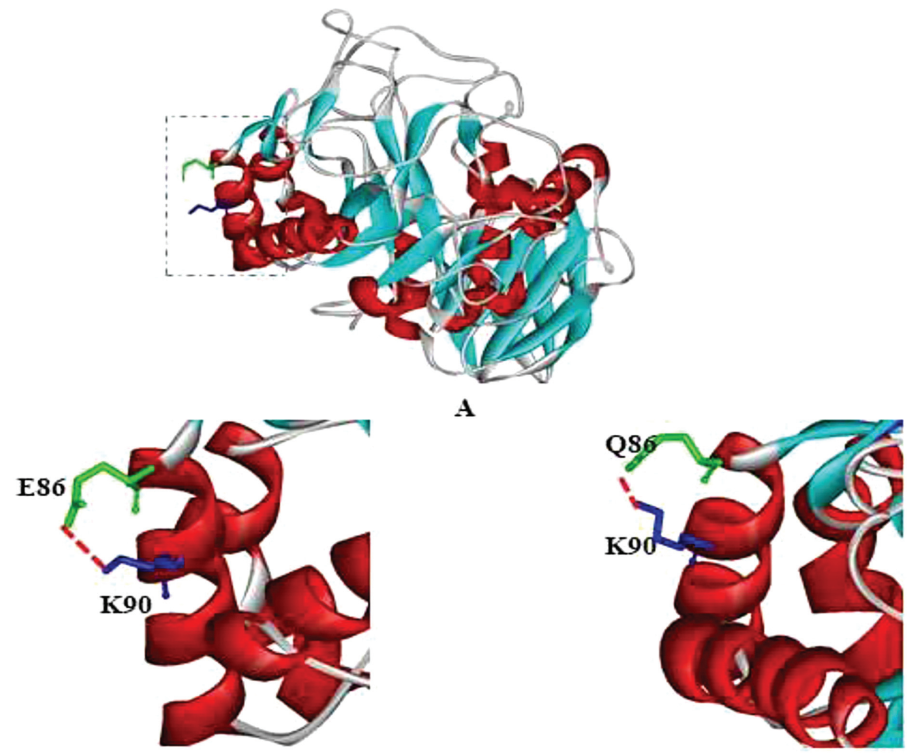
As a concern the 216 residue is close to the catalytic site (Fig. 7a, b) and near the subsites +1 and +2 (Fig. 7b).

Figure 5



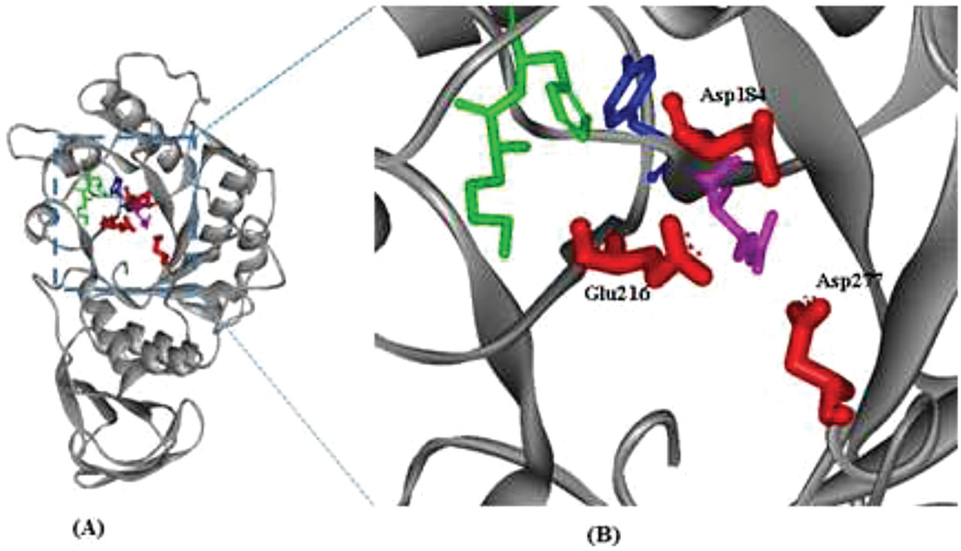
3D structure model of AmyB201. (a) General view: domain A in blue cyan, domain B in blue, domain C in pink; (b): Close-up showing the catalytic triad; (c): model generated by Phyre2 program: domain A in blue cyan, domain B in blue, domain C in red; the missing C-terminal part is in green.

Figure 6



Bond between residues 86 and 90. (a) Overview of the location of the two residues; (b) Salt bridge between Glu86 and Lys90 of AmyB201; (c) Hydrogen bond between Gln86 and Lys90 of amyUS586 and amyUS572.

Figure 7



Position of residue 216. (a) General view of AmyB201; (b): close-up of the AmyB201 active site: the catalytic triad (red); residues Lys 220 and His221 of sub-site +2 (green), Arg215 of sub-site +1 (pink) and residue Tyr216 (blue).

These subsites are formed by Glu317, Asp217, Arg215, His221, and Lys220, respectively, and they control the substrate specificity and hydrolysis products of amylases [32].

Discussion

As mentioned in the results section, the obtained sequence of AmyB201 consists of ~659 amino acids. The uniqueness of this AmyB201 sequence lies in its amino acid count. Indeed, only a few amylases with more than 600 amino acids have been studied and identified [22,23]. This sequence has high alanine content. According to Vielle C and Zeikus GJ [33], alanine significantly contributes to thermostability. Regarding the number of cysteines, AmyB201 has single residue, suggesting that the monomeric form of this AmyB201 lacks disulfide bridges. Hence, studying the secondary and tertiary structures is crucial to understanding this amylase's properties. In fact, the role of any residue in protein stabilization depends on its position in the three-dimensional structure [34].

As a result, the inspection of the secondary structure alignment shows that the majority of the differences between the three amylases are located in the C-terminal part. Additionally, several substitutions located in the helices and sheets were detected and could contribute to the stability of AmyB201 compared to Amy586 and Amy572. For example, substitutions N65H, E119Q, L219P, N229S, A260S, V377A/T, and T381R are located in the helices, while substitutions Y216F, N266D, and S427A are found in the sheets. To better understand the effect and the potential role of some of these substitutions on the properties and stability of the three enzymes, it is useful to analyze the 3D structure.

In fact, *in silico* analyses such as molecular modeling, of α-amylases from various organisms has revealed significant structural features, including catalytic residues and stability-enhancing interactions, which are essential for enzyme functionality [35,36]. In this context, the three-dimensional structure analysis of maltogenic AmyB201 from *Bacillus lehensis* highlighted an aromatic platform crucial for substrate recognition, which was used to improve activity [37]

As mention in the results part, the residue at position 119 belongs to the helix 3 of the A domain, which is located on the surface of the protein. In the AmyB201, this residue forms a salt bridge with Lys123, while in the two other amylases (Amy586 and Amy 572) it forms a hydrogen bond as they have a glutamine residue at position 119. This makes the helix more stable in the AmyB201 than in the other amylases, since the ionic bonds is much stronger than the hydrogen bonds. As described by Vieille C and Zeikus GJ [33], stabilizing the helix on the protein surface is important for the overall stabilization of the protein.

As concern, the residue at position 216 is located near the two subsites +1 and +2, which are responsible of the control of the substrate specificity and the hydrolysis products of amylases. Consequently, any change in this position could influence the enzyme's specificity [37]. This could explain the differences in hydrolysis products between the three enzymes. For instance, AmyB201, which has a tyrosine residue at position 216, primarily hydrolyzes starch into maltose and glucose. In contrast, AmyUS586 and AmyUS572, which have phenylalanine at this position, mainly produce maltotriose and maltose [22,23].

Conclusion

This study was able to describe the origin of some properties of Amy B201, such as thermostability and hydrolysis product of starch and could help us to improve the performance of this enzyme through mutagenesis. In fact, by comparing the sequence of this AmyB201 with other amylases and examining the 3D structures of these proteins, it will be possible to select some key residues involved in the specificity of each enzyme. Hence, the desired property can be modified simply by mutation of the selected residue(s)

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Nil.

Conflicts of interest

There are no conflicts of interest.

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