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in

Kodad O. (ed.), López-Francos A. (ed.), Rovira M. (ed.), Socias i Company R. (ed.). XVI GREMPA Meeting on Almonds and Pistachios

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 119

2016
pages 59-63

Do almonds (*Prunus amygdalus* Mill.) alpha-amylase germinating seedlings have a beta/alpha fold?

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Abstract. Alpha-amylases have been well characterized in microorganisms and mammals. Very little is known about plant amylases. A previous study has allowed us to demonstrate the presence of alpha-amylase in germinated almond seedling (*Prunus amygdalus* Mill.) var 'Tuono Mazzetto' (Rosaceae family). The enzyme activity was optimal at the 4th day of germination with a Vmax = 2.5 UI and Km = 6.32 mM. In the current study, our aim was to propose *in silico*, by comparative modelling, a putative homologous model of three-dimensional (3D) structure of *Prunus persica* alpha-amylase, a species from the same family. The *Prunus persica* alpha-amyalse structure shows a beta/alpha fold with small deviation from the template 3D coordinates even in the loop regions. The geometry of the catalytic site is preserved. The Ramachandran plot confirmed that the proposed model might be of a good quality. Alpha-amylase structure from *Prunus Persica* was also compared to a porcine (*Sus scrofa*) one, indicating the structural homology of these two different species. Combination of bioinformatics and biochemistry would allow us to better understand almond alpha amylase function.

Keywords. Almonds – *Prunus Amygdalus* Mill. – Alpha-amylase – *Prunus Persica* – Beta/alpha fold – Structural homology.

L'alpha-amylase des graines d'amandier* (Prunus amygdalus *Mill.*) *en germination présente-t-elle un repliement bêta / alpha?

Résumé. *Les alpha-amylases ont été bien caractérisées chez les micro-organismes et les mammifères. Peu d'informations concernent les amylases végétales. Une étude précédente nous a permis de mettre en évidence la présence d'alpha-amylase dans des graines d'amandier en germination* (Prunus amygdalus *Mill.*) *var. 'Tuono Mazzetto' (famille des Rosacées). Cette activité de l'enzyme est optimale au 4ème jour de germination avec une Vmax = 2,5 UI et km = 6.32 mM. Dans l'étude actuelle, notre objectif est de proposer in silico, par modélisation comparative, un modèle putatif d'homologie à trois dimensions (3D) de la structure d'une alpha-amylase de* Prunus persica*, une espèce de la même famille. La structure de l'alpha-amylase de* Prunus persica *présente un repliement bêta/alpha avec une déviation par rapport au modèle des coordonnées en 3D, même dans les régions en boucle. La géométrie du site catalytique est bien conservée. Le diagramme de Ramachandran montre que le modèle proposé est de bonne qualité. La structure de l'alpha-amylase de* Prunus persica *est également comparée à celle d'une espèce porcine* (Sus scrofa) *mettant en évidence l'homologie structurale de ces espèces différentes. La combinaison de la bioinformatique et de la biochimie nous permettrait de mieux comprendre le fonctionnement de l'alpha amylase d'amandier.*

Mots-clés. *Amandes* – Prunus Amygdalus *Mill.* – *Alpha-amylase* – Prunus Persica – *Repliement bêta/alpha* – *Homologie structurale.*

I – Introduction

In Tunisia, almond (Family: *Rosaceae*; Genus: *Prunus*) holds a very important place in agriculture after the olive tree. Almond plantations are spread across all the countrie and they are characterized by a relatively high genetic diversity (Gouta *et al.*, 2008, 2010). Moreover, almond studies concerned mainly its ecology as well as its physiology. Little work has concerned the metabolism of almond seeds and their germination. We are interested by enzymes involved in germination (Bahri, 2012) and in particular in the study of alpha amylase. Alpha amylases (EC 3.2.1.1.) are α- (1-4) D-glucan glucanohydrolase which catalyze α- (1-4) linkages in starch and any related oligosaccharides to produce D-glucose, D-maltose and a small amount of maltodextrins (Mercier, 1985; Graber and Combes, 1989). They have been classified in the family of glycosyl hydrolases 13: GH13 (Davies and Henrissat, 1995). Amylases from microorganisms have been extensively studied (Ben Abdelmalek *et al.*, 2009; Jay Kant, 2009). Plant, animal and microbial amylases show significant differences in the primary, secondary and tertiary structure as well as the catalytic mechanism (Tripathi *et al.*, 2007; Ben El Arbi *et al.*, 2009). It is well known in the literature, that the arrangement of the major elements of secondary structures and the topology of the connections between them corresponds to a protein fold (Chothia *et al.*, 1997). Three dimensional structures of proteins give valuable insights into the molecular organization and function (Messaoudi, 2011). Protein structure homology modeling has become a routine technique to generate 3D model for proteins when experimental structure are not available (Biassini *et al.,* 2014). Plant amylases are generally considered to be involved in the metabolism of germinating seedling and Biotechnology (Ben El Arbi *et al.*, 2009; Khady *et al.,* 2013). Another species, *Prunus persica*, of the same genus and in the same family as the almond tree (Dirlewanger *et al.*, 2002) is also much studied and has an importance in traditional medicine and pharmacology (Han *et al.*, 2015). To understand almond alpha-amylase mechanism, we combined bioinformatics and biochemistry. In a first step and in the absence of an almond alpha-amylase sequence in the databases, our aim is to propose, for the first time, a *Prunus persica* alpha amylase fold by homology modeling with Phyre 2 server (Kelley and Sternberg, 2009).

II – Materials and methods

1. Plant material

Samples of Italian almond seeds introduced in Tunisia (*Prunus amygdalus* Mill., var. 'Tuono'), were collected on 2012 and kindly provided by the (Institut de l'Olivier, Sfax-Tunisia). The Almond seeds were germinated at 26 ± 1°C in the darkness for different stages of germination. The alpha-amylase was extracted and identified by both the Somogyi-Nelson and the glucose oxydase methods (Trinder, 1969; Dingeon, 1975; Lott, 1975; Somogyi, 1952).

2. Homology modelling

The procedure for homology modeling involves 4 steps-template selection, target template alignment, model building and evaluation. We firstly selected the primary structure of α-amylase from *Prunus persica (*Uniprot database, code: M5VVU6). Many servers and algorithms are able to predict 3D structures with a good accuracy we used the Phyre 2 (Protein Homology/analogY Recognition Engine) (Kelley and Sternberg, 2009) server as a fully automated based method which can reliably detect up to twice as many distant homologies. This method represents one of the many alternatives available from the structural bioinformatics. The primary structure of our target protein was submitted to the the program server (Phyre 2) which returned a list of candidate 3D structures along the alignment of the target sequences with the corresponding template sequence. The 3D model was selected based on the quality of the alignment. The stereochemical assessment was then achieved by constructing the Ramachandran Plot (Sheik *et al.*, 2002; Kelley and Sternberg, 2009).

III – Results and discussion

A comparative modeling method was used to construct the 3D model of α-amylase from *Prunus persica* which α-amylase sequence contains 401 residues (Fig. 1). The template used for the construction of the model is an orthologous protein sequence of *Hordeum vulgare* alpha amylase (PDB code: 2QPU), a plant member of the Poaceae family. The template selection by Phyre 2 is highly entrusted as judged by the confidence level score (100) which shows that both the target and the template sequences are homologous with an identity value of 65%.

The structure shows a beta/alpha fold with small deviation from the template 3D coordinates even in the loop regions.The model presents 9 alpha helices surrounding a hydrophobic core, consisting mainly on a beta sheet layer. The enzyme also presents two other exposed beta sheets on the protein surface with 5 and 2 strands.

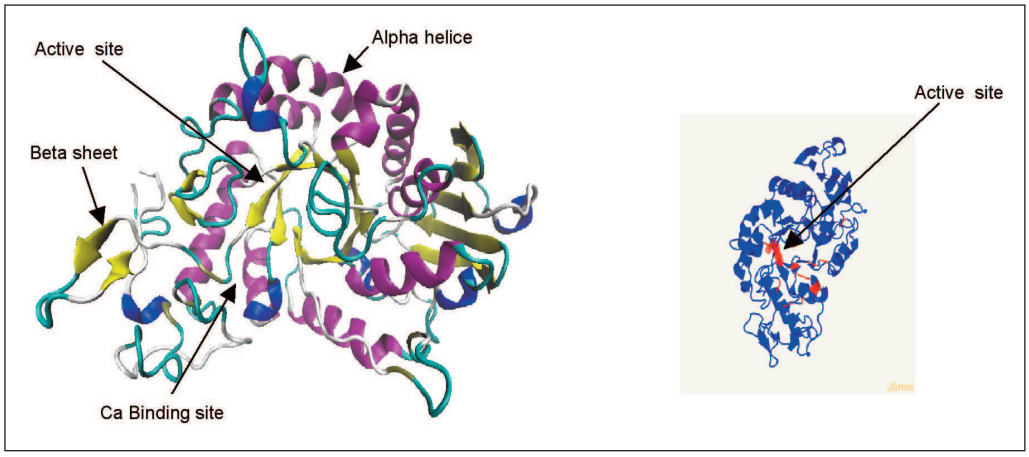

Fig. 1. Three dimensional structure of *Prunus persica*: (a) Ribbon representation of three dimensional structure of *Prunus persica* alpha amylase: strands are shown in light and helices in dark colour. (b) Active site of *Prunus persica* three dimensional structure.

The Ramachandran Plot indicates also a good stereochemical quality of the model. It's a significant result because 96% of all φ/Ψ angles residues are located in the favored regions and 4% in the allowed regions while no residue is located in the outlier zone of the Ramachandran Plot (Fig. 2).

Ramachandran Plot reveals a model of good quality which suggested that alpha amylase is well preserved between these two species *Prunus persica* and *Hordeum vulgare* which belong to two different families: respectively Rosaceae and Poaceae.

We also compared the proposed model to *Sus scrofa alpha-amylase* (Q7M328). Despite the low sequence identity of 14%, we observed that this later, revealed a backbone RMSD value of 3.4 angstrom suggesting the presence of a preserved structure between two different species. This result is in agreement with that described by Svensson (1994) in which diverse alpha amylases contains a characteristic catalytic $(\beta/\alpha)_8$-barrel domain.

In a previous biochemical study (unshown data) we have identified the presence of an alpha amylase during the germination of almond (*Prunus amygdalus* Mill.) seedlings. The enzyme showed an optimal activity at the 4th step of germination and has kinetic parameters Vmax = 2.5 UI and Km = 6.32 mM.

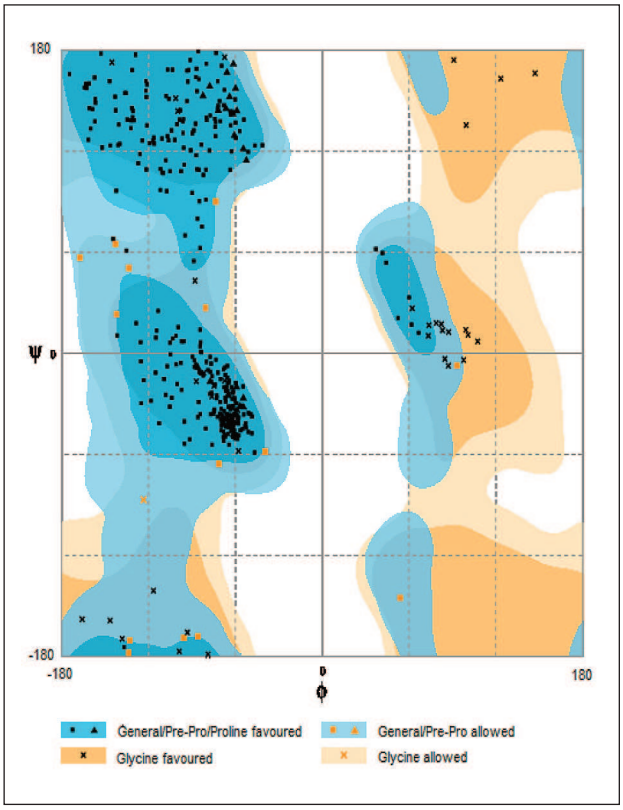

Fig. 2. ***Prunus persica*** **Ramachandran Diagram.**

IV – Conclusions

For the first time, folding of alpha amylase from peach has been proposed. The model shows a beta / alpha fold, as described for microrganisms with small deviation from the template 3D coordinates even in the loop regions. It is well known in literature that the 3D structures of proteins are more highly conserved than their sequences.

The involvment of alpha-amylase in oligosaccharides hydrolysis is well demonstrated among different species. Alpha-amylase structure seems to be preserved between two diverse species (*Prunus persica* and *Hordeum vulgare*). This folding was also observed in an animal species (*Sus scrofa*). This leads to suggest that the enzyme stucture is probably conserved during evolution.

In order to preserve oligosaccharides hydrolysis function, could almond alpha-amylase from germinating seedlings (*Prunus amygdalus* Mill.), has a conserved beta/alpha fold like *Prunus persica* and as described in microorganisms? (Ben Abdelmalek *et al.*, 2009; Tayyaba *et al.*, 2014). Further investigations would allow us to determine it.

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