Evolutionary relationships of Microbial Transglutaminases

Deborah Giordano [1,2] and Angelo Facchiano[1,*]

[1] Istituto di Scienze dell'Alimentazione, CNR, via Roma 64, Avellino, Italy
[2] Dottorato di Ricerca in "Innovazione e management di alimenti ad elevata valenza salutistica", Università di Foggia, Italy
[*] Corresponding author: angelo.facchiano@isa.cnr.it

Introduction

Transglutaminases (TGases) are a class of enzyme widely spread in nature; it is possible to find them in plants, microorganisms, vertebrates and invertebrates. This enzyme is able to catalyze post-translational modification in many proteins by acyl transfer reactions, deamidation and crosslinking (polymerisation) between protein intra- or inter-chain glutamine (acyl donor) and lysine (acyl acceptor) peptide residues (Camolezi Gaspar et al. 2014). More that 50 years of studies on this enzyme family indicate a large interest for its function, in particular for human TGases and their involvement in physiopathological processes (Facchiano et al., 2006; Facchiano et al., 2010; Tabolacci et al., 2013; Cordella et al., 2017).
In bacteria, TGases appear largely present, although the function of this enzyme is still unknown. Microbial TGases (MTG, or MTGase) are of extreme interest in industry and biotechnology fields. Nowadays, a particular MTGase, Streptomyces mobaraensis MTGase, is commercially available and is become an important industrial tool in the biopolymers industry, in cosmetics production, in wool textiles, and in the food processing. The growing interest for these enzymes is determined by the possible role in various biological processes and clinical applications (Csosz et al. 2008; Lorand and Graham 2003) as the prevention of allergy and food intolerance, including celiac disease (Martins et al., 2014; Strop, 2014).
Massive sequencing of microbial genomes continuously increases the number of potential MTGase sequences available in data bases. In this work, we present the results of bioinformatics analysis on MTGases sequences, based on database searching, sequence comparisons and alignments, phylogenetic tree constructions, with the aim of improving the knowledge of MTGases, in the perspective of investigating by protein modelling and simulations techniques the functional features.

Methods

To obtain the classification presented in the following results, a large-scale analysis of all Pfam proteins annotated as "Transglutaminase" has been performed by means of a reiterative procedure. First, sequences have been divided on the basis of their phylum belongs in, and the TGase cores of all the protein sequences belonging to those groups have been analyzed by an integrated procedure including multiple sequences alignments, pairwise comparisons, database searches and pattern searches, by BLAST and Clustal Omega tools. Afterwards the analysis of evolutionary relationships among the examined proteins has been performed by MEGA tool and phylogenic trees have been constructed by means of Maximum Likelihood and Neighbor Joining algorithms. From the constructed trees only the sequences which belongs to the same clade but that are the most distant have been selected, in order to sampling in the wider way all the set of sequences in our availability. Furthermore, to make sure do not lose information, all the sequences selected and discarded have been analyzed by visual inspection and pairwise comparisons, so that sequences judged as relevant have been added to the set of sequences selected, and sequences judged as still too much similar have been discarded. This procedure has been repeated several times until the groups were composed of the best representative sequences. After doing that, all the groups were collect together and the procedure repeated.

Results

Among the almost eight thousand bacterial proteins annotated in Pfam as Transglutaminase, we find different characteristics in terms of length, presence of domains, and position of amino acids with expectable functional roles. However, based on these features, it was possible to make a preliminary

classification that brings to the identification of five main groups. A first group is composed by MTGases extracted by different Flavobacteria and Sphingobacteria that show the characteristics to be very similar to the MTGase of Chryseobacterium sp., a novel form of MTGase, which is very different from all the other MTGase known but whose activity has been experimentally proved by S. Yamaguchi et al. (Yamaguchi S. et al, 2001).
MTGases extracted by bacteria from different Phylum compose a second group. They are very similar to the MTGase of Bacillus Subtilis (Tgl-like) (Fernandes C. G. et al. 2015), but some of them do not preserve all the catalytic residues.
A third group is composed by the most common MTGases, which is the MTGase which preserve the main catalytic triad of Streptomyces mobaraensis MTGase, in the order Cys Asp His. All of them are Actinobacteria and most of them belong to the genus Streptomyces. They also present a good sequence identity.
A little group of proteins from Proteobacteria composes the fourth group. These MTGases differ from all the other MTGases but maybe preserve the catalytic residues in the order Cys His Asp, even if Ser could replace Cys.
The last group is the biggest; it is composed by all the MTGases, which present a similarity to the eukaryotic TGase and preserve the catalytic triad order typical of the eukaryotic TGase, i.e. Cys, His, Asp. Asp in these proteins is often replaced by Glu. Sequences present in this group differ each other also for the sequence length (double in the half of the cases). Whereas it was not possible to obtain a good clustering of these sequences, this last group would need further deep analysis in order to evaluate the possibility to obtain a more detailed classification.

Conclusions

From this classification, a more clear view of microbial Transglutaminases, their subgroups and possibly their similarities in functional properties is now available. This work is part of a more complex project, for which we have ongoing studies on the properties of the 3D structure of selected microbial TGases, and planned studies for experimental validations.

References

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