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Investigation

Gingipains as a virulence factor in the oral cavity

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ABSTRACT

Aim: The objective of this study is to demonstrate the molecular action of *Porphyromonas gingivalis* cysteine proteases such as gingipains (R1, R2 and K) upon human molecules.
Materials and methods: Using the information on protein structure and function available in international databases (UniProtKB and Merops Database), the molecular interactions already described between gingipains and host molecules were clarified.
Results: Possible cleavage sites were identified in host-produced elastase inhibitors and in pro-Matrix MetalloProteinase (MMP)1. Analysis of the results leads to the suggestion that the elastase inhibitor alpha1-antitrypsin is also degraded by interpain A, a cystein protease of *Prevotella intermedia* sharing a high homology with the PrtT and periodontain of *P. gingivalis*.
Conclusion: The information obtained suggests a synergistic molecular mechanism by which cysteine proteases of different bacteria can be responsible for the clinical manifestations of periodontal disease, and illustrates the use of bioinformatics to establish and predict molecular mechanisms.

Gingipains como fatores de virulência na cavidade oral

Palavras-chave:
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RESUMO

Objetivo: O objetivo deste trabalho é verificar o detalhe molecular da ação de proteases de cisteína de *Porphyromonas gingivalis* como as gingipains (R1, R2 e K) em moléculas do hospedeiro.
Material e métodos: Utilizando a informação disponível sobre estrutura e função de proteínas nas bases de dados internacionais (UniProtKB e Merops Database) as interações já descritas entre gingipains e moléculas do hospedeiro são clarificadas.
Resultados: São identificados possíveis locais de corte das gingipains em inibidores naturais das elastases e a identificação molecular de um local de corte na pro MetaloProteinase da Matriz (MMP) 1. A análise dos resultados sugere que a interpain A, uma protease de cisteína de *Prevotella intermedia* com elevada homologia estrutural com as proteases de cisteína PrtT e periodontain de *P. gingivalis*, também degrada o inibidor de elastases, alfa1antitripsina.

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Conclusão: Com a integração da informação obtida, sugere-se um possível mecanismo molecular da sinergia criada entre proteinases de cisteína no sentido de promover a doença periodontal. Este estudo ilustra a forma como as ferramentas bioinformáticas podem ser úteis no esclarecimento dos mecanismos moleculares e na previsão de resultados experimentais, melhorando o desenho de estudos laboratoriais.

Introduction

Periodontitis is an inflammatory and infectious disease affecting the supporting tissues of the tooth. Despite the fact that the microflora associated with periodontal disease has been extensively studied,[1] no species by itself could be identified as the etiologic agent.[2] *P. gingivalis* has been identified as one of the major organisms associated with destructive adult periodontitis. The virulence of this microorganism is particularly related to its proteolytic activity, and the cysteine peptidases gingipains have been extensively studied and characterized.[3,4] Gingipains have been described and studied as being particularly efficient and versatile enzymes, capable of proteolysis of several host molecules associated with physiological processes such as host immune defense, cell adhesion and vascular permeability.[5–9] Studies demonstrating the "*in vitro*" cleavage of host molecules by gingipains often just report a digestion[10] and sometimes identify the resulting fragments either by size[11] or by antibody[5] based techniques, but lack information on the molecular details of the interaction between the gingipains and the host molecules.

Public databases accumulate an enormous quantity of sequences and structural data and several bioinformatics tools have been developed. Some of the tools are integrated in the databases and repositories (*e.g.* Blast search and ClustalW2 alignment algorithms) while others are developed independently such as PyMol.[12,13] In either case, the objective is the same: to extract biological significance of the accumulated data. This knowledge may then be used to direct further scientific research prompting new experimental studies or, in other instances, be applied in the development of chairside innovative tools and/or procedures.

The purpose of this work is to show how the structural information available in public databases such as UniProtKB[14] can be explored with bioinformatics tools such as PyMol[12] and Psipred[13] to produce information on the interactions between host molecules and gingipains. This analysis clarifies the molecular detail of these interactions and proposes new experimental approaches to elucidate other interactions between microbial proteases and host molecules. The knowledge of the molecular interactions is fundamental not only in the development of specific inhibitors for the action of gingipains and other microbial proteases,[15] but also in the development of additional diagnostic tools such as the recently described by Kaman et al.,[16] and therefore provides a basis for improvement and innovation in the development of new therapeutic approaches and accurate diagnosis of periodontal diseases.

Materials and methods

The bioinformatics analysis of the interactions between the gingipains and host molecules was performed using freeware tools and information available in international databases. This study focused on host molecules whose interaction was described in the literature[7] and for which crystallographic structures were available. Because the modeling of membrane proteins still has limitations, we used only molecules which are soluble and not integrated in the membrane. To analyze the interactions the steps followed are described below.

Characterization of gingipains

The amino acid sequence of proteins used in this study was obtained from the UniProtKB database[14] (http://www.uniprot.org/). The 3D model of Gingipain R2 was obtained from crystallographic data available at http://www.pdb.org,[18] with code 1cvr. The remaining models of gingipains were generated through the program MODWEB[19] (http://modbase.compbio.ucsf.edu/ModWeb20-html/modweb.html) and recorded in the program of structural analysis PyMol[12] (http://www.PyMol.org/).

Characterization of host substrates

The information on the host substrates was obtained from the UniProtKB[14] and Merops[17] database by searching the name of the protein. For each of the host substrates we searched at http://www.pdb.org[18] for the crystallographic information available. In many cases the available crystallographic structure was that of the molecule associated to an inhibitor. When this was the case, MODWEB[19] was used to produce the 3D structure of the host substrate.

Whenever the secondary structure of a protein was necessary the Protein Structure Prediction Server (PSIPRED) was used. This is a highly accurate method for protein secondary structure prediction freely available on-line.[13]

Results

To clarify the molecular details of the cleavage of Secretory Leukocyte Protease Inhibitor (SLPI) by gingipains, a determination of the secondary structure of this molecule was done and is presented in Fig. 1. All arginine (R) residues of the molecule are in areas of the corresponding to beta sheets and therefore possible cleavage sites. The analysis of Fig. 1 also reveals that