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Identification of a vaccine against schistosomiasis using bioinformatics and molecular modeling tools

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ABSTRACT

Schistosomiasis is a serious public health problem in Brazil and worldwide. Although the drugs used to treatment schistosomiasis are effective, the disease continues to expand in all endemic countries due to constant reinfection, poor sanitation, and the lack of effective programs for disease control. However, advances generated through genome projects have provided important information that has improved the understanding of the biology of this parasite. These advances, associated with the advent of bioinformatic analysis, are becoming an important tool in reverse vaccinology.

Through database access to the DNA and protein sequences of *Schistosoma mansoni* and the use of bioinformatics programs, fourteen epitopes were identified. Five epitopes were obtained from proteins whose immunogenic potential had already been assessed in other studies (KP), and nine whose immunogenic potential is unknown (UP). To improve stimulation of the host immune system, the selected epitopes were modeled with a sugar moiety. After this addition, all of the epitopes showed structures similar to those observed in the native proteins, but only eleven of the peptides presented thermodynamically stable structures. Prediction analysis and molecular modeling showed that the glycopeptides presented here are important targets in the search for a vaccine against schistosomiasis. Additionally, they suggest that these molecules may be used in immunological assays to evaluate the level of protection, the effect on pathology reduction and the profile of cytokines and antibodies induced by them.

1. Introduction

Schistosomiasis is one of the most widespread parasitic diseases and is found in 74 countries, virtually all of which are underdeveloped. The World Health Organization (WHO) considers schistosomiasis the second most important tropical disease of socioeconomic significance in the world; it is second only to malaria (Andrade, 2002 and Hinrichsen, 2005). This disease is caused by human contact with water that is contaminated with trematodes of the genus *Schistosoma*. These trematodes penetrate the skin and mucosa ascercariae, which are the larvae released by the parasite's intermediate snail host, *Biomphalaria*. The most important species of the genus are *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* (Rollinson and Southgate, 1987).

The development of a schistosomiasis vaccine would be a very efficient intervention method to combat this endemic disease. This is justifiable because of the difficulty of mass population treatment, constant reinfection following treatment, and the emergence of *S. mansoni* strains that are resistant to current drugs (Oliveira et al., 2008).

The irradiated cercariae used in animal models of immunization induce significant protection against infection. However, growing cercariae in large quantities for vaccine preparation is very complex due to difficulty in maintaining the life cycle of the parasite in vitro and the high risk of contamination during immunization (Souza et al., 1987).

Abbreviations: BLAST, alignment search tool; KP, known proteins; MD, molecular dynamics; RMSD, root mean square deviation; UP, unknown proteins; PDB, Protein Data Bank.

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Upon coming into contact with the host's immune system, antigens from *S. mansoni* are phagocytosed and cleaved into smaller pieces called epitopes, which are subsequently presented to T-lymphocytes. During this interaction, the epitopes are bound to major histocompatibility complex (MHC) glycoproteins present on the surface of the antigen presenting cells (APCs) and are recognized by specific receptors on the surface of T-lymphocytes. The MHC molecules, also known as HLAs (human leukocyte antigens) in humans, are highly polymorphic and bind to a limited set of peptides. However, some epitopes are presented by several different HLAs, thus increasing the likelihood of their being recognized by T-lymphocytes (Abbas et al., 2008).

With the advent of bioinformatics, many prediction programs based on specific algorithms for protein analysis have been developed and used in reverse vaccinology, a technique that aims to determine the theoretical capacity to induce an immune response. In the case of this work, these include programs that predict the affinity with which certain peptides bind to and are presented by HLA molecules. This allows the selection of epitopes that have higher chances of being recognized by HLA molecules. Epitopes that bind to multiple HLAs are known as promiscuous epitopes and represent important targets for vaccine production (Zhaoa et al., 2011).

Several studies have evaluated the complexity and immunogenicity of *S. mansoni* surface proteins to identify vaccine targets against schistosomiasis (Bergquist, 1995 and Hotez et al., 2010). The selection of epitopes and their subsequent conjugation to carrier molecules, such as modified sugars, nanotubes, and even their encapsulation within lipid molecules, have proven to be interesting strategies for vaccine production (Ivanoff et al., 1996; Hsu et al., 2007 and Yandar et al., 2008,). During the various stages of its life cycle, *S. mansoni* expresses surface glycoconjugates with a carbohydrate portion, called Le^x, that is composed of L-fucose, D-galactose, and N-acetylglucosamine. Studies have shown that mice and humans produce anti-Le^x antibodies during the immune response against parasite infection (Richter et al., 1996 and Van Roon et al., 2004). Galactose is found at the terminal ends of this oligosaccharide and is therefore responsible for the first recognition interaction by immune cells.

In the present study, fourteen promiscuous epitopes derived from fourteen different proteins, obtained from *S. mansoni* databases, were identified and modeled to evaluate their stability when attached to a galactose motif.

The results presented in this manuscript suggest that eleven of the fourteen promiscuous epitopes that were modeled presented thermodynamically stable structures when modified with the carbohydrate. Therefore, these results indicate that these thermodynamically stable chimeras should be synthesized for use in immunological assays to evaluate the rate of protection and the induced cytokine and antibody profiles.

2. Methods

2.1. Search for protein sequences in public databases

The sequences of the proteins of interest were obtained from the public databases: GeneDB and the National Center for

Fatty acid binding protein [Schistosoma mansoni]

NCBI Reference Sequence: XP_002580432.1

MSSFLGKWKL SESHNFDAVM SKLGVSWATR
QIGNTVTPTV TFTMDGDKMT MLTESTFKNL
SCTFKFGEEF DEKTSDGRNV KSVVEKNSES
KLTQTQVDPK NTTVIVREVD GDTMKTTVTV
GDVTAIRNYK RLS

Putative Na/Ca exchanger [Schistosoma mansoni]

GenBank: CCD79088.1

MDFLKVPSFG WTGSSDQAQL DLLISEKELQ
HNSLTSTMNE NNFIPTTSIC KENHINISSI
NSNVNHSISK SMNQFNQQII NNHQIKCYPC
PKCNYTAKWP TELQKHVMVH ANSRPFICCV
CTTSYKWSWD LGRHFTNSHP NLPNPYKLYS
LLTNLYSINL IIIFCTFLLP TNEIGFRGPV
DSNIFAEIEG DAPNSSICRR MFSNFTEPEI
RCLIAKHFPG CQFDSGFFQY LVFQYCNFDE
RIAPTVFMGV TLLAFGNGAP DVFSAVTAIT
TGDPDAPDEG LGLGFLLGFL VFYLVYVLTT
WVSSTYMHRQ RRNGTHSILP LFLQNTLSKP
LNMLSRKRQN LSQMCGRICP QFMFLTRIRK
KINLYQFKKS KSSQNHVIIE DGMKNLYSNV
VFEQVLPEKT VLSATAKVNG NVELRDREKV
TIPNIHVSDK RINNRQINAP KIEITSPKDI
DDLNSHLDHS SYLHGDENVS TVNNGSLRLS
SDFKNYLNPP DGVKKRASSL VSTEPMSSDR
RLSTEHSGLS RGRCRRAQSV FRRRPSSRMS
STGYELSYMV RWIIEKWAMK GVWHHFAYYM
IPIDVESWPQ QSVFSRFLQI LQTPIFLVFR
LTIPTVIEEL ADDTETQGFA QESNIEQKIN
EPSREISTIP EENKFTLNSS QPELNHSNDF
VPENTDREPV AVNLELMHGW CKPLNVFQCL
LVPALWPMLL TANGKCIGLS PIGKTPIPIF
CPFLASGFII ALTVFFTSKW NQPPRHYHRP
FFATLGFITS IIWIYALAHE LVNSLETLGI
VWEISEAILG LSVMALASSI GDIMSNCLLA
RNGYPRIAYA ACLGSPLFNL LLGAGLSYTV
KIGRADVGYA YLSFTLTQAL LFSCLLAVLT
LNILTALIGR FQFYRYYGIV LIIIYLVFVT
TAILIEVDVI VSPVNWNLAT GTE

Fig. 1. Protein sequences obtained from the databases. Sequences of (A) the Sm14 protein with known immunogenic potential and (B) the Sm149930 protein, whose immunogenic potential is unknown.