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MALATE DEHYDROGENASE PROTEIN OF *ECHINOCOCCUS GRANULOSUS*- A PROMISING CANDIDATE FOR DIAGNOSIS AND SUBUNIT VACCINE DESIGN - AN *IN-SILICO* ANALYSIS

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ABSTRACT

Echinococcus granulosus, a tapeworm, is responsible for causing a deadly disease- Cystic Echinococcosis which is very difficult to diagnose, treat and control. So far, the crude extract (cystic fluid), is used for the diagnosis of cystic echinococcosis. The main problem in using crude extract is that it often shows cross-reactivity with several other helminthic diseases. Thus there is no specific and sensitive method available for the diagnosis of the disease. Malate dehydrogenase (MD) is observed to be highly expressed protein in all stages of *E. granulosus* life cycle, thus indicating it to be an important protein for the parasite survival as well as could be an important marker in diagnosis of the disease. In the present study, promising HLA Class I (HLA-A 02:01) restricted T cell epitopes and B cell epitopes were identified. The identified peptides were confirmed by visualizing their location on the 3D modeled protein. Identification of such immunodominant regions in MD gene of *E. granulosus* will further help the researchers in better understanding the immune response generated in host in response to MD gene of *E. granulosus* and could facilitate in diagnostic kit and subunit vaccine designing.

Keywords: Malate Dehydrogenase, *Echinococcus Granulosus*, subunit vaccine designing

INTRODUCTION

Human cystic echinococcosis (CE), caused by infection with the larval stage(hydatid) of the helminthic parasite, *Echinococcus granulosus*is responsible for considerable morbidity and mortality with around 50 million cases worldwide [1]. Humans are accidental host which acquire infection by ingestion of *Echinococcus* protoscolesces.CE in humans is one of the most lethal and widespread zoonoses caused by anyhelminthic parasite[2]. Immunodiagnosis is an important tool in early detection of the disease. Immunodiagnostic techniques such as ELISA and immunoblotting are currently applied to confirm the presence of an Echinococcus cyst in patient [3].

The hydatid fluid extracted directly from the cyst is currently used as a main antigenic source for the primary immunodiagnosis of human CE[4]. It has a high sensitivity (75 - 95%), but its specificity is often unsatisfactory [5] due to its cross-reactivity with other helminthic parasites, like *E. multilocularis* and *Taenia solium*[6]. Thus, there is no sensitive and specific test available currently for the diagnosis of the disease in humans [7].

It has been suggested that the use of recombinant proteins or synthetic peptides may improve the specificity of diagnosis of CE [8]. Such peptides have already shown there potential in diagnosis of various infectious diseases of viral [9, 10, 11]and parasitic [12, 13, 14] origins. Katoh et al, [15] generated a vaccine based on Eg95 antigen of *E. multilocularis* in order to protect against the larval stage infection. Similarly, Kouguchi et al, [16]observed 74.3% protective immune effect in rats induced by Emy162 recombinant antigen of *E. multilocularis*. Such results indicate that the prevention of CE is quite feasible by a molecular vaccine. Thus, there is a need of characterizing new antigens for improving the sensitivity and specificity of CE.

Thus keeping these points in view, the aim of the study was to identify the immunogenic regionsin MD protein of *E. granulosus* which could be used for the diagnosis and subunit vaccine design.

MATERIAL AND METHODS

Retrieval of amino acid sequence

The amino acid sequence of Malate dehydrogenase protein of *E. granulosus* was retrieved from NCBI with gene ID CAF18421.1.

MNCLRKIGFVLGRSAKLFSTSTQNPQKI
AILGASGGIGQPLALLMKQSLFVSEIAL
YDIANAAGVAADLSHIETRAKVTGHTG
PDNLKAALDGAKVVIIPAGVPRKPGMT

RDDLFSMNASVVADLSRACGKYCSDA
MICIITNPVNSTVPIAAEILKKEGLYNPR
RLFGVTTLDITRSNTFIAEAKGLDVSKV
SCPVIGGHSGNTIVPVLSQCTPSVNFAQ
KAREELVARIQNAGTEVVNAKAGAGSA
TLSMAYAGALFANSLLHAMKGHADIVE
CAFVECDVAETEFFASPVLLGPNGVEK
VFGAGKLNEYEIELVKKAMPELKKSIQ
KGKEFAAAY

Figure 1: Amino acid sequence of Malate Dehydrogenase protein from *E. granulosus*

Identification of T-cell epitopes

IEDB-ANN and IEDB-SMM servers were used for the identification of peptides of HLA class-I T cell epitopes[17, 18]. The identified T cell epitopes were classified on the basis oftheir binding affinity toHLA*A02:01, using the half-maximal inhibitory concentration of a biological substance (IC_{50}) as the unit of measure. Peptides with IC_{50s} of <50 nM were classified as high-affinity binding epitopes; IC_{50s} of <500 nM were intermediate-affinity binding epitopes; and IC_{50s} of <5,000 nM were classified as low-affinity binding epitopes. Only the peptides with high binding affinity were selected for further analysis.

Identification of B cell epitopes

The Linear (continuous) B cell epitopes were identified using IEDB- analysis resource server. Several parameters like antigenicity prediction [19], beta-turn prediction [20], surface accessibility prediction [21], flexibility prediction [22], hydrophilicity prediction [23] and Bepipred Linear Epitope Prediction [24] were analyzed.

Secondary structure prediction

The secondary structure of MD protein was predicted using SOPMA server (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) [25]. Different structural parameters like helices, sheets, coils, and turns were analyzed.

3D modeling of Malate Dehydrogenase protein

The tertiary structure of malate dehydrogenase gene was predicted using I-Tasser server. A template model was obtained by submitting the Fasta sequence of the protein in the server.

Characterization of the identified peptides

The identified Band T cell epitopes were characterized by using different parameters including molecular weight, amino acid composition, theoretical pI, extinction coefficient, atomic composition, estimated half-life, aliphatic, index, instability indexand grand average of hydropathicity (GRAVY) using ExPASy ProtParam tool.

RESULTS

Identified T cell epitopes

The HLA-A 02: 01 restricted T cell epitopes were identified using IEDB-ANN and IEDB SMM servers. The criteria for selecting the promising T cell epitopes, was that it should have least IC_{50} value, should be present on the surface of the protein, should be charged and predicted by both the servers used. Based on the above mentioned criteria four T cell epitopes were identified (Table 1). The position of each predicted epitope was confirmed by visualizing on its 3D modeled protein using Pymol viewer (Figure 4c).

Identified B cell epitopes

The B cell epitopes were identified using Bepipred Linear Epitope Prediction method. Different parameters like Kolaskar&Tongaonkar Antigenicity Prediction, Chou &Fasman Beta-Turn Prediction, Emini Surface Accessibility Prediction, Karplus& Schulz Flexibility Prediction, Parker Hydrophilicity Prediction, Bepipred Linear Epitope Prediction were analyzed (Figure 2). The criteria for selecting the promising B cell epitopes was that they should fulfill the most of the above mentioned parameters and also should be hydrophobic, should have charged residues and should be present on the surface. Based on all these parameters, six promising B cell epitopes were identified (Table 1).The position of each predicted epitope was confirmed by visualizing on its 3D modeled protein using Pymol viewer (Figure 4c).

Secondary structure

In order to assess the antigenic features of the MD protein of *E. granulosus*, secondary structure was predicted using SOPMA Server. A greater proportion of extended strands and random coils present in the structure of the protein corresponded with an increased likelihood of the protein forming an antigenic epitope. The predicted secondary structure results are demonstrated in Figure 3.

3D structure prediction

The tertiary structure of the protein was predicted using I tasser server. The glyoxysomal malate dehydrogenase of *Citrulluslanatus* was identified as a template for homology modeling by I Tasser server. The server predicted 55% similarity between the MD protein of *E. granulosus* and the template identified (Figure 4a). Ramachandran plot assessment was also carried out in order to check the quality of the model prepared. 87.2% residues fall into the favourable region, 8.6% in allowed region and only 4.2% in outlier region (Figure 4b).

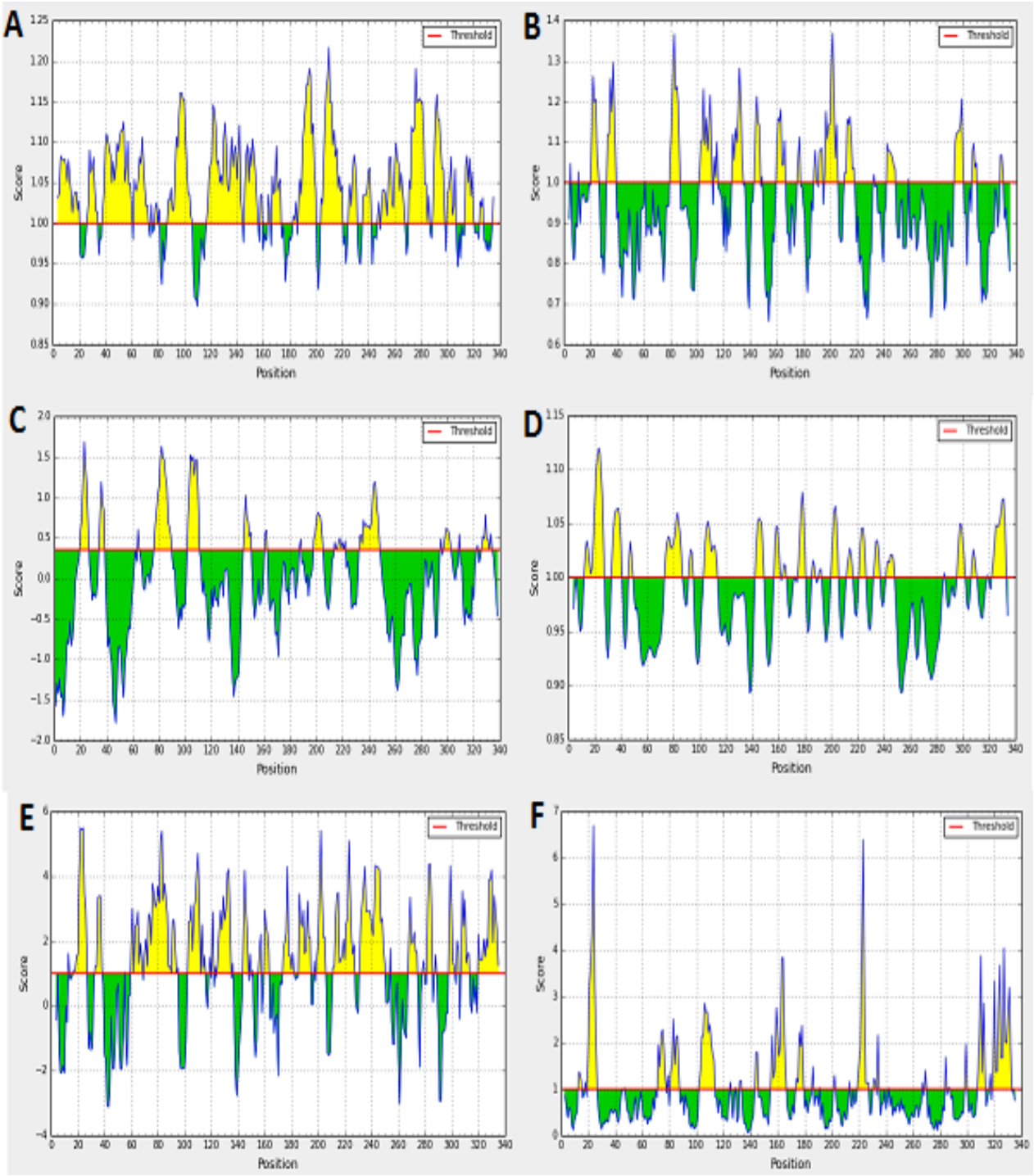

Figure 2: B cell epitope prediction usingKolaskar&Tongaonkar Antigenicity Prediction (A), Chou &Fasman Beta-Turn Prediction (B), Karplus& Schulz Flexibility Prediction (C), Parker Hydrophilicity Prediction (D), Bepipred Linear Epitope Prediction (E) andEmini Surface Accessibility Prediction (F)

Table I: Promising regions within MD protein of *E. granulosus* bearing HLA-Class I T cell epitopes and Linear B cell epitopes

Peptide	Sequence [a]	Mol. Wt.	pI	Instability Index	*Half life	Position
T1	LLMKQSLFV	1078.3	8.75	84.02	5.5 hours	43
T2	VLSQCTPSV	933.0	5.49	79.53	100 hours	209
T3	KLNEYEIEL	1150.2	4.25	61.30	1.3 hours	308
T4	ALYDIANAA	921.0	3.80	35.20	4.4 hours	55
B1	TSTQNPQ	774.7	5.19	21.20	7.2 hours	20-26
B2	GVPRKPGMTRDD	1328.5	8.75	10.25	30 hours	102-113
B3	TTLDITRSNTFIAEAKGL DVSKVS	2566.8	5.79	22.42	7.2 hours	174-194
B4	NFAQKAREEL	1205.3	6.14	26.57	1.4 hours	218-227
B5	NAGTEVVNAKAGAGSA	1416.5	6.00	10.80	1.4 hours	233-248
B6	LKKSIQKGKEF	1305.5	10.00	-14.06	5.5 hours	324-335

***Half-life in mammalian reticulocytes, in vitro**

a- Non polar residues are shown in upper case, polar residues in lower case and charged residues are depicted in bold

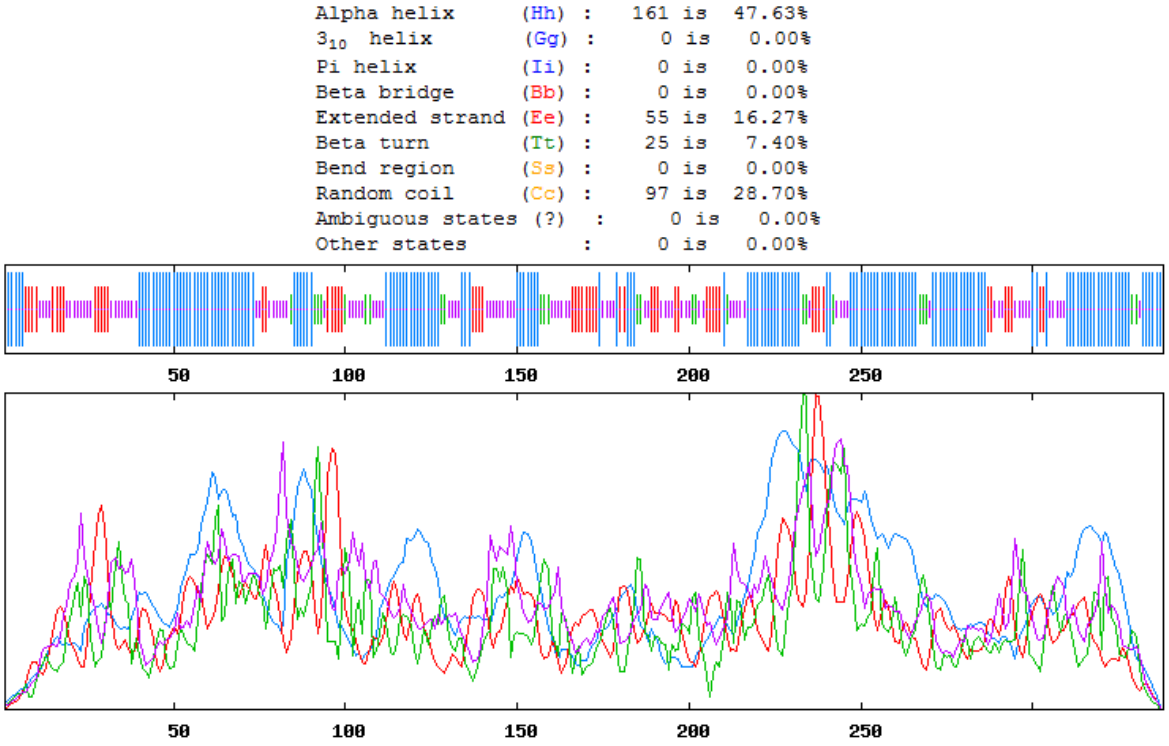

Figure 3: Secondary structure prediction results using SOPMA server. Lines in different colors represent different secondary structures: Blue, α helix; green, β turn; red, extended strand; and purple, random coil.

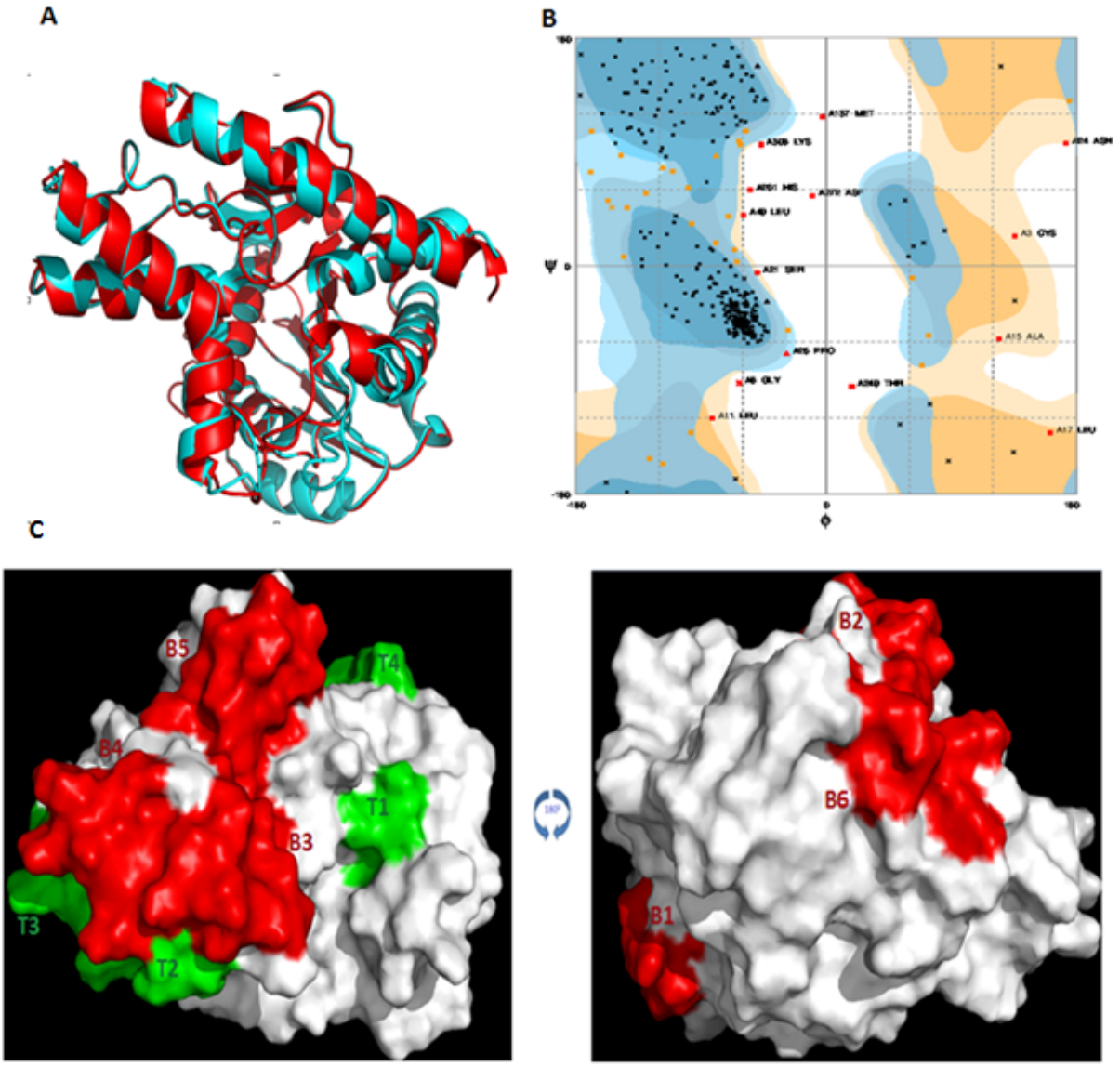

Figure 4:A- showing homology modeling between MD protein of *E. granulosus* andtemplate 1SEV. B- Ramachandran Plot of the model prepared. C- Surface view of the MD protein showing the location of identified T and Linear B cell epitopes. B cell epitopes are shown red in color and T cell epitopes are shown green in color. All the identified epitopes are located on the surface of the protein.

DISCUSSION

During infection, ourimmune system reacts to a number of foreign antigens, for which T and B cells are crucial for generating an efficient immune response. The identification and use of immunodominant peptides/epitopes can be potentially employed as vaccine as they couldhelp in efficient priming of the host immune system,as the immune response is always generated by exposure of such regions.With the accelerating growth of bioinformatics techniques, an immunologist can analyze and identify the immunogenic sites in a protein sequence with potential binding sites for B and T cells, which in turn could lead to the

development of new vaccines. Molecular docking is a key structure-based method of immunoinformatics and has proved to be a rapid and accurate method for evaluating peptide binding to MHCs[26].

The highly immunogenic nature of Malate dehydrogenase protein has already been discussed previously against number of parasitic infections like *Toxoplasma gondii*[27],*Trypanosomacruzi*[28], *Schistosoma mansoni*[29], *Mycobacterium tuberculosis*[30]and *Leishmania* species [31], thus suggesting its potentiality in diagnosis and vaccine development. Keeping these points under consideration, the present study was designed to find the Cytotoxic T cell and B cell epitopes of MD protein of *E. granulosus*using several *in-silico* tools which could develop the adaptive and humoral immunity in host in response to the antigenic protein. First of all, the secondary structure of the protein was predicted in order to obtain the antigenic features of the protein. The primary factors involved in an epitope formation like hydrophilicity, antigenicity, flexibility, the exposed surface area were analyzed. The tertiary structureis a three-dimensional conformation of the naturally folded protein formed by further coiling and folding. It was a useful supplement to the prediction of the MD epitopes.

The criterion for choosing the most promising epitopes was based on the combined results of all the identification servers used, as well as IC_{50} value<50 nM, should be present on the surface of the protein and should have polar and hydrophobic residues. The identified epitopes were found to possess various degrees of polar and non-polar residues, thus implying high solvent accessibility of the predicted peptides. The location of the identified epitopes was viewed on the 3-D modeled template of MD protein.

In conclusion, the following study led to the identification of potential immunogenic epitopes present in the MD protein of *E. granulosus* which should further be tested for their immunoreactivity using *in vitro* and *in vivo* approaches to support the *in-silico* findings that may have an enhanced safety and efficacy.

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