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Research Article

ACTION OF GLYCOTOPE VACCINES ON CANCER CELL LINES - MOLECULAR MODELING AND SIMULATION STUDIES

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ABSTRACT

Melanoma is the most deadly type of skin cancer. Carbohydrate antigens or glycotopes such as GM2,GD2 and GD3 which are structurally diverse are present on the surface of cells in the body. Certain glycotopes are overexpressed during melanoma. The glycotope vaccines GM2,GD2 and were modeled along with suitable adjuvants like Bacillus Calmette Guerin, Detox and QS-21.The dynamic interaction in these potent vaccines were analysed by molecular modeling and simulation and trajectory analysis. Immunoinformatic studies of these vaccines were carried out and hence increasing the survival rate of cancer patients. Among Gm2, Gd2 and Gd3, Gm2 vaccine is most preferred for increasing the survival rate of the melanoma patients.

Keywords: Melanoma, Vaccine, Immunoinformatic

INTRODUCTION

Melanoma is the leading cause of death from skin cancer in industrialized countries. Failure to detect certain clinical and histological parameters such as ulceration, and lymph node status will lead to the gradual progression of melanoma [1]. T-cell activation mechanisms have played a vital role for developments in melanoma research. The presence of carbohydrate antigens on the surface of melanoma cells evades the immune surveillance [2]. These carbohydrate antigens are targets for immune recognition and killing. These carbohydrate antigens help in suppressing the growth of melanoma cells [3].

Protective immunity humoral and cellular responses could be obtained by using carbohydrate antigens as vaccines. Its challenging to develop vaccines for melanoma as it is difficult to break the body's immunological tolerance to antigen [4].

Carbohydrate conjugate vaccines have consistently induced the highest titre IgM and IgG antibodies against tumour cells expressing these antigens. Conjugate vaccine consists of glycoprotein and an immunogenic carrier protein [5].

Vaccination with *Bacillus Calmette-Guérin* (BCG), Detox and Quillaja saponaria-21 as adjuvant induces IgM and IgG antibodies to GM2, GD2 and GD3 [6].

MATERIALS AND METHODS

Extraction of gangliosides

The chicken brain was removed carefully from a dead chicken after half an hour. The brain was put in saline to remove the blood remains .It was again re-suspended in saline to sterilize. The brain was then made into a mixture using motor and pestle. The brain extract was then transferred to a centrifuge tube and to it was added the solvent mixture of methanol and chloroform in the ratio 5:5. The mixture was kept for centrifugation at 5000 rpm for 15 minutes. The organic supernatant was removed and the pellet was re-suspended in the chloroform –methanol solvent and centrifuged for 15 minutes. The supernatant was removed. The pellet was taken and again re-suspended in chloroform-methanol solvent. The supernatant of all the three centrifuges were collected and kept for storage at -4^{o}C.

Thin Layer Chromatography (TLC)

The solvent system Resorcinol- HCl of 100 ml was prepared. The chromatography chamber was kept for saturation. The supernatant was spotted on the baseline of the silica plate. The sample was run for half an hour. The distance travelled by the carbohydrate antigens were observed.

Serological assay of Carbohydrate antigens

The Carbohydrate antigens separated by thin layer chromatography were subjected to serological assay. Mouse induced with melanoma cell lines was injected with Carbohydrate antigens. The IgG and IgM antibodies produced where observed after two week interval.

Immunological adjuvants

Bacillus Calmette Guerin (BCG) derived from cell wall of Mycobacterium *tuberculosis* is a mixture of Phosphatidylinositol dimannoside (PIM2) and Phosphatidylmyoinsitol (PIM6). Ethylenediaminetetraacetic acid (EDTA) was used as detoxifying agent to remove harmful chemicals from the bloodstream. Quillaja Saponaria-21 (QS-21) is a saponin which is extracted from the plant Quillaja saponaria which has been proved to be effective against cancer [7].

I-tasser

The fasta sequence of Keyhole Limpet Hemocyanin (Fig 1) from Megathura *crenulata* whose structure is not predicted was submitted to I-tasser. It consists of 231 amino acids.

Submitted Sequence

```
>protein
DFGHSKKIRKNVHSLTAEEQNSLRRAMDDLQDDKTRGGFQQIAAFHGEPKWCPRPEAEKK
FACCVHGMAVFPHWHRLLTVQGENALRKHGFTGGLPYWDWTRPMSALPHFVADPTYDDSV
SSLEEDNPYSHGHIDSVGHDTTRAVRDDLYQSPGFGHYTDIAKQVLLALEQDDFCDFEVQ
FEIAHNSIHALVGGNEPYGMSTLEYFLYDPIFFLHHSNTDRLWAIWQALQKYRGKPYNTA
NCAIVRHDTYRKPLQPFGLDSVINPDDETREHSVPRDVFNYKDDFNYEYESLNFNGLSIA
QLDRELQRIKSHDRVFAGFLLHEIGQSALVKFYVCKHHVSDCDHYAGEFYILGDEAEMPF
AYDRVYKYEISQALHDLDLHVGDNFHLKYEAFNLNGGSLGGVDLSQPSVIFEPAAGSHTA
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Fig. 1: Fasta sequence of Keyhole Limpet Hemocyanin

RESULT AND DISCUSSIONS

Fourier transform infrared spectroscopy subtraction result

The supernatant obtained was given for FT-IR analysis (Fig 2). The carbohydrate antigens were observed at 725 to 1086 cm^{-1} wavenumber and able to detect the presence of GM2, GD2 and GD3 respectively (Table I).

Separation of Carbohydrate Antigens by Thin Layer Chromatography

It was observed that Gd3 travelled the greatest distance from baseline followed Gd2 and Gm2. The refractive Index of Gd3 was the highest.

Serological Assay Result

The purified carbohydrate antigens mixture was given for immunological assay melanoma infected mouse was used for the assay. It was observed that IgG and IgM production was higher in Gm2 compared to Gd2 and Gd3 (Table II).

Modeling of Components of Vaccine

The components of vaccine GM2 (Fig3), GD2 (Fig4), GD3 (Fig 5), BCG- Phosphatidylinositol dimannoside (PIM2) (Fig 6) and Phosphatidyl myoinositol (PIM6) (Fig 7), EDTA (Fig 8), QS-21 (Fig 9) were modeled using Chemsketch.GM2 consists of Glucose, Galactose, N-Acetylneuraminic Acid and N Acetylgalactosamine. GD2 consists of Glucose, Galactose, two N-Acetylneuraminic Acid and N-Acetylgalactosamine. GD3 consists of Glucose, Galactose and two N-Acetylneuraminic Acid. BCG contains two components PIM2 and PIM6. PIM6 contains two palmitoyl sidechains .QS-21 contains galactose, glucose, xylose, rhamose and fucose. EDTA structure was obtained from pubchem and modelled in Chemsketch. The KLH model was obtained from I-TASSER in which the sequence of KLH was submitted, the model obtained was viewed in VEGA ZZ. The Chou-Fasman Secondary Structure prediction tool help to predict the secondary structures helix, sheet, turn and coil (Fig 13) present in the KLH protein sequence. PDB structure of KLH was predicted by I-tasser. The protein is displayed in tube form using software Vega ZZ (Fig 14).

The Pymol software helped to visualize the secondary structures present in the modeled structure of KLH (Fig 15). The carbohydrate antigens GM2, GD2 and GD2 were modified [9]. They were subjected to ozonolysis and NaHBr was added (Fig 10, 11, 12) so as to allow the KLH sequence for binding with the carbohydrate antigen.

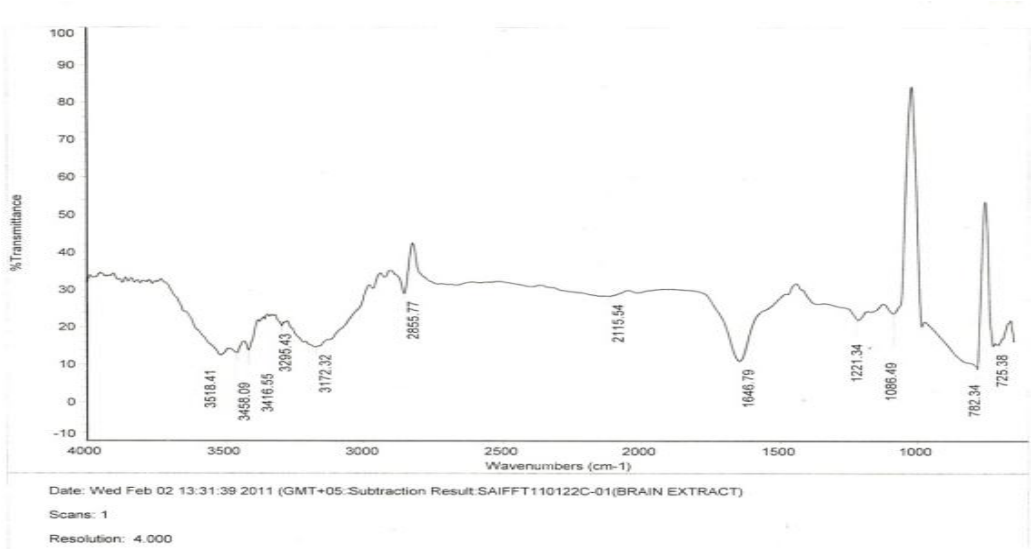

Fig. 2: FT-IR analysis of supernatant

Table I: Carbohydrate antigens preset from 725 – 1086 cm^{-1}

S. No.	Wavenumber (cm-1)	Component
1	3518.41	N-alkalene
2	3458.09	N-alkalene
3	3416.55	N-alkalene
4	3295.43	N-alkalene
5	3172.32	N-alkalene
6	2855.77	N-alkalene
7	2115.54	N-alkane
8	1646.79	Secondary Nitro alkanes
9	1221.34	Alkyl nitrates
10	1086.49	Gd3
11	782.34	Gd2
12	725.38	Gm2

Table II: Immunological assay of Gm2, Gd2 and Gd3

S. No.	Component	Before Immunization		After Immunization	
		IgG	IgM	IgG	IgM
1	Gm2	10	0	80	10
2	Gd2	6	0	30	5
3	Gd3	8	0	40	7

Modeled structure of GM2

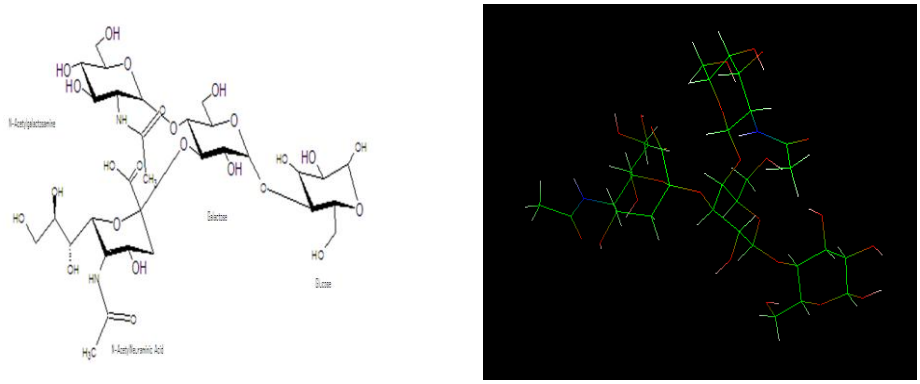

Fig. 3: Gm2 modeled in ChemSketch

Modeled structure of GD2

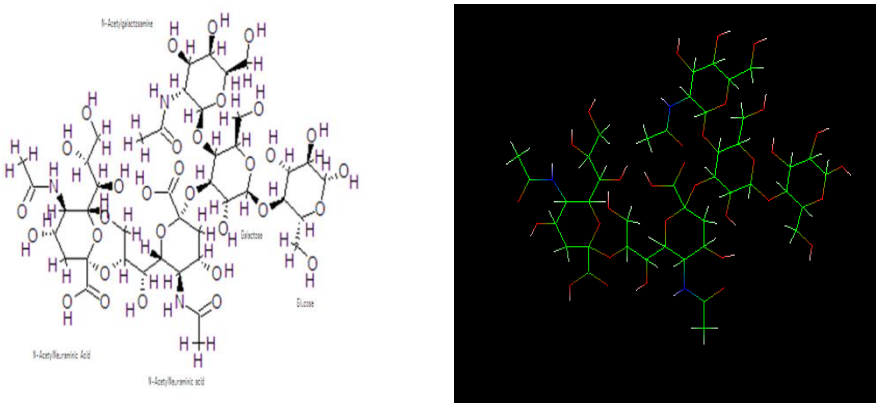

Fig. 4: Gd2 modeled in ChemSketch

Modeled Structure of GD3

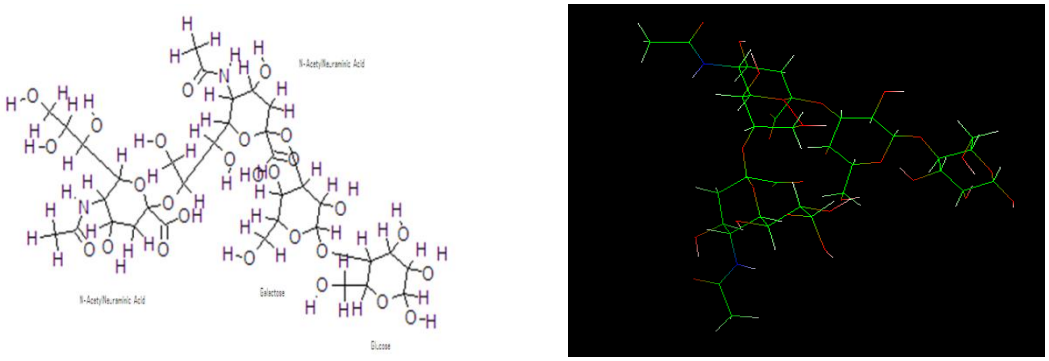

Fig. 5: Gd3 modeled in ChemSketch

Modeled Structure of Phosphatidylinositol dimannoside (PIM2)

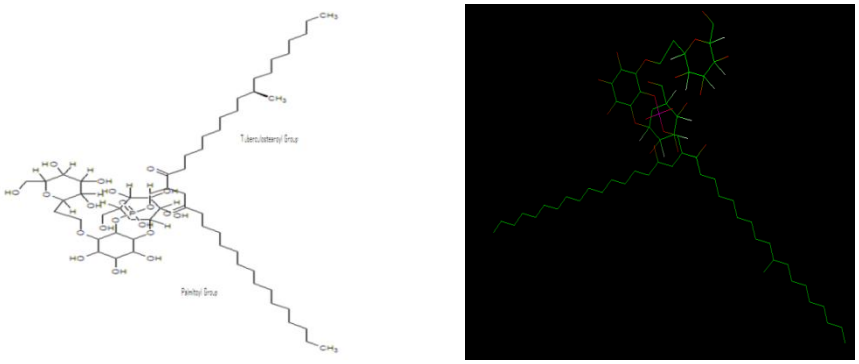

Fig. 6: PIM2 modeled in ChemSketch

Modeled Structure of Phosphatidyl myoinositol (PIM6)

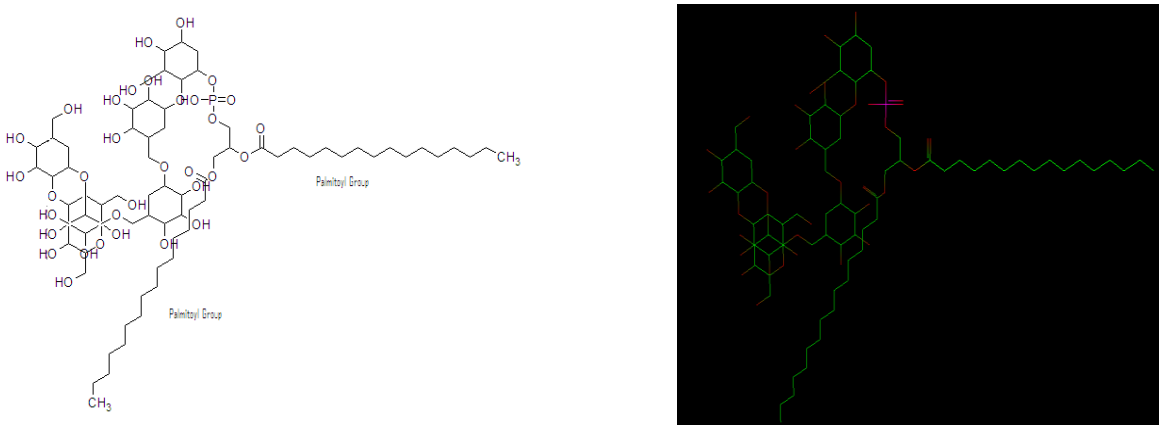

Fig. 7: PIM6 modeled in ChemSketch

Modeled Structure of Ethylenediaminetetra acetic acid (EDTA)

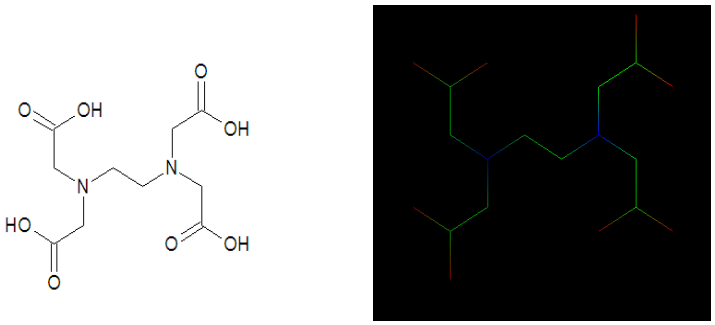

Fig. 8: EDTA modeled in ChemSketch

Modeled Structure of Quillaja saponaria-21 (QS-21)

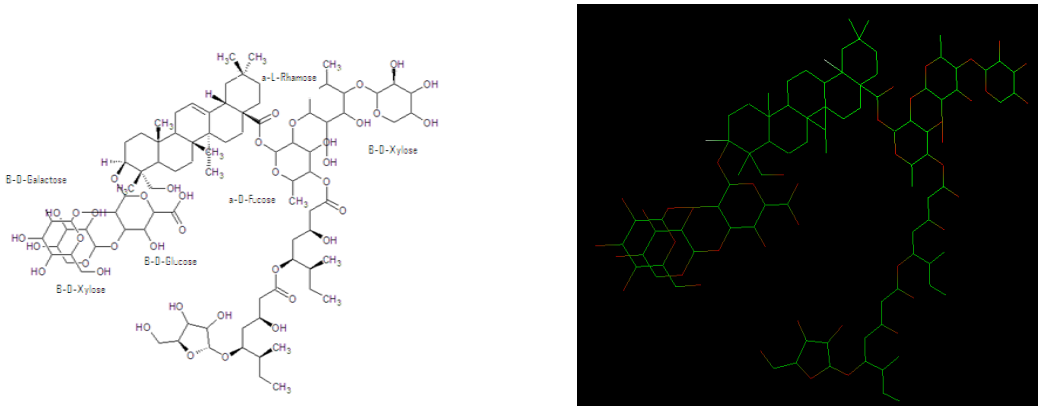

Fig. 9: QS-21 modeled in ChemSketch

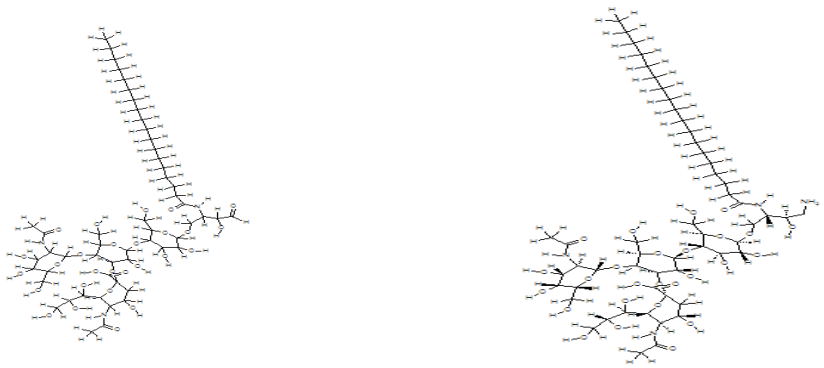

Fig. 10: Gm2 after ozonolysis and adding NaHBr

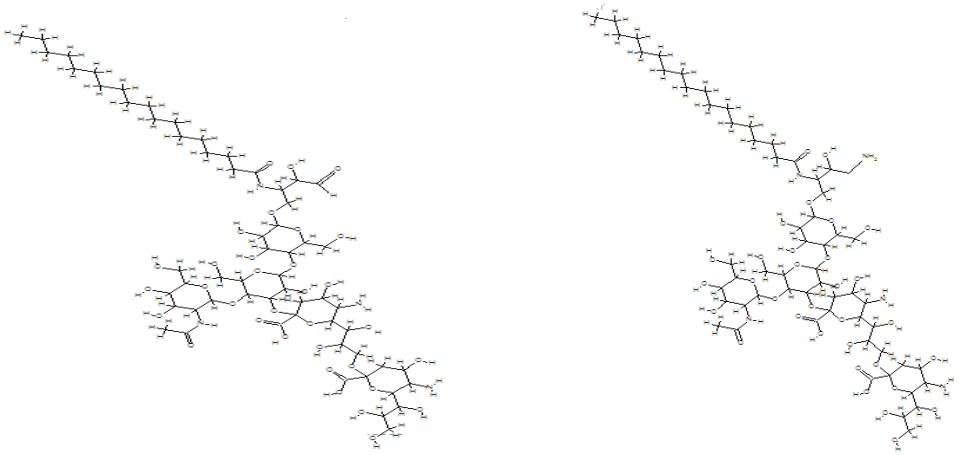

Fig. 11: Gd2 after ozonolysis and adding NaHBr

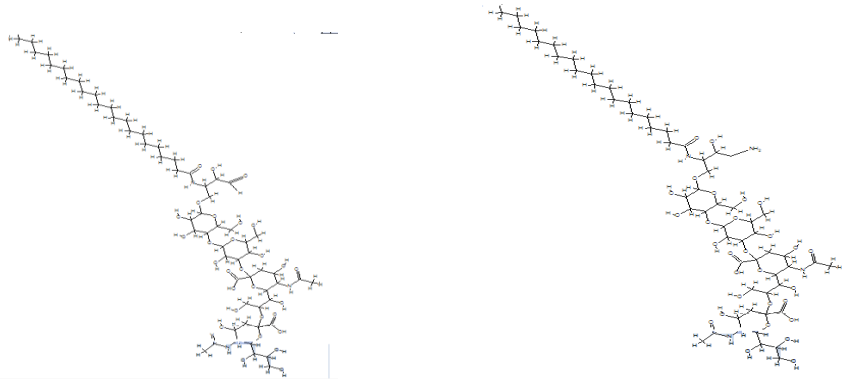

Fig. 12: Gd3 after ozonolysis and adding NaHBr

Chou-Fasman Secondary Structure Prediction (CFSSP)

Target Sequence:

```
DFGHSKKIRK NVHSLTAEEQ NSLRRAMDDL QDDKTRGGFQ QIAAFHGEPK WCPRPEAEKK FACCVHGMAV
FPHWHRLLTV QGENALRKHG FTGGLPYWDW TRPMSALPHF VADPTYDDSV SSLEEDNPYS HGHIDSVGHD
TTRAVRDDLY QSPGFGHYTD IAKQVLLALE QDDFCDFEVQ FEIAHNSIHA LVGGNEPYGM STLEYFLYDP
IFFLHHSNTD RLWAIWQALQ KYRGKPYNTA NCAIVRHDTY RKPLQPFGLD SVINPDDETR EHSVPRDVFN
YKDDFNYEYE SLNFNGLSIA QLDRELQRIK SHDRVFAGFL LHEIGQSALV KFYVCKHHVS DCDHYAGEFY
ILGDEAEMPF AYDRVYKYEI SQALHDLDLH VGDNFHLKYE AFNLNGGSLG GVDLSQPSVI FEPAAGSHTA
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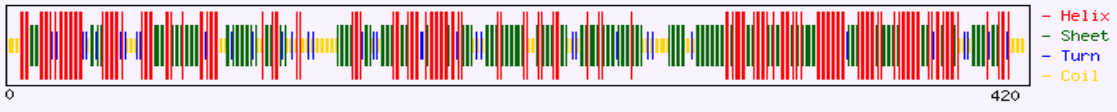

Fig. 13: Chou-Fasman Secondary Structure Prediction (CFSSP) of KLH sequence

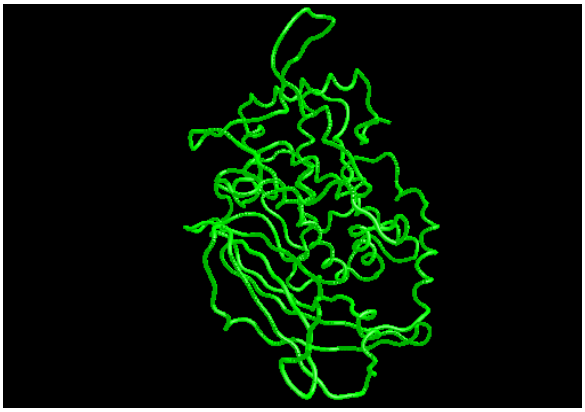

Fig. 14: Best Model Predicted by I-tasser of KLH

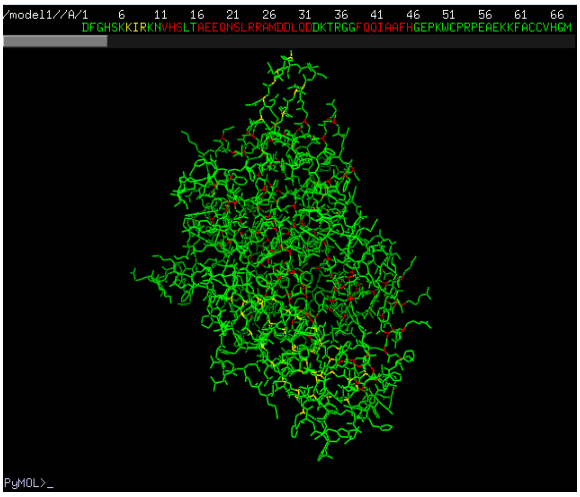

Fig. 15: KLH viewed in Pymol

Result of I-tasser viewed in Pymol. The different colors depict the secondary structures. The green color depicts the loop, red represents helix and yellow color represents sheet.

Result of Molecular Mechanics and Energy Minimization of GM2 using Amber

The carbohydrate antigens were subjected to simulation and energy minimization [8] using Molecular Mechanics Software AMBER. The carbohydrate antigens were run for 20000 Ps and the phi-psi angle of N-acetylgalactosamine was observed for all three antigens. Gm2 showed the least energy hence most stable.GNU plot of N-acetylgalactosamine in Gm2 after energy minimization was plotted (Fig 16).

Result of Molecular Mechanics and Energy minimization using Schrodinger

Gnu plot of the components of vaccine EDTA, PIM2, PIM6, EDTA, QS-21 and KLH were obtained for Preconditioned Conjugate Gradient and Truncated Newton Conjugate Gradient (Fig 17-25). The summary of Energy minimization is given in Table III.

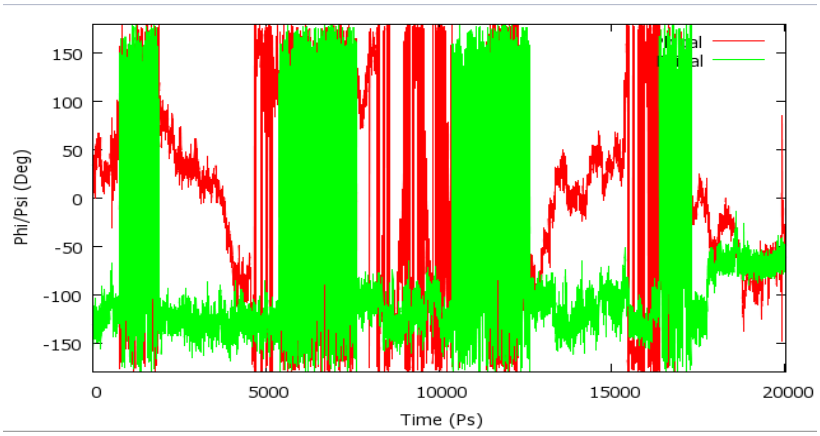

Fig. 16: Phi-psi angle of N-acetylgalactosamine in GM2 after energy minimization using AMBER

Energy Minimization of EDTA

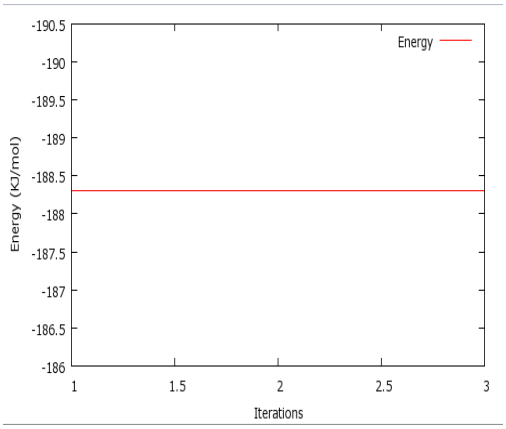

Fig. 17: Gnu plot of Energy versus iteration for Truncated Newton Conjugate Gradient for EDTA

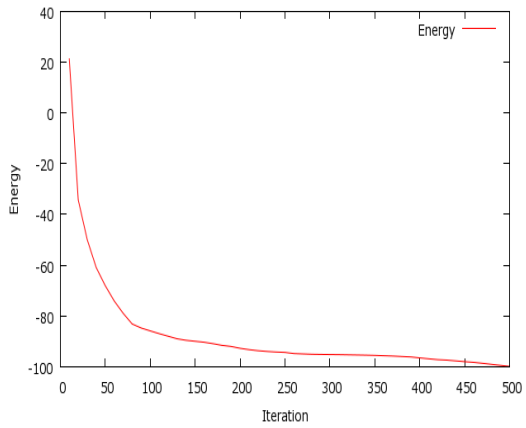

Fig. 18: Energy minimization of phosphatidyl dimanoinositol using preconditioned conjugate gradient

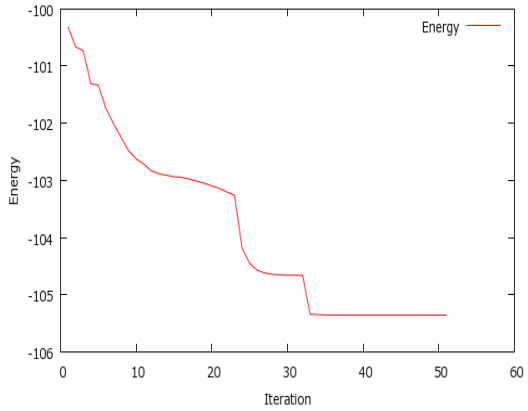

Fig. 19: Energy minimization of phosphatidyl dimannoinositol using Truncated Newton Conjugate Gradient

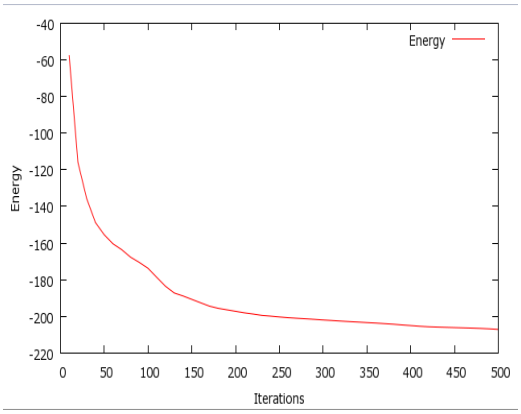

Fig. 20: Energy minimization of phosphatidyl myoinositol using preconditioned conjugate gradient

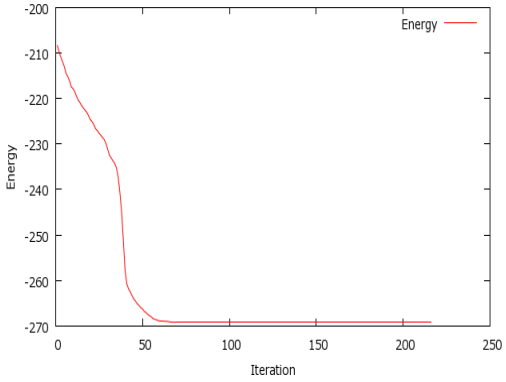

Fig. 21: Energy Minimization of phosphatidyl myoinositol using Truncated Newton Conjugate Gradient

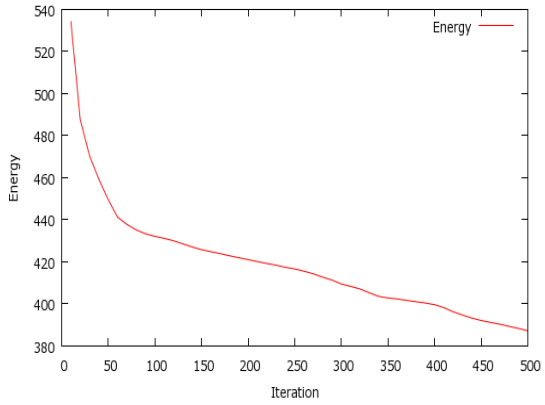

Fig. 22: Energy Minimization of Quillaja saponaria-21 using Preconditioned Conjuagte Gradient

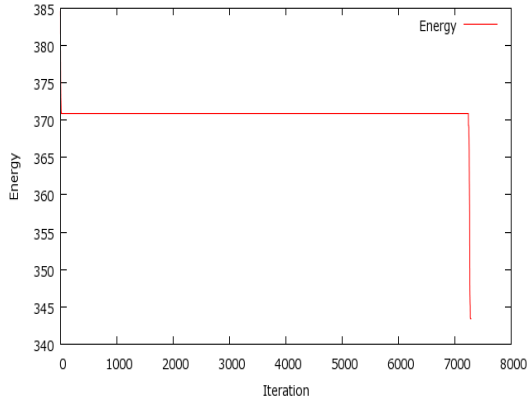

Fig. 23: Energy Minimization of QS-21 using Truncated Newton Conjugate Gradient

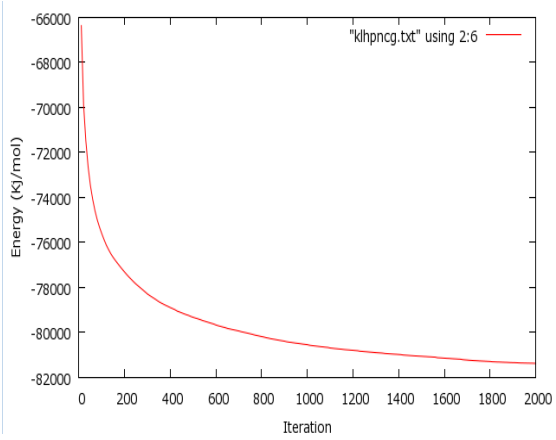

Fig. 24: Energy minimization of Keyhole Limpet Hemocyanin using preconditioned conjugate gradient

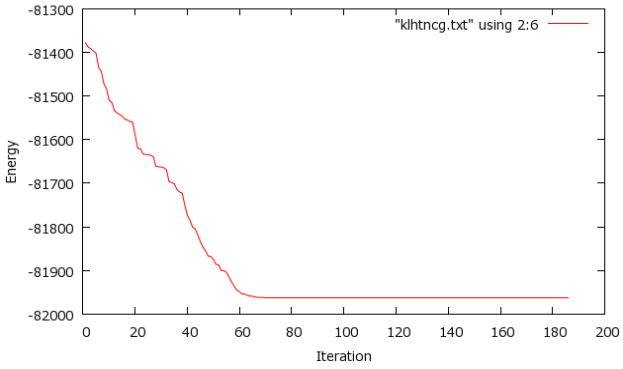

Fig. 25: Energy minimization of Keyhole Limpet Hemocyanin using Truncated Newton Conjugate Gradient

Table III: Summary of Energy Minimization

S. No.	Component	Energy of Pre-conditioned Conjugate Gradient (KJ/mol)	Energy of Truncated Newton Conjugate Gradient (KJ/mol)
1	Phosphatidyl dimannoinositol (PIM2)	-105.0924	-110.5226
2	Phosphatidyl myoinositol (PIM6)	-212.6250	-272.3168
3	Ethylene Diamine Tetraacetic acid	-188.7511	-188.7511
4	Quillaja saponaria-21	383.3672	339.3981
5	Keyhole Limpet Hemocyanin (KLH)	-81368.4922	-81949.4063

Result of Docking of adjuvants against akt3 protein using Schrodinger

The protein akt3 present in homosapien responsible for melanoma was analysed from PDB sum. It was found that the A chain of the protein was where the ligand showed strong interaction. All the other chains were removed and only the chain A was taken into consideration for Glide. The Glide Score from Schrodinger of Extra Precision (Table 4) and Standard Precision (Table 5) for the vaccine components were obtained.

Table IV: Glide Score from Schrodinger of Extra Precision

S. No.	Component	Glide Score (KJ/mol)
1	Gm2	-2.824136
2	Gd3	-2.162340
3	Gd2	-2.019457
4	Gm2+BCG	-2.003083
5	Gm2+Detox	-3.260576
6	Gm2+QS-21	-9.386866
7	Gd3+BCG	-1.341287
8	Gd3+Detox	-2.598780
9	Gd3+QS-21	-8.726070
10	Gd2+BCG	-1.198304
11	Gd2+Detox	-2.455897
12	Gd2+QS-21	-8.583300

Table V: Glide score of Standard Precision

S. No.	Component	Glide Score (KJ/mol)
1	Gm2	-3.376672
2	Gd3	-3.327986
3	Gd2	-3.206453
4	Gm2+BCG	-0.691614
5	Gm2+Detox	-5.858930
6	Gm2+QS-21	-6.940349
7	Gd3+BCG	-0.642928
8	Gd3+Detox	-5.810244
9	Gd3+QS-21	-6.891663
10	Gd2+BCG	-0.521395
11	Gd2+Detox	-5.688711
12	Gd2+QS-21	-6.770130

CONCLUSION

Gangliosides were extracted from chicken brain and its presence was confirmed by Fourier Transform Infrared Spectroscopy. Serological assay was carried out and Gm2 was found to produce maximum IgG antibody in mouse induced with melanoma cell line. The different components of the glycotope vaccine were sketched using chemsketch. Simulation was carried out for the carbohydrate antigens using Assisted Model Building Energy Refinement. The model structure for the carrier protein was obtained from I-tasser. The energy minimization of the individual components was depicted in Gnuplot. Docking was carried out for different components using Schrodinger. The wet lab and the in- silico result were compared. The glycotope vaccine containing Gm2 carbohydrate was proven to have high response in serological assay and the least docking score from glide. Hence among Gm2, Gd2 and Gd3, Gm2 vaccine is most preferred for increasing the survival rate of the melanoma patients.

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