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Molecular Simulation of GABA(A) Receptor to Study of Effects on Nervous Stimulants Inhibitory & Blood Pressure; A Nano Molecular Modeling of GABARAP

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GABA is the most distributed inhibitory neurotransmitter that used by 25-50% of all synapses. Most of the physiological functions of GABA are provided by GABAA receptors which it is a member of the ionotropic receptor family.The purpose of the current study on the energy levels of GABA receptor was to further analyze the effects of molecular structure stability from biophysical point of view. In this study, we worked on the Monte Carlo method with AMBER, BIO+ and OPLS force fields. kinetic energy, potential energy and total energy in 295, 298,305, 310and 315 Kelvin temperatures were used for computation. For kinetic energy ,total energy and potential energy in 310 K temperature(the body normal temperature)amount of energy decreased. This can be interpreted that the molecule is in its most stable condition. The results showed that the chemical structure of GABA is stable in body temperature, so it can be used for proper drug designing.

Key words: GABA receptors, Drug design , Blood pressure, Molecular mechanics, Nervous Stimulants.

In the vertebrate central nervous system (CNS), the most distributed inhibitory neurotransmitter is γ -aminobutyric acid (GABA).1 GABA is used as transmitter by 25-50% of all synapses[2].

Neurons are affected by GABA through a large number of receptor subtypes which are categorized according to their pharmacological characteristics in two major groups of receptors: The ionotropic receptor family and metabotropic receptor family. The aforementioned class is divided into two subfamilies, $GABA_A$ and $GABA_C$ receptors according to their competence of the functional heteromeric and homomeric receptor formation and physiological and pharmacological differences. The second major class of receptors are G protein coupled receptors ($GABA_B$ receptors) which act via second messengers coupled receptors. The molecular diversity of these ligand gated ion channels indicates significant challenges for scientists to design subunit-specific therapeutic agents[3-5].

Most of the physiological functions of GABA are provided by $GABA_A$ receptors[2].

The $GABA_A$ receptor family seemingly is the most complex in terms of the chemical diversity

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of agents interacting with this family and of the protein subunits building these heteromeric receptors[6].

Currently 18 $GABA_AR$ subunits have been identified. Based on sequence homology, these are divided into seven subunit classes[7]. Pharmacological properties of a particular GABA receptor is determined by the subunit combination as previously reported[6].

This theoretical study on the energy levels of $GABA_A$ receptor was done to further analyze the effects of its molecular structure stability from biophysical point of view.

MATERIALS AND METHODS

The first structure of Crystal structure of the GABA(A)-receptor was received from site PDB with PDB ID: 1KJT, that containing 119 amino acid. This protein Classification is Transport Protein. Gene Names is Gabarap.We examine different models for this protein that the results were announced in Table 2. This table have been reported compares the main model with other six models based on RMSD(*figure 2*) and residues(*figure 3*). Features comparison of the three models are in Table 2. The models are noted in Table 1.

Table 1. The structure of the models

PDB ID	Description
1kjtA	Crystal Structure of the GABA(A) Receptor Associated Protein, GABARAP
3dowA	Complex structure of GABA type A receptor associated protein and its binding epitope on calreticulin
3d32B	Complex of GABA(A) receptor-associated protein (GABARAP) with a synthetic peptide
1kotA	Solution Structure of Human GABA Receptor Associated Protein GABARAP
1km7A	Solution Structure and Backbone Dynamics of GABARAP, GABAA Receptor Associated Protein
1klvA	Solution Structure and Backbone Dynamics of GABARAP, GABAA Receptor associated protein
1gnuA	GABA(A) RECEPTOR ASSOCIATED PROTEIN GABARAP

Table 2. Results of Superposition

RMSD of Models	total RMSD	RMSD of final subset
1kjtA to 3dowA	1.3 Å - 114 residues	0.8 Å - 111/114 residues
3d32B to 3dowA	1.3 Å - 116 residues	0.9 Å - 112/116 residues
1kotA to 3dowA	1.9 Å - 117 residues	1.2 Å - 109/117 residues
1km7A to 3dowA	2.4 Å - 100 residues	1.4 Å - 92/100 residues
1klvA to 3dowA	3.2 Å - 100 residues	1.3 Å - 86/100 residues
1gnuA to 3dowA	1.6 Å - 117 residues	0.9 Å - 110/117 residues

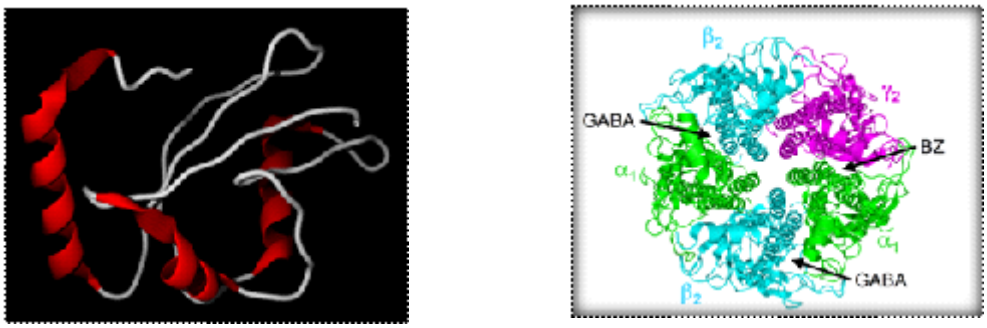

Fig. 1. Crystal Structure of the GABA(A) Receptor Associated Protein, GABARAP

In this study, changes in $GABA_A$ energy levels are discussed according to thermodynamic temperature scale of forces. GABA molecular structure was used to determine thermodynamic terms. First, the structure was optimized by geometric optimization order. Simulation was carried out by Monte Carlo method by means of Chem Office software (Chem3D and Chem Draw) and Hyperchem. AMBER, BIO$^+$ and OPLS were the chosen force fields used in current study. Molecular Mechanics calculations were assessed by Monte Carlo method[9-10].

Three important energy parameters – kinetic energy, potential energy and total energy- in five different simulating temperatures (295, 298,305, 310and 315 Kelvin) were used for computation[16].

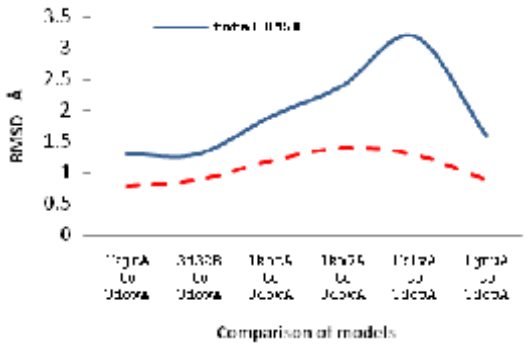

Fig. 2. RMSD of final subset and total RMSD

RESULTS AND DISCUSSION

GABA is the most distributed neurotransmitter in CNS[1]. Most of the physiological actions of GABA are generated via $GABA_A$ receptors[2].

Most $GABA_A$ receptors in the CNS are thought to contain both ± and ² subunits, with one or more of the ³, ´, or µ subunits. Theoretically evaluation of thermodynamic characteristics of this molecule could end to better cognition of its role in biology and to design proper drugs which cause less harm to patients suffering from diseases in which this receptor is involved.

In this study HyperChem software was used to computate desired data. The reason for using this software was its sophisticated and appropriate molecular modeling environment also is known as its flexibility and quality[10].

It has been known that atoms are held together by forces. Function of biological systems arises from interaction of bonds between atoms. In this regard finding the lowest energy level is favorable because, in this case, the molecule is in the most stable condition[11-12].

In current study AMBER, BIO$^+$ and OPLS force fields were chosen. When GABA protein is modeled, it enfaces shaking, rotating, stretching, and so on. The total potential energy is the sum of previously mentioned states according to the force fields. AMBER force field has great application for proteins. It assigns all conformational energies and treats with hydrogen bond energy, and torsion

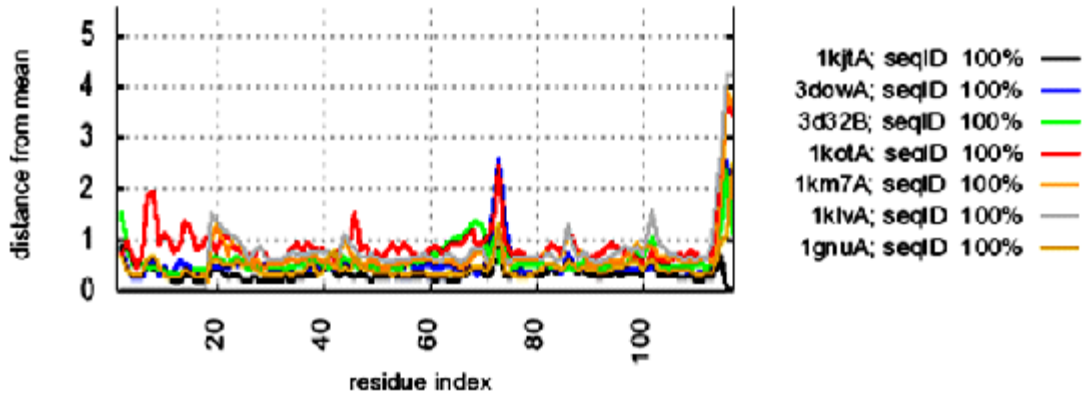

Fig. 3. Local (per residue) deviation of individual models/structures from mean of the ensemble of models/structures based on a distance RMSD (dRMSD)

Table 3. Kinetic energy for AMBER, BIO+, OPLS force fields

Method	**AMBER**					**BIO⁺**					**OPLS**				
Time (PS)	**295k**	**298k**	**305k**	**310k**	**315k**	**295k**	**298k**	**305k**	**310k**	**315K**	**295K**	**298K**	**305K**	**310K**	**315K**
1	1830.77	1823.86	1892.83	1849.388	1954.89	1830.77	1823.86	1892.83	1849.388	1954.89	1246.893	1304.75	1283.706	1254.244	1325.795
5	1830.77	1823.86	1892.83	1849.388	1954.89	1830.77	1823.86	1892.83	1849.388	1954.89	1246.893	1304.75	1283.706	1254.244	1325.795
10	1830.77	1823.86	1892.83	1849.388	1954.89	1830.77	1823.86	1892.83	1849.388	1954.89	1246.893	1304.75	1283.706	1254.244	1325.795
15	1830.77	1823.86	1892.83	1849.388	1954.89	1830.77	1823.86	1892.83	1849.388	1954.89	1246.893	1304.75	1283.706	1254.244	1325.795
20	1830.77	1823.86	1892.83	1849.388	1954.89	1830.77	1823.86	1892.83	1849.388	1954.89	1246.893	1304.75	1283.706	1254.244	1325.795

Table 4. Potential energy calculated by AMBER, BIO+, OPLS methods

Method	AMBER					BIO⁺					OPLS				
Time (PS)	295k	298k	305k	310k	315k	295k	298k	305k	310k	315K	295K	298K	305K	310K	315K
1	4094.199	4064.316	4690.365	4365.592	4506.701	5231.364	5631.932	5712.46	4651.51	5309.451	315.3339	294.378	959.4532	950.3374	743.5843
5	2352.68	2615.413	3244.871	2432.521	2864.665	3854.467	3522.51	3309.607	3846.795	3657.468	-2021.337	-1099.904	-2166.447	-2091.206	-1357.597
10	1932.895	2027.949	2370.871	1953.584	2102.484	3140.709	2727.798	2731.362	3065.882	2855.35	-2656.609	-2273.275	-2920.637	-3054.665	-2817.061
15	1645.846	1714.441	1818.167	1676.552	1730.593	2570.85	2510.539	2426.607	2561.849	2556.34	-3098.395	-3249.931	-3324.384	-3396.877	-3290.425
20	1470.115	1569.326	1571.457	1529.925	1562.39	2243.2	2314.012	2253.942	2299.889	2321.822	-3299.52	-3532.279	-3534.737	-3611.171	-3537.326

Table 5. Total energy computed by AMBER, BIO+, OPLS methods

Method	AMBER					BIO⁺					OPLS				
Time (PS)	295k	298k	305k	310k	315k	295k	298k	305k	310k	315K	295K	298K	305K	310K	315K
1	5924.969	5988.176	6583.195	6214.98	6461.59	7062.134	7555.792	7605.289	6500.898	7264.341	1562.227	2048.335	2243.159	1548.622	2276.132
5	4183.45	4539.272	5137.701	4281.909	4819.555	5685.237	5446.37	5202.436	5696.183	5612.358	-774.4438	-52.8462	-882.7406	154.3403	-765.4112
10	3763.665	3951.809	4263.701	3802.972	4057.374	4971.479	4651.658	4624.192	4915.27	4810.24	-1409.716	-1512.311	-1636.931	-1019.031	-1728.87
15	3476.616	3638.301	3710.997	3525.94	3685.483	4401.62	4434.399	4319.437	4411.237	4511.23	-1851.502	-1985.674	-2040.678	-1995.687	-2071.083
20	3300.885	3493.186	3464.287	3411.778	3484.814	4073.969	4223.748	4146.772	4163.399	4276.712	-2052.627	-2232.576	-2251.031	-2278.035	-2285.376

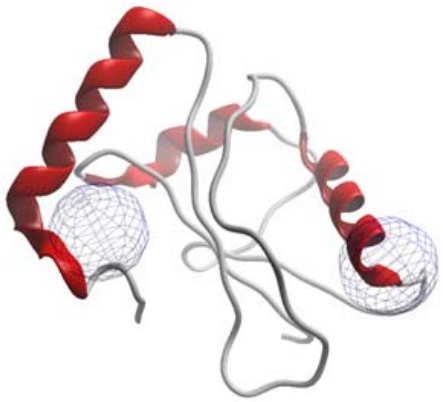

Fig. 4. Crystal Structure of the GABA(A) Receptor & electrostatic Sites(charges)

term.[13]Like AMBER, OPLS is designed for computation of proteins and nucleic acids. In this force field bonded potentials are similar to AMBER and its non-bonded potentials involves vander waals and electrostatics. BIO^{+} filed is an extended form of CHARMM. Similar to AMBER and OPLS it has been designed to study macromolecules[14-15].

GABA protein was simulated in mentioned force fields in 5 different temperatures (295K, 298K, 305K, 310K and 315K). Based on results gathered in table 3 and 5 , calculated energy levels in three different force fields for kinetic and total energy increased when the temperature being elevated. This is the pattern observed till the 310K. In this temperature the amount of energy decreased

Table 6. Single point parameters

Parameters Force field	Electrostatic Fm^{-1}	Vdw $J{\cdot}m^3$ $(kmol^2)^{-1}$	Dihedral $Kcalmol^{-1}$	Angle Kcal (mol per radian^2)$^{-1}$	Bond Kcal (mol perA°^{2})$^{-1}$	Gradient $Kcalmol^{-1}$ Ang^{-1}	Total Energy $Kcalmol^{-1}$
Amber	-1159.48	4102.99	1033.23	524.392	880.457	521.751154	177899.3675
Bio	-1200.65	5160.98	420.234	593.07	853.137	500.268451	157224.7643
Opls	-4857.36	5065.02	174.79	580.641	657.366	631.865037	173637.7985
mm+	-938.662	176.27	211.888	921.271	2208.72	47.916315	2520.360152

in comparison to surrounding temperatures (305K and 315K). 310K is body normal temperature in which all the molecules must be optimized. It can be concluded that, the molecule is stable in this temperature. Table 4 exhibited the potential energy levels calculated in this study. When the molecule has less potential energy, it is less common that it participates in chemical reactions. This can be interpreted that the molecule is in its most stable condition. As it was shown although there is a slight increase in the amount of energy level in this table, a fallen could have been observed in 310K.

CONCLUSION

GABA is the most frequent neurotransmitter exists in vertebrates' CNS. As its inhibitory role in CNS, it can be used as a goal for drug designing. In this study the energy levels were calculated theoretically. The results revealed that the chemical structure of GABA is stable in body temperature. Further studies are recommended to use these data for this structure in association with drug molecules affecting GABA.

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