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In Silico Structure Prediction, Analysis and Energy Calculation of Retinol Binding Protein7 (RBP7) in *Coturnix coturnix japonica* (japanese quail)

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Abstract

The biologically dynamic natural occurring retinoid (retinol and its metabolites, vitamin A) is intermediated by extracellular, intracellular, and nuclear proteins in *Coturnix japonica* or japenese quail. Retinoids mainly transmit by two medium such as Plasma retinoid binding protein (RBP) and Epididymal retinoic acid binding protein (ERABP) carries retinoid in body fluid while cellular retinol binding proteins (CRBPs) and cellular retinoic acid binding proteins(CRABPs) carry retinoids within cells. Accurate description of amino acids of RBP7 is very important to consider when prediction of structure and calculation of energy take place. The aim of the study is prediction the structure of RBP7 in *Coturnix japonica* using Bioinformatics tools and software approach. Ramachandran plot of the φ, ψ values for the amino acids in a RBP7 protein. Using this computational approach the total energy of RBP7 in *Coturnix japonica* is 1047.341 KJ/mol. And total minimum energy is 1026.898 KJ/mol in *Cotunix japonica* or japenese quail. It is also observed that repeating energy trends at each of the molecular, functional group, and atomic levels. Retinols and retinoic acid play essential characters in variation of gene expression and overall growth of embryo in *Coturnix japonica*. This makes RBP7 more significant in terms of expression of adipose tissues in *Coturnix japonica* (japenese quail) because RBP7 works as a novel adipose-specific gene.

Keywords: Retinol; RBP7; Adipose tissue; P value; Computational energy

Introduction

Retinol binding proteins (RBPs) are a family of carriers for fat-soluble retinol (preformed vitamin A). Vitamin A is a significant nutrient that performs a vital role in vision, cell growth and differentiation, and embryonic growth in the *Coturnix japonica* or japenese quail [1]. Retinol binding protein (RBP7) is one of the cellular retinol binding proteins that play a role in cellular metabolism of retinol. Vitamin A is consumed from dietary sources as a retinyl ester or synthesized from β-carotene and is deposited in the liver as a retinyl ester up to it is mobilized for transmission into numerous target tissues. Retinol is one of the forms of vitamin A found from foods of animal origin. Retinal (retinaldehyde), the aldehyde derived from retinol, is essential for vision, while retinoic acid is essential for skin health and bone growth in *Coturnix japonica*. These chemical compounds are mutually recognized as retinoids, having the same structural motif means having all-trans double bonds found in retinol. Structurally, all retinoids possess a β-ionone ring and a polyunsaturated side chain containing an alcohol, an aldehyde, a carboxylic acid group or an ester group [1]. Also the RBP7 contains controlling components for adipose-specific expression. The RBP7 has a novel adipose tissue-specific promoter. RBP7 is recognizing as an adipose-specific gene in the *Coturnix japonica* or japenese quail in adipose tissue under the control of RBP7 promoter expression in adipose tissue. The RBP7 promoter may stimulate expression very strongly in the neck and abdominal adipose tissue of *Coturnix japonica* or japenese quail [2]. Retinoids mostly bind in two ways such as Plasma retinol binding protein (RBP) and Epididymal retinoic acid binding protein (ERABP) carries retinoid in body fluids, while cellular retinol binding proteins (CRBPs) and cellular retinoic acid- binding proteins (CRABPs) carry retinoids within cells [3]. RBP, ERABP, CRBPs i.e. CRBP I, II, III, and IV and CRABPs i.e. CRABP I and CRABP II belong to the lipocalins superfamily in the Structural Classification of Proteins (SCOP) database. The RBP and ERABP belong to their retinol-binding protein-like (RBP) family. CRBPs and CRABPs belong to the fatty acid-binding protein-like (FABP) family [4]. RBP is the specific carrier for retinol (vitamin A alcohol) in the blood [5]. RBP7 plays a key role in terms of development of growth of body weight depends upon the connective tissues known as adipose tissue. The location of an increasing number of new adipose-specific genes has expressively contributed to understand of adipose tissue biology and the etiology of obesity and its related diseases in *Coturnix japonica*. Comparison of gene expression profiles among various tissues performed by analysis of chicken microarray data, leading to identification of RBP7 as a novel adipose-specific gene in Japanese quail. Adipose-specific expression of RBP7 in the avian species was further confirmed at the protein and mRNA levels [2]. The amount of adipose tissue describes the total energy of body, responsible for the metabolism of the body. In this study we are going to predict and analyse the structure of Retinol Binding Protein 7 (RBP7) and calculate the energy of RBP7 in *Coturnix japonica*. The position of residues and the residues radius is predicted in Pymol- v1.8.6.0.tar. The energy calculation and the minimum energy calculation of Retinol Binding Protein 7 (RBP7) calculated in Swiss PDB Viewer4.1.0. The Ramachandran Plot assessment is under the analysis in which the φ, ψ values for the amino acids of RBP7 is study under Molprobity.

Material and Methods

Collection of the data using PUBMED

Collecting the data for structure prediction of protein present in

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Coturnix japonica and retrieving of the sequence from NCBI of retinol binding protein 7 *Coturnix japonica* (Table 1).

RaptorX

A web-based method using RaptorX *(http://raptorx.uchicago.edu/)* for protein secondary structure prediction, template based tertiary structure modelling, alignment quality assessment and probabilistic alignment sampling. Raptor X web server delivers high-quality structural models for many targets with only remote templates. Because of this Computational modelling of the three-dimensional atomic arrangement of the amino acid chain is also possible in determining the role of the protein in biological processes [6].

Swiss PDB Viewer4.1.0

Swiss-PdbViewer is an application method that runs a user friendly interface allowing analysing proteins. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain in form of graphic and menu interface. Moreover, Swiss-PdbViewer is tightly linked to Swiss-Model, an automated homology modelling server accessible from ExPASy [7].

Pymol- v1.8.6.0.tar

PyMOL is an open-source tool to visualize molecules. It runs on Windows, Linux and MacOS equally well. PyMOL has competences in creating high-quality images from 3D structure; it has well established purposes for influencing structures and some elementary functions to evaluate their chemical properties. The opportunities to write scripts and plugins as well as to integrate PyMOL in custom software are vast and superior to most other programs. PyMOL has been written mostly in the Python language (www.python.org), while the time-critical parts of the system have been coded in C. This way, Python programs intermingle most effortlessly with the PyMOL GUI [8].

Ramachandran assessment by Mol probity

A Ramachandran plot is a visual graphic demonstration of the main-chain conformational tendencies of an amino acid. Ramachandran plot based on experimental data is deciding whether scant data represent genuine conformations. Measured the pair-wise distances of main-chain conformational tendencies among amino acids, and showed the conformational relationships of amino acids are well preserved in a two-dimensional map, leading to the conclusion that the conformational diversity space of amino acids is largely two dimensional. Amino acids in early and late evolutionary phases are situated in different zones in the two-dimensional map [9]. The first is a Ramachandran plot or Ramachandran map, which is simply a scatter plot of the φ,ψ values for the amino acids in a single protein structure or a set of protein structures. It may be restricted to a single amino acid type and/or a single structural feature type, such as protein loops. The second is a Ramachandran distribution, a statistical representation of Ramachandran data, usually in the form of a probability density function. A probability density function gives the probability of finding an amino acid conformation in a specific range of φ,ψ values. For instance, if the function is given on a 10° × 10° grid from −180° to +180° in φ,ψ (1296 values), then the distribution may give the probability per 10° × 10° region. It could also be expressed per degree squared or per radian squared. Such distributions may be derived for specific amino acid types and/or for specific structural features. There are numerous significant considerations in developing Ramachandran distributions from structural data, depending on the purpose of the derived distribution. First, while glycine and proline are usually treated separately, the other 18 amino acids are often treated as a single type. However, these amino acids are moderately different in their proportions of residues in the α, β, polyproline II, and left-handed helical regions. Second, relatively different distributions are resolute when either all residues are used or only those outside the regular secondary structures of α-helices and β-sheets. The concluding assumed to be "intrinsic" favourites of the backbone, not subjective by forming specific hydrogen bonds present in regular secondary structures. Third, the quality and quantity of the data are crucial in determining distributions meant to act as eminence filters for newly determined structures or for structure prediction. As more structures have become available at higher resolutions, it is now possible to use quite large datasets with resolution cut-offs of 1.8 Å or even better. Other filters have been used including B-factors and steric clashes to remove residues that may be model improperly or at least with considerable uncertainty within the electron density. For instance, by using higher resolution structures, B-factors, steric overlaps able to determine Ramachandran distributions with smaller "allowed" and "generously allowed" regions than previous efforts. Fourth, most previous efforts have involved density estimation using simple histogram methods – the counts or proportion of counts of residues in non-overlapping square bins of the φ,ψ space. However, even when a large number of proteins are used, the distribution in φ,ψ space may be quite uneven (Figure 1) [10-12].

GenBank	AKC91103.1
LOCUS	AKC91103
DEFINITION	retinol binding protein 7 [Coturnix japonica]
DBSOURCE	accession KP026122.1
PUBMED	25867079
FASTA	>AKC91103.1 retinol binding protein 7 [Coturnix japonica] MPVDFSGTWNLVSNDNFEGYMTALGIDFATRKIAKMLK-PQKVIKQDGDSFSIHTTSTFRDYMLQFKIGEEFEEDNKGLD-NRKCKSLVTWDNDKLICVQAGEKKNRGWTHWLEGDDLHLELR-CENQVCKQVFKRA

Table 1: Collection of the data using PUBMED.

Results

>AKC91103.1 retinol binding protein 7 [*Coturnix japonica*]

MPVDFSGTWNLVSNDNFEGYMTALGIDFATRKIAKMLK-PQKVIKQDGDSFSIHTTSTFRDYMLQFKIGEEFEEDNKGLD-NRKCKSLVTWDNDKLICVQAGEKKNRGWTHWLEGDDLHLEL-RCENQVCKQVFKRA

GDT uSeqID: 92 101 (raptorX)

SeqID ModelName Template(s): 75 6at8A-279444_1 6at8A

Rank P-value Score uGDT: 9.0e-12 158 123 (Figures 2-5).

Discussion

The total energy of retinol binding protein 7 (RBP7) residues in *Coturnix japonica* or japenese quail is 1047.341 KJ/mol by computational calculation. The calculation is based on position of amino acids, bonds, angles, torsion, improper, non-bonded and total energy of amino acids making a retinol binding protein7 (RBP7) in japenese quail (Supplementary Data). The energy minimization of amino acids of RBP7 is also calculated by Swiss Pdb Viewer. In which the minimum energy of Bonds is 936.766 KJ/mol, angles 900.843 KJ/mol, torsion 582.304 KJ/mol, improper 236.255 KJ/mol, nonbonded -1629.27 KJ/mol, and total minimum energy is 1026.898 KJ/mol in japenese quail. This energy is responsible for the threshold value to express the adipose gene and other significant functions in japenese

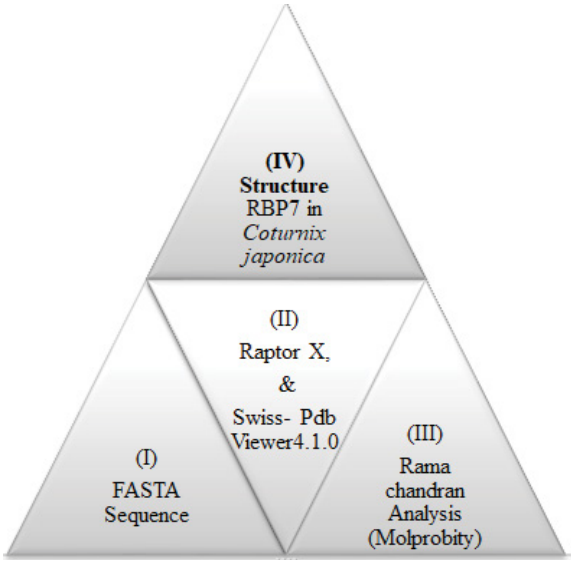

Figure 1: Graphical representation of RBP7 structure prediction.

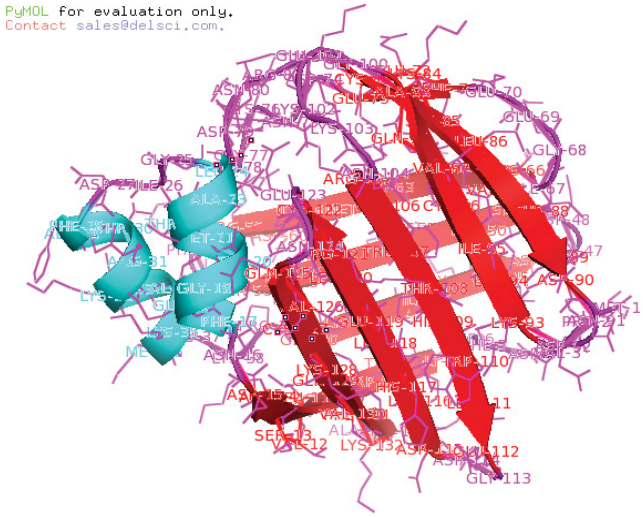

Figure 3: Position of Residues of Retinol binding protein 7 in *Coturnix japonica* using Pymolv-1.8.6.0.

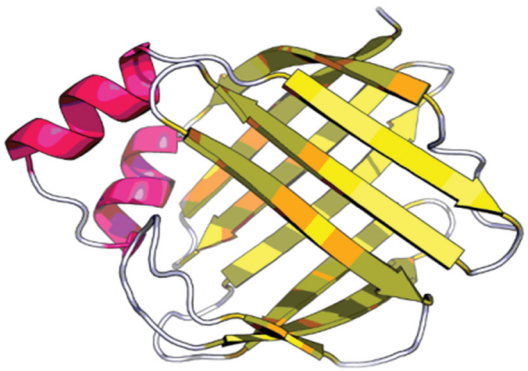

Figure 2: Retinol Binding Protein 7(RBP7) in *Coturnix japonica* using RaptorX.

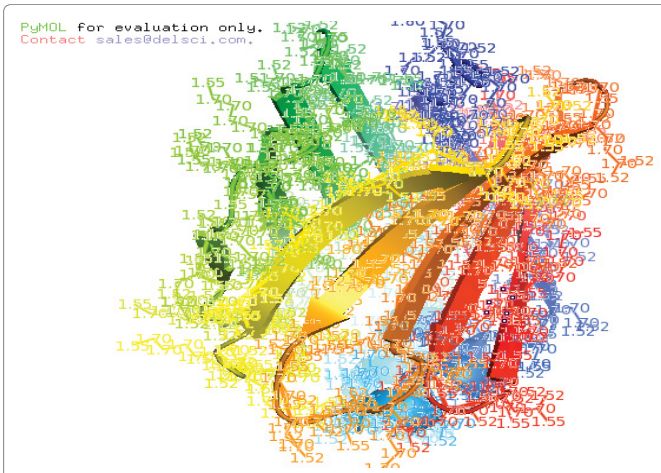

Figure 4: Value of radius of residues of Retinol Binding Protein 7 in *Coturnix japonica* using Pymolv-1.8.6.0.

quail. This RBP7 is responsible for major adipose tissue depositions. This expression is balanced with the help of energy. A scatter plot of the φ,ψ values for the amino acids of RBP7 is also analyse. An statistical representation in the form of a probability density function. A probability density function gives the probability of finding an amino acid conformation in a specific range of φ,ψ values. First glycine and proline are usually treated separately; the other 18 amino acids are often treated as a single type. However, these amino acids are moderately different in their proportions of residues in the α, β, polyproline II, and left-handed helical regions. The "intrinsic" favourites of the backbone, not particular by forming specific hydrogen bonds present in regular secondary structures. 97.0% (128/132) of all residues are in favoured (98%) regions. 99.2% (131/132) of all residues were allowed (>99.8%) regions. One outliers (phi, psi) are 2 PRO (-48.1, -72.3) in validation. The position of amino acids, the Position of residues of amino acids and values of bonds, torsion, improper non bonded and total energy calculation of amino acid of RBP7 in *Coturnix japonica* or japense quail is given below:

Position of amino acids:

1) ATOM 1-8,162-169,275-282,488-495: MET (M).
2) ATOM 9-15,300-306: PRO (P).
3) ATOM 16-22,89-95,325-331, 694-700,777-783,1020-1026,1051-1057: VAL (V)
4) ATOM 23-30,110-117,202-209,358-365,370-377,468-475,592-599,629-636,722-729, 738-745,918-933: ASP (D).
5) ATOM 31-41, 126-136,210-220,384-394,446-456,513-523,563-573,1058-1068: PHE (F)
6) ATOM 42-47, 96-101, 378-383,395-400,433-438,680-685: SER (S).
7) ATOM 48-51,146-149,190-193,366-369,541-544,617-620,798-801,848-851,914-917: GLY (G)
8) ATOM 52-58,170-176,226-232,419-432,439-445,701-707,866-872: THR (T)
9) ATOM 59-72, 708-721,852-865,883-896: TRP (W)
10) ATOM 73-80,102-109,118-125,600-607,637-644,730-737,829-836, 1003-1010: ASN (N).

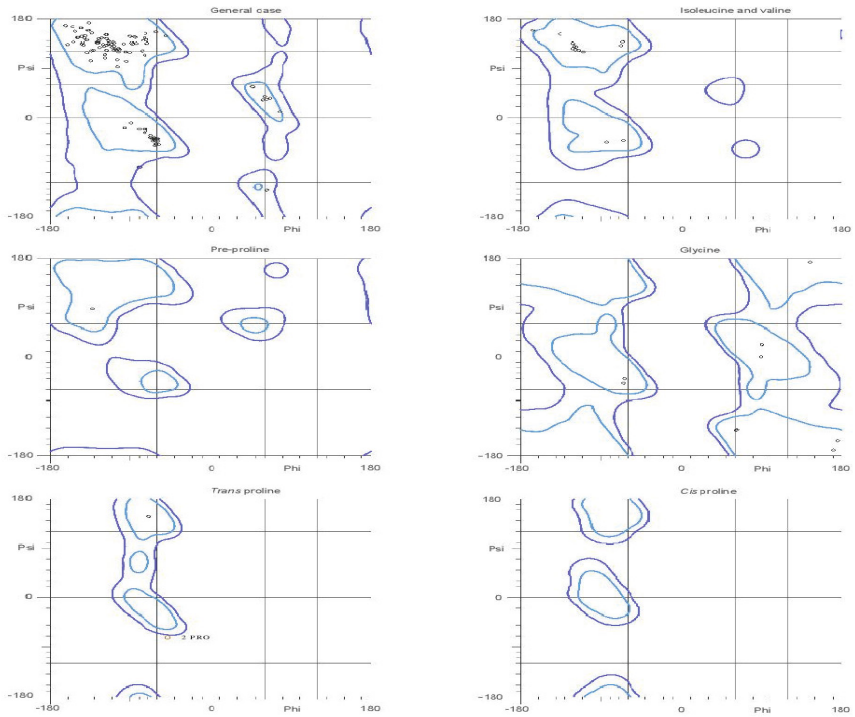

Figure 5: Ramachandran Validation using Mol probily (Vincent B. Chen, W. Bryan Arendall III, Jeffrey J. Headd, Daniel A. Keedy).

RESIDUE		BONDS	ANGLES	TORSION	IMPROPER	NON BONDED	TOTAL
HHT	1	0.000	6.183	7.540	0.000	0.00	13.723
MET	1	7.296	3.333	2.724	0.022	8.09	21.468
PRO	2	6.565	12.317	20.186	11.450	21.70	72.215
VAL	3	7.270	3.375	1.965	0.838	27.28	40.731
ASP	4	4.771	2.227	5.765	1.099	-13.76	0.102
PHE	5	8.323	32.732	2.806	1.078	-25.62	19.322
SER	6	5.114	2.579	3.516	0.241	-18.71	-7.256
GLY	7	5.482	0.405	4.287	1.071	-19.55	-8.306
THR	8	7.386	6.107	4.024	0.209	-24.29	-6.566
TRP	9	24.976	23.874	2.988	7.766	-73.82	-14.216
ASN	10	5.586	3.972	4.228	0.464	-12.09	2.165
LEU	11	4.231	2.096	3.378	1.491	-39.38	-28.182
VAL	12	5.771	6.866	3.376	0.088	-26.27	42.375
SER	13	5.722	2.982	2.872	3.588	-6.86	8.305
ASN	14	7.915	4.480	4.547	3.109	-6.89	13.159
ASP	15	6.024	1.910	0.852	0.705	-18.72	-9.227
ANS	16	5.853	5.626	4.034	0.041	-8.57	6.988
PHE	17	10.332	20.871	2.774	4.272	-25.14	13.112
GLU	18	3.910	6.056	10.853	0.737	-20.73	0.829
GLY	19	3.001	1.041	0.701	1.393	-4.15	1.984
TYR	20	15.231	17.400	1.699	1.258	-32.75	2.834
MET	21	6.828	6.069	2.752	0.894	-39.30	-22.760
THR	22	5.286	2.676	1.776	1.344	24.88	35.962
ALA	23	4.217	1.226	0.468	0.702	-13.39	-6.780
LEU	24	3.890	6.267	1.568	1.124	5.37	18.221
GLY	25	3.451	1.368	2.275	2.873	-4.70	5.262
ILE	26	5.528	2.656	1.097	0.024	55.15	64.458
ASP	27	4.607	5.057	2.678	0.294	3.96	16.599

PHE	28	9.725	18.917	1.770	0.387	-14.78	16.014
ALA	29	4.900	1.783	1.176	1.544	-17.93	-8.525
THR	30	6.896	4.229	0.736	1.205	47.90	60.968
ARG	31	8.268	4.476	6.385	5.537	-30.25	-5.578
LYSH	32	4.380	3.930	4.225	0.693	-17.19	-3.965
ILE	33	6.211	3.609	3.300	0.977	23.25	37.349
ALA	34	3.990	2.007	1.014	1.784	-18.24	-9.447
LYSH	35	4.501	3.532	15.586	0.708	-23.76	0.562
MET	36	7.633	4.483	4.063	0.329	-17.17	-0.657
LEU	37	6.858	5.961	1.665	3.860	1.49	19.838
LYSH	38	6.188	3.957	13.304	1.012	-10.85	13.616
PRO	39	5.646	9.607	22.938	8.381	-14.55	32.022
GLN	40	7.695	4.844	2.318	0.067	-31.93	-17.011
LYSH	41	5.064	8.255	6.318	0.681	-37.22	-16.898
VAL	42	6.913	4.608	2.145	2.177	-17.98	-2.136
ILE	43	6.504	3.330	2.900	2.009	-18.75	-4.006
LYSH	44	5.339	6.054	9.682	0.016	-24.65	-3.562
GLN	45	7.322	6.213	5.264	2.663	-27.38	-5.918
ASP	46	5.416	5.243	7.427	0.114	-8.39	9.814
GLY	47	5.789	1.464	1.116	2.361	-6.30	4.431
ASP	48	5.366	5.073	2.209	0.399	-11.23	1.822
SER	49	4.160	3.674	2.254	0.029	-26.56	-16.447
PHE	50	10.991	18.356	3.992	1.047	-32.70	1.684
SER	51	5.794	6.275	5.026	0.007	-30.51	-13.413
ILE	52	5.004	6.635	3.377	0.495	1.22	16.736
HISB	53	21.754	7.937	1.825	3.255	-45.95	-11.178
THR	54	6.559	5.468	2.582	1.660	16.08	32.352
THR	55	7.702	3.890	2.376	3.924	-18.25	-0.358
SER	56	5.795	4.962	3.653	0.044	-11.11	3.348
THR	57	7.488	2.060	0.596	0.769	15.76	26.670
PHE	58	10.144	18.864	3.846	1.743	-2.63	31.963
ARG	59	10.171	5.888	6.764	6.758	-22.74	6.846
ASP	60	6.042	9.749	3.808	0.272	-19.12	0.749
TYR	61	15.974	27.566	2.572	5.928	-17.52	34.524
MET	62	8.640	5.609	4.029	2.822	-15.54	5.565
LEU	63	5.650	7.812	2.980	1.122	17.27	34.837
GLN	64	7.007	9.276	1.983	0.242	16.58	35.091
PHE	65	10.180	13.054	2.676	3.425	-29.85	-0.518
LYSH	66	5.382	11.110	12.383	0.300	-32.37	-3.254
ILE	67	6.707	4.048	2.720	2.505	12.81	28.786
GLY	68	4.195	2.051	4.285	0.827	-7.76	3.598
GLU	69	4.520	5.361	4.690	3.014	-23.91	-6.322
GLU	70	4.962	6.122	10.216	0.009	-19.53	1.775
PHE	71	10.204	18.300	2.852	2.839	-34.50	0.307
GLU	72	5.411	4.198	7.649	0.702	3.62	21.577
GLU	73	4.415	6.818	3.591	0.045	-24.69	-9.818
ASP	74	3.903	6.312	1.655	0.678	-12.15	0.396
ASN	75	5.666	4.992	3.402	0.137	-1.88	12.314
LYSH	76	3.830	4.171	2.269	2.262	-11.97	0.560
GLY	77	6.458	3.370	1.602	3.910	-5.21	10.130
LEU	78	4.209	8.083	1.976	1.988	36.69	52.947
ASP	79	3.778	9.140	3.573	0.890	-9.26	8.124
ASN	80	6.339	5.021	4.962	0.256	-0.75	15.828
ARG	81	8.597	5.038	7.620	6.895	-19.65	8.499
LYSH	82	5.402	5.370	2.643	0.185	-0.65	12.951
CYSH	83	5.140	1.563	8.269	1.353	-39.28	-22.956
LYSH	84	4.386	10.958	12.507	0.118	-17.09	10.878

SER	85	5.629	2.922	0.962	3.584	-35.69	-22.597
LEU	86	4.778	2.800	4.555	1.291	13.17	26.594
VAL	87	5.481	3.135	1.631	0.876	16.35	27.473
THR	88	6.745	3.925	2.956	0.024	19.75	33.396
TRP	89	27.762	23.674	4.615	7.036	33.69	96.779
ASP	90	7.013	7.770	7.100	1.816	-1.19	22.508
ASN	91	6.568	6.337	3.725	4.281	13.44	34.355
ASP	92	4.723	9.464	1.739	0.218	-12.39	3.751
LYSH	93	4.465	7.499	4.049	2.735	-37.86	-19.109
LEU	94	5.270	4.738	4.318	1.206	8.41	23.944
ILE	95	6.487	3.362	2.454	1.908	-7.17	7.042
CYSH	96	4.664	1.670	1.494	0.120	-32.51	-24.567
VAL	97	5.524	4.379	3.431	0.123	3.07	16.530
GLN	98	6.259	3.091	2.808	1.585	-39.86	-26.166
ALA	99	4.831	1.931	3.354	1.029	-13.82	-2.679
GLY	100	4.760	0.576	0.214	1.199	-7.70	-0.950
GLU	101	4.198	5.449	7.631	0.176	14.13	31.583
LYSH	102	5.752	2.952	2.073	1.215	-34.73	-22.735
LYSH	103	5.353	1.832	4.907	0.700	-14.64	-1.851
ASN	104	6.317	4.923	7.288	0.376	-7.44	11.464
ARG	105	9.666	8.563	13.063	6.657	-30.77	7.180
GLY	106	4.635	0.828	2.008	2.132	-13.24	-3.636
TRP	107	25.936	22.263	3.295	5.287	-53.75	3.034
THR	108	5.658	2.611	4.346	0.933	-15.87	-2.321
HISA	109	20.817	14.094	5.804	0.738	-42.90	-1.442
TRP	110	26.017	24.830	3.897	5.193	-53.38	6.558
LEU	111	4.853	2.927	3.290	2.032	18.60	31.702
GLU	112	6.787	3.192	3.771	0.278	-12.91	1.117
GLY	113	6.152	5.563	2.190	4.078	1.89	19.869
ASP	114	4.973	9.300	11.611	0.443	-5.38	20.946
ASP	115	3.657	3.826	3.974	0.034	-27.80	-16.314
LEU	116	4.730	8.114	3.111	1.603	6.38	23.933
HISA	117	21.379	6.101	1.359	1.908	-42.88	-12.135
LEU	118	5.616	2.163	2.657	0.919	-35.38	-24.029
GLU	119	4.939	5.339	4.877	0.621	-27.11	-11.330
LEU	120	5.114	9.111	2.582	0.476	-31.26	-13.977
ARG	121	9.440	6.283	9.092	8.825	14.77	48.408
CYSH	122	4.770	3.091	2.809	0.035	-30.66	-19.954
GLU	123	3.816	5.983	3.125	0.359	0.99	14.271
ASN	124	4.475	4.749	7.202	0.099	6.52	23.049
GLN	125	5.909	6.835	5.159	1.852	-26.90	-7.149
VAL	126	5.592	3.342	1.663	2.413	9.12	22.127
CYSH	127	5.242	4.001	8.899	0.007	-37.41	-19.256
LYSH	128	4.613	3.755	4.897	0.812	-25.50	-11.420
GLN	129	6.318	4.449	2.326	2.069	-33.84	-18.684
VAL	130	6.558	4.262	1.247	0.482	17.04	29.594
PHE	131	10.004	25.836	2.163	1.491	-46.31	-6.816
LYSH	132	5.256	7.083	6.777	0.003	-22.67	-3.552
ARG	133	9.557	6.536	4.876	5.533	-18.00	8.505
ALA	134	3.468	3.215	3.267	1.098	-12.99	-1.941
OXT	134	0.000	0.000	0.000	0.000	-3.21	-3.213
KJ/mol		**946.199**	**903.096**	**583.115**	**237.443**	**-1622.51**	**1047.341**

Table 2: Position of Residues of Amino Acids and Values of Bonds, Torsion, Improper, Non Bonded and Total Energy Calculation of Amino Acid of RBP7 in *Coturnix Japonica* or japense quail.

11) ATOM 81-88,182-189,283-290,496-503,621-628,686-693,755-762,897-904,934-941, 952-959,969-976:LEU (L)

12) ATOM 137-145, 545-562, 574-591,802-810,905-913,960-968,994-1002:GLU (E)

13) ATOM150-161, 476-487: TYR (Y).

14) ATOM 177-181,221-225,261-265,793-797, 1089-1094:ALA (A)

15) ATOM 194-201,253-260,332-339,401-408,533-540,763-770: ILE (I).

16) ATOM 233-243,257-267,645-655,837-847,977-987, 1078-1088: ARG (R).

17) ATOM244-252,266-274,291-299,316-324,340-348,524-532,608-616,656-664,671-679, 746-754,811-828, 1033-1041, 1069-1077: LYS (K).

18) ATOM 307-315, 349-357,504-512,784-792, 1011-1019, 1042-1050: GLN (Q).

19) ATOM 409-418,873-882,942-951: HIS (H).

20) ATOM 665-670, 771-776,988-993, 1027-1032: CYS (C) (Table 2).

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