International Journal of Advanced Research in IT and Engineering

ISSN: 2278-6244

INSILICO ANALYSIS AND 3D STRUCTURE PREDICTION OF HUMAN GASTRIN PROTEIN

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Abstract: *Human gastrin is the product of a single gene located on chromosome 17 called GAST gene. Excessive secretion of gastrin, or hypergastrinemia, is a well-recognized cause of a severe disease known as Zollinger-Ellison syndrome, which is seen at low frequency in man and dogs. The hallmark of this disease is gastric and duodenal ulceration due to excessive and unregulated secretion of gastric acid. Most commonly, hypergastrinemia is the result of gastrin-secreting tumors (gastrinomas), which develop in the pancreas or duodenum. Physiochemical characterization was done to interpret properties like pI, EC, AI, GRAVY and instability index. The 3D structure of this protein is predicted by homology modeling approach to generate good quality models. The assessment of generated three dimensional structure against structure verification tool showed that model generated by CPHModel was acceptable. The predicted model can be used in structure based drug designing.*

Keywords*: GAST, Gastrin, Hypergastrinemia, Homology modeling, structure based drug designing .*

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INTRODUCTION

Gastrin is a hormone whose main function is to stimulate secretion of hydrochloric acid by the gastric mucosa, which results in gastrin formation inhibition. This hormone also acts as a mitogenic factor for gastrointestinal epithelial cells. Gastrin has two biologically active peptide forms, G34 and G17. Gastrin stimulates the stomach mucosa to produce and secrete hydrochloric acid and the pancreas to secrete its digestive enzymes. It also stimulates smooth muscle contraction and increases blood circulation and water secretion in the stomach and intestine **[1]**. Its existence was first suggested in 1905 by the British physiologist John Sydney Edkins **[2,3]**, and gastrins were isolated in 1964 by Roderic Alfred Gregory and Tracy at the University of Liverpool **[4].** Gastrin is released in response to certain stimuli. These include stomach distension, vagal stimulation (mediated by the neurocrine bombesin, or GRP in humans), the presence of partially digested proteins especially amino acids, hypercalcemia and its release is inhibited by the presence of acid (primarily the secreted HCl) in the stomach (a case of negative feedback), Somatostatin also inhibits the release of gastrin, along with secretin, GIP (gastroinhibitory peptide), VIP (vasoactive intestinal peptide), glucagon and calcitonin **[5,6]**. In the Zollinger-Ellison syndrome, gastrin is produced at excessive levels, often by a gastrinoma (gastrin-producing tumor, mostly benign) of the duodenum or the pancreas. To investigate for hypergastrinemia (high blood levels of gastrin), a "pentagastrin test" can be performed.In autoimmune gastritis, the immune system attacks the parietal cells leading to hypochlorhydria (low stomach acidity). This results in an elevated gastrin level in an attempt to compensate for increased pH in the stomach. Eventually, all the parietal cells are lost and achlorhydria results leading to a loss of negative feedback on gastrin secretion **[7]**.

METHODOLOGY

Retrieval of target sequence

The nuclic acid sequence of the GAST gene of *Homo sapiens* was obtained from the sequence database of NCBI (Accession NC_000017 REGION: 39868578..39872221). The encoded protein is predicted by Genscan server & it was 101 amino acids in length.

Physico-chemical characterization

The values of theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand

average hydropathy (GRAVY) were computed. For physico-chemical characterization Expasy's Protparam server was used. The results were shown in Table No.1.

Secondary structure prediction

SOPMA **[8]** was employed for calculating the secondary structural features of the protein sequence considered for this study. The results were presented in Table No. 2.

Model building and quality assessment

The modeling of the three dimensional structure of the protein was done using two homology modelling programs, CPH Models **[9]**. The overall stereochemical property of the protein was assessed by Ramchandran plot analysis. The evaluation of structure model obtained from tool was performed by using Rampage which were shown in Table No. 3.

RESULTS AND DISCUSSION

In present study, the nucleotide sequence of GAST gene was retrieved from NCBI (Accession NC_000017 REGION: 39868578-39872221) and used as query sequence for Genscan server to predict the coded peptide. For the coded peptide sequence Physiochemical Parameters computed using Expasy's ProtParam tool **[10]** which were represented in Table 1. A protein having instability index smaller than 40 is predicted as stable, on the other hand a value above 40 predicts that the protein may be unstable. Instability index of 67.91 indicates the unstable nature of protein. The low extinction coefficient (24980) indicates presence of low concentration of Cys, Trp and Tyr.

Table No. 1: Parameters computed using Expasy's ProtParam tool

S.NO.	Property	Value
1.	Number of amino acides	101
2.	Molecular weight	11393.7Da Da
3.	Theoretical pI	5.08
4.	Total number of negatively charged residues(Asp+Glu)	15
5.	Total number of positively residues (Arg+Lys)	10
6.	Extinction coefficient	24980
78.	Instability index	67.91
8.	Aliphatic index	65.84
9.	Grand average of hdropathicity	-0.770

The aliphatic index is considered as a positive factor for the increase of thermal stability. High aliphatic index (65.84) of query protein suggests that the protein may be stable for a wide temperature range. The Grand Average hydropathy (GRAVY) value is low (-0.770) and indicates the possibility of better interaction with water.

The secondary structure of protein was predicted by a software namely SOPMA (Self Optimized Prediction Method with Alignment). The results of SOPMA are presented in Table 2. These results show higher number of random coils in comparison to other secondary structure elements (alphahelix, extended strand and beta turns). Default parameters (Window width: 17, similarity threshold: 8 and number of states: 4) were taken by SOPMA for secondary structure prediction.

Table No. 2: Calculated secondary structure elements by SOPMA.

S.NO.	Parameters	Value (%)
1.	Alpha helix	45.54
2.	310helix	00.00
3.	Pi helix	00.00
4.	Beta bridge	00.00
5.	Extended strand	1.98
6.	Beta turn	00.00
7.	Bend region	00.00
8.	Random coil	52.48
9.	Ambiguous state	00.00
10.	Other state	00.00

Three dimensional structure of protein (Figure No. 2) is predicted using the program called CPHModel using 1BKV.B as template for homology modeling. The evaluation of predicted structure generated by CPHModel for the stereochemical quality was done using Ramchandran map calculations with the toll namely Rampage (Figure No. 1). The 86.2% of residues were found in favoured region. 6.9% of residues were found in the allowed & outlier regions . Above results indicate to a good quality of predicted model.

Table No. 3: Ramachandran plot calculation of the model with Rampage.

Server	Parameters	Value (%)
CPHModel	Residues in the most Favoured Region	86.2
	Residues in allowed region	6.9
	Residues in outlier region	6.9

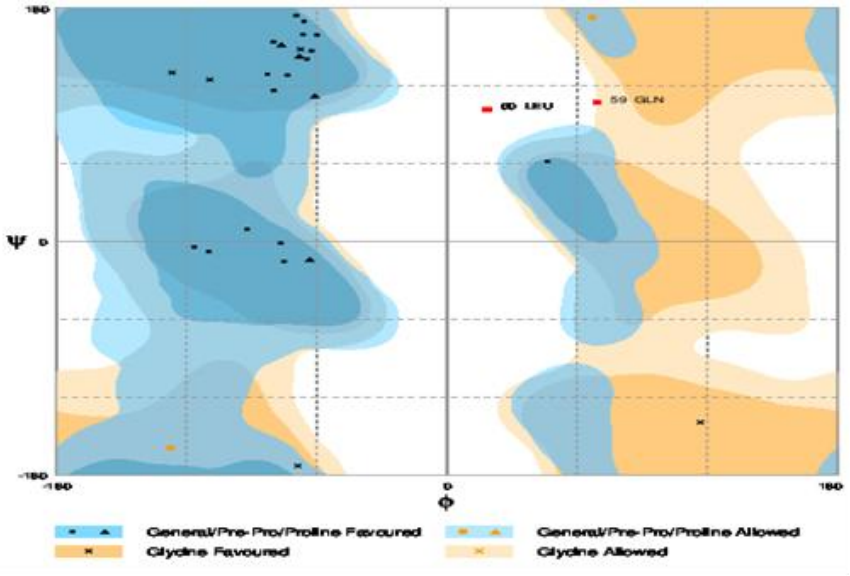

Figure No. 1: Ramachandran plot of GASTRIN protein by Rampage

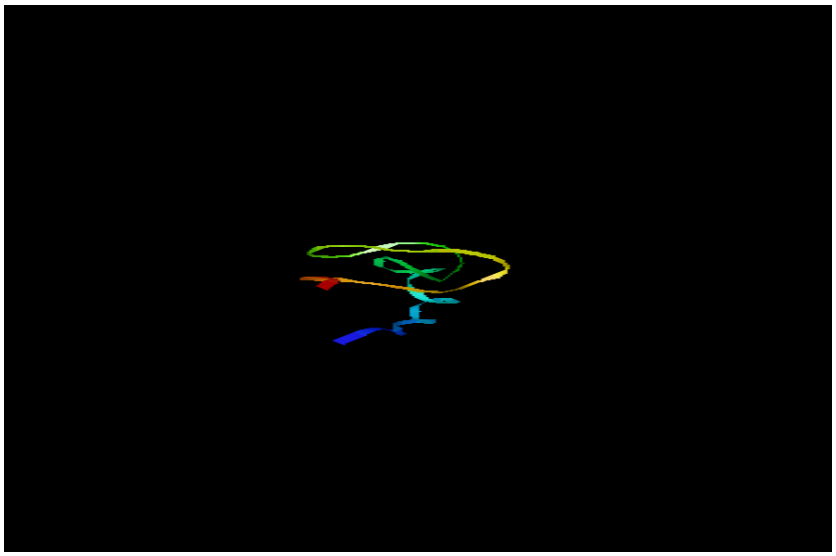

Figure No. 2: Modeled Structure of GASTRIN protein

CONCLUSION

On the basis of various structural and physiochemical parameters assessment, it can be concluded that the predicted three dimensional structure of GASTRIN peptide harmone is stable. Since no effective therapeutic drug is available for gastro intestinal problems, structural information of this model can be effectively used and can be further implemented in future drug designing.

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