Research Article

In silico Comparative Study of Human and Guinea Pig Preproinsulin and its Components

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ABSTRACT

"Guinea pig" (*Cavia porcellus*) has been used as an important experimental model in various clinical studies. Among various metabolic entities it differs from human pathophysiology including carbohydrate metabolism and therefore cannot be deployed for all clinical investigations. Preproinsulin and its respective components i.e. signal peptide, chain B, chain C and Chain A differ in their physico-chemical properties from human insulin specifically in isoelectric point, aliphatic index and instability index. It also lacks an ability to form dimer and in turn to form hexamer. Therefore, it cannot be used for hypoglycemic studies incorporating inactive insulin and its physiology. Receptor binding sites are similar to human insulin; number and position of cysteine residues are conserved in both chain A and chain B but the V loop residues vary at B20 where Gly is replaced by Gln and hence forms the deviated loop and in turn it differs significantly in both human and guinea pig.

Keywords: Guinea pig, preproinsulin, insulin, isoelectric point, instability index, aliphatic index.

INTRODUCTION

"Guinea pig" (*Cavia porcellus*) is used colloquially as test animals and its use in scientific experimentation dates back to 17th century, when Malpighi and Fracassati prepared anatomical vivisections .[1] In 1780, Antoine Lavoisier using guinea pig demonstrated respiratory gas exchange akin to combustion.[2] In the late 19th century they also played a major role in the establishment of germ theory by Louis Pasteur, Émile Roux, and Robert Koch.[3] This rodent has also been deployed in space shuttles as a biological exemplum [4] and has significantly contributed in the discovery of the tuberculosis bacterium, Vitamin C and adrenaline. It has also been used in the development of antibiotics, anticoagulants, asthma medicines, vaccines for diphtheria etc. Currently they are deployed in the research of nutraceuticals, hearing impairment, allergies, carbohydrate maladies and respiratory diseases. The guinea pig is also called the cavy or domestic guinea pig and is a species of rodent of family Caviidae and the genus *Cavia*. Its strains used in scientific research are basically out bred strains. Besides common American or English stock the two main outbred strains i.e Hartley and Dunkin-Hartley are used as an experimental animals although strains are albino but presence of pigmented strains cannot be denied.[5]

Health is an outcome of interrelated metabolic entities and strain or malfunctioning of one effects the complete system adversely. Hence, the selection of the test animal resides on the degree of similarity on molecular level to meet biochemical and physiological amplitudes. In 1972, Zimmerman *et al.*[6] demonstrated the amino acid sequence in guinea pig using assay method and revealed its inability to counter the similar effects as humans. As guinea pig is used as an experimental organism in various research activities therefore, it becomes essential to compare its metabolic entities to human biochemical standards. Following the same array an *in silico* studies were carried out to compare the human insulin and guinea peg insulin so that the physico-chemical properties can be used to evaluate the glycemic potential of targeted drugs.

MATERIALS AND METHODS

1. *Protein sequence retrieval and local alignment*: Preproinsulin and its respective component sequences were retrieved from UniProt (http://www.uniprot.org/) and were locally aligned using BLAST tool with Needleman-Wunsch Global Align.[7]

2. *Analysis of physico-chemical properties*: The ExPASy's ProtParam tool (http://web.expasy.org/protparam/) was used to compute amino acid composition (%), molecular weight, theoretical isoelectric point, number of positively and negatively charged residues, instability index , aliphatic index and grand average of hydropathy .

RESULTS AND DISCUSSION

In silico comparative sequence analysis through local alignment reveals thet guinea pig preproinsulin peptide differ from human preproinsulin in 34 amino acids with 7, 10, 9 and 8 amino acid alteration in signal peptide , chain B, chain C and chain A respectively. Maximum identity was observed in signal peptide whereas chain B differed with highest amino acid residues. Among positive substitution Chain B and Chain A were nearly similar in

their substitution patterns (Figure 1). The convertase target site 55-56 and 88-89 with *Arg-Arg* and *Lys-Arg* from N terminal were constant. In signal peptide the substitution of amino acid is random and a specific pattern cannot be inferred except at 19th position where sequence from human signal peptide contains *Asp* whereas in guinea pig it shows the presence of *Asn*. In chain C *Met* appears as 14th and 28th residue in guinea pig while it is completely absent in respective human peptides. Amino acid substitution in evolutionary course has led to the alterations in secondary structures.[8] Formation of insulin from preproinsulin through cleavages at *Arg-Arg* and *Lys-Arg* is an important attribute as it signifies the sequence length and terminal properties of chain B, chain C and chain A.[9,10] These incision in guinea pig preproinsulin was similar to human sequences and as a result the terminals were similar in both the compared animals.
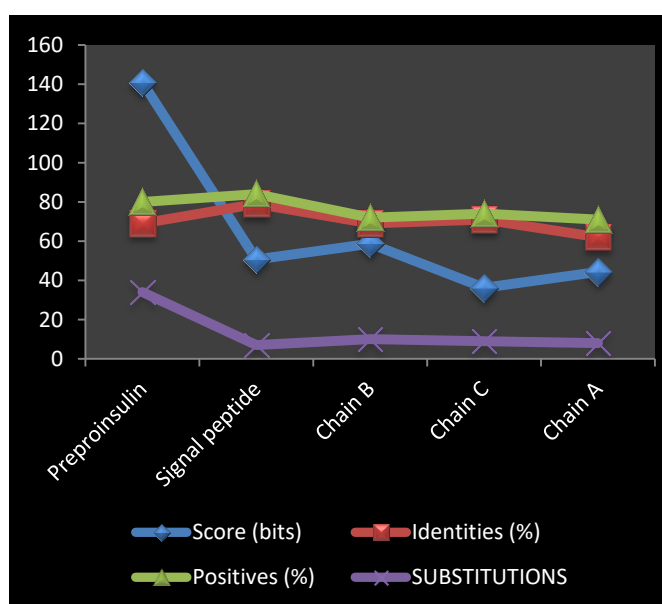

Figure 1: Percent identity, positives, score and substitutions in between humans and guinea pig preproinsulin and its component.

Three conserved regions of insulin form primary receptor-binding surface viz.: (i) the N-terminal and C-terminal segments of the A chain (Gly[A1]-Ile[A2]-Val[A3]-Glu[A4] and Tyr[A19]-Cys[A20]-Asn[A21]), (ii) the central α-helix of the B chain (especially Val[B12]) and (iii) and the C-terminal segment of B chain (Phe[B24]-Phe[B25]-Tyr[B26])[16,17]. In guinea pig all the sites are conserved except A4 at which it has negatively charged *Asp* instead of *Glu* as found in humans. Therefore, it can be presumed that the receptor binding affinity of guinea pig resembles human insulin and in turn leads similar glycemic metabolic routes.

Chain B and chain A forms the chief glycemic regulator hormone-insulin and therefore their comparsion becomes essential for its better insight. The tertiary structure of insulin is stabilized by disulphide bridges. The 6 *Cys*, forming 3 disulphide bridges between A7 - B7, A20 - B19 and A6 -A11 are conserved in guinea pigs as similar to humans. Therefore, the tertiary structure forming capabilities of both are alike.[11] Similarly, smaller *Gly* molecule at B20 and B23 assists to fold into V shape.[12] This brings the C terminal residues B24 *Phe* and B26 *Tyr* into van der waals contact with B15 *Leu* and B11 *Leu* of the alpha-helix.[13] In guinea pig *Gly* at 20 is replaced by uncharged *Gln* and therefore it might deviate from V shaped loop formation whereas the similar *Cys* residues impart same secondary structure as of human insulin. The side chain of B10 *His* coordinates with axial Zn^{2+} ions and in turn it is responsible to form dimers.[14] Additionally, two axial Zn^{2+} atoms lie on the threefold symmetry axis of the hexamer; each exhibits octahedral coordination by three *His*[B10] residues and three water molecules.[15] In guinea pig *Asn* occupies B10 position and results in the failure of ability to form dimer and in turn stable hexamer. The putative insulin surface is proposed to correspond to its hexamer-forming surface, including residues *His*[B10], *Leu*[B17], *Val*[B18], *Ser*[A12], Leu[A13] and *Glu*[A17]. Substitutions among these sites affect the kinetic properties of hormone binding disproportionately to effect its affinity. In guinea pig it is replaced as *Asn* and *Ser* at B10 and B17 in chain B and *Thr*, *Arg* and *Gln* at A12, 13 and 17 respectively (Figure 2).

Table 1: *In silico* physico-chemical comparison of human and guinea pig preproinsulin and its components

S.No	PCP	Preproinsulin		Signal peptide		Chain B		Chain C		Chain A	
		Hs	Cp	Hs	Cp	Hs	Cp	Hs	Cp	Hs	Cp
1	NAA	110	110	23	23	30	30	31	31	21	21
2	MW	11980.9	12244.1	2473.0	2547.0	3429.9	3453.8	3020.3	3238.6	2383.7	2330.6
3	pI	5.22	5.15	5.88	6.79	6.90	4.66	3.45	3.40	3.79	6.71
4	TNNR (D + E)	10	11	1	0	2	4	5	6	2	1
5	TNPR (R + K)	7	7	1	0	2	2	0	0	0	1
6	II	40.33*	57.88*	46.33*	47.34*	9.85**	36.32**	51.27*	64.56*	22.54**	**59.27***
7	AI	102.91	93.09	161.74	148.70	84.33	81.00	103.87	107.10	88.10	50.95
8	GRAVY	0.193	**-0.017**	1.222	0.913	0.220	0.040	**-0.081**	**-0.029**	0.214	**-0.367**

Hs-Homo sapiens and Cp-Cavia porcellus; PCP-Physicochemical parameter; NAA-Number of amino acids; MW-Molecular weight ; pI-Isoelectric point; TNNR (D + E) - Total number of negatively charged residues (Asp + Glu); TNPR (R + K) - Total number of positively charged residues (Arg + Lys); II-Instability index; AI-Aliphatic index and GRAVY- Grand average of hydropathity; *- unstable; **- stable

Figure 2: *In silico* sequence alignment between human and guinea pig's signal peptide, Chain B, Chain C and Chain A

Number of amino acid residues is similar in preproinsulin and all components in guinea pig and humans while molecular weight differed according to the altered amino acids. The isoelectric point varies largely in human and guinea pig's signal peptide, chain B and chain A whereas are about same with minute differentiation in chain C and preproinsulin. In signal peptide and chain A it is more toward neutrality i.e. around 7 in *Cavia* while in humans it is along acidic scale. This can be inferred as total number of negatively charged residues i.e *Asp* and *Glu* are present in humans.[18] Contrary to this in chain B isoelectric point is about to neutral point in humans while acidic in guinea pig.[19,20] According to structural kinetics instability index reveals an estimate of the stability of a protein in the test tube. A protein with instability index smaller than 40 is referred as stable, while value above 40 indicates instability.[21,22] Preproinsulin, signal peptide and chain C has unstable structures in both the compared sequences while insulin basal structural peptides i.e chain B and A are stable as evinced through experimental lines[23] while chain A is unstable in guinea pig resulting in lower stability of insulin molecule. The aliphatic index of a peptide is the relative volume occupied by the amino acids such as - alanine, valine, isoleucine and leucine having an aliphatic side chain.[24] It indicates stability of the globular protein molecule/s similar to instability index,[25] it was observed that aliphatic index was profoundly low in chain A of guinea pig. The GRAVY value for a peptide is estimated by adding the hydropathy values of each amino acid residues and by dividing the number of residues in the sequence.[26] Increasing positive score indicates a greater hydrophobicity. Values of grand average of hydropathicity (GRAVY) were negative in preproinsulin and chain C and chain A of guinea pig indicating presence of non polar amino acid residues (Table 1). As similar to previous findings the guinea pig preproinsulin and its component differed in total 34 amino acids with chain B with highest substitutions of 10 amino acids. Despite of these substitutions the receptor binding affinity has been conserved and no alterations in glucose metabolism can be desked.[27,28]

CONCLUSION

Guinea pig differs from human preproinsulin and insulin in 34 and 18 amino acids respectively but the major difference resides in being inable to form dimer and hexamer. Although *Cys* residues leading to secondary structures are similar to human insulin but secondary configuration in respect to V loop has torsion due to the presence of *Gln.* The receptor binding capacity is similar to human insulin. On the basis of sequence and structural differences it is revealed that guinea pig cannot be recommended as an experimental model for diabetes or insulin related studies as its physico-chemical properties differ as that from human insulin

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