

Research Article



A Computational Functional Prediction of Aldose Reductase Enzyme: A Potential Strategy from Identification of Physicochemical Properties to Transmembrane Helix

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ABSTRACT

The aim of the study is to investigate the beneficial effects of AR such as physicochemical properties, transmembranal helix identification, prediction of motifs, position of aminoacids and its sequencing value identification of Aldose reductase. Today diabetes is an utmost habitual disease in all over the world. The higher presence of AR in polyol pathway increases the risk of nephropathy. The aldo-ketose reductases (AKRs) are classified as oxidoreductases as a consequence are found in prokaryotes as well as in eukaryotes. The superfamily of AKR consists Exceedingly 120 proteins are dispended throughout the 14 families. Aldose reductase is a subclass of NADPH dependent oxido-reductases. This protein is conjointly a focused imaginable drug target for nephropathy. Still the knowledge of this protein is limited. In this elucidation, the authors performed a study to identify the total biological activity of aldose reductase.

Keywords: Aldose reductase, transmembranal helix, motifs, polyol pathway, oxido reductases, physicochemical properties.

INTRODUCTION

For the past two decades we have yielded major advances in finding the pathogenicity that causes diabetic nephropathy. Today nephropathy is a very commonly occurring complication in the patients of diabetes. Complications and trial study strongly believe that the hyperglycemic progression of polyol pathway occurs in diabetic nephropathy¹. Polyol pathway is the most important pathway involved in the pathogenesis of complications occurred in diabetes. Polyol pathway is present in lens of eye, nerve and kidney. Polyol pathway involves two enzymatic reactions². In the first reaction sorbitol is formed by the reduction of glucose in the alacrity of Aldose Reductase and in another reaction sorbitol is oxidized to fructose by the activity of sorbitol dehydrogenase. Aldose reductase (AR) is a reduced monomeric nicotinamide adenine dinucleotide phosphate (NADPH) dependent enzyme belongs to super family of aldo-ketose reductase³. It is present in the inner strip of medulla (where sorbitol is present), proximal tubules. Aldose reductase has glucose reducing activity. AR catalyzes the NADPH dependent glucose to their relative sugar polyols (sorbitol) in polyol pathway. It is the first enzyme which plays a potential role in the early stage of nephropathy⁴. Many scientific studies clearly identify the AR activity increases in the type 1 diabetic patients with nephropathy. Some of the inhibitors are used to step up the AR activity⁵. To overcome the diabetic complications in early stage of nephropathy we perform insilico characterization of AR by using bioinformatics tools. In the present study we clearly explain the structure, physicochemical properties like primary, secondary

structural activity and topological studies of this Aldose Reductase.

MATERIALS AND METHODS

Sequence analysis

The protein sequence of Aldose Reductase (AR) of *Homosapiens* was obtained in FASTA format from the National Centre for biotechnology information (NCBI) database with the accession number NP_001619.1⁶.

Primary Structure Prediction

By using primary structure prediction tool like protparam the physico-chemical properties of Aldose Reductase were identified. For physicochemical characterization molecular weight, instability index, extinction coefficient, aliphatic index etc can also be computed⁷.

Secondary structure prediction

Secondary structure elements like alpha helix, pleated sheets, random coils and beta turns of aldose reductase was figure out by using the secondary structure prediction tools like SOPMA⁸, GOR4⁹ and CHOU FASMAN¹⁰.

Tertiary structure analysis

Tertiary structure characterization, motivation of aldose reductase can be analyzed by using the tool like TLS analysis¹¹.

Protein functional sites

Motif region and signatures in the sequences of aldose reductase were identified by using protein functional site tools like interpro scan and fingerprint scan¹².



Trans membrane Topology identification

Forecasting of trans-membrane spanning regions and their orientations of aldose reductase can be predicted by using TMpred tool¹³.

Signal peptide identification

SignalP tool predicts the presence and location of signal peptide cleavage sites in the amino acid sequence of aldose reductase¹⁴.

RESULTS AND DISCUSSION

Primary structure prediction

In this the amino acid sequence of Aldose Reductase can be retrieved from the uniprot database in FASTA format. The protein Aldose Reductase has 316 amino acids with molecular weight of 1032448.7. The maximum number of amino acids present in this sequence is Threonine (28%) and the least was Alanin with 22%. The theoretical PI of the protein was 4.50, which indicates that the protein is in acidic nature. If the computed PI value is more than 7 it indicates the protein is basic. The extinction coefficient of AR protein is 193625 with the estimated half life is 1.2 hrs in mammalian reticulocytes, more than 10 hrs in E.Coli and greater than 20 hrs in yeasts. The instability index is to be computed as 49.99, which indicates the protein is unstable. At wide range of temperature, the very high aliphatic index indicates the stability of protein, but the AR protein shows aliphatic index as 21.96. The Grand Average Hydropathicity (GRAVY) of AR is 0.731, which indicates the better interaction of protein with water (Table 1).

Table 1: Molecular characterization of AR was predicted from PROTPARAM tool

S.NO	Protein Secondary Structure	Values
1	Aliphatic index	21.96
2	Grand average of hydropathicity	0.731
3	Theoretical PI	4.50
4	Half-life	1.2 hours
5	Molecular Weight	1032448.7
6	The Instability index	49.99
7	Extinction Coefficients	193625

Secondary Structure analysis

The secondary structure elements like alpha helix, random coils, Beta turns and beta sheets can be predicted by SOPMA, GOR4 and CHOU FASMAN tools. Alpha helices are predominately present in all the tools, SOPMA shows 120 with 37.97% (Figure 1), GOR4 shows 107 with 33.86% (Figure 2) and in CHOU FASMAN 237 with 75% (Figure 3). Beta turns are absent in GOR4 but SOPMA and CHOU FASMAN shows 36, 44 with 11.39% and 13.9% respectively. CHOU FASMAN method fails to show random coils while in case SOPMA shows 94 with 29.75% and GOR4 shows 148 with 46.84%. SOPMA displayed 153 with 48.4% of Extended sheets, while GOR4 shows 61 with 19.30% and 153 with 48.4% were present in CHOU FASMAN methods (Table 2).

Table 2: Secondary structure crystallizability predicted from Sopma, Gor4 and Chou Fasman tools:

S.no	Protein Secondary Structure	SOPMA		GOR4		CHOU & FASMAN	
		No. of Residues	% of Residues	No. of Residues	% of Residues	No. of Residues	% of Residues
1	Alpha helix	120	37.97	107	33.86	237	75.0
2	Extended strand	66	20.89	61	19.30	153	48.4
3	Beta turn	36	11.39	-	-	44	13.9
4	Random coil	94	29.75	148	46.84	-	-

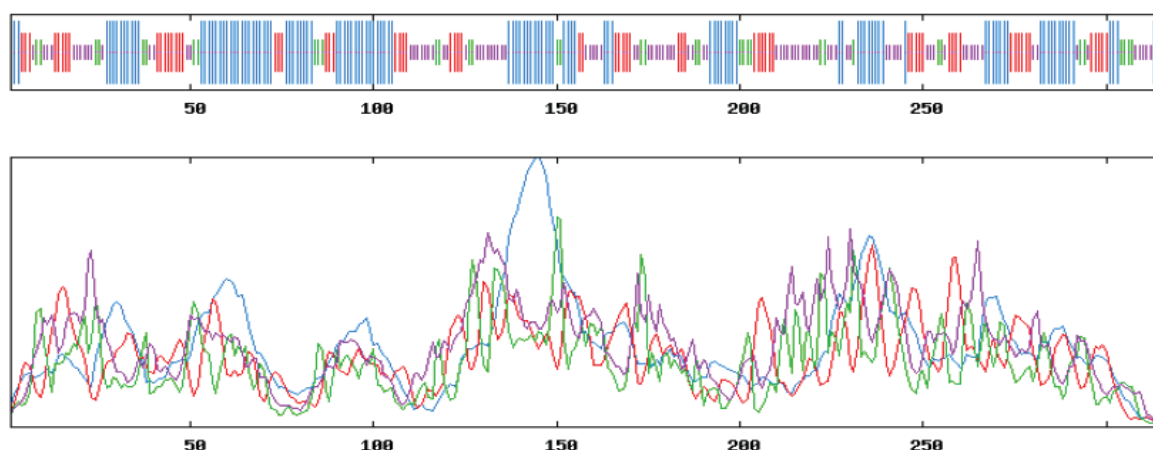


Figure 1: Secondary Structure elements of AR protein by using SOPMA Tool

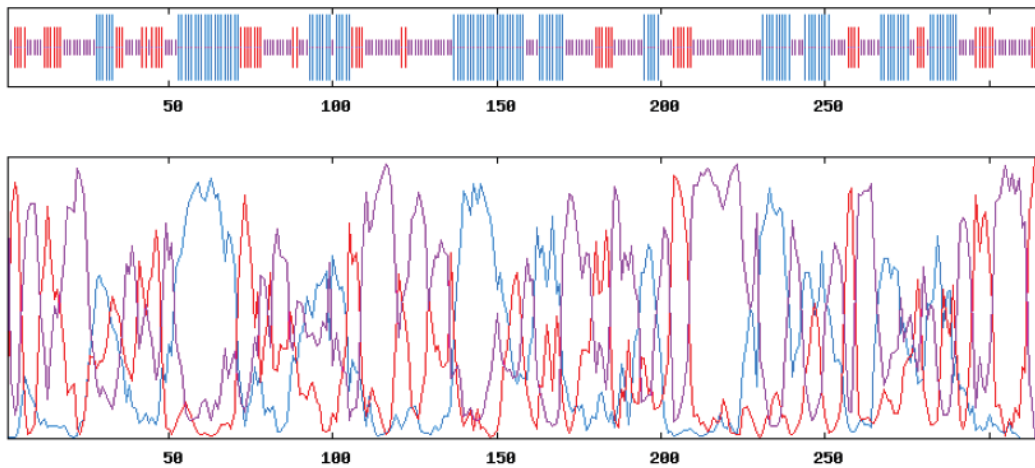


Figure 2: Secondary Structure elements of AR by the help of GOR4 Tool

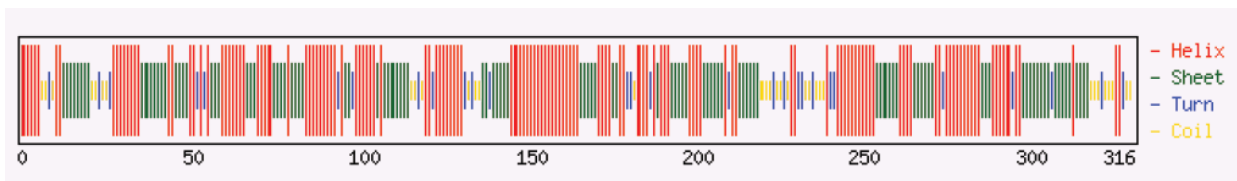


Figure 3: Secondary Structure elements of AR was predicted by CHOU FASMAN Tool

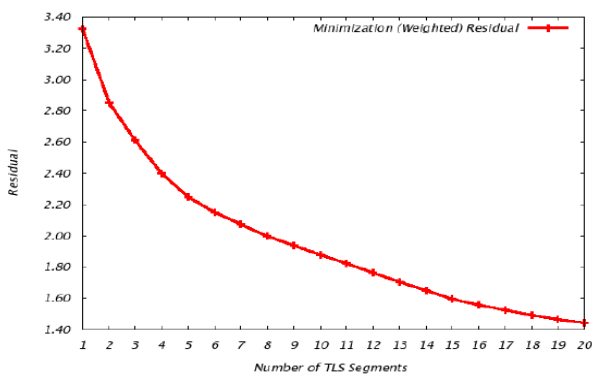


Figure 4: Graphical representation of tertiary analysis by TLS motivational analysis

Tertiary Structure Prediction

TLS motion determination is the tool which is useful for showing the evidence of flexibility of the protein Aldose

Reductase. In this analysis aldose Reductase shows 2610 atoms with RMSD 3.02. By using this tool we can have the graphical representation between the least square residues with number of TLS segment present in AR protein.

Protein Functional site

By using the interproscan tool protein functional sites, like domains, repeats, motifs region in the sequence and signature of the aldose reductase sequence can also be identified.

Transmembrane topology identification

Transmembranal helix present in the Aldose Reductase protein can be identified by TMpred Bioinformatic tool. By the help of this tool we identified the prediction parameters like TM helix, length of the TM Helix sequence of AR protein. The sequence length is from 17 to 33 aminoacid sequences. There is no outside to inside and inside to outside sequences in Aldose Reductase. Figure 6.

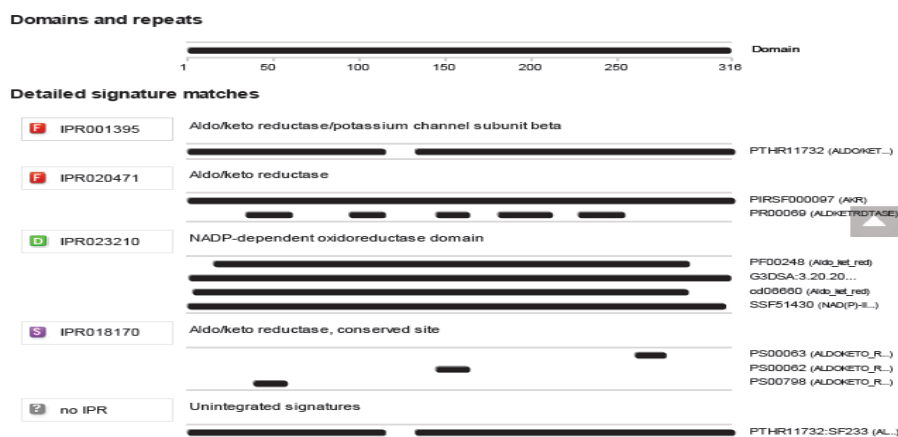


Figure 5: Diagram showing protein functional site analysis of Aldose Reductase

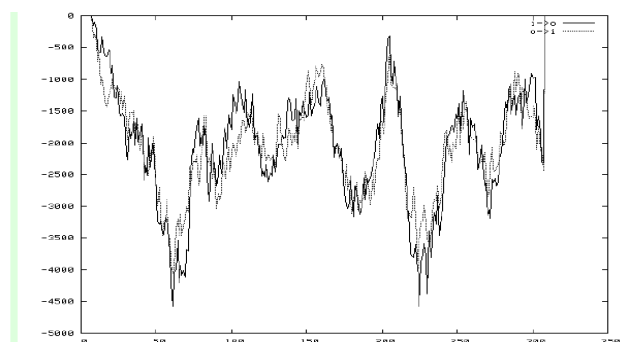


Figure 6: Pattern showing transmembrane helices in Aldose Reductase

Signal P prediction of Aldose Reductase

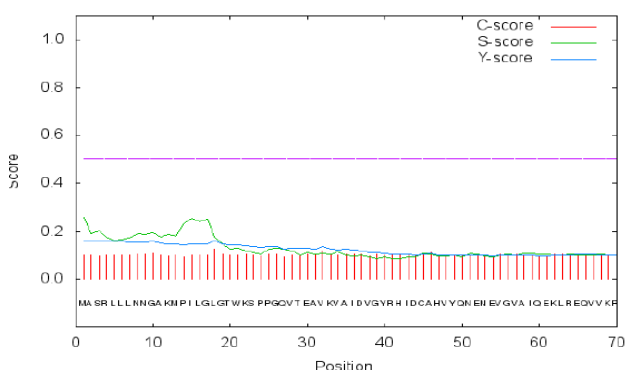


Figure 7: Template showing the position of amino acid in Aldose reductase

From the SignalP tool we have identified the maximum Cystein residues as 0.125 in 18th position and also the range of Serine, Aspartic acid and Tryptophan has 0.258, 0.202 and 0.164 with 18, 1-17, 1 positions in the sequences respectively (Table 3).

Table 3: position of amino acid figured using SignalP server.

S.NO	Measure	Position	Value
1	Maximum Cystein residues	18	0.125
2	Maximum Tryptophan residues	18	0.164
3	Maximum Serine residues	1	0.258
4	Maximum Aspartic acid residues	1-17	0.180

CONCLUSION

We concluded that the present work clearly explains about the physicochemical properties like primary, secondary and tertiary structure analysis by the assist of different

bioinformatic tools simultaneously we also straight forwardly distinguish the motif position of AR protein as well as sequence identification of AR protein by TMpred tool. At lastly we also obviously told that transmembranal helices in the sequences including amino acid position and its values can be measured by the service of SignalP tool. These transmembranal regions are focused for further drug development.

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