

Insilico Analysis Of Cranberry Proanthocyanidin As An Inhibitor For Modelled Afimbrial Adhesin Virulence Protein Of Uropathogenic Escherichia Coli

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Abstract

Fimbrial adhesion is a Virulence Determinant which is classified under Adhesins category of virulence factor of uropathogenic Escherichia coli. Afimbrial adhesin Protein with swissprot accession number P08180 was selected for modeling using Bioinformatics tools. Validated modelled structure obtained from Modeller 8V2 was used as target protein for further analysis. Docking analysis of Epicatechin a Proanthocyanin from cranberry against modelled fimbrial adhesion was carried out using AUTODOCK 4. Some of the commonly used antibiotics to treat urinary tract infections caused by Uropathogenic E.coli which includes Ofloxacin, sulfamethoxazole, Trimethoprim were subjected to docking analysis for comparative studies.

INTRODUCTION

E. coli is responsible for three types of infections in humans: urinary tract infections (UTI), neonatal meningitis, and intestinal diseases (gastroenteritis). These three diseases depend on a specific array of pathogenic (virulence) determinants which renders it pathogenic. One such virulence protein is fimbrial adhesion as seen in Uropathogenic Escherichia coli. Fimbrial adhesion of pathogenic E.coli mediate adherence to the upper urinary tract. These adhesins bind to the Dr blood group antigen and also agglutinate human erythrocytes in the presence of D-mannose (mannose-resistant hemagglutination (MRHA)).

Among various organisms which are known to cause Urinary tract infections, Escherichia coli is the most predominant pathogen being isolated in 70-90% of cases. It has been accepted that UTI caused by E.coli is an ascending infection caused by the strains originating in the intestinal tract, because a high similarity exists between E.coli strains from urine and faeces of infected individuals.

Different virulence factors of E.coli which are thought to have a role in the pathogenesis of Urinary Tract Infections are O Antigens, K Antigens, Hemolysins, Serum resistance, Adhesins, Capacity to produce mannose sensitivity and resistant haemagglutination. fimbrial adhesion is a Virulence Determinant which is classified under Adhesins category of

virulence factor.

ADHESINS-FIMBRIAE

The term adhesin has been described as a microbial surface component that mediates specific attachment to eukaryotic cell membrane and encompasses well known fimbriae as well as other poorly characterized and undefined structures. Adherence is facilitated by E. coli Fimbriae which are proteinaceous fibers on the bacterial cell wall. Fimbriae produce adhesins that bind to specific carbohydrate receptors present on uroepithelial cells (Beachey, 1981).

Many bacteria are known to be adhesive, attaching to and living in close association with various surfaces in their natural habitat. The ability of many pathogenic bacteria to adhere to specific host tissues is a factor of primary importance in diseases such as bacterial diarrhea, gonorrhea and Urinary Tract Infections. This specific adherence plays two important roles

1. It allows the bacteria to resist and circumvent the flushing and cleansing mechanisms that protect many epithelial surfaces in higher animals.
2. It determines the site of microbial infection by facilitating specific surface interaction between the bacteria and host epithelium.

Many studies (Fowler et al 1977, Kallenius et.al 1978) have shown that bacterial adherence is an essential virulence factor in the pathogenesis of community acquired Urinary Tract Infections. Duguid et al (1966) studied fimbriae of E.coli in great detail and classified them into three groups depending upon their haemagglutinating properties as the MSHA(Mannose Sensitive Haemagglutinating) type ,MRHA(Mannose Resistant Haemagglutinating) type and non-fimbrial haemagglutinin type. There is considerable evidence to support the use of cranberries for the prevention of urinary tract infections (Bodel et al., 1959; Moen, 1962; Sternlieb, 1963; Papas et al., 1968; Avorn et al., 1994).

Cranberry juice acts by preventing adhesion which presumably helps urinary flushing of the causative bacteria, preventing their colonization of the urinary tract. This effect is achieved by inhibiting the infecting bacteria, *Escherichia coli*, from adhering to uroepithelial cells (Sobota, 1984; Schmidt and Sobota, 1988; Zafriri et al., 1989 Ofek et al., 1991). Bacterial adherence to mucosal cells plays a key role in the development of infection (Bea-chey, 1981)

Action of Cranberry proanthocyanidins:

- Alter E. coli's cell membranes
- Prevent the bacteria from making contact with cells or attaching to them even if they somehow manage to get close enough
- Change the shape of E.coli from rods to spheres
- Disrupt bacterial communication

MATERIALS AND METHODS

Protein with swiss-prot primary accession number P08180 , of length 161 amino acids was selected for modelling of Afimbrial adhesin AFA-I.

MODELLING

Modelling of target protein was carried out using Swiss-PdbViewer (or SPDBV) and MODELLER 8V2. MODELLER was used for homology or comparative modeling of protein three-dimensional structures .Alignment of a sequence to be modeled is provided with known related structures and MODELLER automatically calculates a model containing all non-hydrogen atoms. MODELLER implements comparative protein structure modeling by satisfaction of spatial restraints ,and can perform many additional tasks, including de novo modeling of loops in

protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc.

VALIDATION OF MODELLED PROTEIN

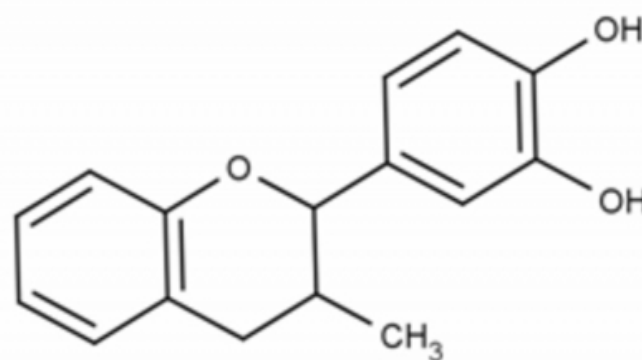
Validation of modelled structure was carried out using Structure Analysis and Validation Server. Structure Analysis and Validation Server greatly simplifies computational analysis of the molecular structure and sequence of proteins. The stereochemical validation of model structures of proteins is an important part of the comparative molecular modeling process.

Active sites present in the protein were identified using Q-SITE FINDER. It is an energy-based method for the prediction of protein-ligand binding sites designed by Laurie AT, Jackson RM.

Epicatechin a Proanthocyanidin from cranberry was selected as lead molecule for insilico analysis of its inhibition activity against modelled fimbrial adhesion .

Figure 1

Figure 1: Epicatechin a from cranberry



DOCKING STUDIES

Docking analysis was carried out using AutoDock 4. Analysis of Epicatechin a Proanthocyanidin from cranberry as an inhibitor against modelled fimbrial adhesion was carried out using AUTODOCK. Further AUTODOCK was also used to visualize Interaction of Epicatechin with modelled fimbrial adhesion protein . Some of the commonly used antibiotics to treat urinary tract infections caused by Uropathogenic E.coli which includes Ofloxacin, sulfamethoxazole, Trimethoprim were subjected to docking analysis for comparative studies using AUTODOCK 4.

RESULTS AND DISCUSSION

Validation of modeled structures obtained from Swiss-PdbViewer and MODELLER 8 V2 was carried out using Structure Analysis and Validation Server. The structure modelled using Modeller 8V2 and Swiss-PdbViewer had over all quality factors of 100% and 77.14 % respectively.

Modelled structure obtained from Modeller 8V2 was used as target protein for further analysis. Prediction of active sites was carried out using Q site finder. It was found that ALA 21, SER 107, MET 108, TYR 122 TRP 159 are potential active sites among several others.

Docking analysis of Epicatechin a Proanthocyanidin from cranberry as an inhibitor against modelled fimbrial adhesion was carried out using AUTODOCK 4. Best Binding energy was found to be -6.47 at the 6 th run. The details of results obtained from AUTODOCK 4 has been mentioned below. Interaction of Epicatechin with modelled fimbrial adhesion protein was analyzed using AUTODOCK 4 as mentioned in figures 2 and 3.

CLUSTERING HISTOGRAM

Figure 2

Table 1: Clustering Histogram of Various Conformations obtained for Epicatechin.

RMSD TABLE

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	6	-6.47	0.00	7.62	RANKING
2	1	8	-6.45	0.00	13.39	RANKING
3	1	4	-6.08	0.00	9.40	RANKING
3	2	7	-5.90	1.35	9.31	RANKING
3	3	9	-5.90	1.19	9.18	RANKING
3	4	3	-5.84	1.08	9.21	RANKING
4	1	5	-5.50	0.00	10.52	RANKING
5	1	10	-5.50	0.00	9.62	RANKING
5	2	1	-5.42	1.05	9.79	RANKING
6	1	2	-4.86	0.00	10.66	RANKING

Figure 3

Figure 2: Interaction of Epicatechin a from cranberry with Modelled fimbrial adhesion virulence protein.

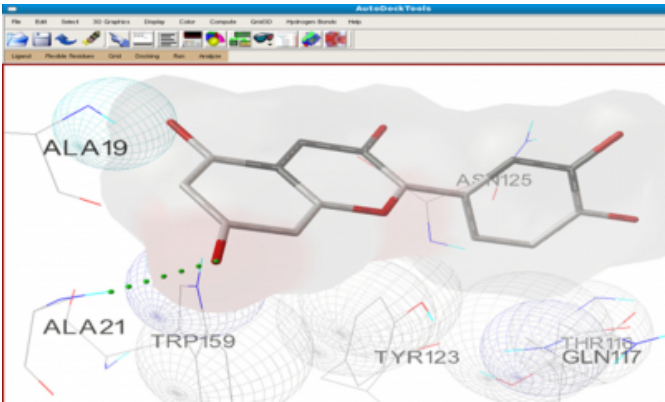
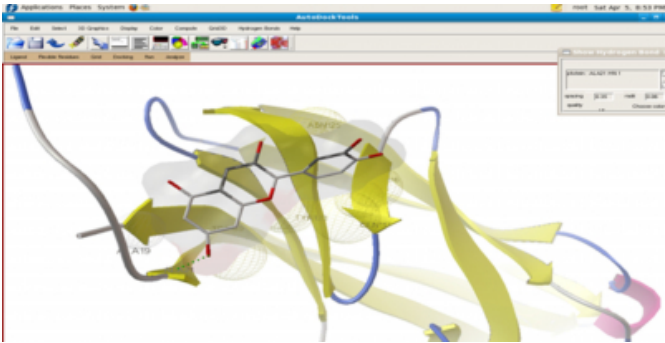


Figure 4

Figure 3: Interaction of Epicatechin a from cranberry with secondary structures of Modelled fimbrial adhesion virulence protein.



Some of the commonly used antibiotics to treat urinary tract infections caused by Uropathogenic E.coli which includes Ofloxacin, sulfamethoxazole, Trimethoprim were subjected to docking analysis for comparative studies. The Binding Energy values obtained for these antibiotics from docking analysis are as follows.

Figure 5

Table 2 : Binding Energy values of antibiotics commonly used for treatment of urinary tract infections caused by Uropathogenic

Antibiotic	Binding Energy kcal/mol
Trimethoprim	-6.22
sulfamethoxazole	-5.33
Ofloxacin	-4.85

CONCLUSION

Since its reported that Uropathogenic E Coli have developed antibiotic resistance(Gupta et al 2002), Epicatechin Proanthocyanidin from cranberry with many evidences of its activity against virulence proteins of Uropathogenic E.coli has a very good prospective of being used as a medicine for Urinary tract infections caused by Escherichia coli. Comparative docking analysis of commonly used antibiotics used for treatment of urinary tract infections caused by Uropathogenic E.coli also suggest that Epicatechin can be an alternative for the treatment.

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