International Journal of Advances in Scientific Research
ISSN: 2395-3616 (Online)
Journal DOI: 10.7439/ijasr
Research Article

In silico analysis of fluoride transport proteins from prokaryotic and eukaryotic organisms

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***Article History:**
Received: 31/12/2016
Accepted: 21/01/2017
DOI: https://dx.doi.org/10.7439/ijasr.v3i2.3822

Abstract

In silico analysis of Fluoride transporter proteins obtained from database are presented in this study. The composition of alanine and glycine was high while low concentrations of aspartic acid, cysteine and glutamine were seen when compared to other aminoacids. Molecular weight of fungal transporters was the highest while the bacterial showed relatively less molecular weights. The instability index of all the proteins was less than 40 showing that all of them are stable except that of Neurospora and Bifidobacterium. Aliphatic index was found to be within a range of 65 to 100.
Keywords: Fluoride Transporter Proteins, Secondary Structural Analysis, Prokaryotes, Eukaryotes.

1. Introduction

Fluorine is the lightest element of the halogen column of the periodic table. Due to rapid urbanization and geo chemical dissolution of fluoride bearing minerals, fluoride concentration has increased in water resources. High fluoride concentration in water (> 1.5mg/L) resources results in the disease called "Fluorosis". Fluorosis is wide spread in certain developing countries like India, Argentina, Morocco, Kenya, China, Algeria Senegal, Turkey and also in developed countries like Japan and USA. In India, many states such as Andhra Pradesh, Karnataka, Tamil Nadu, Haryana, Maharashtra, Gujarat, Rajasthan, Kerala and Himachal Pradesh are affected by fluorosis. Fluoride element is a serious threat to most bacterial and yeast cells. To protect them from fluoride toxicity bacterial cells carry two different types of proteins namely fluoride/hydrogen antiporters and fluoride-specific channels. If bacteria lack Fluc channels, fluoride accumulates in the cells in the form of HF (hydrofluoric acid) which breaks down due to lower acidity inside the cell. Fluc proteins protect bacteria from fluoride toxicity [1,2]. Fluc channels are also being investigated as novel targets for antibiotics [3,4] as bacterial growth can be retarded when they are shut down. In the present study, fluoride transport proteins from different prokaryotic and eukaryotic organisms are analyzed by in silico methods and the results are discussed and communicated.

2. Materials and Methods

Retrieval of complete sequences of the fluc proteins was done from UniProtKB/Swiss-Prot [5]. These sequences were used for further analysis. ExPASy's ProtParam tool was used for the computation of various physical and chemical parameters [6]. SOPMA tool (Self-Optimized Prediction Method with Alignment) server was used to characterize the secondary structural features [7]. The transmembrane regions classified as membrane bound and soluble proteins were predicted by SOSUI server [8].

3. Results and Discussion

The mechanism of fluoride toxicity in prokaryotes or eukaryotes is quite complex and needs a lot investigation. Fluoride inhibits the genes associated with fluoride riboswitches enzymes such as enolase [9]. Increase in fluoride concentration can be detected by a fluoride-responsive riboswitch. Fluoride inhibits phosphoryl-transfer enzymes essential for energy production and nucleic acid synthesis [10,11]. Two phylogenitically unrelated classes of

Fluoride exporters were recently identified in prokaryotes and eukaryotes. Fluc channels decreases the fluoride concentration in the cytoplasm [12]. One cause of fluoride toxicity could be interactions with Mg^{2+} and Ca^{2+} ions which forms complexes with the fluoride ion [13-15]. Fluoride transporter proteins obtained from database are presented in Table 1. Table 2 shows that the amino acid composition of proteins obtained from biological databases. The composition of alanine and glycine was high while low concentrations of aspartic acid, cysteine and glutamine were seen when compared to other aminoacids. The numbers of positively charges are high compared to that of negatively charged proteins (Table 2). Molecular weight of fungal transporters was the highest while the bacterial showed relatively less molecular weights. The instability index of all the proteins was less than 40 showing that all of them are stable except that of Neurospora and Bifidobacterium. Aliphatic index was found to be within a range of 65 to 100. From Table 3, Secondary structural analysis of the proteins showed the dominance of α-helices and random coils followed by extended strands and beta turns. SOSUI server analysis (Table 4) has shown that all the proteins are membrane with transmembrane helices in nature.

Table 1: Physico chemical characteristics of different fluoride ion transporters

S. No	Species	No of Amino acids	MWt	PI	negatively charged residues	Positively charged residues	Extinction coefficients	Instability index	Aliphatic index
1	*Enterobacteriaceae bacterium* (strain FGI 57)	125	13431.95	10.85	2	6	27500 & 27500	27.28	108.56
2	ECOLI Protein crcB	127	13777.31	9.49	3	5	27500	26.13	114.49
3	*Propionibacterium acnes* ATCC 11828	143	15064	11.1	4	12	30605 & 30480	35.5	122.03
4	*Pelodictyon luteolum* 4(strain DSM 273)	129	13471.66	5.57	5	4	12950 & 12950	36.38	103.64
5	*Thermococcus barophilus* (strain DSM 11836 / MP)	118	12979.37	9.3	5	8	8940 & 8940	27.91	118.14
6	*Haloquadratum walsbyi* (strain DSM 16790 / HBSQ001)	113	11416.33	4.82	6	4	3105 & 2980	24.35	120.62
7	*Campylobacter jejuni* subsp. jejuni serotype O:2 (strain NCTC 11168)	122	13514.18	9.81	0	5	11460 & 11460	32.81	131.07
8	*Lactobacillus kefiranofaciens* (strain ZW3)	124	13678.13	9.48	4	8	24410	24.78	113.31
9	*Saccharomyces cerevisiae* (strain ATCC 204508 / S288c)	375	41985.27	8.77	19	26	53455 & 52830	25.85	96.45
10	*Saccharomyces cerevisiae* (strain ATCC 204508 / S288c)	375	41971.24	8.77	19	26	53455 & 52830	25.26	96.19
11	*Neurospora crassa* (strain ATCC 24698 / 74-OR23-1A / CBS 708.71 / DSM 1257 / FGSC 987)	526	57329.11	6.36	52	48	69830 & 69330	44.71	85.53
12	*Scardovia wiggsiae*	180	18620.14	6.69	12	11	27305 & 26930	38.11	75.61
13	*Bifidobacterium longum* (strain DJO10A)	178	17813.32	8.69	9	12	11710 & 11460	26.85	83.6
14	*Bifidobacterium animalis* subsp. lactis (strain AD011)	310	33786.97	7.84	27	28	36565 & 36440	48.5	78.77

Table 2: Amino acid composition of different fluoride ion transporters

S. No	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His
1	11.2	3.2	3.6	0.8	0.8	0.8	0.8	12	2.4
2	10.2	3.1	3.9	0.8	0.8	2.4	1.6	11	1.6
3	11.2	7.7	0.7	1.4	2.1	2.1	1.4	14	1.4
4	11.6	2.3	1.6	0.8	0.8	0	3.1	12.4	0.8
5	5.9	5.1	2.5	1.7	0.8	0.8	2.5	11	0
6	7.1	3.5	1.8	4.4	1.8	1.8	0.9	15	0.9
7	4.9	0.8	7.4	0	0.8	2.5	0	9.8	0.8
8	7.3	3.2	4.8	0	0	0	3.2	14.5	0.8
9	5.9	1.9	5.3	1.9	2.9	1.6	3.2	6.1	2.4
10	5.6	1.9	5.3	1.9	2.9	1.6	3.2	6.4	2.4
11	9.5	6.7	2.7	6.3	1.5	3.8	3.6	9.1	2.5
12	15.6	2.8	1.7	3.3	3.9	1.1	3.3	13.9	4.4
13	18.5	4.5	1.7	3.4	2.8	1.7	1.7	12.9	1.7
14	11	6.1	1.6	4.8	1	2.6	3.9	8.4	3.9

Table 3: Secondary structure analysis of different fluoride ion transporters

S. No	Alpha Helix	310 Helix	Pi Helix	Beta Bridge	Extended Strand	Beta turner	Bend region	Random Coil	Ambiguous State
1	44.8	0	0	0	20	13.6	0	21.6	0
2	48.03	0	0	0	17.32	8.66	0	25.98	0
3	48.25	0	0	0	16.78	14.69	0	20.28	0
4	26.36	0	0	0	33.33	13.95	0	26.36	0
5	49.15	0	0	0	21.19	11.02	0	18.64	0
6	7.08	0	0	0	52.21	7.08	0	33.63	0
7	47.54	0	0	0	23.77	9.02	0	19.67	0
8	35.48	0	0	0	29.03	13.71	0	21.77	0
9	43.2	0	0	0	19.47	8.53	0	28.8	0
10	41.87	0	0	0	20	8.53	0	29.6	0
11	39.73	0	0	0	14.83	8.37	0	37.07	0
12	38.89	0	0	0	12.22	11.11	0	37.78	0
13	40.45	0	0	0	10.67	8.43	0	40.45	0
14	44.19	0	0	0	9.68	5.16	0	40.97	0

Table 4: Nature of the protein using SOSUI server

Name	SOSUI Server analysis
Enterobacteriaceae bacterium (strain FGI 57)	This Amino acid sequence is of a membrane protein which has 4 trans membrane helices.
ECOLI Protein crcB	This Amino acid sequence is of a membrane protein which has 4 trans membrane helices.
Propionibacterium acnes ATCC 11828	This Amino acid sequence is of a membrane protein which has 4 trans membrane helices.
Pelodictyon luteolum (strain DSM 273)	This Amino acid sequence is of a membrane protein which has 4 trans membrane helices.
Thermococcus barophilus (strain DSM 11836 / MP)	This Amino acid sequence is of a membrane protein which has 4 trans membrane helices.
Haloquadratum walsbyi (strain DSM 16790 / HBSQ001)	This Amino acid sequence is of a membrane protein which has 4 trans membrane helices.
Campylobacter jejuni subsp. jejuni serotype O:2 (strain NCTC 11168)	This Amino acid sequence is of a membrane protein which has 4 trans membrane helices.
Lactobacillus kefiranofaciens (strain ZW3)	This Amino acid sequence is of a membrane protein which has 3 trans membrane helices.
Saccharomyces cerevisiae (strain ATCC 204508 / S288c)	This Amino acid sequence is of a membrane protein which has 5 trans membrane helices.
Saccharomyces cerevisiae (strain ATCC 204508 / S288c)	This Amino acid sequence is of a membrane protein which has 5 trans membrane helices.
Neurospora crassa (strain ATCC 24698 / 74-OR23-1A / CBS 708.71 / DSM 1257 / FGSC 987)	This Amino acid sequence is of a membrane protein which has 7 trans membrane helices.
Scardovia wiggsiae	This Amino acid sequence is of a membrane protein which has 3 trans membrane helices.
Bifidobacterium longum (strain DJO10A)	This Amino acid sequence is of a membrane protein which has 3 trans membrane helices.
Bifidobacterium animalis subsp. lactis (strain AD011)	This Amino acid sequence is of a membrane protein which has 4 trans membrane helices.

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