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Secondary Structure of Butyrylcholinesterase

Srinivas K[1], Sridhar GR[2]* and Appa Rao Allam[3]

[1]V R Siddhartha Engineering College, Vijayawada, India
[2]Endocrine and Diabetes Centre, Visakhapatnam, India
[3]C R Rao Advanced Institute for Mathematics, Statistics & Computer Science (AIMSCS), University of Hyderabad Campus, India

Abstract

Objective: Butyrylcholinesterase, a protein from the esterase family of enzymes, has been shown to modulate the expression of insulin resistance syndrome. In order to identify related proteins with more well established functions, the current *in silico* work was done to delineate the secondary structure of the enzyme and compare it with other proteins of similar structure. The purpose was to predict possible role(s) of BchE in comparison to related proteins based on their secondary structures.

Methods: With the input as amino acid sequences of BchE, we obtained the secondary structure using the SOPM tool. From the Protein Data Bank (PDB) database we compared the secondary structure of BchE with those having 65% or more similarity.

Results: We obtained 13 sequences: Acetylcholinesterase (EC 3.1.1.7, PDB 1gqr; score 72.134), Fasciculin 2 mouse acetylcholinesterase complex 1MAH3, PDB 1mah; score 71.806), AT sulfurase from penicillium chrysogenum, 1i2d; score 67.9727), pyruvate kinase (1a3w; score 67.5503), thronine synthase from Arabidopsis thaliana (1e5x; score 67.426), DNA repair UVRB in complex with ATP (1d9z; score 66.778), hyperthermophilic tungstopterin enzyme (1aor; score 66.28), xanthine oxidase from bovine milk (1fiq; score 66.147), alpha D-glucoronidase from Bacillus stearothermophius (1k9d; score 65.959), ribonuclease inhibitor-angiogenin complex (1a4y; score 65.913), hemochromatosis protein HFE complexed with transferrin receptor (1de4; score 65.723), K217e variant of Klebsiella aerogenes urease (1a5k; score 65.667), phosphoenolpyruvase carboxykinase in complex with ADP, A1F3 and pyruvate (1k3c; score 65.5233). Their functions ranged from catalyzing acetylcholine to sulfate asimilation, glycolysis, nucleic acid binding, oxidoreductase activity, iron sulfur cluster binding, xanthine oxidation, cation binding, urease activity and phosphoenolpyruvate carboxykinase activity. They are present in both the cytoplasm, extracellular compartment and cytoplasmic membranes.

Conclusion: We compared the predicted secondary structure of butyrylcholinesterase and obtained 13 proteins with at least 65% similarity that are found in the cytoplasm and extracellular regions, with catabolic, synthetic, electron transport and immune processing.

Keywords: In silico; Protein Data Bank (PDB)

Introduction

Proteins are key components in communication, metabolism and structure in biological processes. The structure of proteins is conventionally obtained by eleborate and complex methods such as X-ray crystallography, NMR and Raman spectroscopy. Though difficult to execute, they form the gold standard for comparison.

The omics revolution has provided an abundance of publicly available data. It is not practical to apply traditional biological methods to classify and annotate them structurally and functionally.

More rapid, automated *in silico* methods have therefore been developed to derive meaning from the sea of data. Given that the amino acid sequences are known and the force character of each molecule is available, physical and chemical computational methods should be able to predict the protein structure based on the amino acid sequences.

In this study we employed *in silico* method to predict the secondary structure of butyrylcholinesterase, a protein that is well characterized structurally, but with poorly defined physiological functions. Comparisons can be performed at various levels in biological organization, eg comparing the nucleotide sequences and constructing phylogenic trees to ascertain possible evolutionary origin and function [1].

Butyrylcholinesterase is an enzyme that is involved in the phenotypic expression of insulin resistance and metabolic syndrome [2]. It belongs to the esterase family of enzymes, in which acetylcholinesterase (AChE) is an important regulator of neuromuscular activity. The two members share structural similarity, although the functional significance of BChE is not as well characterized as it is for AChE. Other than its specific role in hydrolyzing succinylcholine, a muscle relaxant given in general anesthesia, the other functions generally relate to hydrolysis of cocaine, of pesticides and as a prophylactic agent in future exposure to biochemical warfare agents [3-5]. Because it is produced in the liver, circulates in the blood stream, and is present at higher levels than AChE, a toxicological role for BChe has been attributed.

In addition it is affected by dietary lipids, changes in body weight and in diabetes mellitus [2,3,6,7]. The development of succinylcholine induced apnea in individuals with variant forms of the enzyme is the only well established phenotypic expression of the enzyme [6]. Despite its functional relationship to the neuromusclular enzyme acetylcholinesterase, the physiological roles of BchE are not well established. The advent of the genomics era allowed *in silico* studies to compare the re-

***Corresponding author:** Sridhar GR, Endocrine and Diabetes Centre, 15-12-15 Krishnanagar, Visakhapatnam 530 002, India, E-mail: sridharvizag@gmail.com

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lationship of proteins with other proteins with known functions, and infer their possible physiological roles. Using phylogenetic analysis, we had shown that BchE exists in life forms across the spectrum, implying it could have an evolutionarily conserved role [1]. We also showed that it could play a role in the etiology of insulin resistance and the coexistence of Alzheimer's disease and type 2 diabetes mellitus through oxidative stress [8,9].

In this presentation we predict the secondary structure of BchE; by using bioinformatics tools to compare the structure with other proteins in Protein Databank (PDB) and ascertain its possible role vis a vis other proteins with similar structure(s).

Methods and Results

Secondary structure prediction

BChE protein information and its sequence is retrieved from the UniProtKB/Swiss-Prot with entry P06276. Figure 1 shows the BChE sequence and its information. FASTA formatted BChE human protein sequence was entered into self-optimized prediction method (SOPM) to obtain the secondary structure. Figure 2 shows the secondary structure of BChE along with its protein sequence.

The following sequence of BChE (i.e P06276)/ Structure (PDB id: 2pm8) was shown to interact with ApoE (PDB id: 1nfn), PON1 (PDB id: 1v04) and ATP (sequence id: Q9Y487):

>sp|P06276|CHLE_HUMAN Cholinesterase OS=Homo sapiens GN=BCHE PE=1 SV=1

MHSKVTIICIRFLFWFLLLCMLIGKSHTEDDIIIATKNG-KVRGMNLTVFGGTVTAFLGIP

YAQPPLGRLRFKKPQSLTKWSDIWNATKYANSCCQNIDQSFP-GFHGSEMWNPNTDLSEDC

LYLNVWIPAPKPKNATVLIWIYGGGFQTGTSSLHVYDGK-FLARVERVIVVSMNYRVGALG

FLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKS-VTLFGESAGAASVSLHLLSPG

SHSLFTRAILQSGSFNAPWAVTSLYEARNRTLNLAKLTGCS-RENETEIIKCLRNKDPQEI

LLNEAFVVPYGTPLSVNFGPTVDGDFLTDMPDILLELGQFK-KTQILVGVNKDEGTAFLVY

GAPGFSKDNNSIITRKEFQEGLKIFFPGVSEFGKESILFHYTD-WVDDQRPENYREALGDV

VGDYNFICPALEFTKKFSEWGNNAFFYYFEHRSSKLPWPEW-MGVMHGYEIEFVFGLPLER

RDNYTKAEEILSRSIVKRWANFAKYGNPNETQNNSTSW-PVFKSTEQKYLTLNTESTRIMT

KLRAQQCRFWTSFFPKVLEMTGNIDEAEWEWKAGFHRWN-NYMMDWKNQFNDYTSKKESCV

GL

Comparison of secondary structure

In the current presentation we have compared the secondary structure of BChE with other proteins to impute a common biological function, considering the similarity of secondary structures. However, the authors realize this is a hypothesis-generating proof of concept *in silico*

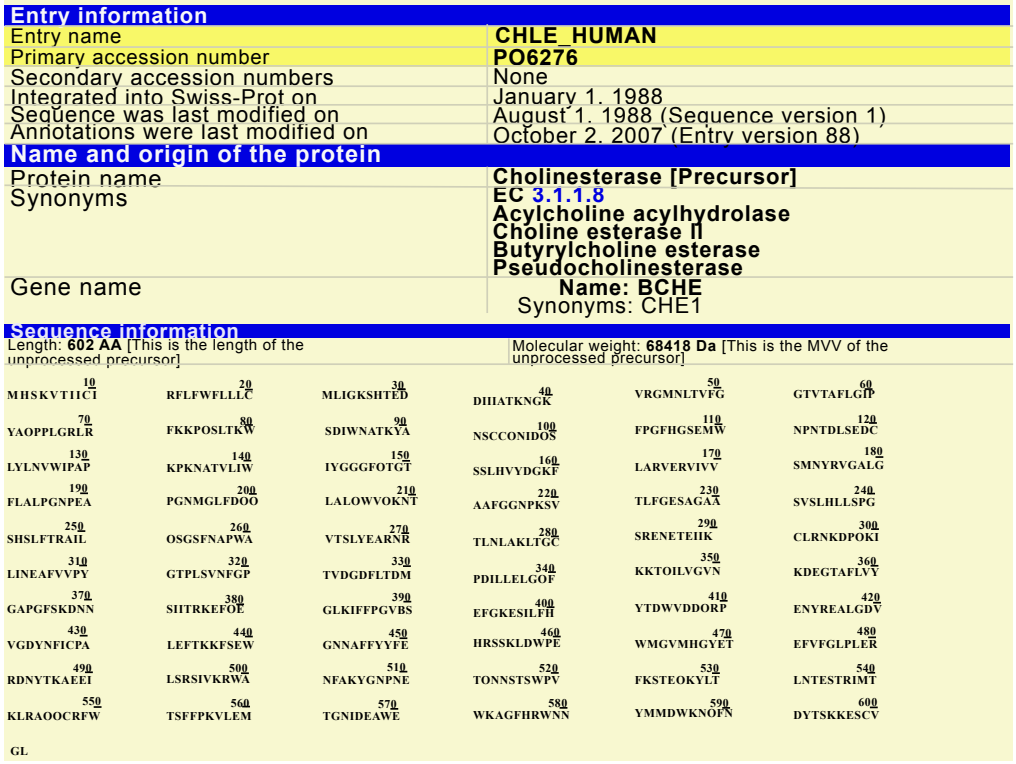

Figure 1: UniPortKB/Swiss-prot entry for BChE Human.

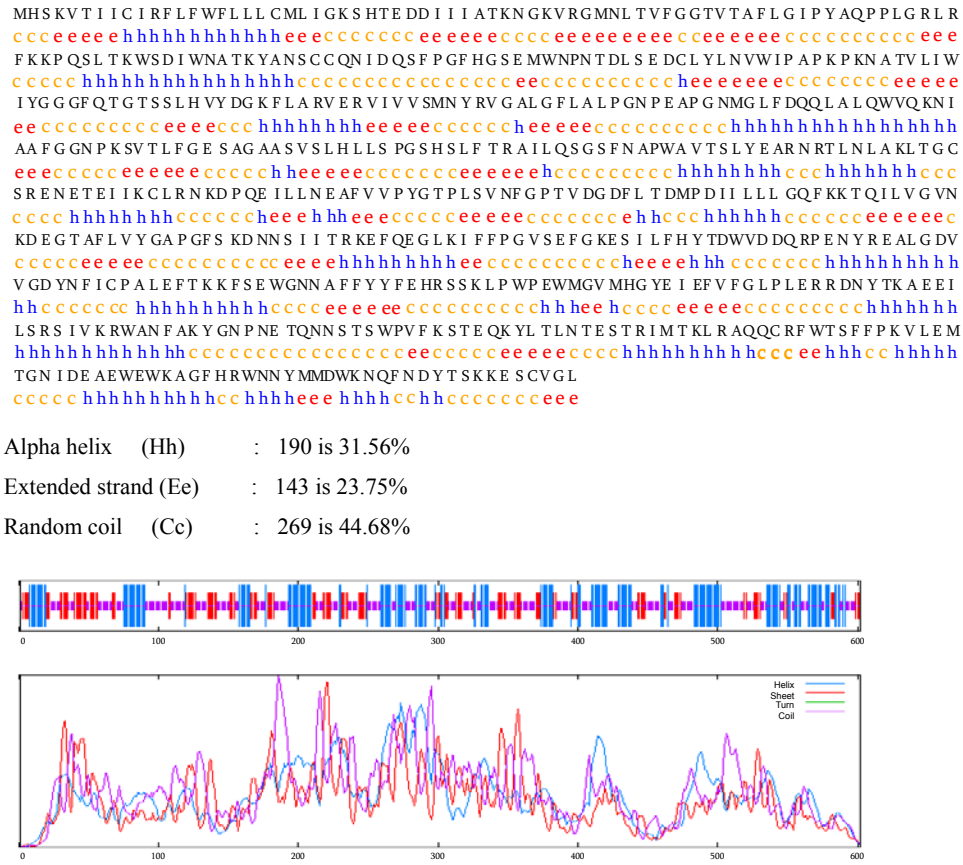

Figure 2: BChE Secondary Structure as well as its sequence.

study, which is to our knowledge, is the first comparison published for this purpose. This must be further studied both *in silico* by comparison of tertiary and quarternary structures, as well as pathway analysis and protein-protein interaction to further refine the possible functional role that can be verified by biological methods.

By using secondary structure of BChE to search for similar structures in PDB library, thirteen structures with different species have been identified. To infer the functionality for assigning BChE protein, in accordance with the similarity and Z-score values, pair-wise alignment has been implemented between BChe structure with all these thirteen structures. Figure 3 shows the pair wise alignment of BChE secondary structure with 1gqr secondary structure and their structure similarity.

>1gqr ACETYLCHOLINESTERASE (E.C. 3.1.1.7) COMPLEXED WITH RIVASTIGM...

Z-Score: 2.20

Normalized score: 72.134

Experimental residue alignment:

Length 602

Length 532

By using the literature survey, the functionalities of thirteen species have been collected, analyzed and given in tabular form. The Table gives information about functionalities of these species.

Discussion

Our approach to secondary structure prediction is based on using the sequence of proteins as an interlingua between the different identifiers. This strategy allows, our secondary structure prediction platform to integrate data from multiple sources into a single structure, while allowing the user to control which sequences are used in the prediction.

Genomic data from sequencing projects can be used for biological and clinical research only if the functional information can be extracted from them and biological data can be converted into 'knowledge of biological systems' [10]. Butyrylcholinesterase lends itself as a prototype for such analysis.

Here we have shown that the secondary structure of butyrylcholinesterase had similar structures distributed across the biological spectrum ranging from plants to fish, bacteria, fungi and mammals [11-20]. This is consonant with our earlier report where BchE related sequences were shown to be present in a similar wide spectrum of life forms [1].

The known functional roles of proteins ranged across a broad sweep of physiological processes including the catalysis of final steps in glycolysis [17], threonine formation [11], urate formation [19] and the binding of a key protein in angiogenesis [20]. The active sites of proteins also ranged from acetylcholinesterase with a deep narrow gorge [12] to that of hyperthermophilic aldehyde ferredoxin oxidoreductase, which shares the properties of multicentric redox proteins [13].

CCCCCCHHHHHHHHHHHHEEEEECCCCCCCCCEEEEECCCCCCCCEEE--CCCCCEEEEE-
CCC--------------EEEE-CC-------EEEE-C-------EEEEECC---EEEEEE

----CCCCCCCCCCCCCCCCCCCCCCCCCE--HCCCCCCCCCCCCCCCCCCCCCCCCCC
EEEECCCCCCCCCCCCCCCCCCCCCC---EEE-CCCCCCCCCCCCCCCCCC--------

CCCCCCCCH---EEEEEECCCCCCCCC----EEEEEECCCCEECCCCC--EE-----CCC
--------HHHH------CCCCCCCCCCCCCEEEEEE------CCCCCCCEEEEEEECCC

C------------HHHHH--CCCEEEEEECCCCCEEEEEECCCCCCCCCCCCHHHHHHH
CCCCCCCCCCCCCCHHHHHHHCC-EEEE--CCCCCHHHHH-CCCCCCCCCCCCHHHHHHH

HHHHHHHH-EECCCCCCEEEEEE-----CCCCCCHHHH-------EEHCCCCCCHHHHHE
HHHHHHHHH--CCCCC-EEEEEEEEEEC-----HHHHHHHHHHH---C-----HHHH--

ECCCCCCCCEEEE-CCC---------HHHHHHHHHHHHH-CCCCCC-HHHHHHHHH-CCC
-CCCC----EEEEECCCCCCCCCCCCHHHHHHHHHHHHHHHHCCCCCCCHHHHHHHHHHCC-

CHHHHHHHHHHCCCCCEEECCCCCCCCCCCCCCC----------HHHHH--CCCCCCC
-HHHHHHH----------CCCCCCCCCCCCCCCCCCCCCCCCCCHHHHHHHCCCCCCC

EEEEEECCCCCCEEEEE--CCCCCCCCCCCCCHHHHHHHHHHHHHCCCCCCHHHHHHHHHH
EEEEEECCCC--HHHHHHHCCCCCCCCCCCCCHHHHHHHHHHH-CCCCC-HHHHHHHHHH

HCCCCCCCCHHHHHHHHHHH----CCCEECCHHHHHHHHHH----CCCCEEEEEECCCC
HCCCCCCCCHHHHHHHHHHHHHHHH-----C-HHHHHHHHHHHHHHCCC-EEEEEECCCC

CCCCCC---------HHH-CCCCCCCEEEEECCCCCCCCCC-HHHHHHHHHHHHHHHH
CCCCCCCCCCCCCCCCHHHH----------CCCCCCCCCCCHHHHHHHHHHHHHHHH

HH--CCCCCCCCCCCCCCCCCCCCCCEEEEECCCCCC------HHHHHHHHHHHHHHHH
HHHHCCCCCCCCCCCCCCCCCCC--EEEEECCCCCEEECCCHHHHHHHH--------

HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH-CCCCCCCCCCCCCC-
---------CHHHHHHHH-------C

Figure 3: Pair wise alignment of BChE with 1gdr.

Evolutionarily, a study of related proteins shows they are conserved from bacteria to humans and have homologous structure across various sources [19], and have vestigial features of bifunctional ancestor of fungal sulfurylase [16]. Divergence of active site is suggested, as well as sequence identify at the erythroid cells compared to others [18].

Structural similarities among proteins are associated with functional relationship and with being part of the same functional network [21]. Earlier we showed that butyrylcholinesterase formed a network with related proteins including dehydrogenases (ALDH9A1, PDHX), ATPase (ATP6VOA2) and peraoxanase (PON1), besides acetylcholinesterases [19]. Based on their similarity, butyrylcholinesterase may have putative roles in maintaining cell growth and a supplementary role to acetylcholinesterase in neural function [22,8].

Evidence is available for the modulatory role of butyrylcholinesterase in different components of the metabolic syndrome [23]. The current study provides further leads to understanding its relation to other proteins similar in their secondary structure. A confluence of *in silico* and in vitro methods will be able to increase possibly new indications for therapeutic use of the protein [2].

The information has been annotated from published biological investigations. The purpose of this *in silico* work is to provide proof-of-con [7,8] and that it may be applied for the study of other proteins with poorly characterized functions.

In summary proteins present in the cytoplasm, extracellular compartment and cytoplasmic membranes share secondary structure simi-

PDB id	Species Name	Score	Z-score	Biological Functions	Molecular Functions	Cellular Components
1gqr	SPECIES OF ELECTRIC RAY	72.134	2.20	acetylcholinesterase activity cholinesterase activity	acetylcholine catabolic process in synaptic cleft	
1mah	MOUSE	71.8062	2.18	cholinesterase activity		Extracellular region
1i2d	CRYSTAL STRUCTURE OF ATP SULFURYLASE FROM PENICILLIUM CHRYSOGENUM	67.9727	1.92	Sulfate adenylyltransferase (ATP) activity ATP binding kinase activity transferase activity, transferring phosphorus-containing groups	sulfate assimilation	
1a3w	PYRUVATE KINASE FROM SACCHAROMYCES CEREVISIAE COMPLEXED WITH FBP, PG, MN2+ AND K+	67.5503	1.89	magnesium ion binding pyruvate kinase activity potassium ion binding	glycolysis	
1e5x	STRUCTURE OF THREONINE SYNTHASE FROM ARABIDOPSIS THALIANA	67.4264	1.89	catalytic activity threonine synthase activity pyridoxal phosphate binding	amino acid metabolic process metabolic process threonine biosynthetic process	
1d9z	CRYSTAL STRUCTURE OF THE DNA REPAIR PROTEIN UVRB IN COMPLEX WITH ATP	66.7785	1.84	nucleic acid binding DNA binding helicase activity ATP binding excinuclease ABC activity hydrolase activity	nucleotide-excision repair	cytoplasm excinuclease repair complex
1aor	STRUCTURE OF A HYPERTHERMOPHILIC TUNGSTOPTERIN ENZYME, ALDEHYDE FERREDOXIN OXIDOREDUCTASE	66.28	1.81	oxidoreductase activity, acting on the aldehyde or oxo group of donors, iron-sulfur protein as acceptor iron-sulfur cluster binding	electron transport	
1fiq	CRYSTAL STRUCTURE OF XANTHINE OXIDASE FROM BOVINE MILK	66.147	1.80	xanthine dehydrogenase activity xanthine oxidase activity electron carrier activity oxidoreductase activity metal ion binding FAD binding iron-sulfur cluster binding	electron transport	
1k9d	A CRYSTAL STRUCTURE OF ALPHA-D-GLUCURONIDASE, A FAMILY-67 GLYCOSIDE HYDROLASE FROM BACILLUS STEAROTHERMOPHILUS T-1	65.9591	1.79	catalytic activity cation binding alpha-glucuronidase activity	carbohydrate metabolic process xylan catabolic process	extracellular region
1a4y	RIBONUCLEASE INHIBITOR-ANGIOGENIN COMPLEX	65.9134	1.79	protein binding nucleic acid binding pancreatic ribonuclease activity		
1de4	HEMOCHROMATOSIS PROTEIN HFE COMPLEXED WITH TRANSFERRIN RECEPTOR	65.7235	1.77		immune response antigen processing and presentation	membrane MHC class I protein complex
1a5k	K217E VARIANT OF KLEBSIELLA AEROGENES UREASE	65.6678	1.77	urease activity nickel ion binding hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	nitrogen compound metabolic process urea metabolic process	
1k3c	PHOSPHOENOLPYRUVATE CARBOXYKINASE IN COMPLEX WITH ADP, ALF3 AND PYRUVATE	65.5233	1.76	phosphoenolpyruvate carboxykinase activity phosphoenolpyruvate carboxykinase (ATP) activity ATP binding purine nucleotide binding	gluconeogenesis	

Table 1: Protein with similar secondary structures.

larity with butyrylcholinesterase and participate in catabolic, synthetic, electron transport and immune processing.

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