Biochimica et Biophysica Acta 1864 (2016) 706–714

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Tetramer-organizing polyproline-rich peptides differ in CHO cell-expressed and plasma-derived human butyrylcholinesterase tetramers

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ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 12 February 2016
Accepted 2 March 2016
Available online 3 March 2016

Keywords:
Polyproline
Mass spectrometry
Recombinant butyrylcholinesterase
Tetramer-organizing peptide
CHO cells

ABSTRACT

Tetrameric butyrylcholinesterase (BChE) in human plasma is the product of multiple genes, namely one BCHE gene on chromosome 3q26.1 and multiple genes that encode polyproline-rich peptides. The function of the polyproline-rich peptides is to assemble BChE into tetramers. CHO cells transfected with human BChE cDNA express BChE monomers and dimers, but only low quantities of tetramers. Our goal was to identify the polyproline-rich peptides in CHO-cell derived human BChE tetramers. CHO cell-produced human BChE tetramers were purified from serum-free culture medium. Peptides embedded in the tetramerization domain were released from BChE tetramers by boiling and identified by liquid chromatography–tandem mass spectrometry. A total of 270 proline-rich peptides were sequenced, ranging in size from 6–41 residues. The peptides originated from 60 different proteins that reside in multiple cell compartments including the nucleus, cytoplasm, and endoplasmic reticulum. No single protein was the source of the polyproline-rich peptides in CHO cell-expressed human BChE tetramers. In contrast, 70% of the tetramer-organizing peptides in plasma-derived BChE tetramers originate from lamellipodin. No protein source was identified for polyproline peptides containing up to 41 consecutive proline residues. In conclusion, the use of polyproline-rich peptides as a tetramerization motif is documented only for the cholinesterases, but is expected to serve other tetrameric proteins as well. The CHO cell data suggest that the BChE tetramer-organizing peptide can arise from a variety of proteins.

1. Introduction

Butyrylcholinesterase (BChE[1], P06276) and acetylcholinesterase (AChE, P22303) are typically found as tetramers of 4 identical subunits. However, a large amount of dimeric AChE is found anchored to the red cell membrane. The tetramers are held together by a novel supercoil motif. Amphipathic, tryptophan-rich, α-helices from the C-terminals of four monomers coil around a polyproline type II helix from an exogenously introduced proline-rich, tetramer-organizing peptide to create the tetramer. The tryptophans are located on the inner surface of the α-helix bundle, where they interact with the prolines from the tetramer-organizing peptide. The structure of the supercoil was elucidated crystallographically [1]. A model of tetrameric BChE showing the supercoil structure is presented in Fig. 1 [2].

Tetrameric forms of AChE and BChE are found in both the cholinergic synapses of the brain and in the neuromuscular junction. In the neuromuscular junction, they are anchored to the extracellular matrix by a collagenous protein, referred to as ColQ [3]. A proline-rich N-terminal domain of ColQ promotes formation of tetramers thereby linking ColQ to the cholinesterase [4]. In the brain (and in the neuromuscular junction), AChE and BChE are anchored to membranes via a transmembrane protein, referred to as PRiMA for proline-rich membrane anchor [5]. This protein also carries a proline-rich domain that promotes formation of tetramers, thereby linking PRiMA to the cholinesterase.

Soluble, tetrameric forms of AChE and BChE are found in serum (human serum, equine serum, and fetal bovine serum). Proline-rich peptides are responsible for the formation of these tetramers, but the tetramer-organizing peptide is neither the one from ColQ nor the one from PRiMA. Rather, the major source of the tetramer-organizing peptide is "Ras-associated and pleckstrin domains-containing protein 1" (lamellipodin, Q70E73), though minor amounts of tetramer-organizing peptides could be traced to other proteins [6–9]. In the

Abbreviations: rBChE, recombinant butyrylcholinesterase; MALDI TOF, matrix-assisted laser desorption–ionization time-of-flight; CHO, Chinese hamster ovary; ATCC, American Type Culture Collection; AChE, acetylcholinesterase; NCBI, National Center for Biotechnology Information; BLASTp, Basic Local Alignment Search Tool for proteins from NCBI; NCBInr, National Center for Biotechnology Information non-redundant protein database; ColQ, a collagenous protein membrane anchor; PRiMA, proline-rich membrane anchor.

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http://dx.doi.org/10.1016/j.bbapap.2016.03.003
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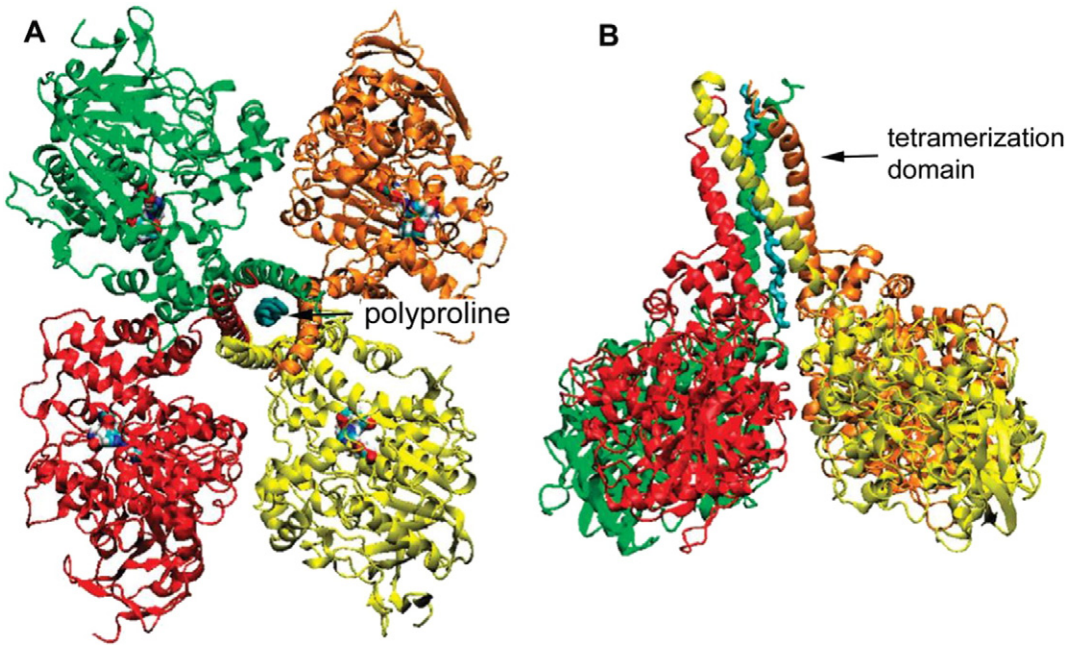

Fig. 1. Model of the tetrameric structure of human BChE. Panel A; top view showing the polyproline peptide in the center of the coiled-coil formed by the C-terminal tetramerization domains of BChE monomers. Panel B; side view showing the coiled-coil motif of the tetramerization domains.
Reproduced with permission from Pan et.al [2]. Copyright 2009 American Chemical Society.

current study, we have examined the tetramer-organizing peptides obtained from recombinant BChE expressed in Chinese Hamster Ovary (CHO) cells. We found that 60 proteins contribute tetramer-organizing peptides and that no single protein dominates the field.

2. Materials and methods

2.1. Expression and purification of recombinant butyrylcholinesterase tetramers

Full-length human butyrylcholinesterase (rBChE) was expressed in CHO-K1 cells (Chinese-hamster ovary cells; ATCC 61-CCL) using serum-free medium without added polyproline and without plasmids encoding proline-rich peptides, as previously described [10]. Soluble human rBChE secreted into the growth medium consisted predominantly of dimers and monomers, but included some tetramers. Tetrameric BChE was separated from monomers and dimers by chromatography on a procainamide-Sepharose affinity gel (prepared by Dr. Yakov Ashani as described in [11]), followed by Q-Sepharose Fast Flow anion exchange chromatography (cat# 17-0510-01, GE Health Sciences/Pharmacia, Pittsburgh, PA) and hupresin affinity chromatography (hupresin synthesized by Dr. Emilie David at the Universite de Rouen; Mont St. Aignan, Cedex, France). Monomers and dimers eluted early from the procainamide affinity gel using a sodium chloride gradient from 0 to 1 M NaCl, whereas tetramers eluted with 0.7 to 1 M NaCl. The elution positions were reversed on the anion exchange column, where gradient elution with 0 to 0.5 M NaCl yielded tetramers in the first half of the gradient, and monomers and dimers in the second half. Activity stained native PAGE gels (performed as previously described [10]) showed the purified rBChE to be predominantly tetrameric.

2.2. Isolation of tetramer-organizing peptides

Purified rBChE tetramers in 20 mM Tris/Cl pH 7.3 containing 0.05% sodium azide, 100 mM sodium chloride, and 500 mM tetramethylammonium bromide were desalted into water, depleted of small peptides, and concentrated to 24 μM (tetramer) in a final volume of 200 μl, using a Centricon-30 centrifugal filter (30,000 MW cutoff, cat# 4209, Amicon/Millipore, Bedford MA). The product was boiled for 5 min to release the tetramer-organizing peptides which then were separated from residual protein by filtration through an Ultracel YM 10 membrane (10,000 MW cutoff, cat# MRCPRT010, EMD/Millipore, Billerica, MA) followed by a 100 μl water wash to give a 300 μl preparation of peptides equivalent to 16 μM rBChE tetramer (assuming no losses during handling). This calculation takes into account that there is one polyproline peptide per tetrameric BChE.

A 20 μl sample of the 16 μM peptide preparation was used for electrospray ionization mass spectrometry. The remainder was prepared for MALDI TOF mass spectrometry. It was dried by vacuum centrifugation (Savant SpeedVac SC100, Thermo Scientific) and resuspended in 20 μl of 60% acetonitrile plus 0.1% trifluoroacetic acid to give a solution of about 240 pmol per μl.

2.3. Mass spectrometry of tetramer-organizing peptides

Peptides were subjected to both MALDI TOF and electrospray ionization mass spectrometry.

MALDI TOF mass spectrometry was performed on an ABSciex 4800 instrument (Sciex, Framingham MA). One microliter of the peptide preparation (about 240 pmol per μl) was air dried onto the MALDI target plate and overlaid with 1 μl of α-cyano-4-hydroxy cinnamic acid (10 mg/ml in 50% acetonitrile/water plus 0.3% trifluoroacetic acid, saturated) (cat# 70990, Sigma-Aldrich, St. Louis, MO). Mass spectra were taken in positive reflector mode using a 450 ns delay with the laser set to 4500 V. Data from 3000 laser shots were averaged. Fragmentation of selected masses was performed via post-source decay (i.e. collision induced dissociation was off) using an absolute precursor window of ±25 Da with metastable suppression on, timed-ion selection on, and delay times of 450 ns for DE1 and 43,860 ns for DE2. The laser was set to 5100 V. Data from 3000 laser shots were averaged.

Electrospray ionization mass spectrometry was performed on an ABSciex 5600 Triple TOF instrument (Sciex, Framingham MA). The peptide preparation (about 16 pmol per μl) was acidified with 0.1% formic acid. Five microliters were subjected to HPLC separation using a cHiPLC Nanoflex microchip column (Eksigent, Dublin CA) attached to a splitless Ultra 1D Plus ultra-high pressure chromatography system (Eksigent,