Chemico-Biological Interactions 187 (2010) 49–55

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Structure–function relationships of the α/β-hydrolase fold domain of neuroligin: A comparison with acetylcholinesterase

Philippe Leone[a], Davide Comoletti[b], Palmer Taylor[b], Yves Bourne[a,**], Pascale Marchot[c,*]

[a] Architecture et Fonction des Macromolécules Biologiques (AFMB), CNRS/Universités Aix-Marseille, Campus Luminy, Marseille, France
[b] Department of Pharmacology, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, CA, USA
[c] Centre de Recherche en Neurobiologie-Neurophysiologie de Marseille (CRN2M), CNRS/Universités Aix-Marseille, Institut Fédératif de Recherche Jean Roche, Faculté de Médecine-Secteur Nord, Marseille, France

ARTICLE INFO

Article history:
Available online 25 January 2010

Keywords:
Acetylcholinesterase
α/β-Hydrolase
Catalysis
Cell adhesion
Crystal structure
Fasciculin
Neurexin
Neuroligin
Synapse

ABSTRACT

The neuroligins are postsynaptic cell adhesion proteins whose extracellular domain belongs to the α/β-hydrolase fold family of proteins, a family characterized through the enzyme acetylcholinesterase (AChE) and other enzymes with various substrate specificities. Neuroligin associations with the pre-synaptic neurexins participate in synapse maturation and maintenance. Alternative splicing of the neuroligin and neurexin genes results in multiple isoforms and presumably regulation of activity, while mutations appear to be associated with autism spectrum disorders. The crystal structures of the extracellular, cell adhesion domain of three neuroligins (NL1, NL2 and NL4) revealed features that distinguish the neuroligins from their enzyme relatives and could not be predicted by homology modelling from an AChE template. The structures of NL1 and NL4 bound with a soluble β-neurexin domain (Nrxβ1) revealed the precise position and orientation of the bound Nrxβ1 and the Ca^{2+}-dependent interaction network at the complex interface. Herein we present an overview of the unbound and Nrxβ1-bound neuroligin structures and compare them with structures of AChEs with and without a bound fasciculin partner. This study exemplifies how an α/β-hydrolase fold domain tailored for catalysis varies to acquire adhesion properties, and defines three surface regions with distinctive locations and properties for homologous or heterologous partner association.

1. Introduction

The neuroligins belong to a subfamily of cell adhesion molecules that form heterophilic adhesive complexes via an extracellular α/β-hydrolase fold domain similar to that found in several esterases and lipases [1,2]. In the cell adhesion molecules, this domain lacks the characteristic enzymatic activity due to mutation of one or more residues in the catalytic triad; rather, it has acquired adhesive surface determinants to mediate cell–cell interaction. Different neuroligin isoforms (five identified in humans and four in rodents) [3–6] are expressed at the surface of post-synaptic neurons, where their interaction with pre-synaptic neurexins is necessary and sufficient for maturation and maintenance of functional synapses [7–9]. In fact, a *trans*-synaptic association is established between the α/β-hydrolase fold domain of neuroligins and the extracellular laminin–neurexin–sex hormone-binding-protein (LNS) domains of neurexins-α (which encompass six different LNS domains) and neurexins-β (a single LNS domain corresponding to the sixth domain of neurexin-α). The interaction between the different isoforms of neuroligins (NL1–5) and neurexins (Nrxα1–3 and Nrxβ1–3) is controlled by an intricate recognition code that involves alternative splicing of either partner, N-linked glycosylation, and recognition through Ca^{2+} and ionic strength-dependencies [10–15]. The essential role of neuroligins in neuronal connectivity and circuitry formation is further highlighted by the identification of mutations related to autism spectrum disorders and mental retardation [16–21].

The extracellular domain of neuroligin displays ~35% sequence identity with that of the enzyme acetylcholinesterase (AChE) (Fig. 1). Besides substitution of a Gly residue for the catalytic Ser of AChE, comparison of neuroligin and AChE sequences reveals other differences, such as a distinct linkage for the third disulfide bridge (cf. below), different positions for N-glycosylation and, in the neuroligins, the presence of alternatively spliced inserts (A and B) that can generate yet additional isoforms.

* Corresponding author at: Dépt. Signalisation Neuronale, CRN2M UMR-6231, Faculté de Médecine-Secteur Nord, CS80011, Bd. Pierre Dramard, 13344 Marseille Cedex 15, France. Tel.: +33 491 698 908; fax: +33 491 698 839.
** Corresponding author at: AFMB UMR-6098, Case 932, 163 avenue de Luminy, 13288 Marseille Cedex 9, France. Tel: +33 491 825 566; fax: +33 491 266 720.
E-mail addresses: Yves.Bourne@afmb.univ-mrs.fr (Y. Bourne), pascale.marchot@univmed.fr (P. Marchot).

0009-2797/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2010.01.030

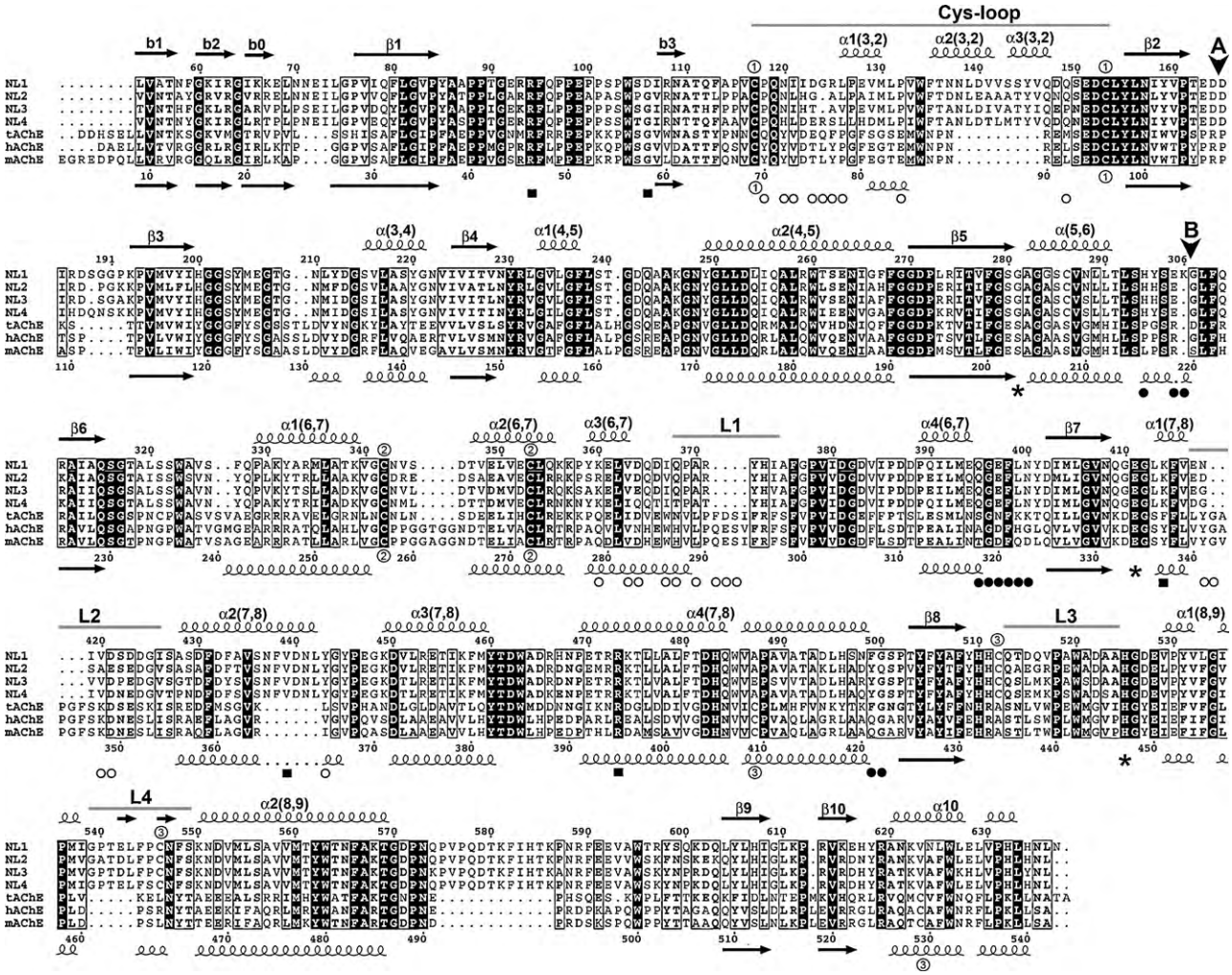

Fig. 1. Sequences conservation within the α/β-hydrolase fold domains of neuroligin and AChE. The NL1 and mouse AChE (mAChE) respective numberings and secondary structure elements are displayed above and below the alignment. Secondary structure elements are labelled according to [51]. The Cys residues forming the disulfide bridges are indicated by numbers in open circles. The positions for spliced inserts A and B in NL1–3 are indicated by black arrowheads and the Cys-loop and loops L1–4 by grey bars above the alignment. The NL1 or NL4 residues buried at the Nrxβ1 binding interface are indicated by filled circles and the NL3 or NL4 residues mutated in some patients with autism spectrum disorders by filled squares. The AChE catalytic triad residues are indicated by asterisks and the AChE residues buried at the fasciculin binding interface by open circles.

The architecture of the AChE subunit is characterized by a buried active site where the catalytic triad is located at the bottom of a deep and narrow gorge. The face of the subunit where the active center gorge opens also bears the peripheral anionic site (PAS) that binds positively charged, non-competitive inhibitors of AChE such as the peptidic toxin from snake venom, fasciculin, and several organic inhibitors, such as propidium or gallamine. In fact, due to an anisotropic repartition of surface charges this face of the subunit displays a strong electronegative surface potential insuring attraction of the positively charged substrate, acetylcholine [22]. The gorge itself is lined predominantly by aromatic groups that participate in 'aromatic guidance' of the substrate toward the active center [23]. The outer wall of the gorge is formed in part by the long Ω-loop, as named from its particular conformation. This surface loop, which corresponds to the mobile lid of lipases, restricts substrate and product trafficking into and out of the gorge. In AChE there is no evidence for opening of the Ω-loop as a rigid-body to mimic the lid opening process associated with lipase activation. Rather, the AChE loop is believed to undergo rapid conformational fluctuations allowing diffusion-limited substrate access to the active center [24].

The recently solved structures of the extracellular domains of NL1, NL2 and NL4 provide new examples of the structural variability of the α/β-hydrolase fold domain along with an initial view of members involved in cell surface recognition and adhesion [25–28]. Moreover, structures of the Nrxβ1–NL1 and Nrxβ1–NL4 complexes clearly identified the neurexin binding site and orientation and provided initial clues on the determinants for partner recognition and complex formation [25–27]. Herein, we describe the main structural features of neuroligin with emphasis on the vestigial active center and the neurexin binding site, compared with the active center gorge and the fasciculin binding site on AChE.

2. Methods

Structural comparisons used program COOT [29] and structures of rat NL1 (PDB code 3BIX, 1.8 Å [25]), mouse NL2 (3BL8, 3.3 Å [28]), human NL4 (3BE8, 2.2 Å [26]), rat NL1 bound with rat Nrxβ1 (3BIW, 3.5 Å [25]), mouse NL1 bound with human Nrxβ1 (3B3Q, 2.4 Å [27]), human NL4 bound with rat Nrxβ1 (2VH8, 3.9 Å [26]), mouse AChE (1JO6, 2.35 Å [30]), human AChE (1B41, 2.76 Å [31]), *Torpedo californica* AChE (2ACE, 2.5 Å [23]), mouse AChE bound