Journal of Chromatography B 1102–1103 (2018) 109–115

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Purification of recombinant human butyrylcholinesterase on Hupresin®

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ARTICLE INFO

Keywords:
Hupresin®
Affinity chromatography
Procainamide-Sepharose
Butyrylcholinesterase
Acetylcholinesterase

ABSTRACT

Affinity chromatography on procainamide-Sepharose has been an important step in the purification of butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) since its introduction in 1978. The procainamide affinity gel has limitations. In the present report a new affinity gel called Hupresin® was evaluated for its ability to purify truncated, recombinant human butyrylcholinesterase (rHuBChE) expressed in a stably transfected Chinese Hamster Ovary cell line. We present a detailed example of the purification of rHuBChE secreted into 3940 mL of serum-free culture medium. The starting material contained 13,163 units of BChE activity (20.9 mg). rHuBChE was purified to homogeneity in a single step by passage over 82 mL of Hupresin® eluted with 0.1 M tetramethylammonium bromide in 20 mM TrisCl pH 7.5. The fraction with the highest specific activity of 630 units/mg contained 11 mg of BChE. Hupresin® is superior to procainamide-Sepharose for purification of BChE, but is not suitable for purifying native AChE because Hupresin® binds AChE so tightly that AChE is not released with buffers, but is desorbed with denaturing solvents such as 50% acetonitrile or 1% trifluoroacetic acid. Procainamide-Sepharose will continue to be useful for purification of AChE.

1. Introduction

Human butyrylcholinesterase (HuBChE) is an efficient bioscavenger of organophosphorus nerve agents. Animals pretreated with tetrameric HuBChE are protected from the toxicity of nerve agents [1]. Plasma-derived HuBChE is being developed as a prophylactic to protect humans from nerve agent toxicity. Crystal structure studies of rHuBChE in complex with small molecules contribute to the design of compounds that release covalently bound nerve agent [2], or identify ligands for treatment of Alzheimer's disease [3]. The rHuBChE used for crystal structure studies is different from plasma-derived HuBChE (UniProt accession #P06276) in that it is a 529-residue monomer, whereas plasma HuBChE is a tetramer of 574 residues per subunit. The C-terminal tetramerization domain was deleted from rHuBChE to facilitate crystallization [4,5]. Another difference is the number of N-linked glycans. Plasma HuBChE has 9 N-linked glycans per subunit [6]. The rHuBChE in this report has 5 N-linked glycans. The structural differences between rHuBChE monomers and plasma-derived HuBChE tetramers require development of separate purification strategies.

Affinity chromatography on procainamide Sepharose 4B beads has been successfully used as part of purification protocols for BChE since 1978 [7–11]. Though chromatography on procainamide beads greatly enriches samples for BChE, the resultant partially-purified BChE requires additional manipulations to make the BChE homogeneous. A new affinity matrix, introduced by Brazzolotto et al., yielded pure truncated rHuBChE from the culture medium of transfected insect cells [12] in a single step. All 9 native N-linked glycosylation sites were present on the rHuBChE secreted by insect cells, but the glycans were not capped with sialic acid. The present work demonstrates that affinity chromatography on Hupresin® can yield highly purified rHuBChE suitable for crystallization trials. The truncated rHuBChE was expressed in Chinese hamster ovary (CHO) cells, where at least 72% of the glycans are capped with sialic acid [13].

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CHO, Chinese hamster Ovary cells; rHuBChE, recombinant human butyrylcholinesterase; TMA, tetramethylammonium bromide

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https://doi.org/10.1016/j.jchromb.2018.10.026
Received 19 September 2018; Received in revised form 21 October 2018; Accepted 24 October 2018
Available online 25 October 2018