Biochemical Pharmacology 136 (2017) 24–31

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Corrector VX-809 promotes interactions between cytoplasmic loop one and the first nucleotide-binding domain of CFTR

Tip W. Loo, David M. Clarke *

Department of Medicine, University of Toronto, Toronto, Ontario M5S 1A8, Canada
Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada

ARTICLE INFO

Article history:
Received 30 January 2017
Accepted 28 March 2017
Available online 31 March 2017

Chemical compounds studied in this article:
VX-770 (Ivacaftor, Kalydeco) (PubChem CID: 16220172)
VX-809 (Lumacaftor) (PubChem CID: 16678941)
15Jf (PubChem CID: 11958611)
Cycloheximide (PubChem CID: 6197)

Keywords:
CFTR
Cystic fibrosis
VX-809 (Lumacaftor)
Bithiazole correctors
Transmembrane- and nucleotide-binding domains
Cytoplasmic loop
Cysteine cross-linking

ABSTRACT

A large number of correctors have been identified that can partially repair defects in folding, stability and trafficking of CFTR processing mutants that cause cystic fibrosis (CF). The best corrector, VX-809 (Lumacaftor), has shown some promise when used in combination with a potentiator (Ivacaftor). Understanding the mechanism of VX-809 is essential for development of better correctors. Here, we tested our prediction that VX-809 repairs folding and processing defects of CFTR by promoting interactions between the first cytoplasmic loop (CL1) of transmembrane domain 1 (TMD1) and the first nucleotide-binding domain (NBD1). To investigate whether VX-809 promoted CL1/NBD1 interactions, we performed cysteine mutagenesis and disulfide cross-linking analysis of Cys-less TMD1 (residues 1–436) and ΔTMD1 (residues 437–1480; NBD1-R-TMD2-NBD2) truncation mutants. It was found that VX-809, but not bithiazole correctors, promoted maturation (exited endoplasmic reticulum for addition of complex carbohydrate in the Golgi) of the ΔTMD1 truncation mutant only when it was co-expressed in the presence of TMD1. Expression in the presence of VX-809 also promoted cross-linking between R170C (in CL1 of TMD1 protein) and L475C (in NBD1 of the ΔTMD1 truncation protein). Expression of the ΔTMD1 truncation mutant in the presence of TMD1 and VX-809 also increased the half-life of the mature protein in cells. The results suggest that the mechanism by which VX-809 promotes maturation and stability of CFTR is by promoting CL1/NBD1 interactions.

1. Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR, ABCC7) is an ABC (ATP-binding cassette) cAMP-regulated chloride channel. It is located on the apical surface of epithelial cells that line the lung airways and ducts of various glands where it functions to regulate salt and water homeostasis [1].

Cystic fibrosis (CF) is caused by genetic mutations that lead to reduced activity or expression of CFTR at the cell surface. Loss of CFTR function in the airway epithelia leads to obstructive airway disease and chronic bacterial infections. The majority of CF patients express CFTRs that contain processing mutations (such as ΔF508 (most common), V232D, H1085R, and others located throughout the molecule) that impair folding, trafficking, stability and activity of the protein [1]. Processing mutations trap CFTR in the endoplasmic reticulum (ER) as a partially folded protein with incomplete domain interactions [2] and incomplete packing of the transmembrane (TM) segments [3,4]. Partial rescue of ΔF508-CFTR by expression at low temperature [5] or in the presence of correctors [6,7] suggests that it should be possible to prevent CF by coaxing enough of the CFTR processing mutants to complete the folding process to yield functional channels at the cell surface using a drug-rescue approach. Clinical trials showed that monotherapy with the best corrector identified to date (VX-809) did not significantly improve lung function or sweat chloride concentration [8]. In addition, immunoblot analysis of rectal biopsy specimens from patients showed no maturation of ΔF508-CFTR [8]. A combination of VX-809 with VX-770 (a potentiator that improves channel function) shows some promise. A problem however, is that it has been reported that VX-770 destabilized ΔF508-CFTR [9,10]. Another group found that VX-770 also destabilized wild-type CFTR [9]. A

* Corresponding author at: Department of Medicine, University of Toronto, 1 King's College Circle, Rm. 7342, Medical Sciences Building, Toronto, Ontario M5S 1A8, Canada.
E-mail address: david.clarke@utoronto.ca (D.M. Clarke).

http://dx.doi.org/10.1016/j.bcp.2017.03.020
0006-2952/

potential solution would be to develop better correctors to improve the efficiency of ΔF508-CFTR maturation into a stable conformation.

The 1480 amino acids of CFTR are organized into two transmembrane domains (TMDs), two nucleotide-binding domains (NBDs), and an R domain [11] (Fig. 1A). The protein contains two N-glycosylation sites in the external loop connecting TM segments 7 and 8 of TMD2. Each homologous half contains an N-terminal TMD followed by an NBD. The secondary structure predicts that each TMD is linked to each NBD via cytoplasmic loops (CLs) (see below). Domain interactions are predicted to be an important feature of CFTR maturation, inhibition of maturation by processing mutations, and rescue of processing mutations by correctors such as VX-809 (reviewed in [12]). CFTR is first synthesized in the ER as a core-glycosylated immature 170 kDa protein. The term "maturation" refers to the process whereby immature CFTR leaves the ER and traverses the Golgi where addition of complex carbohydrates to the pair of N-glycosylated sites in the external loop connecting TM segments 7 and 8 converts it to the 190 kDa mature protein. Mature CFTR is then delivered to the cell surface. While folding of much of CFTR occurs cotranslationally, some folding steps such as packing of the TM segments and incorporation of NBD2 into the structure appear to occur post-translationally [13,14]. Studies on CFTR truncation mutants showed that a mutant lacking NBD2 could mature but those lacking TMD1, TMD2 or NBD1 do not [15]. Therefore, studies of the CFTR truncation mutants suggest that TMD1-TMD2 or TMD1-NBD1 interactions may be particularly important for maturation.

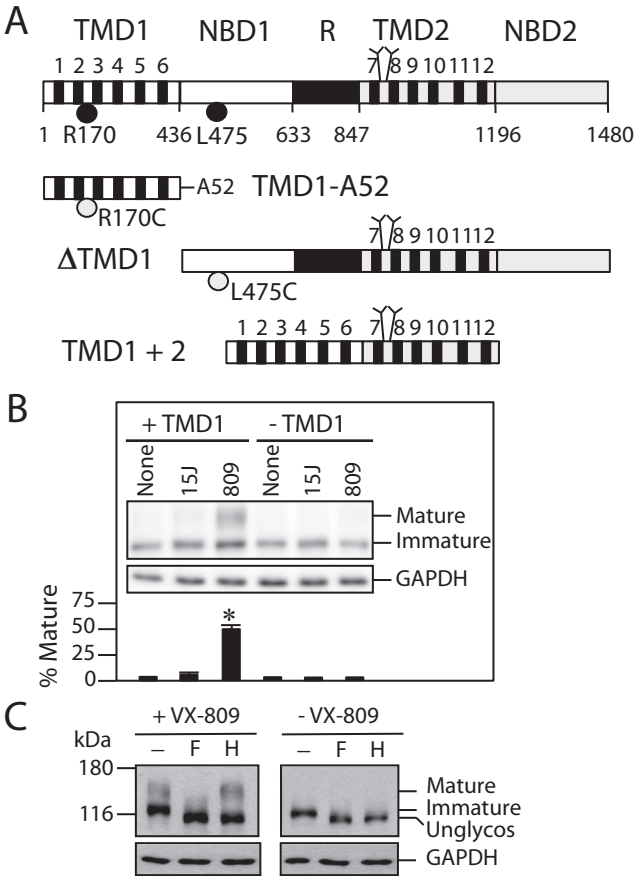

Fig. 1. *VX-809 promotes maturation of ΔTMD1 in the presence of TMD1.* (A) The boundaries of the five domains (TMD1, NBD1, R, TMD2 and NBD2) of CFTR and the composition of truncation mutants TMD1, ΔTMD and TMD1 + 2 are shown. An A52 epitope tag was added to the C-terminal end of TMD1. The cysteines (R170C and L475C) introduced into Cys-less constructs used for cross-linking are shown. The numbered and filled rectangles represent TM segments. The branched lines between TM segments 7 and 8 represent N-glycosylation sites. (B) Rescue of CFTR ΔTMD by correctors and TMD1. HEK 293 cells were transfected with cDNAs encoding ΔTMD1 plus A52-tagged TMD1 (+TMD1) or ΔTMD1 alone (-TMD1). The cells were incubated in the absence (None) or presence of 5 μM 15JF or VX-809 for 48 h. Whole cell SDS extracts were subjected to immunoblot analysis. The positions of mature and immature forms of ΔTMD1 are indicated. The amount of mature ΔTMD1 relative to total (immature plus mature) was determined. Each value is the mean ± S.D. ($n = 4$). An asterisk indicates a significant ($P < 0.001$) increase when each sample treated with corrector was compared to expression in the absence of correctors. (C) To determine the glycosylation state of ΔTMD1, it was expressed TMD1-A52 for 24 h in the presence or absence of 5 μM VX-809. Samples of SDS cell extracts were treated without (–) or with endoglycosidases F (F) or H (H) prior to immunoblot analysis. The positions of immature, mature and unglycosylated (Unglycos) forms of ΔTMD1 are shown.

Although many correctors identified to date are nonspecific, VX-809 is particularly important because it is more specific (also rescued processing mutants of ABCA4 [16] but not P-glycoprotein [17] or hERG potassium channel [7] processing mutants), restores domain assembly [18], and has the ability to promote maturation of CFTR mutants with processing mutations in different domains [17] [18]. In a previous study using CFTR domains expressed as separate proteins, we found that VX-809 stabilized TMD1 (residues 1–402) but not TMD2, NBD1 or NBD2 [19]. Expression of TMD1 in the presence of VX-809 increased its half-life in intact cells from 1.5 h to 8 h. Ren et al. [20] also reported that VX-809 stabilized a TMD1 truncation mutant consisting of residues 1–380. The result suggested that the VX-809 binding site was located in TMD1.

Knowledge of the mechanism of VX-809 is needed to develop better correctors. One advantage of VX-809 compared to other correctors is that it promotes maturation of ΔF508-CFTR and stabilizes the protein [21]. We previously observed that human ABC proteins defective in processing showed defects in TMD-NBD interactions [2]. Here, we used CFTR truncation mutants to test our prediction that VX-809 promotes maturation and stability of CFTR by promoting domain interactions between the first cytoplasmic loop (CL1) of TMD1 and NBD1. This is the major TMD1/NBD1 contact point identified recently in the atomic structure of CFTR [22].

2. Materials and methods

2.1. Chemicals

Corrector 3-(6-{[1-(2,2-Difluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarbonyl]-amino} -3-methyl-pyridin-2-yl)-benzoic acid (VX-809, Lumacaftor™) was obtained from Selleck Chemicals LLC (Houston, TX). Corrector N-(2-(5-chloro-2-methoxyphenylamino-4′-methyl-4,5′-bithiazol-2′-ylpivalamide (15Jf) and potentiator N-(5-Hydroxy-2,4-*bis*(2-methyl-2-propanyl)phenyl]-4-oxo-1,4-dihydro-3-quinolinecarboxamide (VX-770, Ivacaftor™, Kalydeco™) were obtained from Cystic Fibrosis Foundation Therapeutics, Inc. and Dr. Robert Bridges (Rosalind Franklin University, Chicago, IL, USA). Dulbecco's modified Eagle's media and calf serum were obtained from Wisent Inc. (St. Bruno, Quebec). Cycloheximide was obtained from BioShop Canada (Burlington, ON). Monoclonal antibody against GADPH (1:2500 dilution of a 100 μg/ml stock) was obtained from Santa Cruz Biotechnology (Dallas, TX). 1, 10-phenanthroline and sodium butyrate were obtained from Sigma Aldrich Canada (Oakville, ON). Mouse monoclonal antibody A52 (1:1000 dilution of a 100 μg/ml stock) and rabbit polyclonal antibody against NBD2 of CFTR (1:5000 dilution of serum) were generated as described previously [3,23]. Endoglycosidase H_f (used at 20 Units/μμl) and endoglycosidase F (PNGase F; used at 10 Units/μl) were obtained from New England Biolabs (Whitby, Ontario, Canada). Peroxidase-labeled secondary antibodies to mouse or rabbit IgGs were obtained from KPL (Gaithersberg, MD). Luminata Forte Western HRP substrate was obtained from Millipore.