Biochemical and Biophysical Research Communications 446 (2014) 857–862

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Molecular interplays involved in the cellular uptake of octaarginine on cell surfaces and the importance of syndecan-4 cytoplasmic V domain for the activation of protein kinase Cα

Ikuhiko Nakase [a,b,*], Katsuhiro Osaki [a], Gen Tanaka [a], Atsushi Utani [c], Shiroh Futaki [a,*]

[a] Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan
[b] Nanoscience and Nanotechnology Research Center, Research Organization for the 21st Century, Osaka Prefecture University, Naka-ku, Sakai, Osaka 599-8570, Japan
[c] Department of Dermatology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki, Nagasaki 852-8501, Japan

ARTICLE INFO

Article history:
Received 27 February 2014
Available online 12 March 2014

Keywords:
Cell-penetrating peptides
Syndecan-4
PKCα
Macropinocytosis

ABSTRACT

Arginine-rich cell-penetrating peptides (CPPs) are promising carriers for the intracellular delivery of various bioactive molecules. However, many ambiguities remain about the molecular interplays on cell surfaces that ultimately lead to endocytic uptake of CPPs. By treatment of cells with octaarginine (R8), enhanced clustering of syndecan-4 on plasma membranes and binding of protein kinase Cα (PKCα) to the cytoplasmic domain of syndecan-4 were observed; these events potentially lead to the macropinocytic uptake of R8. The cytoplasmic V domain of syndecan-4 made a significant contribution to the cellular uptake of R8, whereas the cytoplasmic C1 and C2 domains were not involved in the process.

1. Introduction

Arginine-rich cell-penetrating peptides (CPPs) such as the human immunodeficiency virus type 1 (HIV-1) Tat (48–60) peptide and oligoarginine peptides are promising carriers for the intracellular delivery of various bioactive molecules. CPPs hold particular promise for carrying molecules that are otherwise difficult for cells to internalize, such as proteins, peptides, and nucleic acids [1,2]. Although a detailed understanding of cellular uptake mechanisms of arginine-rich CPPs should rationally lead to the design of improved delivery systems, efforts to achieve this have been hampered by the involvement of multiple and complicated pathways in the internalization of CPPs into cells, which include physiological cellular uptake (*i.e.*, endocytosis) and direct permeation through the plasma membranes [1,2]. Macropinocytosis (accompanied by actin reorganization, plasma membrane ruffling, and the stimulated engulfment of large volumes of extracellular fluid) [3,4] has been shown to be an important pathway for the physiological cellular uptake of arginine-rich CPPs [5–9]. We previously reported that the accumulation of arginine-rich CPPs at proteoglycans on plasma membranes leads to the activation of Rac1 and induction of macropinocytosis [8]. The induction of macropinocytosis by arginine-rich CPPs is significantly suppressed by a deficiency in glycosaminoglycans (GAGs) in the plasma membrane due to reduced CPP accumulation on cell membranes [8]. The formation of divalent hydrogen bonds and electrostatic interactions between the arginines and sulfates in GAGs are considered important for the accumulation of CPPs on plasma membranes [1].

Syndecans are type I transmembrane heparan sulfate proteoglycans (HSPGs). Syndecan-4 (Syn-4) is expressed on the surface of nearly all cell types, whereas syndecan-1 (epithelial and plasma cells), syndecan-2 (fibroblasts, endothelial cells, smooth muscle cells, and mesenchymal cells), and syndecan-3 (neuronal cells) are expressed on the membranes of specific cell types [10–12]. Syn-4 is an integral membrane protein with attached heparan sulfate chains, which are linear polysaccharides comprised of glucuronic/iduronic acid and glucosamine residues. The Syn-4 core protein is composed of an extracellular domain, a transmembrane region, and a conserved short C-terminal cytoplasmic domain (CD) [10]. The CD harbors structural features that contribute to signal

Abbreviations: CPPs, cell-penetrating peptides; GAGs, glycosaminoglycans; HSPGs, heparan sulfate proteoglycans; Syn-4, syndecan-4; CD, cytoplasmic domain; PKCα, protein kinase C-alpha; PtdIns[4,5]P_2, phosphatidylinositol (4,5) bisphosphate; Syn-4ΔV, V domain-lacking syndecan-4; Syn-4ΔC1, C1 domain-lacking syndecan-4; Syn-4ΔC2, C2 domain-lacking syndecan-4; FITC-dex, fluorescently labeled dextran; PDZ, postsynaptic density 95, disc large and zona occludens-1.

* Corresponding authors. Address: Nanoscience and Nanotechnology Research Center, Research Organization for the 21st Century, Osaka Prefecture University, Naka-ku, Sakai, Osaka 599-8570, Japan. Fax: +81 72 254 9895 (I. Nakase). Fax: +81 774 32 3038 (S. Futaki).

E-mail addresses: i-nakase@21c.osakafu-u.ac.jp (I. Nakase), futaki@scl.kyoto-u.ac.jp (S. Futaki).

http://dx.doi.org/10.1016/j.bbrc.2014.03.018
0006-291X/

transduction across cell membranes [10], which act by binding to and activating protein kinase C-alpha (PKCα) [11–13]. *In vitro* experiments have suggested that the multimerization of the core proteins of Syn-4 significantly enhances binding to PKCα; thus, the clustering of Syn-4 that is induced by ligand interactions should control signal transduction [14,15]. The importance of HSPGs for cellular uptake of arginine-rich CPPs has been previously shown [1,2,8,9,16]. The contribution of Syn-4 and potential involvement of PKCα in the cellular uptake of representative arginine-rich CPPs, HIV-1 TAT (48–60) and octaarginine (R8), has been suggested from a study that employed Syn-4 overexpressing cells and inhibitors of a macropinocytosis and PKCα [16]. However, the details of the molecular interplays involved in this activation step of R8 cellular uptake remain unclear.

In the present study, we shed light on the molecular interplays that accompany the initiation of cellular uptake, especially during macropinocytic uptake of R8 on the membrane. We found that the Syn-4 cytoplasmic V domain makes a significant contribution to the cellular uptake of the R8 peptide. Our results indicate that the clustering of Syn-4 on plasma membranes after they interact with R8 and the eventual binding of PKCα to the Syn-4 cytoplasmic V domain should lead to the cellular uptake of arginine-rich CPPs, which encompasses macropinocytic uptake. Furthermore, we found that the C1 and C2 CDs of Syn-4 are not involved in the cellular uptake of R8 peptide.

2. Materials and methods

2.1. Cell culture

Human cervical cancer-derived HeLa cells were purchased from Riken BRC Cell Bank (Ibaraki, Japan) and cultured in α-minimal essential medium containing 10% heat-inactivated bovine serum (α-MEM(+)) (Invitrogen, Eugene, OR). The cells were grown on 100 mm dishes and incubated at 37 °C under 5% CO_2.

2.2. Confocal microscopy

HeLa cells (in glass-bottom dishes) overexpressing Syn-4 and V domain-lacking Syn-4 (Syn-4ΔV) were washed with α-MEM(−) and treated with Alexa488-labeled peptides in α-MEM(−) (200 μL) for 30 min at 37 °C under 5% CO_2. After washing the cells with 0.5 mg/mL heparin in phosphate-buffered saline (PBS), confocal microscopy was conducted using a FV300 confocal scanning laser microscope (Olympus, Tokyo, Japan) equipped with a ×60 objective without fixing the cells.

In the case of examinations for the clustering of Syn-4 derivatives by peptides, the cells (in glass-bottom dishes) were washed with α-MEM(−) (Fig. 2A and B) and incubated with the medium for 15 min at 4 °C before peptide treatment (Fig. 2A). Next, the cells were treated with non-fluorescently labeled peptides in α-MEM(−) (200 μL) for 30 min at 4 °C (Fig. 2A) or for 15 min at 37 °C (Fig. 2B). The cells were fixed with 4% paraformaldehyde (200 μL) for 30 min at 4 °C, then treated with 0.01% Triton X-100 in PBS (200 μL) for 5 min at room temperature. After blocking the cells with 2% fetal bovine serum in PBS (200 μL) for 1 h at 4 °C, the cells were treated with anti-syndecan-4 (5G9; Santa Cruz Biotechnology, Santa Cruz, CA) (5 μg/mL, 200 μL), anti-HA (3F10; Roche Diagnostics, Indianapolis, IN) (5 μg/mL, 200 μL), or anti-PKCα (Enzo Life Sciences, Farmingdale, NY) (4 μg/mL, 200 μL) (30 min, room temperature), followed by treatment with Alexa Fluor 488 goat anti-mouse IgG or Alexa Fluor 568 goat anti-rat IgG (Invitrogen; each 4 μg/mL, 200 μL) (30 min, room temperature). After washing the cells with PBS, confocal microscopy was conducted.

2.3. Flow cytometry

HeLa cells (in 24-well microplates) overexpressing Syn-4, C1 domain-lacking Syn-4 (Syn-4ΔC1), Syn-4ΔV, or C2 domain-lacking Syn-4 (Syn-4ΔC2) were washed with α-MEM(−), and the cells were treated with Alexa488-labeled peptides in α-MEM(−) (200 μL) for 30 min at 37 °C under 5% CO_2 prior to washing with 0.5 mg/mL heparin in PBS. The cells were then treated with 0.01% trypsin at 37 °C for 10 min prior to the addition of PBS (200 μL) and centrifuged at 3000 rpm (800*g*) for 3 min at 4 °C. After the supernatant was removed, the cells were washed with PBS (400 μL) and centrifuged at 3000 rpm for 3 min at 4 °C. After this washing cycle was repeated, the cells were suspended in PBS (400 μL) and subjected to fluorescence analysis on a FACSCalibur (BD Biosciences, Franklin Lakes, NJ) flow cytometer using 488-nm laser excitation and a 515- to 545-nm emission filter.

2.4. Western blot analysis

HeLa cells overexpressing Syn-4, Syn-4ΔC1, Syn-4ΔV, or Syn-4ΔC2 (6-well microplates) were washed with α-MEM(−) and the cells were treated with peptides in α-MEM(−) (500 μL) for 30 min at 37 °C under 5% CO_2. After peptide treatment, the cells were scraped into lysis buffer (200 μL) (1% octylphenyl-polyethylene glycol, 1% digitonin, and 0.5% Triton X-100) supplemented with protease inhibitor cocktail reagent (Complete, EDTA-free; Roche Diagnostics GmbH, Mannheim, Germany) as described in the manufacturer's protocol. Syn-4 immunoprecipitation was then conducted using Dynabeads Protein G (Invitrogen) bound to anti-Syn-4 antibodies, as described in the manufacturer's protocols. The boiled antigen samples were separated by 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, then transferred to polyvinylidene fluoride membranes (GE Healthcare, Waukesha, WI) and treated with anti-PKCα (Enzo Life Sciences). Secondary antibodies labeled with horseradish peroxidase (anti-mouse IgG HRP-linked whole antibody donkey; GE Healthcare) were then used, and immunoreactive species were detected using the ECL Plus Western Blotting Detection System (GE Healthcare).

3. Results and discussion

3.1. Effect of Syn-4 expression on the cellular uptake of R8

R8 is a representative CPP [1,2,8] that has been employed in the delivery of bioactive molecules into various types of cells. We validated the importance of Syn-4 in the cellular uptake of R8 by overexpressing Syn-4 in HeLa cells transfected with a Syn-4 expression vector (Syn-4-HeLa). The cellular uptake of fluorescently labeled R8 (R8-Alexa488) peptides by Syn-4-HeLa was compared to wild-type (WT) non-transfected HeLa cells (Fig. 1A and B). Cells were treated with 5 μM R8-Alexa488 for 30 min at 37 °C (Fig. 1A and B). Punctuated endosome-like signals from R8-Alexa488 peptides were observed in both Syn-4-HeLa and WT cells (Fig. 1A). However, Syn-4-HeLa cells displayed a more intense signal than WT cells treated with R8-Alexa488 peptides. Fluorescence-activated cell sorting (FACS) analysis also indicated a two-fold increase in peptide internalization by Syn-4-HeLa cells (Fig. 1B).

3.2. Syn-4-dependent induction of macropinocytosis by R8

Macropinocytosis has been shown to be one of the major pathways for the cellular uptake of R8 [6–8]. We examined the contribution of Syn-4 to the induction of macropinocytic cellular uptake of R8 (Fig. 1C). When wildtype HeLa cells were treated with Fluorescein isothiocyanate-dextran (FITC-dex, 70 kDa), a marker of

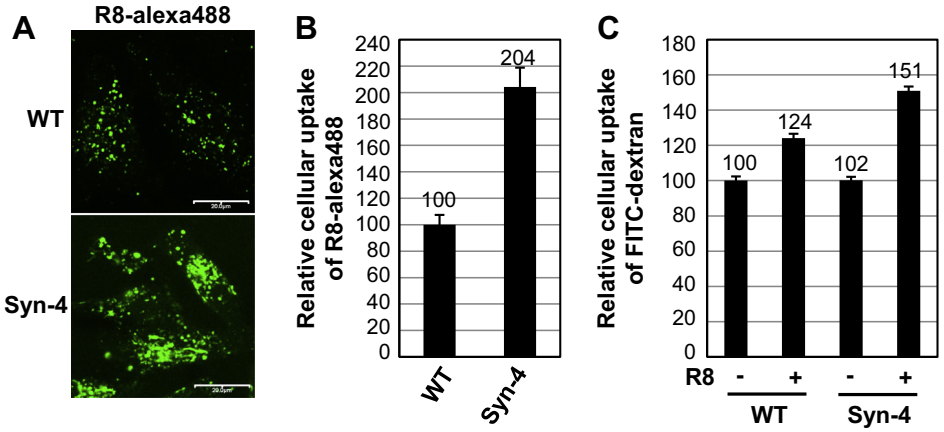

Fig. 1. Syn-4 expression significantly increases the cellular uptake of R8 and FITC-dextran. (A and B) Wildtype (WT)- or Syn-4-overexpressing (Syn-4) HeLa cells were treated with R8-Alexa488 (5 μM) for 30 min at 37 °C, prior to confocal microscopy (A) and FACS analysis (B). Scale bars, 20 μm. The data represent the averages (±SD) of three experiments. (C) WT- or Syn-4 HeLa cells were treated with R8 (5 μM) for 15 min at 37 °C, and were then incubated with FITC-dex (2 mg/mL) for 30 min at 37 °C prior to FACS analysis. The data represent the averages (±SD) of five experiments.

macropinocytosis, R8 (5 μM) enhanced the internalization of FITC-dex by 24% when compared to the case where R8 was not added (Fig. 1C). Conversely, in Syn-4-HeLa cells, R8 strongly stimulated the cellular uptake of FITC-dex (51% higher than that measured in wildtype HeLa cells; Fig. 1C). These results suggest that Syn-4 significantly contributes to the R8-mediated induction of macropinocytosis.

3.3. Induction of Syn-4 clustering on plasma membranes by R8

The effects of R8 on Syn-4 clustering at the plasma membrane are presented in Fig. 2A. Syn-4-HeLa cells were treated with non-fluorescently labeled R8 (10 μM) for 30 min at 4 °C, and immunostaining for Syn-4 was conducted to visualize their locations at the plasma membrane using anti-hemagglutinin epitope (HA) antibodies to detect the HA-tag sequence fused to Syn-4 (Fig. 3A). Under this experimental condition, a low temperature treatment (4 °C) effectively prevented the internalization of membrane molecules by endocytosis. In the presence of R8, comparatively larger-sized fluorescence signals from Syn-4 on the plasma membrane were observed when compared with non-peptide-treated cells (Fig. 2A, Supplementary Fig. S1A). This observation suggests that R8 enhances Syn-4 clustering on plasma membranes during this short period of 30 min. R8-Alexa488 and Syn-4 co-localization was also observed at the plasma membrane (Supplementary Fig. S1B). Conversely, tetraarginine (R4), which has a significantly lower internalization efficiency into cells than R8 [6–17], was largely unable to induce Syn-4 clustering on plasma membranes (Supplementary Fig. S1A). Likewise, Syn-4 did not enhance the cellular uptake of R4 (Supplementary Fig. S2). The observations from our co-localization experiments suggest a strong correlation between Syn-4 clustering and the cellular uptake of arginine-rich CPPs.

3.4. R8 enhances the binding of PKCα to Syn-4 in the cytosol

Next, we examined the effects of R8 on the localization of PKCα inside cells (Fig. 2B). As described in Section 1, PKCα binding to Syn-4 has previously been found to activate PKCα, leading to signal transduction [15]. However, the details regarding the correlations between the extent of Syn-4 clustering, binding of PKCα, and its activation remained unclear. In the present study, Syn-4-HeLa cells were treated with or without R8 (10 μM) for 15 min at 37 °C, and immunostaining for Syn-4 and PKCα was conducted prior to confocal microscopy. Treatment with R8 significantly induced Syn-4 clustering on the plasma membrane, as observed in Fig. 2A, and obvious co-localization of Syn-4 clustering and PKCα

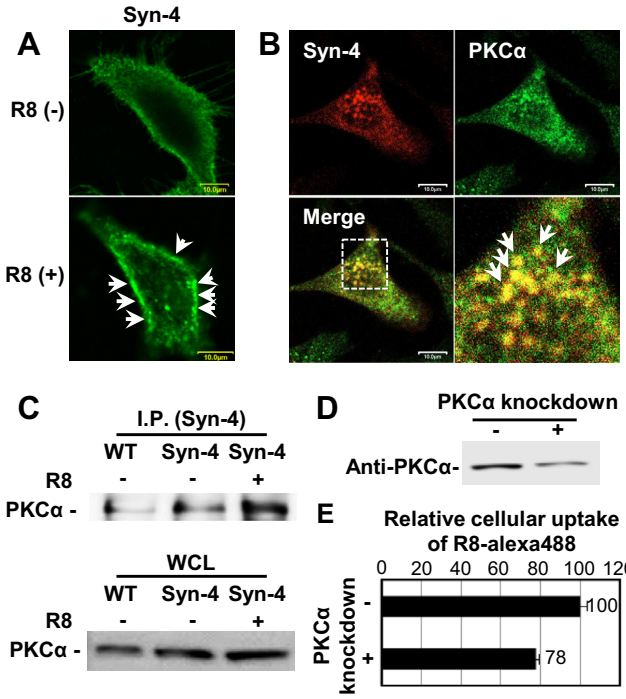

Fig. 2. R8 induces the clustering of Syn-4 on plasma membranes, leading to enhanced binding of PKCα to Syn-4 inside cells. (A) Syn-4-HeLa cells were treated with or without R8 (10 μM) for 30 min at 4 °C prior to immunostaining for Syn-4 (green) and confocal microscopy. Arrows show representative induced clustering of Syn-4. (B) Syn-4-HeLa cells were treated with R8 (10 μM) for 15 min at 37 °C, and immunostaining for Syn-4 (red) and PKCα (green) was conducted prior to confocal microscopy. Arrows show representative co-localization of Syn-4 and PKCα. Scale bar, 10 μm. (C) PKCα binding to Syn-4 was analyzed by Western blotting as described in Section 2. Wildtype (WT) or Syn-4-HeLa cells were treated with R8 (10 μM) for 30 min at 37 °C, then immunoprecipitation (IP) for Syn-4 was conducted from whole-cell lysates (WCL). (D and E) The knockdown of PKCα decreases cellular uptake of R8. (D) The expression level of PKCα in wildtype (WT) or PKCα-knockdown HeLa cells as analyzed by Western blotting. (E) PKCα-knockdown HeLa cells were treated with R8-Alexa488 (5 μM) for 30 min at 37 °C prior to FACS analysis. The data represent the averages (±SD) of three experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

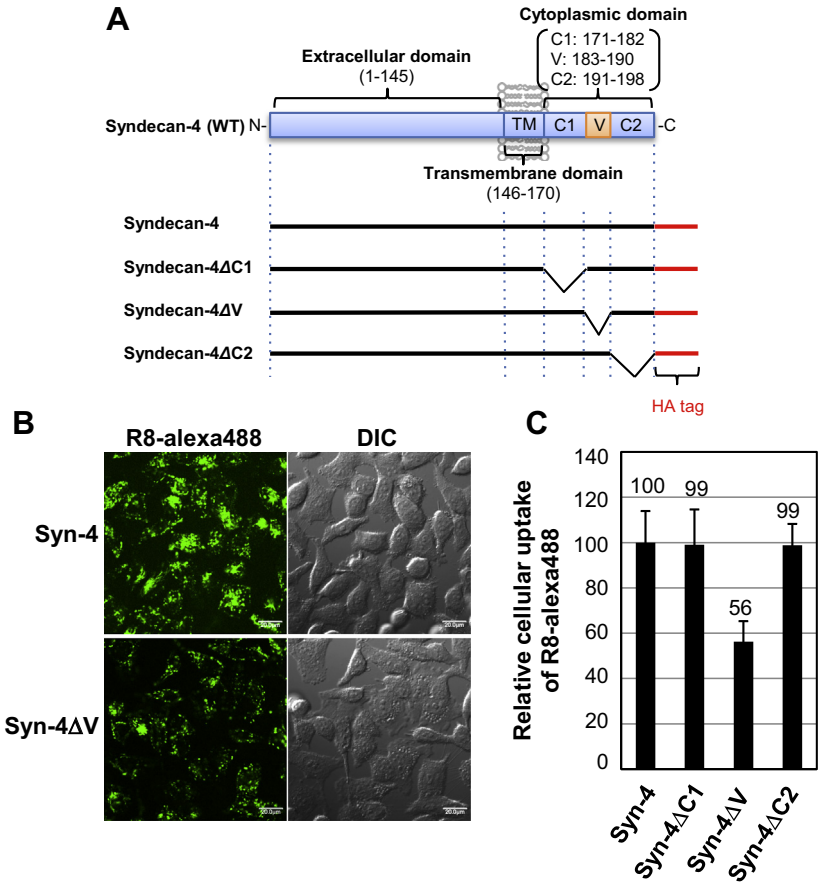

Fig. 3. The V domain of Syn-4 is crucial for the cellular uptake of R8. (A) Schematic representation of Syn-4 derivatives. (B and C) Syn-4- or Syn-4ΔV-HeLa cells were treated with R8-Alexa488 (5 μM) for 30 min at 37 °C prior to confocal microscopy (B). The uptake of R8-Alexa488 (5 μM) in HeLa cells expressing Syn-4 derivatives after 30 min at 37 °C was analyzed by FACS (C). Scale bars, 20 μm. The data represent the averages (±SD) of three experiments.

was also detected (Fig. 2B). In the case of no peptide treatment, uniform staining for Syn-4 at the plasma membrane and diffuse PKCα staining in the cytosol were observed (data not shown). Additionally, in the case of R4, peptide treatment under the same condition did not affect the localization of PKCα inside cells (data not shown). These results suggest that the clustering of Syn-4 by R8 effectively recruits PKCα to Syn-4.

Immunoprecipitation was also conducted using anti-Syn-4 antibodies to detect the interaction between Syn-4 and intracellular molecules as described in Section 2. Western blot analysis showed that the treatment of Syn-4-HeLa cells with R8 (10 μM) for 30 min significantly enhanced the binding of PKCα to Syn-4 (Fig. 2C, upper panel). In whole-cell lysates (WCL), PKCα expression was unaffected by treatment with R8 (Fig. 2C, lower panel), indicating that the expression level of PKCα was not affected during treatment with R8. These results strongly suggest that the induced clustering of Syn-4 after treatment with R8 leads an enhanced intracellular interaction between PKCα and Syn-4.

3.5. PKCα knockdown influences the internalization efficiency of R8 into cells

To confirm the role of PKCα in the cellular uptake of R8, we analyzed the effects of PKCα knockdown by short hairpin RNA (shRNA) treatment on the internalization of the peptide (Fig. 2D and E). In PKCα-knockdown cells, PKCα expression was reduced to about 50% of the levels in non-shRNA-treated cells (Fig. 2D). When the PKCα-knockdown cells were treated with R8-Alexa488 (5 μM) for 30 min at 37 °C, the cellular uptake efficiency of the peptide was decreased by approximately 20% when compared to the control (non-shRNA-treated) cells (Fig. 2E), suggesting that PKCα and its associated signal pathways are involved in the cellular uptake of R8.

3.6. The cytoplasmic V domain of Syn-4 is essential for the cellular uptake of R8

PKCα binds to the V domain of the Syn-4 cytoplasmic region via phosphatidylinositol (4,5) bisphosphate (PtdIns[4,5]P_2) [18,19], which leads to PKCα activation [14,18,20]. To examine how cytosolic PKCα binding to Syn-4 affects the cellular uptake of R8, we constructed a plasmid to express a mutated form of Syn-4 in plasma membranes that lacks the V domain (Syn-4ΔV) (Fig. 3A, Supplementary Fig. S3). Syn-4ΔV-expressing HeLa cells (Syn-4ΔV-HeLa cells) were prepared by transfection of the plasmid as described in the Supplementary materials and methods. We also constructed plasmids to express the C1 domain lacking Syn-4 (Syn-4ΔC1) to prevent the binding of cytoskeletal proteins to Syn-4 inside the cell [21], and to express the C2 domain lacking Syn-4 (Syn-4ΔC2) in plasma membranes to prevent the binding of proteins containing a PDZ domain (postsynaptic density 95, disc large, and zona occludens-1) to Syn-4 (Fig. 3A, Supplementary Fig. S3) [21]. PDZ proteins are known to be important for the induction of Rac1 activation through their binding to Syn-4 (e.g. FGF2) [22,23]. In the present study, Syn-4, Syn-4ΔV, Syn-4ΔC1, and Syn-4ΔC2 expression in HeLa cells was shown to be similar by Western blotting (Supplementary Fig. S3). Immunostaining for heparan sulfate on the plasma membranes of the cells that had

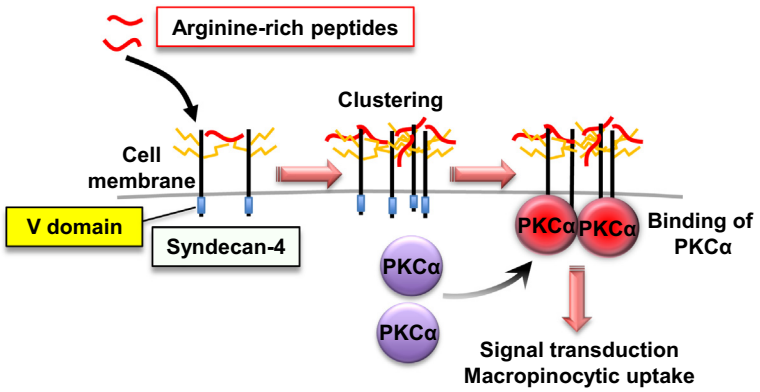

Fig. 4. Schematic diagram of Syn-4 clustering and binding to PKCα inside cells induced by arginine-rich peptides.

been treated with each Syn-4-mutant-expressing plasmid showed similar expression levels (data not shown).

Each mutated Syn-4-expressing HeLa cell line was treated with R8-Alexa488 (5 μM) for 30 min at 37 °C, and the cellular uptake efficiency of the peptide was analyzed using confocal microscopy and FACS (Fig. 3B and C). Deletion of the V domain significantly reduced cellular uptake of the peptide by 44% (Syn-4ΔV-HeLa cells) when compared with Syn-4-HeLa cells (Fig. 3B and C), although R8 enhanced clustering of Syn-4ΔV on the plasma membrane (Supplementary Fig. S4). This suggests a significant contribution of the V domain of Syn-4 to the cellular uptake of R8. Conversely, deletion of C1 (Syn-4ΔC1-HeLa cells) and C2 (Syn-4ΔC2-HeLa cells) domains did not affect the cellular uptake of R8 (Fig. 3C). Moreover, the internalization efficiency of R8-Alexa488 (20 μM), which was expected to enhance cytosolic release of the peptide, was also reduced in Syn-4ΔV-HeLa cells (24%) as determined by FACS (Supplementary Fig. S5B). Confocal microscopic images showed that the signal intensity of R8-Alexa488 that diffusely localized in the cytosol and the nucleus was significantly diminished (Supplementary Fig. S5A). Conversely, the internalization efficiency of the peptide into Syn-4ΔC1- and Syn-4ΔC2-HeLa cells under this experimental condition was similar to that in Syn-4-HeLa cells (Supplementary Fig. S5B). These results suggest that the V domain of Syn-4 is important for the cellular uptake and membrane penetration of R8, and the binding of PKCα to the V domain of Syn-4 is considered to be important as a first cellular event that prompts cellular uptake of the peptide. Additionally, an increase in FITC-dex uptake by Syn-4ΔV-HeLa cells was only 9% of that demonstrated by Syn-4-HeLa cells when treated with R8 (data not shown), suggesting that the induction of macropinocytosis by R8 through the binding of PKCα and Syn-4 is important for cellular uptake of the peptide.

To summarize, our results elucidate the molecular interplays that take place on plasma membranes and contribute to R8 cellular uptake. Specifically, we found that the R8-mediated clustering of Syn-4 leads to PKCα activation, and that the V domain in Syn-4 is critical for this activation (Fig. 4). Although further studies are necessary to obtain more detailed information on the cytoplasmic events following PKCα activation, these findings should have an impact on our understanding of the biological significance of membrane-associated proteoglycans as well as the internalization methods of other macromolecules and viruses.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and Takeda Science Foundation. This work was also supported by the Collaborative Research Program of Institute for Chemical Research, Kyoto University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.03.018.

References

[1] S. Futaki, Special theme issue on membrane permeable peptide vectors: chemistry and functional design for the therapeutic applications, Adv. Drug Deliv. Rev. 60 (2008) 447–614.
[2] I. Nakase, H. Akita, K. Kogure, et al., Efficient intracellular delivery of nucleic acid pharmaceuticals using cell-penetrating peptides, Acc. Chem. Res. 45 (2012) 1132–1139.
[3] J.A. Swanson, C. Watts, Macropinocytosis, Trends Cell Biol. 5 (1995) 424–428.
[4] J.A. Swanson, Shaping cups into phagosomes and macropinosomes, Nat. Rev. Mol. Cell Biol. 9 (2008) 639–649.
[5] J.S. Wadia, R.V. Stan, S.F. Dowdy, Transducible TAT-HA fusogenic peptide enhances escape of TAT-fusion proteins after lipid raft macropinocytosis, Nat. Med. 10 (2004) 310–315.
[6] I. Nakase, M. Niwa, T. Takeuchi, et al., Cellular uptake of arginine-rich peptides: roles for macropinocytosis and actin rearrangement, Mol. Ther. 10 (2004) 1011–1022.
[7] I.M. Kaplan, J.S. Wadia, S.F. Dowdy, Cationic TAT peptide transduction domain enters cells by macropinocytosis, J. Control. Release 102 (2005) 247–253.
[8] I. Nakase, A. Tadokoro, N. Kawabata, et al., Interaction of arginine-rich peptides with membrane-associated proteoglycans is crucial for induction of actin organization and macropinocytosis, Biochemistry 46 (2007) 492–501.
[9] I. Nakase, H. Hirose, G. Tanaka, et al., Cell-surface accumulation of flock house virus-derived peptide leads to efficient internalization via macropinocytosis, Mol. Ther. 17 (2009) 1868–1876.
[10] K. Lambaerts, S.A. Wilcox-Adelman, P. Zimmermann, The signaling mechanisms of syndecan heparan sulfate proteoglycans, Curr. Opin. Cell Biol. 21 (2009) 662–669.
[11] E. Okina, T. Manon-Jensen, J.R. Whiteford, J.R. Couchman, Syndecan proteoglycan contributions to cytoskeletal organization and contractility, Scand. J. Med. Sci. Sports 19 (2009) 479–489.
[12] J.R. Couchman, Syndecans: proteoglycan regulators of cell-surface microdomains?, Nat Rev. Mol. Cell Biol. 4 (2003) 926–937.
[13] M. Simons, A. Horowitz, Syndecan-4-mediated signaling, Cell. Signal. 13 (2001) 855–862.
[14] E.S. Oh, A. Woods, J.R. Couchman, Multimerization of the cytoplasmic domain of syndecan-4 is required for its ability to activate protein kinase C, J. Biol. Chem. 272 (1997) 11805–11811.
[15] E.S. Oh, A. Woods, J.R. Couchman, Syndecan-4 proteoglycan regulates the distribution and activity of protein kinase C, J. Biol. Chem. 272 (1997) 8133–8136.
[16] T. Letoha, A. Keller-Pintér, E. Kusz, et al., Cell-penetrating peptide exploited syndecans, Biochim. Biophys. Acta 1798 (2010) 2258–2265.

[17] M. Kosuge, T. Takeuchi, I. Nakase, et al., Cellular internalization and distribution of arginine-rich peptides as a function of extracellular peptide concentration, serum, and plasma membrane associated proteoglycans, Bioconjug. Chem. 19 (2008) 656–664.
[18] E.S. Oh, A. Woods, S.T. Lim, et al., Syndecan-4 proteoglycan cytoplasmic domain and phosphatidylinositol 4,5-bisphosphate coordinately regulate protein kinase C activity, J. Biol. Chem. 273 (1998) 10624–10629.
[19] D. Lee, E.S. Oh, A. Woods, et al., Solution structure of a syndecan-4 cytoplasmic domain and its interaction with phosphatidylinositol 4,5-bisphosphate, J. Biol. Chem. 273 (1998) 13022–13029.
[20] A. Horowitz, M. Murakami, Y. Gao, M. Simons, Phosphatidylinositol-4,5-bisphosphate mediates the interaction of syndecan-4 with protein kinase C, Biochemistry 38 (1999) 15871–15877.
[21] E. Tkachenko, J.M. Rhodes, M. Simons, Syndecans: new kids on the signaling block, Circ. Res. 96 (2005) 488–500.
[22] Y. Gao, M. Li, W. Chen, M. Simons, Synectin, syndecan-4 cytoplasmic domain binding PDZ protein, inhibits cell migration, J. Cell. Physiol. 184 (2000) 373–379.
[23] E. Tkachenko, A. Elfenbein, D. Tirziu, M. Simons, Syndecan-4 clustering induces cell migration in a PDZ-dependent manner, Circ. Res. 98 (2006) 1398–1404.