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CD38/ADP-ribosyl cyclase in the rat sublingual gland: Subcellular localization under resting and saliva-secreting conditions

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ABSTRACT

CD38 is a 42–45 kDa transmembrane glycoprotein that exhibits ADP-ribosyl cyclase enzyme activity. In the rat, we have previously reported strong ADP-ribosyl cyclase activity in the sublingual salivary gland (Masuda W. and Noguchi T. Biochem. Biophys. Res. Commun. (2000) 270, 469–472). Here, we have examined the specific localization of CD38/ADP-ribosyl cyclase activity in this gland and whether that localization changes upon saliva-secretary stimulation. Under resting conditions, CD38/ADP-ribosyl cyclase activity in the post-nuclear fraction of SLG homogenates was separated into two major peaks by sucrose density gradient centrifugation. The first peak included the plasma membrane proteins Na^+/K^+ ATPase and aquaporin 5, while the second peak included mucous secretory protein mucin and vesicle-associated membrane protein 2. When rats were subjected to the muscarinic agonist pilocarpine, the CD38/ADP-ribosyl cyclase activity disappeared from the second peak, as did mucin and vesicle-associated membrane protein 2. Pre-treatment of rats with the muscarinic antagonist atropine before pilocarpine administration, or adrenergic stimulation with isoproterenol, the sucrose density gradient separation profiles were same as that seen under resting condition. Using an immunofluorescent strategy, we observed the preferential localization of CD38 in the basolateral plasma membrane and intracellular granule-like membrane in sublingual acinar cells under resting conditions.

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Introduction

CD38 is a 42–45 kDa transmembrane glycoprotein that has a short cytosolic N-terminal region, a long extracellular C-terminal region, and a transmembrane domain; it is ubiquitously expressed in various cells and tissues including hematopoietic cells and organs such as spleen, liver, thymus, salivary gland, pancreas, heart, thyroid gland, ileum, colon, jejunum, brain, and eye [1–10]. In mammals, CD38 has been reported to localize in both the plasma membrane and the intracellular membrane, including the nuclear envelope, depending on the cell types and tissues [10–22]. Experiments to elucidate the physiological function of CD38 in various systems have revealed that its biological roles can be categorized into two functions.

Firstly, it functions as a receptor on the plasma membrane. In hematopoietic cells in particular, CD38 has been shown to be an important surface immunoregulatory molecule with a multitude of diverse functions, including the induction of B- and T-cell proliferation [23], regulation of the humoral immune response [24], apoptosis [25], tyrosine phosphorylation of various proteins [26], activation of various kinases [27], and cytokine release [28]. CD38 also has adhesion properties and has been suggested to mediate selectin-type adhesion between different blood populations and vascular endothelial cells via its putative ligand CD31, or hyaluronic acid [29–31]. Expression of CD38 in the plasma membrane is essential for these functions.

Secondly, it functions as an enzyme. CD38 has a catalytic domain in its long extracellular region that produces not only cyclic ADP-ribose (cADPR)[1] and ADP-ribose (ADPR) from NAD^+ but also NAADP from $NADP^+$ [32–36]. It should be noted that all of these products could enhance intracellular Ca^{2+} concentration in different ways. cADPR has been reported to mobilize Ca^{2+} from endoplasmic reticulum (ER) by affecting the ryanodine receptor on ER membrane [34–36]. NAADP also induces Ca^{2+}-release from the intracellular Ca^{2+}-store, but not, it is thought, from ER [34–36]. Both are independently regulated by the intracellular Ca^{2+}-release system driven by inositol 1,4,5-trisphosphate ($InsP_3$); in contrast, ADPR is thought to bind with the cytosolic domain of TRPM2 cation channels to promote Ca^{2+}-influx from the extracellular region [37]. Despite evidence of physiological function of molecules produced by CD38 in many

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[1] *Abbreviations used:* Ca^{2+}, calcium; ADPRC, ADP-ribosyl cyclase; cADPR, cyclic adenosine diphosphoribose; InsP3, inositol; 1,4,5-triphosphate; cGDPR, cyclic guanosine diphosphoribose; NAD^+, nicotinamide adenine dinucleotide; NGD^+, nicotinamide guanine dinucleotide; SLG, sublingual gland; SMG, submandibular gland; PG, parotid gland.

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cells and tissues, there is a topological paradox between its catalytic domain and the space where its products would function: since the catalytic domain of CD38 is localized in the extracellular or intraluminal region, its products must be transported to the cytosolic area to function. Zocchi et al. suggested that binding extracellular substrate to CD38 located in the plasma membrane triggers transport of the substrate/enzyme intermediate into the intracellular membrane [31,38]. It has also been suggested that CD38 molecules could interact to form a complex, which builds a "membrane hole" that makes it possible for extracellular cADPR to access the cytosol [39]. Recently, Chang and colleagues reported the localization of CD38 in the nuclear envelope and cADPR-induced release of Ca^{2+} [17]. Even though most CD38 molecules localize in the plasma membrane, a small number were recovered from the intracellular membrane fraction of some cells/tissues including osteoclast, lung, liver, eye, spleen, and heart [10,13–21]. These results clearly show that knowing the location of CD38 in the target cells or tissue could be important in elucidating its physiological role.

Saliva is important in keeping the oral cavity healthy, with roles including buffering, digestion, antibiotic, and protection/smoothing effects. Saliva is mainly secreted from the three large salivary glands (parotid, submandibular, and sublingual); some is also secreted by the small salivary glands. Saliva secretion is triggered by an increase in intracellular Ca^{2+} concentration in the salivary acinar cells after parasympathetic stimulation. The rise of intracellular Ca^{2+} activates the Cl^- channel in the apical membrane, following Cl^- secretion into the lumen and then inducing Na^+ and water secretion by electrical and osmotic driving force [40,41].

We have previously studied the activity of ADP-ribosyl cyclase (ADPRC), which is catalytic in the conversion of NAD^+ into cADPR, and discovered its enzyme activity to be strong in the rat sublingual gland (SLG) [42]. ADPRC activity in the rat SLG transiently increased at 3 weeks of age (approximately 500 nmol/min/g tissue), then decreased and remained at 200 ~ 300 nmol/min/g tissue. In contrast, ADPRC activity in the other two major salivary glands (submandibular gland (SMG) and parotid gland (PG)) remains low from postnatal day (approximately 30–40 nmol/min/g tissue). These results suggest the important physiological role(s) of ADPRC in the rat SLG development. However, cADPR appeared to be unable to induce Ca^{2+}-release from the microsome fraction prepared from SLG [42].

Here, we have examined the intracellular localization of CD38 in the rat SLG. CD38 and ADPRC activity were observed to be separated into the same two fractions after sucrose density gradient centrifugation. One of the fractions also contained Na^+/K^+ ATPase and aquaporin 5 (AQP5), whereas mucin and vesicle-associated membrane protein 2 (VAMP2) were found in the other fraction. Muscarinic stimulation induced salivary secretion from rat SLG, which resulted in the disappearance of the fraction including mucin, VAMP2, CD38, and ADPRC activity; this was completely inhibited by pre-treatment with the muscarinic antagonist atropine. Adrenergic stimulation did not induce any changes in the sucrose density gradient fractionation profile. These results indicate that CD38 is present in both the plasma and secretary granule membrane. This idea is supported by immunofluorescent images using an anti-CD38 polyclonal antibody.

Materials and methods

Chemicals

NGD^+ and cGDPR were purchased from Sigma (Japan). All other chemicals used in this study were of analytical grade.

Animals

Adult male Wistar rats (7 weeks old) were purchased from Kyudou Co., (Fukuoka, Japan) and kept in a constant 12-h light/12-h dark cycle (lights on at 8:00 AM) with free access to food and water. All rats were maintained at the Animal Resource Center, and experimental procedures were approved by the Animal Care and Use Committee of Kyushu Dental College.

Sublingual duct ligation

Adult male Wistar rats were anesthetized using sodium pentobarbital (50 mg/kg i.p.). The excretory duct of the right sublingual gland was ligated using a silk thread near the hilum of the SLG, with the ligature remaining in place for 2 weeks. The other side of the SLG was sham operated.

Sucrose density gradient centrifugation

After the perfusion of a rat with physiological saline (0.9% NaCl solution) under sodium pentobarbital anesthesia (50 mg/kg i.p.), bilateral SLG were removed immediately, minced with razors, and homogenized using a Dounce-type glass tissue homogenizer in PIPES/NaOH buffer (3 ml, 20 mM, pH 7.0) containing sucrose (250 mM), EDTA (1 mM), phenylmethylsulfonyl fluoride (100 μM) and leupeptin (10 μM). A post-nuclear extract was obtained as supernatant by centrifugation (800 × g, 10 min, 4 °C) and then subjected to sucrose density gradient centrifugation as described previously [43]. After sucrose density gradient centrifugation, the sample was separated into 20 fractions from bottom to top. The density of each fraction was calculated from the refraction index of sucrose.

Assay for enzyme activities

For measurement of ADPRC activity, we used the spectrofluorometric assay with NGD^+ as substrate, according to Graeff et al. [44]. Suitable aliquots of sample were incubated in the reaction mixture containing PIPES/NaOH (20 mM, pH 7.0), $MgCl_2$ (1 mM), and NGD^+ (1 mM) at 37 °C. Fluorescence increase was converted to rate of cGDPR production using a calibration curve obtained from known concentrations of commercially available cGDPR. Activities of other enzymes (glutamate dehydrogenase (GDH) [45], catalase [46], lactate dehydrogenase (LDH) [47], NADPH/cytochrome c reductase [48], acid phosphatase [49], and gamma glutamyl transpeptidase (GGT) [50]) were colorimetrically measured as described elsewhere.

Western blotting

Samples were separated on 7.5% polyacrylamide gels according to Laemmli [51], and electrophoretically transferred to Hybond ECL nitrocellulose membranes (Amersham Pharmacia Biotech). The nitrocellulose membranes were blocked in phosphate-buffered saline (PBS) containing 5% skim milk and 0.1% Tween 20 and then probed with antibody (anti-CD38 goat polyclonal antibody (Santa Cruz Biotechnology, CA, USA), anti-AQP5 rabbit polyclonal antibody (Alpha Diagnostic International, TX, USA), anti-Na^+/K^+ ATPase mouse monoclonal antibody (Upstate, NY, USA), anti-VAMP2 rabbit polyclonal antibody (Affinity BioReagents, CO, USA) overnight at 4 °C. The membranes were then washed with PBS containing 0.1% Tween 20 and incubated with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, CA, USA) for 1 h: immunoreactive bands were detected using enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech).

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