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Perilipin 1 binds to aquaporin 7 in human adipocytes and controls its mobility *via* protein kinase A mediated phosphorylation

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ABSTRACT

Accumulating evidence suggests that dysregulated glycerol metabolism contributes to the pathophysiology of obesity and type 2 diabetes. Glycerol efflux from adipocytes is regulated by the aquaglyceroporin AQP7, which is translocated upon hormone stimulation. Here, we propose a molecular mechanism where the AQP7 mobility in adipocytes is dependent on perilipin 1 and protein kinase A. Biochemical analyses combined with *ex vivo* studies in human primary adipocytes, demonstrate that perilipin 1 binds to AQP7, and that catecholamine activated protein kinase A phosphorylates the N-terminus of AQP7, thereby reducing complex formation. Together, these findings are indicative of how glycerol release is controlled in adipocytes, and may pave the way for the future design of drugs against human metabolic pathologies.

1. Introduction

Storage and mobilization of lipid energy are fundamental processes of eukaryotic cells. Primary human adipocytes store lipids as triacylglycerols (TAG) in a single large lipid droplet that takes up around 90% of the cytoplasm, displacing nucleus and cytosol to the periphery of the cell. The lipid droplet is surrounded by a monolayer of phospholipids, which is decorated with adherent proteins [1]. In particular, perilipin 1 (PLIN1) is abundantly expressed on the surface of the lipid droplet [2]. During states of increased energy demands, such as fasting and exercise, adipocyte lipolysis is increased causing increased release of glycerol and free fatty acids (FFA). In adipocytes, PLIN1 is a key regulator of TAG mobilization. While feeding suppresses lipolysis, fasting leads to protein kinase A (PKA)-dependent phosphorylation of PLIN1 and activation of lipolysis [3,4]. The generated FFA and glycerol are released from the cell to support energy demands in other tissues, and the efflux of glycerol from adipocytes is facilitated by aquaglyceroporin AQP7 [5]. Inter-

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estingly, disruption of PLIN1 in mice leads to a considerable enhancement of basal glycerol release, suggesting that PLIN1 is a central regulator of lipolysis [6].

The aquaglyceroporins are integral membrane proteins, which are known to transport glycerol in addition to other solutes like water and arsenic trioxide [7]. The metabolic impact of AQP7 in adipocytes has been highlighted by phenotypes observed in two different lines of AQP7 null mice [8,9]. In both cases, knockout of AQP7 resulted in increased accumulation of glycerol and TAG, leading to adipocyte hypertrophy, suggesting that AQP7 is a key element in regulation of fat accumulation. In human adipose tissue, AQP7 is known to translocate from the lipid droplet to the plasma membrane upon catecholamine stimulation, while the presence of AQP7 around the lipid droplet is increased upon insulin treatment [10,11]. Yet, the detailed mechanism controlling AQP7 translocation in human adipocytes is still poorly understood. In the current study, we sought to identify proteins involved in controlling AQP7 trafficking in human primary adipocytes. We investigated the putative contacts between AQP7 and PLIN1 in adipocytes using a combination of fluorescence confocal microscopy and biochemical analyses. Our data show that PLIN1 and AQP7 are in physical contact, which is mediated by the cytosolic termini of AQP7. Under lipogenic conditions, AQP7 and PLIN1 are in close proximity, while complexation between AQP7 and PLIN1 is significantly reduced under lipolytic conditions. *In vitro* experiments indicate that the observed effect might be due to PKA phosphorylation of the N-terminus of AQP7. These results suggest that AQP7 localization is tightly regulated by PKA and PLIN1 in human primary adipocytes.

2. Materials and Methods

2.1. Human Subjects

The study was approved by the local ethical committee (Dnr: 2013/298) and performed after written informed consent from all volunteers. Female patients in the study had a BMI between 21.5 and 28.6 and were without known diabetes (type 1 or 2), or thyroid gland dysfunction. The Declaration of Helsinki protocols were followed.

2.2. Cloning, Protein Expression and Purification of PLIN1

A synthetic gene encoding human PLIN1 was obtained from commercial sources (Genescript) and sub-cloned into the XhoI and NdeI sites of the pETMBP_1a *E. coli* plasmid (Protein Expertise Platform, Umeå University, Sweden) creating a construct encoding PLIN1 with an N-terminal maltose binding protein and a 6× histidine tag (pETMBP_1a:PLIN1). To produce the PLIN1 expressing *E. coli* lysate (PLIN1-lysate), the pETMBP_1a:PLIN1 plasmid was transformed into BL21 (DE3) *E. coli* cells. The cells were cultured at 37 °C with 225 rpm orbital shaking in terrific broth medium supplemented with 50 μg/ml kanamycin. At an OD_{600} of 0.5 the culture temperature was down shifted to 18 °C, and the protein expression induced by adding isopropyl β-D-1-thiogalactopyranoside to a final concentration of 1 mM. Following overnight protein expression, the cells were harvested by centrifugation at 5000*g* for 5 min followed by cell re-suspension in 20 mM NaPi; pH 8.0, 100 mM NaCl, 5% *v/v* glycerol, 1% *w/v* n-decyl-β-D-maltopyranoside (DM) supplemented with EDTA-free protease inhibitor cocktail (Roche). Cell lysis was performed at 4 °C by incubation with lysozyme (Sigma), DNAse1 (Sigma), 5 mM $MgSO_4$ and 1 mM $CaCl_2$, followed by mild sonication (Branson M1800E). The homogenate was clarified by centrifugation at 50.000*g* for 20 min at 4 °C, and the resulting lysate was flash frozen in liquid nitrogen and stored at −80 °C until use.

2.3. Cloning, Protein Expression and Purification of AQP7

P. pastoris strains without endogenous aquaporins and aquaglyceroporins were created for the production of AQP7. *P. pastoris* strains used in this study derives from the X33 strain. A protein encoded by the gene PAS_chr4_0784 in *P. pastoris* GS115 (HIS4$^-$) was identified as an endogenous aquaglyceroporin based on 35% sequence identity to *S. cerevisiae* Fps1 glycerol facilitator protein in a BLASTp query. This protein was named aquaglyceroporin 1 (Agp1). The double deletion strain *P. pastoris* GS115 *aqy1Δ::HIS4 agp1Δ::NatMX* was generated by homologous recombination, inserting a deletion cassette into *P. pastoris* GS115 *aqy1Δ::HIS4* [12]. The double cloning approach used for deleting *AGP1* was previously described by Ternes and co-workers [13]. A ~670 bp fragment upstream of *AGP1* containing restriction sites for XbaI and NotI and a ~800 bp downstream fragment reaching into the *AGP1* gene containing NotI and XbaI restriction sites was amplified from genomic DNA by PCR. Both fragments were simultaneously ligated into the NotI/XbaI site of a pPICZ B vector. The antibiotic nourseothricin-resistance gene was sub cloned from the pSLnat plasmid [13] into the NotI site of pPICZB plasmid flanked by up and down stream regions to the *AGP1* gene. The construct was confirmed by sequencing. The deletion cassette was amplified by PCR and transformed into *P. pastoris* GS115 *aqy1Δ::HIS4* [14]. Transformed cells were plated on YPD-agar and grown for 2 days at room temperature before replica plating onto YPDS-agar plates + clonNAT (15 μg/ml). These plates were incubated at 30 °C for 3–4 days. Transformants were re-streaked for single colonies on YPD-agar plates + clonNAT (15 μg/ml). Successful deletion of the *AGP1* gene was verified by PCR. The produced double deletion strain is henceforth referred to as strain *aqy1agp1Δ P. pastoris* and was used throughout for the production of AQP7. For AQP7 expression, cDNAs encoding wild type AQP7 (aa1-342), AQP7-ΔN (aa33-342), AQP7-ΔC (aa1-279) and AQP7-ΔNΔC (aa33-279), all with a C-terminal 6× histidine tag, were amplified by PCR and cloned into the Not1 and EcoRI sites of the pPICZB plasmid using standard molecular biology techniques. The double point mutant AQP7-S10A/T11A was created from wild type AQP7 using the quick change protocol according to the manufacturer's instructions. All constructs were verified by sequencing (MWG biotech, Germany). Generation of *P. pastoris* strains expressing the AQP7 and the AQP7 variants followed the standard protocol provided by the manufacturer (Invitrogen). Briefly, plasmids were linearized by digestion with PmeI and transfected by electroporation into the *aqy1agp1Δ P. pastoris* strain. Transformants were selected on yeast extract peptone dextrose medium (YPD: 1% *w/v* yeast extract, 2% *w/v* peptone, 2% *w/v* dextrose, 1% *w/v*