The International Journal of Biochemistry & Cell Biology 41 (2009) 1269–1275

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

Human glutaredoxin-1 catalyzes the reduction of HIV-1 gp120 and CD4 disulfides and its inhibition reduces HIV-1 replication

Joeri Auwerx[a,b,1], Ola Isacsson[a], Johan Söderlund[a], Jan Balzarini[b], Magnus Johansson[a], Mathias Lundberg[a,*]

[a] Division of Clinical Microbiology, Karolinska University Hospital Huddinge, F68, Karolinska Institute, Stockholm, S-14186, Sweden
[b] Rega Institute for Medical Research, K.U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 10 July 2008
Received in revised form 24 October 2008
Accepted 31 October 2008
Available online 6 November 2008

Keywords:
HIV-1 gp120
Thioredoxin-1
Glutaredoxin-1
Disulfide reduction
Protein disulfide isomerase

ABSTRACT

Reduction of intramolecular disulfides in the HIV-1 envelope protein gp120 occurs after its binding to the CD4 receptor. Protein disulfide isomerase (PDI) catalyzes the disulfide reduction *in vitro* and inhibition of this enzyme blocks viral entry. PDI belongs to the thioredoxin protein superfamily that also includes human glutaredoxin-1 (Grx1). Grx1 is secreted from cells and the protein has also been found within the HIV-1 virion. We show that Grx1 efficiently catalyzes gp120, and CD4 disulfide reduction *in vitro*, even at low plasma levels of glutathione. Grx1 catalyzes the reduction of two disulfide bridges in gp120 in a similar manner as PDI. Purified anti-Grx1 antibodies were shown to inhibit the Grx1 activity *in vitro* and block HIV-1 replication in cultured peripheral blood mononuclear cells. Also, the polyanion PRO2000, that was previously shown to prevent HIV entry, inhibits the Grx1- and PDI-dependent reduction of gp120 disulfides. Our findings suggest that Grx1 activity is important for HIV-1 entry and that Grx1 and the gp120 intramolecular disulfides are novel pharmacological targets for rational drug development.

1. Introduction

The human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins gp120 and gp41 undergo major conformational changes during viral entry (Chan and Kim, 1998; Moore and Doms, 2003). This process is initiated by the binding of gp120 to the CD4 receptor on the target host cell. The interaction between gp120 and CD4 results in structural changes in gp120 that results in the exposure of regions of the protein that bind to the chemokine co-receptors CXCR4 or CCR5. Co-receptor binding subsequently triggers additional structural changes that lead to exposure of the envelope gp41 fusion protein that allows its insertion into the cell membrane and mediates fusion of the viral envelope with the host cell. The structural changes in the envelope proteins during HIV-1 entry require reduction of gp120 intramolecular disulfides (Fenouillet et al., 2007; Ryser and Fluckiger, 2005).

Ryser and co-workers first showed that agents that interfere with thiol-disulfide reduction on the cell surface inhibit HIV-1 infection. They identified protein disulfide isomerase (PDI) located on the cell surface as a candidate enzyme to catalyze this reaction (Ryser et al., 1994). Intramolecular disulfides in gp120 are reduced by PDI *in vitro* and the inhibition of PDI activity using either anti-PDI antibodies or chemical PDI inhibitors has been shown to decrease HIV-1 replication by inhibiting viral entry in cultured cells (Barbouche et al., 2003; Fenouillet et al., 2001; Gallina et al., 2002; Markovic et al., 2004; Ryser et al., 1994). These findings imply that PDI activity is important for HIV-1 entry by reduction of gp120 intramolecular disulfides.

PDI constitutes a family of structurally related enzymes that catalyzes reduction, formation and isomerization of disulfide bonds (Turano et al., 2002). PDI enzymes are predominantly located in the endoplasmic reticulum where they act as chaperones and facilitate protein folding. However, PDI enzymes are also present on the cell surface and can be secreted extracellularly (Terada et al., 1995; Turano et al., 2002; Yoshimori et al., 1990). The PDI enzyme family belongs to the thioredoxin superfamily of enzymes that also include the human thioredoxins and glutaredoxins (Turano et al., 2002). Human thioredoxin-1 (Trx1) is secreted from cells in a similar way as PDI and the enzyme has been shown to be located on the cell surface (Martin and Dean, 1991; Rubartelli et al., 1992; Tagaya et al., 1989). The secreted Trx1, or a truncated part of the

Abbreviations: HIV-1, human immunodeficiency virus type-1; Grx1, glutaredoxin-1; Trx1, thioredoxin-1; GSH, reduced glutathione; PDI, protein disulfide isomerase; GR, glutathione reductase.

* Corresponding author at: Mitochondrial Medicine Center, Karolinska Institute, Novum, S-14186, Sweden. Tel.: +46 8 58583648; fax: +46 8 7795383.
E-mail address: Mathias.Lundberg@mbb.ki.se (M. Lundberg).
[1] Temporarily at Division of Clinical Microbiology for a post-doctoral stay.

1357-2725/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2008.10.031

protein, is a cytokine that induces cell proliferation. A role of extracellular Trx1 during HIV-1 infection has also been suggested by a study showing that disulfide reduction in the cellular CD4 receptor is required for HIV-1 entry and that Trx1 catalyzes this reaction (Matthias et al., 2002). We have previously reported that human glutaredoxin-1 (Grx1) is secreted from cells in a similar way as PDI and Trx1 (Lundberg et al., 2004). The overlapping properties of Grx1 and other members of the thioredoxin enzyme superfamily as well as the presence of Grx1 in the extracellular space suggest a possible role of this enzyme in reduction of extracellular disulfides. Grx1 has also been shown to be located in the HIV-1 virion (Davis et al., 1997), but these observations have not yet been confirmed by other investigators. Based on the similarities between Grx1 and PDI as well as the association of Grx1 with the HIV-1 virion, we hypothesized that Grx1 may be important for HIV-1 entry and infection.

We have shown in this study that recombinant Grx1 efficiently reduces intramolecular disulfides in the virus envelope protein gp120 as well as in the cellular CD4 receptor. We used anti-Grx1 antibodies that inhibit the activity of the enzyme and showed that HIV-1 replication in cultured target cells decreases when Grx1 activity is inhibited. In addition, we showed that PRO2000, a previously described inhibitor of HIV infection (Rusconi et al., 1996), could have an additional inhibitory effect on HIV entry due to its interference with the reduction of the disulfides mediated by Grx1 and/or PDI. Our findings suggest that extracellular Grx1 may have an important role in HIV-1 viral entry.

2. Materials and methods

2.1. Materials

Bovine liver protein disulfide isomerase (PDI), NADPH, yeast glutathione reductase (GR) and reduced glutathione (GSH) were purchased from Sigma. Glutaredoxin-1 (Grx1) was obtained from IMCO Cooperation (Stockholm, Sweden). Human CD4 was purchased from Nordic Biosite, Sweden. Recombinant glycosylated HIV-1_{IIIB} gp120 produced in CHO cells was purchased from ImmunoDiagnostics (MA, USA).

2.2. Production and purification of goat anti-human Grx1 polyclonal antibody

An anti-human Grx1 antibody has been generated by immunizing goats with recombinant Grx1 and was a kind gift from Dr. Arne Holmgren (Karolinska Institute, Stockholm, Sweden). The highly specific anti-Grx1 antibody was then purified from the IgG fraction of the anti-sera by affinity chromatography using a column with recombinant human Grx1 coupled to cyanogen bromide-activated sepharose as described previously (Lundberg et al., 2004).

2.3. Determination of GSH-dependent reduction of gp120 and CD4 by human Grx1 or bovine PDI

96-well Maxi-Sorp ELISA plates (Nunc Inc.) were coated with 2 μg/ml CD4 or gp120 in carbonate buffer pH 9.6 for 60 min at 37 °C. Subsequently, the plates were blocked with PBST (phosphate buffered saline, pH 7.4 with 0.05% Tween-20) for 60 min at 37 °C. After four washes with PBST, Grx1 or PDI activity was determined incubating the wells with following assay mix for a fixed time: PBST + 0.5 μM Grx1 or 1 μM PDI + 1 mM EDTA + 200 μg/ml NADPH + 6 μg/ml yeast GSH reductase (GR) and different amounts of GSH as indicated in the different experiments. Subsequently the wells were washed four times with PBST and then incubated for 30 min at room temperature (RT) with 2 μM *N*-etylmaleimide-biotin (NEM-biotin) that specifically reacts with thiols (Sigma). After incubation the wells were washed four times with PBST. Thereafter alkaline phosphatase conjugated streptavidine (MABTECH, Sweden) diluted 1:1000 was added to each well. After 30 min incubation at RT the wells were washed four times with PBST and subsequently the substrate buffer (1 mg/ml *p*-nitrophenyl-phosphate (Sigma) dissolved in 10% diethanolamine pH 9.8 with 0.5 mM $MgCl_2$) was added. The absorbance at 405 nm was determined using a Victor3 microtiter plate reader (PerkinElmer). Specific absorbance was calculated subtracting the value obtained for the assay mix in the absence of enzymes or GSH (approximately 65 mOD) from the obtained value.

2.4. Comparison of the GSH-dependent reduction of disulfides in CD4 or gp120 between human Grx1 and bovine PDI

96-well Maxi-Sorp ELISA plates were coated with gp120 and CD4, respectively as described above. Subsequently, the proteins were incubated with the previously described reaction mixture including 0.5 μM Grx1 or 1 μM PDI (both separately or together in a mixture), PBS or dithiotreitol (DTT), for 30 and 35 min at RT, after which free-SH groups were detected.

2.5. Inhibition of Grx1-dependent reduction of CD4 disulfides by anti-Grx1 antibody

Purified goat anti-human Grx1 (final concentration 0–0.8 μM) was mixed with 0.5 μM Grx1 (final concentration). After 1 h incubation at RT the Grx1 activity was assayed as described above using CD4 as a substrate.

2.6. Inhibition of Grx1- and PDI-dependent reduction of HIV-1 gp120 by polyanions and pradimicin A

Dextran sulfate (M_r 5000) (DS-5000) and suramin were purchased from Sigma (St. Louis, MO). The sulfonated polyvinyl alcohol (PVAS) was synthesized by Dr. S. Görög (Budapest, Hungary). Pradimicin A (PRM-A) was obtained from Prof. T. Oki and Prof. Y. Igarashi (Toyama, Japan). PRO2000 was kindly provided by Dr. Albert Profy, Indevus Pharmaceuticals, Inc., Lexington, MA, USA.

HIV-1 gp120 was immobilized as described above and incubated with serial dilutions of several test compounds for 30 min at 37 °C. Subsequently, 0.5 μM Grx1 or 1 μM PDI in the appropriate reaction mixture, including 1 mM GSH, was added to the compound dilution. Grx1 and PDI activity was further assayed as described above. An additional 10 mM Ca^{2+} was used when PRM-A was evaluated in the assay.

2.7. Inhibition of HIV-1 replication by anti-Grx1 antibodies

Equal volumes of anti-Grx1 antibodies and non-immune goat IgG (Sigma) as the negative control were diluted to a final concentration of 1, 10 and 40 μg/ml and mixed with primary isolate HIV-1RW009 ($TCID_{50}$ between 10 and 50) supplemented with RPMI-1640 containing 10% foetal calf serum (FCS) and incubated for 1 h at 37 °C. Subsequently, the virus mixtures (150 μl) were added to PBMCs that were pre-activated with 75 μl PHA/IL-2 for 3 days. After 16 h incubation at 37 °C, the cells were washed twice to remove extracellular HIV-1. Fresh RPMI-1640 medium containing 10% FCS was supplied at day 3. After an additional 6 days incubation at 37 °C the supernatant of the cell cultures was collected and the amounts of p24 antigen were analyzed by ELISA (Murex HIV Antigen Mab, Abbott, Abbott Park, IL, USA). In all experiments, sera from HIV-1 seropositive individuals were used as positive controls and showed more than 70% inhibition (data not shown).