Biochimica et Biophysica Acta 1830 (2013) 3816–3827

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Secreted glyceraldehye-3-phosphate dehydrogenase is a multifunctional autocrine transferrin receptor for cellular iron acquisition

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ARTICLE INFO

Article history:
Received 21 December 2012
Received in revised form 13 March 2013
Accepted 18 March 2013
Available online 28 March 2013

Keywords:
Glyceraldehyde-3-phosphate dehydrogenase
Transferrin receptor
Iron
Trafficking
Multifunctional protein
Urokinase plasminogen activator receptor

ABSTRACT

Background: The long held view is that mammalian cells obtain transferrin (Tf) bound iron utilizing specialized membrane anchored receptors. Here we report that, during increased iron demand, cells secrete the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) which enhances cellular uptake of Tf and iron.
Methods: These observations could be mimicked by utilizing purified GAPDH injected into mice as well as when supplemented in culture medium of model cell lines and primary cell types that play a key role in iron metabolism. Transferrin and iron delivery was evaluated by biochemical, biophysical and imaging based assays.
Results: This mode of iron uptake is a saturable, energy dependent pathway, utilizing raft as well as non-raft domains of the cell membrane and also involves the membrane protein CD87 (uPAR). Tf internalized by this mode is also catabolized.
Conclusions: Our research demonstrates that, even in cell types that express the known surface receptor based mechanism for transferrin uptake, more transferrin is delivered by this route which represents a hidden dimension of iron homeostasis.
General significance: Iron is an essential trace metal for practically all living organisms however its acquisition presents major challenges. The current paradigm is that living organisms have developed well orchestrated and evolved mechanisms involving iron carrier molecules and their specific receptors to regulate its absorption, transport, storage and mobilization. Our research uncovers a hidden and primitive pathway of bulk iron trafficking involving a secreted receptor that is a multifunctional glycolytic enzyme that has implications in pathological conditions such as infectious diseases and cancer.

1. Introduction

Iron is an essential element for the growth of all organisms [1]. Practically all extracellular iron is transported in the blood by the iron carrier protein transferrin, which facilitates cellular iron uptake via interaction with specific membrane receptors, transferrin receptors 1 and 2 (TfR1 and TfR2). Membrane bound Tf receptors play a key role in the process by which cells acquire iron for growth and other vital functions [2].

After binding to cell surface receptor, the Tf-receptor complex is internalized into endosomes where iron is released and apo-transferrin receptor complex is recycled [3,4]. Both TfR1 and 2 are transmembrane glycoproteins. TfR1 is a high affinity ubiquitously expressed receptor while expression of TfR2 is restricted to certain cell types and is not regulated by intracellular iron concentrations. Although TfR1 mediated iron uptake is the major pathway for iron acquisition by most cells and especially developing erythrocytes [5–8], several studies have indicated that the uptake mechanism varies depending upon the cell type. It is also reported that Tf uptake, independent of these TfRs exists although the mechanisms are not well characterized [8–13].

Glyceraldehyde-3-phosphate dehydrogenase, a ubiquitously present glycolytic enzyme is predominantly localized in the cytosol. In addition to its well reported role for glycolysis, this protein exhibits numerous diverse functions [14].

Earlier we had reported that, GAPDH expressed on the surface of macrophage cells in an iron dependent manner, functions as a

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; sGAPDH, secreted GAPDH; Tf, transferrin; TfR, transferrin receptor; DFO, desferrioxamine; CTX, cholera toxin; SFM, serum free medium

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http://dx.doi.org/10.1016/j.bbagen.2013.03.019

Table 1
Fold change in GAPDH secreted into medium upon iron depletion as compared to control cells at 24 h, see also Fig. S1.

S. no.	Cell type	Fold change
1.	Mouse enterocytes	1.21
2.	Mouse spleen lymphocytes	1.46
3.	Mouse spleen macrophages	1.72
4.	Mouse peritoneal macrophages	1.76
5.	Mouse hepatocytes	0.87
6.	Mouse bone marrow cells	0.97
7.	Human peripheral blood lymphocytes	1.71
8.	J774	6.05
9.	J774 (iron depletion by incubation in Chelex treated media)	8.18
10.	K562	5.76
11.	NS	2.41
12.	THP1	1.92
13.	GAPDH knock down THP1	0.92
14.	CHOTrVb	1.59
15.	CHO	1.18
16.	Hut 78	1.19
17.	A20	0.97
18.	L1210	0.92
19.	Jurkat	0.80
20.	Cos	0.72
21.	N2A	0.70
22.	Hela	0.49

No change in cell viability of iron depleted cells or level of LDH in supernatant as compared to controls was observed.

transferrin receptor [15]. Recently we have established that, apart from macrophages, GAPDH also functions as an additional transferrin receptor on numerous cell types and upon iron depletion many of them prefer to utilize their surface localized GAPDH for Tf-iron uptake instead of TfR1. The kinetics of transferrin associated iron delivery, by this pathway, have been characterized [16]. Recent investigations by proteomic based analysis of primary murine macrophage cells have confirmed the role of cell surface GAPDH in cellular iron homeostasis [17].

Apart from cytosolic localization, studies have reported the secretion of GAPDH (sGAPDH) by mammalian cells in culture [15,18]. It also constitutes a normal component of serum [19]. As transferrin is an iron carrier molecule abundantly present in serum along with GAPDH, we decided to investigate the role of sGAPDH in Tf mediated iron uptake.

In the present study, we demonstrate that, upon iron depletion, GAPDH secretion from cells is enhanced. This can be correlated to an increase in the trafficking of transferrin and iron into cells. To understand the significance of this pathway for transferrin acquisition, we evaluated transferrin delivery in cell lines and primary cells that express known membrane bound transferrin receptors. Cell types that play a key role in mammalian iron homeostasis, such as hepatocytes and cells of the reticuloendothelial system were also selected. Enhanced Tf delivery by sGAPDH to mouse peritoneal macrophages and lymphocytes was also confirmed *in vivo*. The binding, internalization, recycling and degradation kinetics of this soluble receptor were studied in CHO-TRVb cells that lack TfR1 and 2 (a knock out system). Our findings reveal the dual role of GAPDH as a membrane associated and soluble receptor in Tf iron uptake.

2. Materials and methods

2.1. Iron depletion in cells

Cells were cultured in RPMI-1640 supplemented with 10% FCS. For iron depletion, cells were cultured for 24 h in either, complete medium supplemented with 100 μM DFO (Sigma) or with Chelex-100 treated medium [15,16]. Controls were set up in parallel with normal media. Iron depletion had no significant effect on cell viability which was confirmed by five independent methods as described earlier [16], in addition, elevated GAPDH levels in the medium due to leakage of cytosolic protein were ruled out by measuring the levels of lactate dehydrogenase (LDH) using a commercial kit (Roche).

2.2. Evaluation of GAPDH secretion

Supernatants from cultured cells were assayed using the kDalert™ GAPDH assay kit (Applied Biosystem/Ambion, USA) as per manufacturer's instructions.

2.3. FACS analysis

Cell surface staining of GAPDH, CD71 as well as binding and uptake of Tf, GAPDH and BSA fluorescent conjugates by FACS was carried out in triplicate as described previously [15,16].

2.4. Labeling of Tf with ^{55}Fe

^{55}Fe (American Radio Chemicals, USA) was incorporated into Human apo-Tf as described earlier [16].

2.4.1. Culture supernatant of iron depleted cells enhances transferrin and iron uptake

Culture supernatant from control or iron depleted cells was added to fresh cells and their uptake of Tf evaluated by FACS after control cell supernatants were supplemented with an equivalent concentration of DFO as the iron depleting media. Iron uptake was evaluated using ^{55}Fe labeled Tf and liquid scintillation counting.

2.5. Relative trafficking of transferrin in cells by sGAPDH and via surface receptors

Cells were incubated with either; (i.) only transferrin Alexa-633 (10 μg), (ii.) only GAPDH-FITC (20 μg) or (iii.) both (i.) and (ii.) and analyzed by FACS for internalization of transferrin and GAPDH. A two-color quadrant plot was established by plotting events for internalized Tf and GAPDH signals along the x and y axes respectively. Autofluroscence in either channel determined the threshold of labeling. The lower right quadrant represents cells that are positive for only transferrin (uptake only via surface receptors) while cells in the upper right quadrant represent cells that have co-internalized both signals (Tf internalization via surface receptors + internalization mediated by sGAPDH). A comparison of, % cell population in each quadrant and signal intensity of Tf between the two quadrants provides information on the extent of Tf internalization by the surface receptor or surface receptor + sGAPDH receptor route. As the extent of cells co-internalizing both signals was essentially 100% in several cases, the comparison for increase in Tf signal in presence of added GAPDH had to be made with cells where GAPDH was omitted. For *in vivo* uptake BALB/c mice were injected i.p with fluorescent labeled Tf and GAPDH. After 1 h macrophages and lymphocytes from perotineal lavage were analyzed.

2.6. Co-localization and interaction of GAPDH and transferrin

Co-localization and interaction of GAPDH and transferrin inside J774 and K562 cells as well as in endosomes purified from K562 cells were demonstrated using confocal microscopy, acceptor photobleaching FRET analysis and negative staining electron microscopy as described previously [15,20]. Membrane un-roofing and transmission electron microscopy of J774 cells in the process of internalizing GAPDH and transferrin conjugated to gold particles were carried out as described earlier [21].