Redox Biology 20 (2019) 146–156

Contents lists available at ScienceDirect

Redox Biology

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

Research paper

Diphthamide affects selenoprotein expression: Diphthamide deficiency reduces selenocysteine incorporation, decreases selenite sensitivity and predisposes to oxidative stress

Klaus Mayer[a], Olaf Mundigl[a], Hubert Kettenberger[a], Fabian Birzele[b], Sebastian Stahl[a], Ira Pastan[c], Ulrich Brinkmann[a,*]

[a] Roche Pharma Research & Early Development, Large Molecule Research, Roche Innovation Center Munich, Penzberg, Germany
[b] Roche Pharma Research & Early Development, Pharmaceutical Sciences, Roche Innovation Center Basel, Basel, Switzerland
[c] Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

ARTICLE INFO

Keywords:
Eukaryotic translation elongation factor 2
Selenocysteine
Oxidative stress
Diphtheria toxin

ABSTRACT

The diphthamide modification of translation elongation factor 2 is highly conserved in eukaryotes and archaebacteria. Nevertheless, cells lacking diphthamide can carry out protein synthesis and are viable. We have analyzed the phenotypes of diphthamide deficient cells and found that diphthamide deficiency reduces selenocysteine incorporation into selenoproteins. Additional phenotypes resulting from diphthamide deficiency include altered tRNA-synthetase and selenoprotein transcript levels, hypersensitivity to oxidative stress and increased selenite tolerance. Diphthamide-eEF2 occupies the aminoacyl-tRNA translocation site at which UGA either stalls translation or decodes selenocysteine. Its position is in close proximity and mutually exclusive to the ribosomal binding site of release/recycling factor ABCE1, which harbors a redox-sensitive Fe-S cluster and, like diphthamide, is present in eukaryotes and archaea but not in eubacteria. Involvement of diphthamide in UGA-SECIS decoding may explain deregulated selenoprotein expression and as a consequence oxidative stress, NFkB activation and selenite tolerance in diphthamide deficient cells.

1. Introduction

The post-translational diphthamide modification of eukaryotic translation elongation factor eEF2 is highly conserved in eukaryotes as well as in the archaeal eEF2 counterpart [1–5]. It consists of a histidine in elongation factor 2 (His 715 in human eEF2), modified by the concert action of diphthamide synthesis enzymes encoded by DPH genes DPH1–7 in humans, [6–12]. High conservation of diphthamide and -synthesis genes would suggest that this modification may be rather important for eEF2 functionality and hence for protein synthesis. Reports indicate that it contributes to translation fidelity and avoidance of frameshifting during elongation [13–16], as well as IRES-dependent translation events [41]. Because diphthamide deficient cells are viable [17], lack of diphthamide does not generally affect the synthesis and function of proteins essential for metabolism, propagation and replication of cells.

We have recently generated a set of MCF7 derivatives which lack diphthamide as consequence of gene editing inflicted destruction of individual DPH genes [12,17]. All copies of the DPH1 gene were inactivated in MCF7-DPH1ko, all copies of DPH2 in MCF7-DPH2ko, of DPH4 in MCF7-DPH4ko, and of DPH5 in MCF7-DPH5ko. Because diphthamide deficiency is inflicted by knockout of different genes, this set of cell lines can be applied to address the function of diphthamide by itself: phenotypes that are common among all these cell lines are attributable to diphthamide deficiency itself and not to potential other functionalities of the different inactivated individual genes.

One common observation in all these cell lines was resistance to Diphtheria and Pseudomonas toxin, an expected phenotype as diphthamide is the molecular target of toxin-mediated eEF2 inactivation. In addition, all diphthamide deficient cell lines had NFkB pathway genes activated (without active stress-trigger) and all were hypersensitive to TNF. TNF hypersensitivity is probably due to NFkB pathway activation, but the underlying reason for diphthamide mediated modulation of NFkB activity is still unexplained [17].

Abbreviations: eEF2, eukaryotic translation elongation factor 2; DPH gene, diphthamide synthesis gene; SeCys, selenocysteine; SECIS, selenocysteine incorporation stemloop; DT, diphtheria toxin; NFkB, nuclear factor kappa B; Dio1, deiodinase 1

* Corresponding author.
E-mail address: ulrich.brinkmann@roche.com (U. Brinkmann).

https://doi.org/10.1016/j.redox.2018.09.015
Received 6 June 2018; Received in revised form 17 September 2018; Accepted 24 September 2018
Available online 26 September 2018

Diphthamide is part of the translation elongation factor eEF2. 'Occam's razor' (simplest explanation most likely being the right one) would therefore imply effects on protein translation as likely explanation for diphthamide deficiency associated phenotypes. Because overall protein synthesis and generation of proteins essential for cell growth was not affected by diphthamide deficiency, we considered the possibility that expression of only certain proteins or of a subset of 'special' proteins is affected by diphthamide deficiency. Such proteins would not be essential for general growth, survival and propagation, yet (as that is a phenotype of lack of diphthamide) nevertheless be involved in NFkB signaling.

Selenocysteine containing proteins are a small group of proteins whose translation involves a special incorporation process at the elongation-vs-termination decision point [18–23]. Their mRNAs harbor UGA codons which are not recognized as termination signals but instead decoded as selenocysteine, dependent on presence of 3′UTR-SECIS elements. Most selenoproteins are involved in redox-processes to manage or detoxify oxidative stress [21–23] and oxidative stress is a known trigger of NFkB activation [24–26]. Therefore, we have analyzed if diphthamide deficiency influences the expression of selenoproteins and if it affects (as downstream consequence) cellular sensitivity towards oxidative stress.

2. Results

2.1. Diphthamide deficiency affects transcript levels of cytoplasmic amino-acid tRNA-synthetase genes and of genes that encode selenoproteins

A common phenotype of cells carrying DPH gene knockouts resulting in lack of diphthamide is induction of NFkB pathway-associated genes under normal growth conditions. This phenotype correlates with TNF hypersensitivity due to NFkB triggered induction of TNF-sensitivity genes [17]. The underlying reason for the association of diphthamide deficiency with NFkB activity, however, is still unexplained. Diphthamide is part of the essential translation elongation factor eEF2, yet its deficiency does not interfere with overall protein synthesis. Diphthamide may, however, play a role in regulating translation. NFkB induction would be a consequence of direct or indirect modulation of protein synthesis in diphthamide deficient cells. Evidence that diphthamide deficiency affects some aspects of protein synthesis is provided by transcriptional profiling of different DPH-knockout derivatives in comparison to parent MCF7 cells. Table 1A shows that the transcript levels of nuclear encoded cytoplasmic tRNA-synthetases are changed significantly. Alteration of tRNA-synthetase transcript levels was observed in all analyzed diphthamide deficient cell lines (DPH1, DPH2, DPH4 or DPH5). We found changes in > 50% of the synthetases that charge cytoplasmic tRNAs (i.e. those that utilize eEF2 for incorporation by 80 S ribosomes). The general pool of tRNA-synthetases for eEF2-independent mitochondrial protein synthesis was not affected when compared to overall transcriptional changes (the only mito-synthetase affected was prolyl-tRNA synthetase). Also, the levels of tRNA-synthetase pseudogene transcripts were not changed. In summary, diphthamide deficiency triggers changes in transcript levels of those tRNA-synthetases that utilize diphthamide-eEF2 for chain elongation.

Another set of genes whose mRNA levels change in diphthamide deficient cells encode selenoproteins (Table 1B): mRNA levels for 7 out of 23 selenoproteins were altered in DPH1ko cells, 8 were altered in DPH2ko cells, 5 were altered in DPH4ko cells and 9 were altered in DPH5ko cells. Thus, diphthamide deficiency changes the transcript levels of a large portion of selenoproteins.

2.2. Diphthamide deficiency affects the translation of selenocysteine containing proteins

Because diphthamide is not part of the transcriptional machinery, altered selenoprotein transcript levels in diphthamide deficient cells may be a feedback/compensation in response to altered selenoprotein translation. Selenocysteine is incorporated at UGA codons of mRNAs that carry SECIS elements in their 3′ untranslated region. We expressed UGA-selenocysteine containing cDNAs accompanied by 3′ SECIS sequences in parent MCF7 and DPH1ko cells and compared their translation products. Fig. 1 shows Western blot analyses of deiodinase 1 (Dio1) protein [27] in extracts of MCF7 cells, and in DPH1ko cells that lack diphthamide. Dio1 is composed of 249 amino acids with a selenocysteine at position 126. Termination at that position generates a ~15 kDa truncated protein; successful selenocysteine incorporation results in a ~30 kDa full length protein. To facilitate detection of full length Dio1, we have added a His6 tag to its C-terminus, only full length Dio1 carries this tag (Fig. 1A). Detection of full length Dio1 via His6-binding antibody (Fig. 1B) indicates that full length Dio1 translation is strictly dependent on presence of the SECIS element. Cells carrying constructs without SECIS do not produce His-tagged Dio1. A comparison of the levels of expressed Dio1-His6 revealed higher levels of full length Dio1 in parent MCF7 compared to DPH1ko cells. Fig. 1C shows additional Western blots that were probed with antibodies that detect full length as well as truncated Dio1 protein. Polyclonal antibodies from two different sources revealed differences in Dio1 protein content and composition between parent MCF7 and DPH1ko cells. The 30 kDa Dio1 protein band corresponding in size to full length protein was more pronounced in extracts of MCF7 cells than in DPH1ko extracts and the 15 kDa Dio1 fragment (indicative of premature termination at SeCys-UGA) was more pronounced in DPH1ko than in parent cells.

To confirm the influence of diphthamide on selenoprotein expression, we compared the expression of a different selenoprotein in MCF7 and DPH1ko cells. SelenoMabs are antibody derivatives that contain in their mRNA an UGA codon and 3'SECIS for selenocysteine incorporation, for site-directed payload attachment, [28,29]. SelenoMabs are secreted into culture supernatants and can be purified by protein A -chromatography. This allowed us to assess and quantify selenocysteine incorporation on purified protein samples. Fig. 2A shows the composition of the SelenoMab and of the expression cassettes used for its production. Selenocysteine-UGA is positioned at the C-terminus of the L-chain, followed by a His6 stretch and the 3′-SECIS element of human Dio1 mRNA. Reading UGA at this position as 'stop' generates a normal IgG, selenocysteine incorporation generates a SeCys-His6 extended antibody. Both antibody forms (irrespective of presence or absence of His6) are secreted into medium from which they were purified by proteinA and subsequently quantified (Fig. 2). Total IgG protein can subsequently be separated via NiNTA-chromatography into a 'normal IgG' fraction (UGA is read as 'stop', protein does not bind to NiNTA) and a fraction with His6 extension (captured on NiNTA).

Quantification of total IgG and individual fractions revealed that MCF7 as well as DPH1ko cells produced similar amounts of total IgG. This confirms that overall protein synthesis is not significantly affected in diphthamide deficient cells. A comparison of the relative content of normal and His6 extended IgG showed that in parent MCF7 cells approximately 4% of total IgG had the UGA codon decoded followed by His6. This ratio of read-through vs stop is similar in order of magnitude as previously reported for SelenoMabs (approx.10% in systems optimized for expression, [29]. In contrast to that, DPH1ko cells (despite expressing the same overall amount of IgG) contained almost exclusively IgG terminated at UGA. Less than 1% of the total IgG contained His6. Thus, diphthamide deficient cells decode selenocysteine codons (UGA-SECIS) less efficient than parent MCF7 cells. Reduced production of full length Dio1 and reduced selenocysteine-UGA read-through of SelenoMabs in diphthamide deficient cells indicates that diphthamide plays a role in the 'special' translation of selenoproteins.

To determine if diphthamide deficiency affects chromosome encoded selenoprotein transcripts, we analyzed chromosome encoded selenoprotein P (SELENOP, SEPP1) secreted into supernatants of MCF7 cells and of DPHko derivatives. SELENOP harbors multiple selenocysteins as well as an oligoHis stretch. The latter enables enrichment of full