Free Radical Biology and Medicine 83 (2015) 305–313

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Protein disulfide–isomerase, a folding catalyst and a redox-regulated chaperone

Lei Wang [1], Xi Wang [1], Chih-chen Wang *

National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

ARTICLE INFO

Article history:
Received 20 November 2014
Received in revised form
4 February 2015
Accepted 7 February 2015
Available online 17 February 2015

Keywords:
Chaperone
Endoplasmic reticulum
Oxidative protein folding
Protein conformation
Protein disulfide–isomerise
Redox regulation

ABSTRACT

Protein disulfide–isomerase (PDI) was the first protein-folding catalyst to be characterized, half a century ago. It plays critical roles in a variety of physiological events by displaying oxidoreductase and redox-regulated chaperone activities. This review provides a brief history of the identification of PDI as both an enzyme and a molecular chaperone and of the recent advances in studies on the structure and dynamics of PDI, the substrate binding and release, and the cooperation with its partners to catalyze oxidative protein folding and maintain ER redox homeostasis. In this review, we highlight the structural features of PDI, including the high interdomain flexibility, the multiple binding sites, the two synergic active sites, and the redox-dependent conformational changes.

© 2015 Elsevier Inc. All rights reserved.

Introduction

Remarkable early work by Hsien Wu [1] and others established the first theory of protein denaturation. Further, pioneering investigations by the Nobel Prize laureate Christian B. Anfinsen in the late 1950 s of the last century created a new field of protein science—"protein folding"—which was specified by Anfinsen's proposition that "the amino acid sequence of a polypeptide chain contains all of the information for its three dimensional structure" [2], so called "spontaneous self-assembly" principle of protein folding. In the 1980 s, a new concept for protein folding emerged along with the identification of a "molecular chaperone" by John Ellis [3]. The refined definition of molecular chaperone is "a large and diverse group of proteins that share the property of assisting noncovalent folding and unfolding, and assembly and disassembly, of other macromolecular structures, but are not permanent components of these structures when they are performing their normal biological functions" [4]. The concept of chaperone-assisted protein folding/unfolding/assembly/disassembly, i.e., the "assisted self-assembly" principle, expands the protein folding problem with a kinetic viewpoint, and does not conflict with Anfinsen's proposition as a thermodynamic hypothesis.

Oxidative protein folding characterized by intramolecular disulfide bond formation is probably the most complicated protein folding problem, as the number of all possible disulfide-bonded isomers of a protein skyrockets with the increase of the number of constituent cysteines, but only one form imparts correct protein function [5]. It is known now that nearly one-third of human proteins are secretory and membrane proteins, which usually possess intra- and/or intermolecular disulfide bonds. Disulfide bonds are very important for the structure, function, and regulation of these proteins. The formation of disulfide bonds mainly occurs in the endoplasmic reticulum (ER) of eukaryotic cells and in the periplasm of prokaryotic cells. The pioneering work in the 1960 s by Anfinsen's group led to the discovery of protein disulfide–isomerase (PDI) (for review, see Ref. [6]), which is a key and abundant enzyme in the ER for catalyzing oxidative protein folding. About 20 years later the amino acid sequence of rat PDI was deduced through sequencing of cDNA, suggesting that the enzyme comprised two distinct regions homologous with *Escherichia coli* thioredoxin (Trx) [7]. However, the accurate identification of the domain boundaries took almost another 20 years. It is now known that PDI is composed of four Trx-like domains in the order of **a**, **b**, **b**′, and **a**′, with a C-terminal acidic extension **c** and an **x**-linker between domains **b**′ and **a**′ [8,9]. Domains **a** and **a**′ each contain a -CGHC- active site responsible

Abbreviations: C_P, peroxidatic cysteine; C_R, resolving cysteine; ER, endoplasmic reticulum; ERAD, ER-associated degradation; Ero1, ER oxidoreductin 1; GAPDH, glyceraldehyde phosphate dehydrogenase; GPx, glutathione peroxidase; MTP, microsomal triglyceride transfer protein; SAXS, small angle X-ray scattering; P4H, prolyl 4-hydroxylase; PDI, protein disulfide–isomerase; Prx, peroxiredoxin; QSOX1, quiescin sulfhydryl oxidase 1; Trx, thioredoxin; UPR, unfolded protein response; VKOR, transmembrane vitamin K epoxide reductase

* Correspondence to: National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, China. Fax: +86 10 64840672.

E-mail address: chihwang@sun5.ibp.ac.cn (C.-c. Wang).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.freeradbiomed.2015.02.007
0891-5849/© 2015 Elsevier Inc. All rights reserved.

for a thiol–disulfide interchange reaction [10], and domain **b′** provides the principal substrate-binding site [8,11].

PDI is ubiquitously expressed in different tissue and cell types of mammals, with a great quantity (0.4% of total cellular protein) in professional secretory tissues [12]. Possessing a C-terminal ER retention sequence of KDEL, PDI is primarily located in the ER lumen where its concentration can approach the millimolar range [13]; nevertheless it has also been found in other intracellular compartments, such as mitochondria, nucleus, cytosol, and even at the cell surface and extracellular space [14]. To date, more than 20 members of human PDI family have been characterized, which share a common structural feature of having at least one Trx-like domain [15]. The modular combinations of Trx-like catalytic domains and noncatalytic domains give rise to structural and functional versatilities of PDI family members (for review, see Refs. [6,15,16]). The most well-studied PDI from lower eukaryotes is yeast *Saccharomyces cerevisiae* Pdi1p. Although human PDI and yeast Pdi1p share a sequence identity of 31% (BLAST) with similar overall structures and *in vitro* oxidoreductase activities, they differ in their molecular dynamics, *in vivo* redox states, and interactions with client proteins. In this review, we will focus on the structural basis of human PDI both as an enzyme and as a molecular chaperone, its activity regulation, and its interactions with client proteins. In terms of these aspects, differences between human PDI and yeast Pdi1p will also be discussed.

Chaperone activity of PDI

PDI is both an enzyme and a molecular chaperone

Given the enzyme classification number EC 5.3.4.1, PDI can catalyze the reactions of thiol–disulfide interchange (oxidation/reduction) and the rearrangement of disulfide bonds (isomerization) in proteins, depending on the redox states of its active sites (Fig. 1). In each active site (-CGHC-) of PDI the two vicinal thiol groups can either form an intramolecular disulfide (oxidized PDI) or exist in free dithiol form (reduced PDI) during the catalytic cycle. PDI catalyzes disulfide formation in substrates by transferring oxidizing equivalents to reduced substrates as an oxidoreductase but not an oxidase, because it does not use molecular oxygen as the electron acceptor. Conversely, PDI catalyzes disulfide reduction in a substrate by transferring reducing equivalents to the oxidized substrate. The disulfide isomerization process could be achieved by two modes: the N-terminal cysteine in the active site of PDI attacks the mispaired disulfide in substrate and then directly shuffles the intramolecular disulfide rearrangement; alternatively, repeated cycles of substrate reduction followed by reoxidation are completed by mixed reduced/oxidized PDI [17]. PDI was recognized only as an oxidoreductase and excluded as a chaperone [18] or a general chaperone [19] at the beginning of the 1990 s. However, chaperone is a functional rather than a structural concept, and PDI was deduced to meet the main requirements of being a chaperone for the followings: (1) the spontaneous folding and formation of disulfide bonds within a polypeptide *in vitro* are often slow processes, and PDI-promoted oxidative folding with elevated efficiency does not need the presence of recognized chaperones; (2) PDI exists in the ER at high concentrations [13], and thus can function at stoichiometric levels as a chaperone; (3) PDI is notable in its capacity of nonspecific peptide binding through hydrophobic interaction [20,21], a prominent feature for a chaperone.

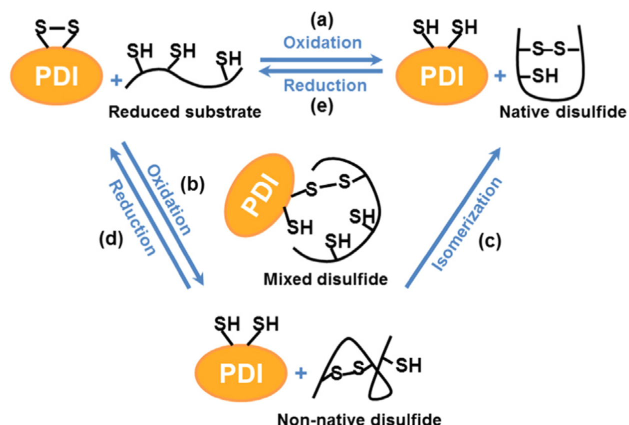

Fig. 1. PDI-catalyzed thiol–disulfide interchange reactions. PDI-catalyzed disulfide formation occurs when oxidizing equivalents are transferred from the active site of oxidized PDI to reduced substrate, resulting in either a native disulfide (a) or a nonnative disulfide (b) in the substrate and reduced PDI. The mispaired disulfide can be isomerized by reduced PDI through direct intramolecular disulfide rearrangement (c). Alternatively, the nonnative disulfide can be converted to the native one via the pathway of reduction (d) and reoxidation (a). In some cases, the disulfide in native substrate can be reduced by reduced PDI (e) to facilitate substrate unfolding. Note that in all the thiol–disulfide interchange reactions, a mixed disulfide is formed between the N-terminal cysteine in the PDI active site and a cysteine in the substrate. Only one catalytic domain of PDI is shown for simplicity.

“PDI is both an enzyme and a molecular chaperone” was originally proposed as a hypothesis [22]. This hypothesis has now been strongly supported by numerous *in vitro* and *in vivo* experimental data and widely accepted [4,23]. For oxidative protein folding, the chemical formation or disruption of disulfide bonds is accompanied by the processes of “conformational folding” of the polypeptide chain. These processes are connected intimately and affected interdependently. Therefore, to explore the chaperone activity of PDI explicitly distinct from its known enzyme activity, proteins without disulfide, such as glyceraldehyde phosphate dehydrogenase (GAPDH) [24] and rhodanese [25], were selected as target proteins. The presence of PDI in the folding system at stoichiometric instead of catalytic amounts indeed greatly increased the reactivation yield of the guanidine hydrochloride-denatured GAPDH or rhodanese on dilution and suppressed their aggregation during refolding, without being a part of the final functional structure. In addition, PDI suppressed aggregation of rhodanese during thermal denaturation. These properties are entirely consistent with the definition of chaperones by Ellis [18] and fully meet the four criteria proposed by Jakob and Buchner [26] for characterization of a protein as a molecular chaperone. As the reactivation of those target proteins has nothing to do with the formation of disulfides, the folding promoting effects of PDI cannot be attributed to its enzyme activity but only to its intrinsic chaperone activity.

The intrinsic chaperone activity of PDI has also been characterized in physiological disulfide-containing proteins. The oxidative refolding yield of denatured lysozyme was either increased or decreased in the presence of different concentrations of PDI [27]. PDI also increased the reactivation yield of denatured and reduced antibody fragments enormously, with a maximum effect at near stoichiometric amounts [28]. Later a set of elegantly designed experiments [29] showed that the maximal refolding and reactivation of denatured and reduced acidic phospholipase A2, a protein composed of 124 amino acid residues with seven disulfide bonds, were achieved in the presence of stoichiometric amounts of PDI, and 90% of PDI in the refolding reaction can be replaced by alkylated PDI [30] with only chaperone but no enzyme activity. Catalytic amounts of PDI only catalyzed very limited reactivation, and alkylated PDI alone, even at stoichiometric amounts, showed no effect on the reactivation of acidic phospholipase A2. These experiments unambiguously discriminated the chaperone and oxidoreductase activities of PDI in the oxidative folding of

Download English Version:

https://daneshyari.com/en/article/1907939

Download Persian Version:

https://daneshyari.com/article/1907939

Daneshyari.com