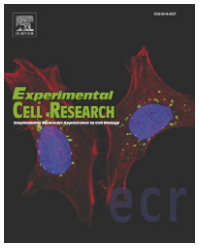
available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/yexcr



Review

Selenoproteins—What unique properties can arise with selenocysteine in place of cysteine?

*Elias S.J. Arnér**

Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-171 77 Stockholm, Sweden

ARTICLE INFORMATION

Article Chronology:
Received 14 January 2010
Accepted 24 February 2010

Keywords:
Selenoprotein
Selenocysteine
Cysteine
Nucleophilicity
Reactivity

ABSTRACT

The defining entity of a selenoprotein is the inclusion of at least one selenocysteine (Sec) residue in its sequence. Sec, the 21st naturally occurring genetically encoded amino acid, differs from its significantly more common structural analog cysteine (Cys) by the identity of a single atom: Sec contains selenium instead of the sulfur found in Cys. Selenium clearly has unique chemical properties that differ from sulfur, but more striking are perhaps the similarities between the two elements. Selenium was discovered by Jöns Jacob Berzelius, a renowned Swedish scientist instrumental in establishing the institution that would become Karolinska Institutet. Written at the occasion of the bicentennial anniversary of Karolinska Institutet, this mini review focuses on the unique selenium-derived properties that may potentially arise in a protein upon the inclusion of Sec in place of Cys. With 25 human genes encoding selenoproteins and in total several thousand selenoproteins yet described in nature, it seems likely that the presence of that single selenium atom of Sec should convey some specific feature, thereby explaining the existence of selenoproteins in spite of demanding and energetically costly Sec-specific synthesis machineries. Nonetheless, most, if not all, of the currently known selenoproteins are also found as Cys-containing non-selenoprotein orthologues in other organisms, wherefore any potentially unique properties of selenoproteins are yet a matter of debate. The pK_a of free Sec (approximately 5.2) being significantly lower than that of free Cys (approximately 8.5) has often been proposed as one of the unique features of Sec. However, as discussed herein, this pK_a difference between Sec and Cys can hardly provide an evolutionary pressure for maintenance of selenoproteins. Moreover, the typically 10- to 100-fold lower enzymatic efficiencies of Sec-to-Cys mutants of selenoprotein oxidoreductases, are also weak arguments for the overall existence of selenoproteins. Here, it is however emphasized that the inherent high nucleophilicity of Sec and thereby its higher chemical reaction rate with electrophiles, as compared to Cys, seems to be a truly unique property of Sec that cannot easily be mimicked by the basicity of Cys, even within the microenvironment of a protein. The chemical rate enhancement obtained with Sec can have other consequences than those arising from a low redox potential of some Cys-dependent proteins, typically aiming at maintaining redox equilibria. Another unique aspect of Sec compared to Cys seems to be its efficient potency to support one-electron transfer reactions, which, however, has not yet been unequivocally shown as a Sec-dependent step during the natural catalysis of any known selenoprotein enzyme.

* Fax: +46 8 31 15 51.
E-mail address: Elias.Arner@ki.se.

0014-4827/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2010.02.032

Contents

Introduction

This mini review, written at the occasion of the bicentennial jubilee of Karolinska Institutet and the 60th anniversary of Experimental Cell Research, will discuss the potential unique properties that may arise from the inclusion of a selenium atom in a selenoprotein in the form of selenocysteine (Sec). This subject is clearly suitable at this particular occasion, having firm historic links to Karolinska Institutet through Berzelius (see Box 1).

The single atom of selenium in Sec as found in selenoproteins, is tremendously energy costly for organisms to synthesize, because of a number of dedicated cellular factors that redefine specific UGA codons from termination of translation to Sec insertion. This *de facto* expansion of the genetic code and the intricate pathways of selenoprotein synthesis have been described in many reviews [1–6] and will not be dealt with here. Instead, we shall focus on the potential biochemical differences between Sec and Cys that may underlie any unique aspects of utilizing Sec in proteins. What could the truly unique selenium-dependent properties of Sec be, which could help us understand the existence of selenoproteins and their special features?

Through several bioinformatic analyses, essentially all performed by Vadim Gladyshev et al., the number of described selenoproteins has rapidly expanded in recent years. This includes the 25 human and 24 mouse selenoprotein genes [7], at least 15 different types of selenoproteins encoded by completely sequenced bacterial and archeal genomes [8], more than 300 different selenoprotein genes found in samples from the Sargasso Sea [9] and more than 3600 distinct selenoprotein genes derived from 58 selenoprotein families, as globally sampled from different marine microbial organisms [10], as well as the single selenoprotein (thioredoxin reductase) that seems to be encoded in the genome of *Caenorhabditis elegans* [11]. Striking to note in these analyses of different "selenoproteomes" is the, at least at first glance, apparently sporadic occurrence of selenoproteins. Several organisms have Cys-dependent non-selenoprotein versions of the selenoproteins found in other genomes. No generally accepted reason or model has yet been formulated that could explain when or where a selenoprotein is maintained in a genome, in place of some less energetically costly Cys-dependent non-selenoprotein orthologue. It seems clear that selenoproteins most often utilize Sec as a catalyst of redox reactions, while Cys residues in proteins obviously can be used for either redox reactions or for other purposes, such as metal ion coordination, maintenance of structural disulfides or other functions [12,13]. It should also be noted that several organisms, such as higher plants, some fungal species or certain insects, seem to completely lack selenoproteins [14]. Still, although Cys-dependent oxidoreductases are significantly more common in nature than their selenoprotein counterparts, and although selenoprotein expression appears to be rather sporadic, further in-depth evolutionary analyses strongly indicate that the exchange rate between Cys and Sec in evolution is very low [15,16]. This was interpreted not necessarily to reflect an evolutionary pressure or an advantage for selenoproteins, but rather being an illustration of the fact that there must be some qualitatively distinct functional difference between Cys and Sec in terms of properties, even if it is not yet clear what such these qualitative differences should be [15–17]. Let us therefore briefly discuss what is evident regarding the similarities, complementary roles and potential biochemical differences between Sec and Cys when acting as redox active residues in proteins.

Physicochemical properties of selenium vs. sulfur

Directly comparing the physicochemical properties of sulfur and selenium, as Berzelius immediately realized (Box 1), the two compounds are very similar. In terms of electronegativity, oxidation state, atomic radius, etc. the differences between selenium and sulfur are rather slight, but selenium could perhaps be viewed upon as a slightly "exaggerated" form of sulfur. The different chemical and physical properties of the two elements were rather recently summarized in considerable detail [17]. The combined effects of the, albeit rather slight, physicochemical differences between the two elements, i.e. selenium having a bit longer atomic radius and bond lengths than sulfur, being more polarized and having lower diatomic bond energies, determine the genuine biochemical differences between Sec and Cys as found in proteins. Let us therefore now discuss the more prominent of those differences, asking how they could translate into any unique properties of Sec that could explain the low exchangeability between Sec and Cys in evolution [15,16].

A note on pK_a and generally lower catalytic efficiencies of Cys-dependent non-selenoproteins

One of the more evident and often cited differences between Sec and Cys are their highly divergent pK_a values. With Sec having a determined p$K_a \approx 5.2$ for the selenolate while Cys has a p$K_a \approx 8.5$ for its thiolate [18], this certainly has major implications for the

Please cite this article as: E.S.J. Arnér, Selenoproteins—What unique properties can arise with selenocysteine in place of cysteine? Exp. Cell. Res. (2010), doi:10.1016/j.yexcr.2010.02.032

Box 1
Berzelius—the discovery of selenium and its similarities with sulfur

Jöns Jacob Berzelius (1779–1848) was one of the founding professors of the establishment that would later develop into Karolinska Institutet. He studied medicine in Uppsala, where he received his doctoral degree. After his exam and thesis he assumed an honorary position ("oavlönad adjunct") in medicine and pharmacy at a surgical school in Stockholm, a position which was converted to a professorship in 1807. In 1810, that school was modernized and inaugurated by King Carl XIII on December 13th, 1810, as an institute with a mission to educate surgeons, "Institut för danande av skickelige fältläkare", which later developed into what we today know as Karolinska Institutet. Berzelius fought for the notion that medicine should be based upon solid ground in the natural sciences. At the time of the inauguration of the new school, the position held by Berzelius was renamed to a professorship in chemistry and pharmacy. Among many other major accomplishments holding this position, Berzelius discovered selenium.

Together with two associates, Gottlieb Gahn and H.P. Eggertz, Berzelius bought a sulfuric acid production factory in Gripsholm (a small Swedish village by the lake Mälaren). Unclean preparations of pyrite (an iron disulfide mineral, called "svavelkis" or "kattguld" in Swedish) were used as sulfur source, obtained from a mine in Falun (a city in Dalarna, some 250 km north of Stockholm). When the pyrite preparation was heated in lead chambers and the bottom sludge that remained from the sulfuric acid preparation was recovered, Berzelius felt a strong odor of black radish ("rättika"), the source of which he wished to analyze further. He rapidly realized that there was a substance in the preparation that was similar to tellurium, which had been discovered already in the 1780's and named after *Tellus* (Earth)—an element well known to Berzelius. He realized that he might have found a new basic element that was somewhat similar to tellurium. As he analyzed it further, his efforts led to the discovery of selenium. Already in his very first studies, he noted the close similarities between selenium and sulfur. Berzelius wrote[1]:

"Det bruna ämnet, som vid ammoniumsalternas sönderdelning afskiljt sig, blef nu ett föremål för undersökningen, och befanns, genom de försök, som i det följande skola beskrifvas, vara en egen, hittills okänd, brännbar mineralkropp, hvilken jag, för att utmärka dess slägtskap i egenskaper med tellurium, kallat Selenium, af Σελήνη, måna. Den ligger för öfrigt i detta hänseende midt emellan svafvel och tellurium, och har nesten flere af svaflets caracterer än af tellurens."

Attempting a translation into English, his words were phrased as follows:

"The brown substance, which the decomposition of the ammonium salts yielded, now became an object of investigation, and was found, through the experiments, which in the following will be described, to be a separate, hitherto unknown, combustible mineral, which I, to mark its akin properties with tellurium, have named Selenium, from Σελήνη, moon (goddess). What is more, it is in this regard, midway between sulfur and tellurium, and has almost more characters of sulfur than of tellurium."

Selenium, positioned just between sulfur and tellurium in the chalcogen group of the periodic system (group 16), is, in most of its properties, indeed highly similar to sulfur. How thrilled would Berzelius not have been, had he been able to learn that the life of many organisms depend upon selenoproteins, with selenium as a basic constituent, while the much more common sulfur-containing amino acid Cys has almost—but not exactly—the same features as the selenium-carrying Sec entity.

[1] Berzelius, J. J. (1818) Undersökning af en ny Mineral-kropp, funnen i de orenare sorterna af det i Falun tillverkade svaflet. *Afhandlingar i fysik, kemi och mineralogi* 6, 42–144; a scanned copy of this book is at present freely available on internet through a search in Google Books.

protonation state of the two amino acid side chains when present in free form in a water-based solution. At a physiological pH of 6.5–7.5, most of all Cys molecules having a $pK_a \approx 8.5$ will, naturally, at any given moment be found in their protonated and thereby rather inert forms, while Sec molecules (although not believed to exist in free form in cells) would mainly be deprotonated and thus more prone to engage in chemical reactions. Importantly, however, it must be noted that these pK_a values only relate to the free amino acids as studied in water solution. The situation can be highly distorted when Sec or Cys residues are present in the microenvironment of a protein structure. An example of this is the well known case with a low pK_a of Cys32 of *E. coli* thioredoxin (and corresponding active site Cys residues of many other proteins in the thioredoxin-fold family), with its pK_a lowered by the combined effects of a number of other residues in the protein including buried such as Asp26 and Lys57 [19]. In another example involving "Cys activation", the activity of thioredoxin reductase from *Drosophila melanogaster* being a non-selenoprotein orthologue of a mammalian selenoprotein was found to be surprisingly high [20]. With that enzyme having a –SCCS carboxyterminal active site motif, instead of –GCUG (where U is Sec) as found in the mammalian orthologue, the activity was found to be enhanced by the two flanking Ser residues in that motif, activating the two redox active Cys residues [21]. However, the very same –SCCS motif was not active when introduced in place of the –GCUG motif in the mammalian selenoprotein orthologue [22], thus showing that additional features of the *Drosophila* enzyme are needed to enable the flanking Ser residues to exert Cys activation. In this particular case, those features must facilitate the oxidative half reaction of the enzyme, since it was this step that became exceedingly slow in the mammalian enzyme mutant variant [22]. It was also recently emphasized, in a review of Sec-dependent

Please cite this article as: E.S.J. Arnér, Selenoproteins—What unique properties can arise with selenocysteine in place of cysteine? Exp. Cell. Res. (2010), doi:10.1016/j.yexcr.2010.02.032

and Cys-dependent glutathione peroxidases, that different pK_a values between different active site Cys or Sec residues, cannot easily explain the very high peroxidase velocity seen with many glutathione peroxidases [23]. Furthermore, when 16 different Cys-containing model peptides were synthesized, having pK_a values for their Cys residues ranging from 7.35 to 9.08, the reactivity with some disulfide substrates correlated well with the hypothetical Brønsted correlations between reaction rate and pK_a (based upon chemical reactivity due to proton transfer propensity), while the reactivity with other disulfide substrates showed no such correlation at all, potentially due to charge effects and steric hindrances [24]. The lessons learnt from these, and many other studies with similar results, show that a lower pK_a of Sec as compared to Cys can hardly be used as the sole explanation why selenoproteins are expressed in nature, simply because redox active Cys residues can also obtain lowered pK_a values and be "activated" in the context of active site microenvironments and because pK_a values of redox active residues are not the sole determinants for their reactivity.

Some inherent overall higher catalytic efficiency of Sec-dependent redox active selenoproteins as compared to Cys-dependent non-selenoprotein orthologues, are also unlikely the reasons why selenoproteins are found in so many different organisms. The arguments against this are several. First, if higher catalytic efficiencies of selenoproteins would be necessary for an efficient metabolism of some type of substrate, why are non-selenoprotein orthologues apparently found in other organisms for virtually any selenoprotein of choice [7–10,12,13,25]? Second, as already mentioned above, Cys-dependent orthologues of selenoproteins are not necessarily much less efficient in catalysis than their selenoprotein counterparts [20,21,23]. One proposal has been that selenoprotein variants are better peroxidases than non-selenoprotein variants, but this seems unlikely considering that many enzymes with peroxidase activity, or other efficient "antioxidant" reductase activity, such as peroxiredoxins, catalases, thioredoxins, glutaredoxins, methionine sulfoxide reductases, and many more, are widespread in nature in the form of non-selenoprotein variants (even if also selenoprotein orthologues of some of these enzymes are found as well). Third, simply expressing higher levels of a Cys-dependent enzyme with somewhat lower catalytic efficiency than a selenoprotein variant, should likely be more energy conserving than the expression of the selenoprotein, considering the total energy required for the whole selenoprotein translation machineries. Fourth, since many organisms lack selenoproteins completely, most (all?) metabolic pathways can evidently be supported by Cys-dependent non-selenoproteins as well. These arguments collectively suggest that "solely" a higher catalytic efficiency of selenoproteins as compared to Cys-dependent non-selenoprotein variants, can hardly be the reason why selenoproteins are found in nature. It thus seems plausible that selenoproteins should have some other feature(s) that may be unique in terms of properties, in comparison to those found in Cys-dependent orthologues. Let us discuss some of these potential features.

Higher nucleophilicity of Sec as compared to Cys

An interesting and perhaps truly unique aspect of selenoproteins may involve the significantly higher nucleophilicity of Sec as compared to Cys. Nucleophilicity (or nucleophilic reactivity), i.e. the propensity to donate electrons to a foreign atomic nucleus or to supply a pair of electrons to form a new bond with another atom [26], is a complex chemical property. Nucleophilicity derives from a combination of factors such as pK_a, polarizability, electronegativity and atomic radius, where attempts to formulate scales of nucleophilicity for diverse compounds have yet mainly been based upon empirical measurements [26–31]. It was earlier found that nucleophilic reactivity is guided, independently, by at least the three elemental properties of Lewis basicity, polarizability, and the accessibility of the unshared pairs of electrons in reactivity with any substrate molecule [28], although at least up to seventeen different factors have been proposed to influence the degree of nucleophilicity [26]. Being similar to, but not equivalent with, the properties and effects of a Lewis base, nucleophiles engage in chemical reactions utilizing accessible electrons "in search of nuclei". These "nuclei", presented by electrophilic substrates, may be distinct target sites on diverse molecular substrates, including the somewhat exposed nuclei of polarized covalent bonds between two atoms of different electronegativity. Although the physicochemical features guiding nucleophilicity are not yet completely understood, it is clear that Sec (selenium) is far more nucleophilic than Cys (sulfur). Comparing the chemical reactivity of 2,6-Dimethoxyphenyl derivatives of either sulfur, selenium or tellurium, the selenium compounds typically showed higher nucleophilicity and thus reactivity compared to the sulfur compounds (and less than those containing tellurium) [32]. When thiol/disulfide exchange reactions were compared to selenol/diselenide exchange with NMR spectroscopy, it was found that at physiological pH, the higher nucleophilicity of selenium, possibly together with its higher propensity to act as a leaving group, could yield more than 10^7 times faster reaction rates than with the corresponding sulfur compounds [33]. It has even been suggested that the high nucleophilicity of selenium as present in pyrite (see Box 1) may have played an important catalytic role in the evolution of life itself, but that this high nucleophilicity would be too deleterious due to "exhaustive hydrogenation" and that the occurrence of Sec would serve to control the reactive selenium in an organic sense, while selenomethionine could protect life forms from this reactivity as a detoxification mechanism [34].

Nucleophilicity is mainly considered to guide *initial rates* in chemical reactions, while basicity with Lewis or Bjørnsted bases mainly directs the *extent of thermodynamic equilibria* and thus the final proportion of reduced or oxidized end products at equilibrium, although the two concepts are, in most cases, closely related [30]. Since Sec is significantly more nucleophilic than Cys, while Cys is more basic than Sec, this difference might imply that selenoproteins, involving the highly nucleophilic Sec residue, could have a greater impact in facilitating initial reactions with high rates in redox chemistry under non-equilibrium states. This would hold true if some steps of a selenoprotein-catalyzed reaction involved nucleophilic substitution reactions or some other reaction that makes use of a high nucleophilicity. Non-selenoproteins, on the other hand, that would have redox activity (thereby depending upon Cys as a weaker nucleophile but stronger base than Sec) would suffice as well (or perhaps even be better than selenoproteins) in maintaining redox equilibria, or at least in striving towards this end. This potential functional difference in qualitative terms between selenoproteins and Cys-dependent non-selenoprotein orthologues, i.e. either rapidly facilitating initial high rates in catalysis and thus reacting with electrophiles (selenoproteins) as compared to striving to maintain redox equilibria (Cys-dependent non-selenoproteins) is at this

Please cite this article as: E.S.J. Arnér, Selenoproteins—What unique properties can arise with selenocysteine in place of cysteine? Exp. Cell. Res. (2010), doi:10.1016/j.yexcr.2010.02.032

stage purely theoretical as well as hypothetical, although the notion has some solid base in the findings cited herein. However, although this theory is hypothetical, as of today, it should possibly be testable. Metabolic redox networks set up to depend upon either selenoproteins or non-selenoprotein Cys-dependent orthologue enzymes could perhaps be possible to assess experimentally regarding their speed of recovery from perturbations (dependent upon initial rates and thereby potentially better with selenoproteins), sturdiness in redox equilibria (thus potentially better with Cys-dependent non-selenoproteins) or their dependence upon parameters such as temperature or concentrations (that according to this theoretic framework might affect selenoproteins less than non-selenoproteins). Tracing these types of effects it would perhaps be possible to use radiolabeled redox active substrates or probes with pulse-chase measurements, or some other faster "snap shot" techniques visualizing redox states. Future studies may show whether the notion of a qualitative difference in the role of selenoproteins vs. non-selenoproteins can be validated and whether this would have any useful implications.

A direct consequence of the higher nucleophilicity of Sec as compared to Cys is the extraordinarily efficient targeting of Sec by electrophiles. Already in one of the classic studies determining the pK_a values of Sec and Cys, it was noted that Sec reacts with electrophilic compounds such as chloroacetic acid or chloroacetamide more rapidly than Cys, and even more so with iodoacetic acid or iodoacetamide. Extraordinarily, these electrophiles derivatized Sec highly efficiently at very acidic pH, corresponding to an apparent pK_a of around 2, although Sec based upon the regular acid–base titration would then have been presumed to be protonated and less reactive [18]. The conclusion by the authors was that Sec apparently may react in a haloderivative-specific manner with electrophiles also in its protonated form, due to its high inherent nucleophilicity [18]. This propensity of Sec to efficiently be derivatized by electrophilic compounds may have significant biological relevance. This property is certainly reflected by the fact that mammalian thioredoxin reductase, with a highly accessible Sec residue in the reduced enzyme [35], is easily targeted by a large number of electrophiles—many of them used as anticancer agents [36]. This targeting should clearly be dependent upon the nature of Sec and not only upon the fact that the thioredoxin reductase active site is simply easily accessible. This was demonstrated by the fact that the corresponding enzyme from *D. melanogaster* was not easily derivatized by auranofin, an electrophilic gold compound, but when the redox active dithiol motif at the C-terminus was changed into a Sec-dependent motif, the enzyme indeed became easily inactivated by auranofin thereby presumably reacting rapidly with the nucleophilic Sec residue [21]. The same type of effect was seen when a Sec-containing *Sel-tag*, used as a handle for labeling of proteins with electrophilic probes, was compared to different Cys-containing counterparts; only the Sec variants were rapidly derivatized due to the inherent high nucleophilicity of Sec [37,38]. It is thus possible that the biological relevance of some of the selenoproteins found in nature specifically utilize the nucleophilicity of Sec, either for reactions with naturally occurring electrophiles, perhaps in some pathways of "redox signaling", or during the formation of catalytic intermediates using nucleophilic substitution reactions, where Cys-containing orthologues would not be able to efficiently mimic such properties even if having lowered pK_a values. However, it should also be noted that all types of enzymatic catalysis are complex events and for thioredoxin reductases, specifically, it has also been proposed that some of its catalyzed reactions may be Sec-involving reactions while others may be non-Sec involving, and that the properties of substrate/product leaving groups would also be of major importance [39,40]. Nonetheless, it may still hold true that the significantly higher nucleophilicity of Sec as compared to Cys could be a major feature telling selenoproteins apart from their non-selenoprotein orthologues, both when it comes to the mechanistic features of their catalytic cycles and their biological roles.

Redox potential in relation to nucleophilicity

The redox potential of a redox active protein determines its capacity to propel reducing reactions in relation to catalyzing the reverse oxidizing reactions, with a lower (more negative) redox potential resulting in more reduced over oxidized products at equilibrium state. With all members of the thioredoxin-fold superfamily of proteins that share a redox active –CXXC– motif, these still display highly varying redox potentials, ranging from −270 mV for the strongly reducing *E. coli* thioredoxin to −124 mV for oxidizing DsbA [41]. This vividly displays how purely Cys-dependent redox active proteins may still obtain highly divergent redox potentials. Their redox potential will depend both upon the actual pK_a values of their active site Cys residue(s), guided by the active site microenvironment (see discussion above), as well as other features of both the active site itself and the overall thermodynamic features of the protein. Changing the Cys residue(s) in the active site of a Cys-dependent thioredoxin-fold protein to Sec can yield highly interesting effects on the redox potential. Although it is technically difficult to achieve this type of study, it has been done for recombinant *E. coli* thioredoxin using selenium incorporation through the Cys anabolic pathways using a Cys auxotrophic host strain [42], as well as using synthesis of Sec-substituted glutaredoxins of *E. coli* by chemical means [43,44]. When comparing the Cys-to-Sec-substituted variants of these proteins, it was found that the redox potential had been lowered compared to the native Cys-containing proteins [42,43] and that dithiol/disulfide exchange activities were indeed increased [43,44]. The question was whether the catalytic activities were increased because of the lowered redox potential as such (and why this became lowered), or whether other features of these selenium substituted proteins were more important. The studies of Cys-to-Sec-substituted variants of glutaredoxin 3 from *E. coli* indeed strongly suggested that the most important feature explaining the increased activity of these proteins was the higher nucleophilicity of Sec as compared to Cys, rather than Sec being more active as a central atom or as leaving group during the catalysis. This was reflected by highly increased rate constants in the "reverse" reaction (i.e. reducing the active site disulfide of thioredoxin) compared to the forward reaction (being reduced by thioredoxin), with the authors commenting their study as follows: "Significantly, the effects of Sec on the reaction kinetics suggest that the difference in nucleophilicity between selenolate and thiolate groups could provide the bulk of the rate enhancement observed in many selenoenzymes." [43]. Thus, while selenoproteins may gain lower redox potentials than their Cys-containing non-selenoprotein counterparts, their generally higher reactivity and increased *initial rates* (rather than the lowered redox potentials

Please cite this article as: E.S.J. Arnér, Selenoproteins—What unique properties can arise with selenocysteine in place of cysteine? Exp. Cell. Res. (2010), doi:10.1016/j.yexcr.2010.02.032

as such) may likely be explained by, or at least certainly involving, the higher nucleophilicity of Sec compared to Cys.

It is a well known fact that selenoproteins changed into Sec-to-Cys substituted variants typically lose activity, which was elsewhere reviewed in more detail [45]. It is hard to draw strong conclusions from this fact, since Cys-dependent non-selenoproteins may have "activated" Cys residues yielding a higher turnover (see above), while selenoproteins may not necessarily involve such activation mechanisms and thereby the activity will be lost when the Sec residue is exchanged for Cys. However, some interesting observations can still be made, such as noting a complete loss of peroxidase activity but "only" a 100-fold loss in disulfide reductase activity in a Sec-to-Cys substituted thioredoxin reductase [46]. In studies of Sec-to-Cys substituted formate dehydrogenase H of *E. coli*, the maximal turnover of the mutant enzyme became significantly diminished (about 300-fold), mainly due to the rate of formate oxidation in the catalytic cycle being lowered by about three orders of magnitude [47]. Although the exact role of Sec in formate dehydrogenase catalysis is yet unclear, with the selenium atom coordinating a molybden atom, the crystal structure of the enzyme has suggested the involvement of Sec in a proton transfer reaction [48]. Another structure of a [NiFeSe] hydrogenase also suggested Sec involvement in proton transfer reactions, in this case coupled with heterolytic cleavage of hydrogen [49]. Since Sec is typically worse as a base than Cys, it is not evident how Sec would facilitate proton transfer reactions unless the *release* of the proton from Sec would be the most important aspect in this role. Interestingly, however, the type of proton transfer reaction in hydrogenases may potentially also be favored by the higher nucleophilicity of selenium as compared to sulfur, at least when the reaction is considered as a nucleophilic addition reaction thus explaining why Sec-substituted Cys-dependent hydrogenases may gain increased activities [50].

One- vs. two-electron transfer reactions

Although Sec-involving redox couples typically have much lower (more reducing) redox potential than their Cys-involving counterparts and selenium is more nucleophilic than sulfur, additional qualitative differences can also exist in reactions catalyzed by Sec compared to Cys. This includes the capacity of Sec to catalyze one-electron reactions, as well as two-electron reactions, much more efficiently than Cys [51,52]. Although radical-based (or one-electron transfer) chemistry has not yet been proposed to form a firm basis of any natural selenoprotein reaction mechanism, some one-electron transfer reactions can be catalyzed by the mammalian selenoprotein thioredoxin reductase. This includes one-electron reduction of ascorbyl radicals [53], juglone and other quinones [54], some nitroaromatic compounds [55] and a redox cycling with dinitrohalobenzenes producing superoxide [56]. However, in all of these cases it is not yet clear if the Sec residue of the enzyme participated in one-electron transfer reactions, or whether the FAD moiety of the enzyme was solely catalyzing these reactions *via* flavin semiquinone formation. However, future studies may possibly reveal whether the Sec residue of any selenoprotein indeed could be involved in catalyzing one-electron transfers during any physiological processes; in terms of chemistry this amino acid is at least prone to this effect.

Conclusions

The enigmas of selenoproteins are many, including their unique pathways of synthesis, their roles in nature, their catalytic properties and their curious appearance and expression patterns throughout evolution. Is there really anything truly unique in terms of the properties arising from that single selenium atom, as found in a selenoprotein, as compared to the sulfur of its non-selenoprotein Cys orthologue? It would seem so, but what is it? Herein we have focused upon the extraordinarily high nucleophilicity of Sec as one potential unique feature found in selenoproteins and, potentially, also the facile catalysis of one-electron transfer reactions by Sec. Future studies are needed to ascertain the biological importance of these features. The selenium and selenoprotein research field, initiated by Berzelius about 200 years ago, has evidently only just begun.

Acknowledgments

The author wishes to warmly acknowledge all excellent coworkers and colleagues in the selenoprotein field and is furthermore thankful for financial support from Karolinska Institutet, the Swedish Research Council (Medicine), the Swedish Cancer Society, Vinnova and the Swedish Foundation for Strategic Research.

REFERENCES

[1] S. Yoshizawa, A. Böck, The many levels of control on bacterial selenoprotein synthesis, Biochim. Biophys. Acta 1790 (2009) 1404–1414.
[2] C. Allmang, L. Wurth, A. Krol, The selenium to selenoprotein pathway in eukaryotes: more molecular partners than anticipated, Biochim. Biophys. Acta 1790 (2009) 1415–1423.
[3] M.J. Berry, G.W. Martin III, R. Tujebajeva, E. Grundner-Culemann, J.B. Mansell, N. Morozova, J.W. Harney, Selenocysteine insertion sequence element characterization and selenoprotein expression, Methods Enzymol. 347 (2002) 17–24.
[4] D.L. Hatfield, B.A. Carlson, X.M. Xu, H. Mix, V.N. Gladyshev, Selenocysteine incorporation machinery and the role of selenoproteins in development and health, Prog. Nucleic Acid Res. Mol. Biol. 81 (2006) 97–142.
[5] D.L. Hatfield, V.N. Gladyshev, How selenium has altered our understanding of the genetic code, Mol. Cell. Biol. 22 (2002) 3565–3576.
[6] D.M. Driscoll, P.R. Copeland, Mechanism and regulation of selenoprotein synthesis, Annu. Rev. Nutr. (2003).
[7] G.V. Kryukov, S. Castellano, S.V. Novoselov, A.V. Lobanov, O. Zehtab, R. Guigo, V.N. Gladyshev, Characterization of mammalian selenoproteomes, Science 300 (2003) 1439–1443.
[8] G.V. Kryukov, V.N. Gladyshev, The prokaryotic selenoproteome, EMBO Rep. 5 (2004) 538–543.
[9] Y. Zhang, D.E. Fomenko, V.N. Gladyshev, The microbial selenoproteome of the Sargasso Sea, Genome Biol. 6 (2005) R37.
[10] Y. Zhang, V.N. Gladyshev, Trends in selenium utilization in marine microbial world revealed through the analysis of the Global Ocean Sampling (GOS) project, PLoS Genet. 4 (2008) e1000095.
[11] K. Taskov, C. Chapple, G.V. Kryukov, S. Castellano, A.V. Lobanov, K.V. Korotkov, R. Guigo, V.N. Gladyshev, Nematode selenoproteome: the use of the selenocysteine insertion system

Please cite this article as: E.S.J. Arnér, Selenoproteins—What unique properties can arise with selenocysteine in place of cysteine? Exp. Cell. Res. (2010), doi:10.1016/j.yexcr.2010.02.032

to decode one codon in an animal genome? Nucleic Acids Res. 33 (2005) 2227–2238.
[12] Y. Zhang, H. Romero, G. Salinas, V.N. Gladyshev, Dynamic evolution of selenocysteine utilization in bacteria: a balance between selenoprotein loss and evolution of selenocysteine from redox active cysteine residues, Genome Biol. 7 (2006) R94.
[13] D.E. Fomenko, S.M. Marino, V.N. Gladyshev, Functional diversity of cysteine residues in proteins and unique features of catalytic redox-active cysteines in thiol oxidoreductases, Mol. Cells 26 (2008) 228–235.
[14] A.V. Lobanov, D.L. Hatfield, V.N. Gladyshev, Eukaryotic selenoproteins and selenoproteomes, Biochim. Biophys. Acta 1790 (2009) 1424–1428.
[15] S. Castellano, A.M. Andres, E. Bosch, M. Bayes, R. Guigo, A.G. Clark, Low exchangeability of selenocysteine, the 21st amino acid, in vertebrate proteins, Mol. Biol. Evol. (2009).
[16] S. Castellano, On the unique function of selenocysteine—insights from the evolution of selenoproteins, Biochim. Biophys. Acta (2009).
[17] L.A. Wessjohann, A. Schneider, M. Abbas, W. Brandt, Selenium in chemistry and biochemistry in comparison to sulfur, Biol. Chem. 388 (2007) 997–1006.
[18] R.E. Huber, R.S. Criddle, Comparison of the chemical properties of selenocysteine and selenocystine with their sulfur analogs, Arch. Biochem. Biophys. 122 (1967) 164–173.
[19] H.J. Dyson, M.F. Jeng, L.L. Tennant, I. Slaby, M. Lindell, D.S. Cui, S. Kuprin, A. Holmgren, Effects of buried charged groups on cysteine thiol ionization and reactivity in *Escherichia coli* thioredoxin: structural and functional characterization of mutants of Asp 26 and Lys 57, Biochemistry 36 (1997) 2622–2636.
[20] S.M. Kanzok, A. Fechner, H. Bauer, J.K. Ulschmid, H.M. Muller, J. Botella-Munoz, S. Schneuwly, R. Schirmer, K. Becker, Substitution of the thioredoxin system for glutathione reductase in *Drosophila melanogaster*, Science 291 (2001) 643–646.
[21] S. Gromer, L. Johansson, H. Bauer, L.D. Arscott, S. Rauch, D.P. Ballou, C.H. Williams Jr., R.H. Schirmer, E.S.J. Arnér, Active sites of thioredoxin reductases—why selenoproteins? Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 12618–12623.
[22] L. Johansson, D. Arscott, D.P. Ballou, C.H. Williams Jr., E.S.J. Arnér, Studies of an active site mutant of the selenoprotein thioredoxin reductase: the Ser-Cys-Cys-Ser motif of the insect orhologue is not sufficient to replace the Cys-Sec dyad in the mammalian enzyme, Free Radic. Biol. Med. 41 (2006) 649–656.
[23] S. Toppo, L. Flohe, F. Ursini, S. Vanin, M. Maiorino, Catalytic mechanisms and specificities of glutathione peroxidases: variations of a basic scheme, Biochim. Biophys. Acta 1790 (2009) 1486–1500.
[24] G. Bulaj, T. Kortemme, D.P. Goldenberg, Ionization–reactivity relationships for cysteine thiols in polypeptides, Biochemistry 37 (1998) 8965–8972.
[25] A.V. Lobanov, D.E. Fomenko, Y. Zhang, A. Sengupta, D.L. Hatfield, V.N. Gladyshev, Evolutionary dynamics of eukaryotic selenoproteomes: large selenoproteomes may associate with aquatic life and small with terrestrial life, Genome Biol. 8 (2007) R198.
[26] J.F. Bunnett, Nucleophilic reactivity, Ann. Rev. Phys. Chem. 14 (1963) 271–290.
[27] A. Cedillo, R. Contreras, M. Galván, A. Aizman, J. Andrés, V.S. Safont, Nucleophilicity index from perturbed electrostatic potentials, J. Phys. Chem. A 111 (2007) 2442–2447.
[28] J.I. Edwards, R.G. Pearson, The factors determining nucleophilic reactivities, J. Am. Chem. Soc. 84 (1962) 16–24.
[29] P. Jaramillo, P. Pérez, R. Contreras, W. Tiznado, P. Fuentealba, Definition of a nucleophilicity scale, J. Phys. Chem. A 110 (2006) 8181–8187.
[30] P. Jaramillo, P. Pérez, P. Fuentealba, Relationship between basicity and nucleophilicity, J. Phys. Org. Chem. 20 (2007) 1050–1057.
[31] T.B. Phan, M. Breugst, H. Mayr, Towards a general scale of nucleophilicity? Angew. Chem. Int. Ed. 45 (2006) 3869–3874.
[32] M. Wada, S.-I. Nobuki, Y. Tenkyuu, S. Natsume, M. Asahara, T. Erabi, Bis(2,6-dimethoxyphenyl) sulfide, selenide and telluride, and their derivatives, J. Organomet. Chem. 580 (1999) 282–289.
[33] J.C. Pleasants, W. Guo, D.L. Rabenstein, A comparative study of the kinetics of selenol/diselenide and thiol/disulfide exchange reactions, J. Am. Chem. Soc. 111 (1989) 6553–6558.
[34] G. Wächtershäuser, Groundworks for an evolutionary biochemistry: the iron–sulphur world, Prog. Biophys. molec. Biol. 58 (1992) 85–201.
[35] Q. Cheng, T. Sandalova, Y. Lindqvist, E.S.J. Arnér, Crystal structure and catalysis of the selenoprotein thioredoxin reductase 1, J. Biol. Chem. 284 (2009) 3998–4008.
[36] E.S.J. Arnér, Focus on mammalian thioredoxin reductases—important selenoproteins with versatile functions, Biochim. Biophys. Acta 1790 (2009) 495–526.
[37] Q. Cheng, S. Stone-Elander, E.S.J. Arnér, Tagging recombinant proteins with a Sel-tag for purification, labeling with electrophilic compounds or radiolabeling with carbon-11, Nat. Protoc. 1 (2006) 604–613.
[38] Q. Cheng, L. Johansson, J.-O. Thorell, A. Fredriksson, E. Samén, S. Stone-Elander, E.S.J. Arnér, Selenolthiol and dithiol C-terminal tetrapeptide motifs for one-step purification and labeling of recombinant proteins produced in *E. coli*, ChemBioChem 7 (2006) 1976–1981.
[39] A.P. Lothrop, E.L. Ruggles, R.J. Hondal, No selenium required: reactions catalyzed by mammalian thioredoxin reductase that are independent of a selenocysteine residue, Biochemistry 48 (2009) 6213–6223.
[40] R.J. Hondal, Using chemical approaches to study selenoproteins-focus on thioredoxin reductases, Biochim. Biophys. Acta 1790 (2009) 1501–1512.
[41] F. Åslund, K.D. Berndt, A. Holmgren, Redox potentials of glutaredoxins and other thiol-disulfide oxidoreductases of the thioredoxin superfamily determined by direct protein–protein redox equilibria, J. Biol. Chem. 272 (1997) 30780–30786.
[42] S. Müller, H. Senn, B. Gsell, W. Vetter, C. Baron, A. Böck, The formation of diselenide bridges in proteins by incorporation of selenocysteine residues: biosynthesis and characterization of (Se)2-thioredoxin, Biochemistry 33 (1994) 3404–3412.
[43] N. Metanis, E. Keinan, P.E. Dawson, Synthetic seleno-glutaredoxin 3 analogues are highly reducing oxidoreductases with enhanced catalytic efficiency, J. Am. Chem. Soc. 128 (2006) 16684–16691.
[44] G. Casi, G. Roelfes, D. Hilvert, Selenoglutaredoxin as a glutathione peroxidase mimic, ChemBioChem 9 (2008) 1623–1631.
[45] L. Johansson, G. Gafvelin, E.S.J. Arnér, Selenocysteine in proteins—properties and biotechnological use, Biochim. Biophys. Acta 1726 (2005) 1–13.
[46] L. Zhong, A. Holmgren, Essential role of selenium in the catalytic activities of mammalian thioredoxin reductase revealed by characterization of recombinant enzymes with selenocysteine mutations, J. Biol. Chem. 275 (2000) 18121–18128.
[47] M.J. Axley, A. Böck, T.C. Stadtman, Catalytic properties of an *Escherichia coli* formate dehydrogenase mutant in which sulfur replaces selenium, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 8450–8454.
[48] J.C. Boyington, V.N. Gladyshev, S.V. Khangulov, T.C. Stadtman, P.D. Sun, Crystal structure of formate dehydrogenase H: catalysis involving Mo, molybdopterin, selenocysteine, and an Fe4S4 cluster, Science 275 (1997) 1305–1308.
[49] E. Garcin, X. Vernede, E.C. Hatchikian, A. Volbeda, M. Frey, J.C. Fontecilla-Camps, The crystal structure of a reduced [NiFeSe] hydrogenase provides an image of the activated catalytic center, Structure 7 (1999) 557–566.
[50] S. Niu, M.B. Hall, Modeling the active sites in metalloenzymes 5. The heterolytic bond cleavage of H2 in the [NiFe] hydrogenase of *Desulfovibrio gigas* by a nucleophilic addition mechanism, Inorg. Chem. 40 (2001) 6201–6203.

Please cite this article as: E.S.J. Arnér, Selenoproteins—What unique properties can arise with selenocysteine in place of cysteine? Exp. Cell. Res. (2010), doi:10.1016/j.yexcr.2010.02.032

[51] T. Nauser, S. Dockheer, R. Kissner, W.H. Koppenol, Catalysis of electron transfer by selenocysteine, Biochemistry 45 (2006) 6038–6043.
[52] D. Steinmann, T. Nauser, J. Beld, M. Tanner, D. Gunther, P.L. Bounds, W.H. Koppenol, Kinetics of tyrosyl radical reduction by selenocysteine, Biochemistry 47 (2008) 9602–9607.
[53] J.M. May, C.E. Cobb, S. Mendiratta, K.E. Hill, R.F. Burk, Reduction of the ascorbyl free radical to ascorbate by thioredoxin reductase, J. Biol. Chem. 273 (1998) 23039–23045.
[54] N. Cenas, H. Nivinskas, Z. Anusevicius, J. Sarlauskas, F. Lederer, E.S.J. Arnér, Interactions of quinones with thioredoxin reductase—a challenge to the antioxidant role of the mammalian selenoprotein, J. Biol. Chem. 279 (2004) 2583–2592.
[55] N. Cenas, S. Prast, H. Nivinskas, J. Sarlauskas, E.S.J. Arnér, Interactions of nitroaromatic compounds with the mammalian selenoprotein thioredoxin reductase and the relation to induction of apoptosis in human cancer cells, J. Biol. Chem. 281 (2006) 5593–5603.
[56] J. Nordberg, L. Zhong, A. Holmgren, E.S.J. Arnér, Mammalian thioredoxin reductase is irreversibly inhibited by dinitrohalobenzenes by alkylation of both the redox active selenocysteine and its neighboring cysteine residue, J. Biol. Chem. 273 (1998) 10835–10842.

Please cite this article as: E.S.J. Arnér, Selenoproteins—What unique properties can arise with selenocysteine in place of cysteine? Exp. Cell. Res. (2010), doi:10.1016/j.yexcr.2010.02.032