Published in final edited form as:

Arch Biochem Biophys. 2014 April 15; 548: 60–65. doi:10.1016/j.abb.2014.02.001.

Site-specific insertion of selenium into the redox-active disulfide of the flavoprotein Augmenter of Liver Regeneration

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Abstract

Augmenter of liver regeneration (sfALR) is a small disulfide-bridged homodimeric flavoprotein with sulfhydryl oxidase activity. Here, we investigate the catalytic and spectroscopic consequences of selectively replacing C145 by a selenocysteine to complement earlier studies in which random substitution of ~90% of the 6 cysteine residues per sfALR monomer was achieved growing *Escherichia coli* on selenite. A selenocysteine insertion sequence (SECIS) element was installed within the gene for human sfALR. SecALR2 showed a spectrum comparable to that of wild-type sfALR. The catalytic efficiency of SecALR2 towards dithiothreitol was 6.8-fold lower than a corresponding construct in which position 145 was returned to a cysteine residue while retaining the additional mutations introduced with the SECIS element. This all-cysteine control enzyme formed a mixed disulfide between C142 and β -mercaptoethanol releasing C145 to form a thiolate-flavin charge transfer absorbance band at ~530 nm. In contrast, SecALR2 showed a prominent long-wavelength absorbance at 585 nm consistent with the expectation that a selenolate would be a better charge-transfer donor to the isoalloxazine ring. These data show the robustness of the ALR protein fold towards the multiple mutations required to insert the SECIS element and provide the first example of a selenolate to flavin charge-transfer complex.

Keywords

Augmenter of Liver Regeneration; Charge-transfer; Disulfide; Flavin; Selenium; Selenocysteine insertion sequence

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Introduction

Augmenter of liver regeneration (ALR¹) is a FAD-linked flavoprotein that functions as a sulfhydryl oxidase and a cytokine [1–6]. It exists in two spliced forms: the long-form ALR (lfALR) is involved in the disulfide bond formation pathway in the mitochondrial intermembrane space [6–8], while the short-form (sfALR) is implicated in liver regeneration [4, 5]. Both forms share a core flavin-binding domain, which contains a redox-active CXXC motif proximal to the isoalloxazine ring [2, 3, 9, 10]. The first of these cysteine residues (C142) faces the solvent and participates in disulfide exchange reactions with thiol substrates (Fig. 1). Its redox partner, C145, communicates directly with the flavin; its sulfur atom is ~3.2 Å from the C4a position of the isoalloxazine ring [2, 9, 10]. A schematic representation of the flow of reducing equivalents in sfALR from thiol substrate to terminal oxidant is shown in Fig. 1 [3, 6, 10, 11].

We recently demonstrated that it is possible to randomly substitute sulfur for selenium in sfALR [12]. In that study, we characterized this selenium-containing sfALR and demonstrated that the structure of sfALR was essentially unaltered by the substitution of 90% of the sulfur atoms by selenium. Intriguingly, a new long-wavelength intermediate species was observed in the UV-VIS spectrum when DTT was added to the selenium-containing protein. Such a spectral feature might represent a charge-transfer interaction between a selenolate (E-Se[−]) at position 145 and the flavin ring [12]. However the extensive substitution of sulfur for selenium in all the cysteines and methionines of sfALR made this assignment tentative. Here we report investigations of this long-wavelength feature by site-specific incorporation of selenocysteine (Sec, U) at position 145 of sfALR (Fig. 1). This C145U mutation allowed us to differentiate the spectral features that arise from the individual contribution of a CXXU motif from those made by the predominant population of UXXU generated when sulfur is indiscriminately substituted for selenium in sfALR [12]. In addition, the C145U mutation allowed the catalytic consequences of substitution of a selenium at the charge-transfer thiol to be investigated without confounding additional insertion of selenocysteine and selenomethionine associated with overall substitution of sulfur to selenium.

In order to insert Sec at position 145, we employed the native genetic incorporation machinery of *Escherichia coli*, which relies on the authentication of the UGA codon as Sec-encoding rather than as a termination codon (Fig. 2A) [13, 14]. Here, a special hairpin RNA loop, termed the selenocysteine insertion sequence (SECIS), recruits the Sec-specific elongation factor (SelB) and tRNA^{Sec} to mediate the Sec insertion [15]. To communicate with SelB, the SECIS stem-loop must be placed in the immediate vicinity of the Sec codon (UGA) and carry specific nucleotides in its upper stem [15–17]. To accommodate the requirements associated with the SECIS element, genetic incorporation of Sec into sfALR in *E. coli* required introducing several mutations to the primary amino acid sequence of sfALR.

¹Abbreviations used: ALR, augmenter of liver regeneration; sfALR, short-form augmenter of liver regeneration; lfALR, long-form augmenter of liver regeneration; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; IPTG, isopropyl β-D-thiogalactopyranoside; βME, β-mercaptoethanol; SECIS, selenocysteine insertion sequence.

Here we report the successful expression and purification of a C145U mutant of sfALR. While the protein was generated at modest levels, it proved stable and enzymatically active with the model substrate dithiothreitol (DTT). Spectrophotometric experiments provided clear evidence that the long wavelength band, previously observed by random selenium incorporation, was a charge-transfer interaction between U145 and the oxidized flavin prosthetic group of ALR.

Materials and methods

Reagents

Chemicals and reagents were supplied by Sigma-Aldrich, Acros Organics, Fisher Scientific, GE Healthcare Bio-Sciences and GoldBio as before [11, 12]. Enzymes used for molecular biology were acquired from New England Biolabs. The pSUABC plasmid was generously provided by Professor Arnér from the Karolinska Institutet [18].

Expression plasmids

A human sfALR gene (GenBank AAH28348.2) was codon optimized for expression in *E. coli* and the gene synthesized by DNA2.0. The gene (SecALR1) was provided in the expression vector pJexpress414 fused to a C-terminal His₆ affinity tag. The following primers (obtained from IDT or Sigma) were used for subcloning the SecALR1 gene into the pTrcHisA and pET-28a vectors: (NheI site) 5'-AAA TTT GCT AGC ATG CGC ACC CAA CAA -3' and (HindIII site) 5'-ACC GAA AAG CTT AAT CGC AGG AAC CG -3'. SecALR2 was generated using site-directed mutagenesis with SecALR1 as the template. The following mutagenesis primers were used for converting SecALR1 to SecALR2: 5'-GAC CTG GTT GCA CGC ACC GGA ACC AC -3' and 5'-GTG GTT CCG GTC CGT GCA ACC AGG TC -3'. To generate the SecALR2 U145C the following mutagenesis primers were used: 5'-CCG TGT GAG GAG TGC GCT GAA GAC CTG G -3' and 5'-CCA GGT CTT CAG CGC ACT CCT CAC ACG G -3'. The rationale for the design of both SecALR constructs is presented in the Results section. Additionally, the programs SECISDesign [19] and RNAfold [20] aided in SECIS element construction. The amino acid and nucleotide sequences of all constructs are provided in Supplementary Figures S1 and S2.

Expression and purification of SecALR1 and SecALR2

For protein expression, the appropriate plasmid was co-transformed into *E. coli* BL21(DE3), with the pSUABC plasmid expressing *E. coli* Sela, SelB, and SelC under the control of their endogenous promoters in order to increase Sec incorporation [18]. Cells were grown in 1.2 L aliquots of Terrific Broth that were inoculated with 10 mL overnight cultures, with 100 µg/mL ampicillin (to maintain the pJexpress414 vector) and 34 µg/mL chloramphenicol (to maintain the pSUABC vector). Cells were grown at 37 °C to an OD₆₀₀ of ~2.2 and then the temperature was reduced to 18 °C approximately 30 min prior to induction. Protein expression was induced with 1 mM IPTG added with 5 µM Na₂SeO₃ and 100 µg/mL L-cysteine. The cells were grown for 24 hours, harvested at 5000g for 10 min and re-suspended in 50 mM potassium phosphate (pH 7.5) supplemented with 500 mM NaCl (binding buffer) before freezing. Purification was as previously reported [12] with the

exception that approximately 100 μ L of Ni-IDA (Invitrogen) resin was used per 3 L of culture medium to minimize non-specific binding. The resin was washed with 10 mL of binding buffer, followed by five 1 mL volumes of binding buffer containing 20 mM imidazole, four 1 mL volumes containing 200 mM imidazole, two 1 mL volumes containing 500 mM imidazole and two 1 mL volumes containing 1 M imidazole. Yellow fractions were combined and dialyzed against 50 mM potassium phosphate, pH 7.5, containing 1 mM EDTA. Following dialysis, the sample was analyzed for purity by reducing and non-reducing 16% tris-tricine SDS-PAGE. SecALR2 showed a final purity, as determined by SDS-PAGE analysis, of about 90%. The minor contaminant was chloramphenicol acetyltransferase, a 25 kDa protein that binds to IMAC resins. Chloramphenicol acetyltransferase does not bind cofactors and has no competing activity. Expression and purification of the all-cysteine control, SecALR2 U145C, in the pJexpress414 vector was similar to that of the wild-type protein, as previously reported [9]. The all-cysteine control was obtained in >95% purity. All enzymes, like the wild-type protein, were stable at 4 °C in 50 mM potassium phosphate (pH 7.5) containing 1 mM EDTA.

Spectral analysis

UV–Vis spectra were recorded using an HP8453 diode-array spectrophotometer (Hewlett-Packard, Palo Alto, CA). Where necessary, turbidity correction software supplied with the diode-array spectrophotometer was used to correct for slight light-scattering. Spectrophotometric experiments with β -mercaptoethanol (β ME) utilized ~30 μ M ALR with 20 mM thiol in either 50 mM phosphate buffer, pH 7.5, or 20 mM Tris, pH 9.0. Both buffers contained 1 mM EDTA.

Turnover with DTT

The enzymatic activity was measured using a Clark-type oxygen electrode as described previously [3]. Dithiothreitol (DTT) was freshly prepared in 50 mM potassium phosphate, pH 7.5, containing 1 mM EDTA and standardized prior to use.

Results

Insertion of a SECIS element into sfALR

Incorporation of Sec at a unique protein location can be carried out by chemical synthesis, chemical biology based-methods, or by heterologous expression [21]. While the diversity of Sec incorporation methods is expanding [22], the native selenium incorporation machinery of *E. coli* is still one of the most commonly employed for the heterologous expression of selenoproteins. To insert Sec into sfALR, we designed a SECIS element based on that from the *E. coli* formate dehydrogenase H (*fdhF*) gene (Fig. 2B, panel 1). This element has been extensively characterized and its sequence requirements for effective communication with SelB have been identified. Thus the conserved upper loop of the SECIS element must be spaced 11 nucleotides after the UGA codon (see Fig. 2B, panel 1, where U is nucleotide number 1) [23]. The upper stem contains the nucleotides necessary for recognition and binding of SelB: U17, U18, G23, and U24 [17, 23–25]. While the nucleotides at the lower stem need not be base-paired, the stem-loop structure should be thermodynamically stable [17].

Thus, to adopt *fdhF* SECIS for Sec insertion in ALR, it is necessary to ensure that the stem-loop structure is a stable, preferred, secondary structure and that the conserved upper loop nucleotides communicating with SelB are in place. An additional consideration is that since bacterial SECIS elements occur within the protein coding region, additional changes to the sequence of the wild-type sfALR are unavoidable. We sought to minimize the number of these additional mutations to increase the chances of obtaining a folded, stable, protein product containing a Sec at position 145. Two constructs were created; one with a substantially-modified *fdhF* SECIS sequence called SecALR1, and a version with a more conserved *fdhF*-like SECIS sequence named SecALR2. We briefly explain the reasoning behind these designs in the next paragraph.

In both SecALR designs, a G22C mutation in the *fdhF* SECIS element was introduced to retain Arg152 (Fig. 3A) because this residue contacts the carboxylate moieties of Asp148, within the same helix, and of Glu176 on an adjacent helix in sfALR and thus is part of the architecture that dictates the active site alignment (Fig. 3B). In *fdhF*, the guanine nucleotide at position 22 was shown to be essential for efficient read-through of the UGA codon [23]. However, a cytosine at that location was tolerated by cytochrome P450cam [26]. This demonstrates the challenge of designing successful bacterial SECIS elements: the vast majority of studies focus on formate dehydrogenase H, so it is difficult to differentiate between those requirements that are inherent to SECIS fold stability and SelB binding, and those constraints that are specific to formate dehydrogenase H structure and stability. The success of experiments that introduce SECIS elements in the coding regions of target proteins depends on the impact of the corresponding mutations on protein stability and folding, together with effects on SECIS element stability and, possibly, factors associated with translation on the ribosome [26–32].

In addition to the G22 to C22 switch, we also introduced an inversion of the nucleotide pair C20/G27 to G20/C27 and G19/C28 to C19/G28 into SecALR1 (see circled nucleotides in Fig. 2B). This mutation maintained a positively charged surface residue at this position with a K151R mutation (Fig. 3B). The inversion of the nucleotides C20/G27 to G20/C27 in the SECIS element was previously reported to decreased protein expression by 70% [23]. Compared to these mutations, the inversion of G19/C28 to C19/G28 showed a lesser impact on formate dehydrogenase H expression [16, 23, 25].

Overall, the SecALR1 and -2 designs introduce 5 amino acid substitutions compared to the sequence of wild-type sfALR. The placement of these residues is highlighted in red in Figure 3A. Two non-conserved, non-essential, and surface-accessible cysteine residues (C145 and C165; Fig. 3A) in the human sfALR are routinely mutated to alanine residues in *in vitro* work to avoid disulfide-mediated aggregation [3, 6, 7, 9–12]. In SecALR1 these non-conserved cysteines were substituted with the corresponding amino acid residues found in the rat sfALR sequence (C154D and C165S). In the SecALR2 SECIS design C154 and C165 become histidine and serine respectively. Of the remaining 3 mutations, both SecALRs share the C145U and R150V mutations. Finally, K151 in SecALR1 and -2 is replaced by arginine and alanine respectively. With the exception of C145U the additional mutations introduced for the SECIS element appear located at solvent-exposed positions and would not be expected to impact catalysis directly (Fig. 3).

Expression and purification of SecALR

The expression levels and ease of purification of the two SecALR constructs were tested using different vectors, promoters and affinity tag locations. The expression of SecALR was initially followed using small-scale expression and purification tests. In order to increase selenium incorporation efficiency, a second vector, pSUABC, was also transfected into the BL21(DE3) cell line. pSUABC allows for the co-expression of SelA (selenocysteine synthase), SelB (elongation factor SelB), and SelC (tRNA^{Sec}) [18]. The use of this additional vector is reported to increase translation efficiency by up to 5-fold [33]. We initially tried an N-terminal His₆ affinity tag (pTrcHisA) because this vector routinely affords yields of about 20 mg/L of sfALR [3]. We also tested a pET-28a vector with an N-terminal His₆ affinity tag. However placement of the His-tag at the N-terminus proved ultimately impractical because much of the protein that was bound to the affinity resin was truncated following misrecognition of the UGA codon as a termination signal. In contrast, premature termination of a SecALR carrying a C-terminal His tag would result in a failure of the truncated protein to bind to the nickel resin and hence allow ready purification of the full-length selenoprotein product.

We found that while both SecALR proteins were expressed at a similar level, the majority of SecALR1 was in the insoluble fraction (not shown). Because refolding of the protein after exposure to denaturants proved to be problematic, we focused instead on characterizing SecALR2. SecALR2 was workably expressed at 0.7 mg/L from a pJexpress414 vector with the C-terminal His₆ tag construct and purified with little to no aggregation. We then constructed the corresponding all-cysteine control protein, SecALR2 U145C, in which a cysteine was restored at position 145 while keeping the other four mutations in place. A comparison between SecALR2 U145C and SecALR2 will then highlight the impact of the insertion of Sec at position 145. As might be expected, this quadruple all-cysteine mutant expressed with a much better yield (8.4 mg/L) than its selenoprotein version.

Fig. 4 shows that the UV-Vis spectrum of SecALR2 is similar to that of sfALR. The inset shows SDS-PAGE analysis of SecALR2 run under reducing and non-reducing conditions. We observed bands at the expected molecular weight of the monomer and covalent disulfide-bridged dimer (15.8 kDa and 31.6 kDa, respectively) for SecALR2. Premature termination at the UGA codon would generate a truncated product at 7.7 kDa and, if disulfide-paired with a full-length subunit, would yield a heterodimer of 23.5 kDa. These species were not evident in our purified fractions, but the truncated protein was clearly visualized in the insoluble fraction (not shown). Hence, we have demonstrated that it is possible to express and purify SecALR2 and that the resulting protein is stable. We next characterized the activity of this selenium-containing protein.

Catalytic activity of SecALR2 and its all-cysteine variant

Table 1 compares the steady state kinetic parameters using DTT for wild-type sfALR with those of the SecALR2 U145C and SecALR2 mutants. The mutations accompanying the introduction of the SECIS element in the all-cysteine control result in a 3.4-fold decline in k_{cat}/K_m with this model substrate. This modest decrease may reflect the impact of the R150V and K151A mutations in the context of a highly charged third helix in the ALR fold

(Fig. 3B). In addition the guanidino moiety of R150 is hydrogen bonded to the backbone amide oxygen from serine 137 in helix 2. Fortunately, the robustness of the ALR fold [3, 9, 11] tolerates these mutations with minimal catalytic impact. The replacement of the charge-transfer cysteine by Sec effects an additional approximate 7-fold lowering of catalytic efficiency stemming from a 2-fold decline in k_{cat} and an approximately 3.5-fold increase in K_m over that of the all-cysteine control. With these data as preliminaries, we then explored spectrophotometric experiments following the flavin chromophore of ALR upon the aerobic addition of DTT and β ME.

Spectral evidence for the formation of a selenolate to oxidized flavin charge-transfer complex

Mixing wild-type sfALR with DTT leads to the rapid formation of a weak wedge-shaped charge-transfer absorbance (followed at 530 nm) that is maximal at 10 ms at mM levels of reductant [11]. In manual mixing experiments this species is obscured by the subsequent development of an intense blue semiquinone absorbance extending to 700 nm when wild-type ALR turns over thiol substrates under aerobic conditions [2, 3, 11, 12]. However, when sfALR in which 90% of the cysteines were randomly substituted by selenium was mixed with DTT under aerobic conditions a new species persisted for seconds before accumulation of the blue semiquinone [12]. This newly-observed species showed a prominent long wavelength absorbance maximal at 590 nm and extending to 800 nm. While this feature resembled a charge-transfer complex the almost complete substitution of both redox-active cysteine residues and the other cysteine and methionine residues by selenium made this assignment tentative. The experiment shown in Fig. 5 provides a definite resolution of this issue. However rather than using DTT, with the consequent confounding rapid generation of the blue semiquinone, we incubated sfALR with β ME. Although this monothiol is a very poor substrate of sfALR, β ME has recently been shown to readily form mixed disulfides with C142 of the oxidase [11]. As a control experiment, Fig. 5 shows addition of 20 mM β ME to the all-cysteine enzyme, SecALR2 U145C, at pH 7.5 and 9.0. At the higher pH value where approximately 25% of the β ME would be deprotonated (pK_a 9.6 [34]), a noticeable charge-transfer band is observed centered at about 530 nm for the U145C enzyme. This feature is qualitatively similar to that observed with wild-type sfALR [11] although it is approximately half the intensity at both pH 7.5 and 9.0. These data are in line with the steady-state kinetics (Table 1) and again suggest a modestly adverse impact accompanying the SECIS mutations introduced in the SecALR2 U145C control. However when these experiments were repeated with the SecALR2 enzyme, the CxxU motif yields a well resolved long-wavelength feature at 585 nm upon the addition of β ME (Fig. 5B). These data clearly show that the 585 nm long-wavelength band observed on reduction of sfALR that was randomly substituted by selenium [12] solely reflects the placement of a selenocysteine at position 145 immediately proximal to the C4a position of the isoalloxazine ring.

Conclusions

Herein we have demonstrated the design, expression, purification and characterization of a single selenocysteine-containing ALR. Substitution of the charge-transfer cysteine of the

proximal redox-active disulfide with a selenocysteine generates an enzyme with a ~7-fold lower catalytic efficiency over its all-cysteine counterpart. The work provides, to the best of our knowledge, the first demonstration of a charge-transfer complex between a selenide and oxidized flavin. It should be noted that in studies of sarcosine oxidase, Jorns and colleagues investigated the spectral effects of replacing the oxygen atom of the ether functional group in methyl sarcosine by sulfur, selenium, and tellurium [35]. They found an approximate 60 nm red shift of the charge-transfer complex on substitution of sulfur by selenium. In our case, the selenium at position 145 would be positioned ~3.2 Å directly above the C4a position of the isoalloxazine ring [12]. The ~55 nm red-shift in the charge-transfer absorbance is certainly consistent with the expectation that the increased electron density and polarizability of selenium over sulfur would make the selenide a better charge-transfer donor [36].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by NIH P30 GM103519 and NSF MCB-1054447 “CAREER: Reactivity of Selenoproteins” (S.R.), GM26643 (C.T.) and 1-T32-GM008550 (S.S.-R.). We acknowledge service from the University of Delaware’s protein characterization core facility. We thank Ms. Brittany Riggio, Yuliya Pepelyayeva, Fei Li and Dr. Shawn Gannon for help during the early phase of this work. Professor Elias Arnér generously provided the pSUABC plasmid used in this work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

Highlights

- Short-form augments liver regeneration (sfALR) is a FAD-linked cytokine
- Selenocysteine was introduced using bacterial selenoprotein synthesis
- The modification in the active site showed ~7-fold lower catalytic efficiency
- Scission of C142-U145 (S-Se bond) yields a U145 to flavin charge-transfer complex
- This is the first spectrum of a selenolate to flavin charge-transfer complex

Fig. 1.

Flow of reducing equivalents in the active site of mammalian ALR. The isoalloxazine ring is bound at the mouth of a bundle of four helices. C142 forms mixed-disulfide intermediates with thiol substrates. Upon scission of the proximal disulfide, C145 interacts with the oxidized flavin via a charge-transfer complex. The structure shown is that of the human sfALR (PDB 3MBG).

Fig. 2.

Selenocysteine biosynthesis and incorporation in *E. coli* and the SECIS elements used in this study. Panel A diagrams a dedicated machinery for the incorporation of selenocysteine in bacteria; it includes a tRNA specific for selenocysteine, selenocysteine-tRNA^{Sec} (SelC), a selenocysteine synthase (SelA), and an elongation factor (SelB) that recognizes a unique stem-loop RNA structure proximal to the UGA codon. The tRNA^{Sec} is charged with a serine by serine-tRNA synthetase. This is followed by the conversion of seryl-tRNA^{Sec} into selenocysteinyl-tRNA^{Sec} by selenocysteine synthase. The source of selenium is selenophosphate, which is synthesized by selenophosphate synthetase (SelD). SelB binds specifically to selenocysteinyl-tRNA^{Sec} and the loop of the SECIS element. Panels B1–B4 show the SECIS elements used in this study. B1) Represents the SECIS element from *E. coli* formate dehydrogenase H. Bases are numbered starting with the U1 in the bolded UGA codon (bottom left). B2) wild-type sfALR primary sequence oriented in a stem-loop for comparison. B3) SecALR1 nucleotide sequence arranged as before, and B4) the comparable sequence for SecALR2. Bases in circles are different between constructs SecALR1 and SecALR2.

Fig. 3.

Position of mutations in the primary and tertiary structure of sfALR. Panel A highlights the mutations in red font that were introduced into wild-type sfALR to generate SecALR1 and SecALR2. Panel B shows the location of key residues for this work using the human sfALR structure (PDB entry 3MBG). The redox active site disulfide (C142/C145) is in contact with the non-covalently bound FAD cofactor (yellow).

Fig. 4.

UV-VIS spectrum of SecALR2. The absorbance spectrum of SecALR2, as purified, shows a typical flavin envelope when recorded in 50 mM phosphate buffer (pH 7.5), containing 1 mM EDTA (solid line panel A; the dotted line is that of the all-cysteine variant). The inset shows SDS-PAGE analysis of SecALR2. Lane 1, SecALR2 run under reducing conditions; lane 2, protein molecular mass standards (the molecular weights in kDa is noted on the gel); lane 3, SecALR2 run under non-reducing conditions. sfALR is a disulfide-bridged homodimer under native conditions. Hence the expected molecular weights of full-length SecALR2 in pJexpress414 vector are 31.6 kDa under non-reducing conditions and 15.8 kDa following reduction.

Fig. 5.

Formation of a charge-transfer intermediate in SecALR2 C145U and SecALR2 following the addition of β ME. Panel A shows the spectrum of 30 μ M of the all-cysteine control construct at pH 7.5 (solid line). The spectrum was then recorded 10 s after the addition of 20 mM β ME (dotted line). A separate experiment at pH 9.0 yielded the dashed line after β ME addition (dashed line).

Panel B is the corresponding experiment using the selenoprotein SecALR2. The insets depict the mixed disulfide formed between the outermost cysteine, C142, and β ME.

Table 1

Steady state kinetic parameters for the turnover of DTT by wild-type sfALR, SecALR2 U145C and SecALR2 with the corresponding U145 insertion.

ALR construct	k_{cat} (min^{-1})	K_{m} (mM)	$k_{\text{cat}}/K_{\text{m}}$ ($\text{M}^{-1} \text{s}^{-1}$)
sfALR ^a	108 ± 7	1.6 ± 0.4	1100 ± 300
SecALR2 U145C	60 ± 5	3.0 ± 0.7	330 ± 80
SecALR2	30 ± 3	10 ± 2	50 ± 10

^aTaken from ref. [9]