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Natural and synthetic selenoproteins

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Once considered highly toxic, the element selenium is now recognized as a micronutrient essential for human health. It is inserted co-translationally into many proteins as the non-canonical amino acid selenocysteine, providing the resulting selenoprotein molecules with a range of valuable redox properties; selenocysteine is also increasingly exploited as a structural and mechanistic probe in synthetic peptides and proteins. Here we review topical investigations into the preparation and characterization of natural and artificial selenoproteins. Such molecules are uniquely suited as tools for protein chemistry and as a test bed for studying novel catalytic activities.

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Introduction

Selenocysteine (Sec, U), the twenty-first genetically encoded amino acid, extends the properties of natural proteins in all domains of life. In humans, 25 selenoproteins have been identified [1]. These selenoproteins promote a panoply of chemical transformations important for regulating reactive oxygen species, redox homeostasis, and thyroid hormone metabolism (Figure 1). In other organisms, selenoproteins are involved in the reduction of CO_2 to methane and the production of energy-rich molecules such as formate and glycine (Figure 1). The chemical and biophysical properties that make selenocysteine biologically useful are also being exploited for novel applications outside the cell. In this review, we highlight work on natural and artificial selenoproteins, focusing on recent advances in the production of these molecules and their utility for protein folding applications.

Selenoprotein biosynthesis

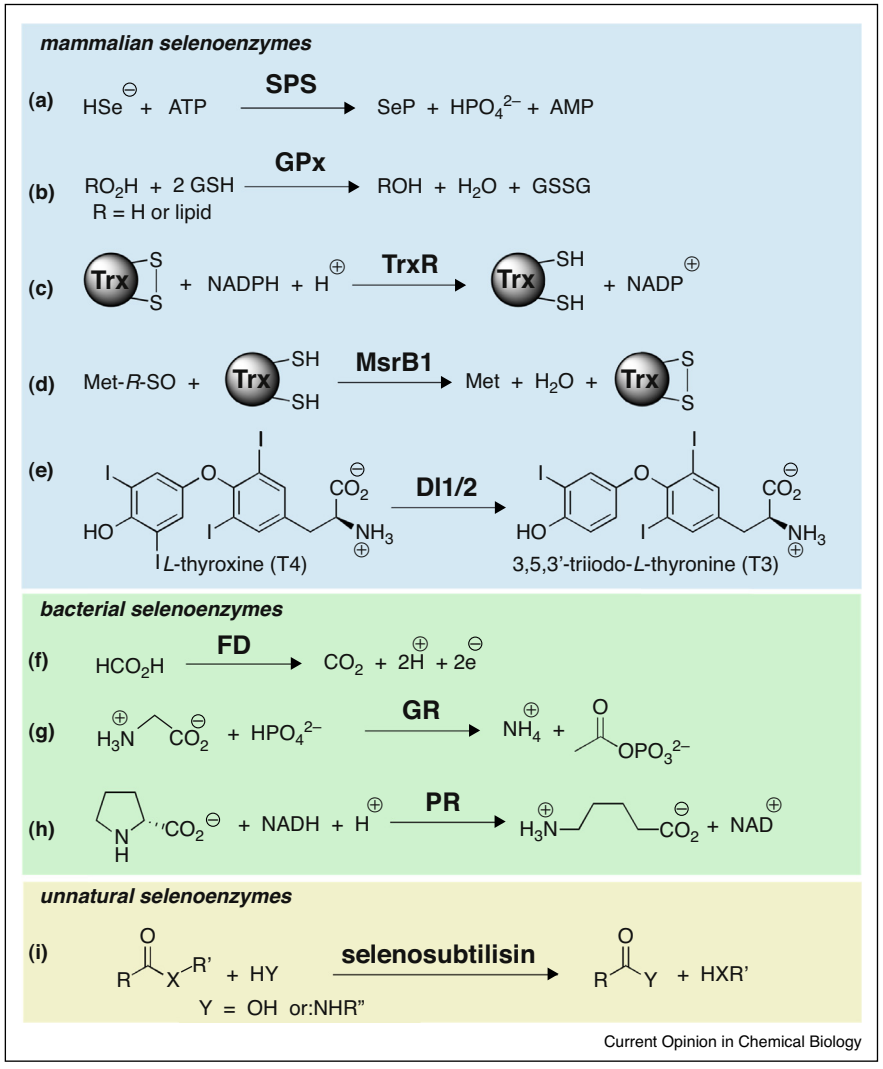
Selenocysteine is inserted into proteins cotranslationally by a natural recoding of the UGA stop codon (Figure 2a, see [2–5] for comprehensive reviews). Codon reassignment is made possible by a specific stem-loop structure called a selenocysteine-insertion sequence (SECIS), located in the 3′ untranslated region of archaeal and eukaryotic mRNA transcripts and immediately downstream of the UGA codon in prokaryotes. The SECIS element is recognized by a specialized elongation factor, called SelB (bacteria) or EF-Sec (archaea and eukaryotes), which delivers the aminoacylated suppressor tRNA, selenocysteinyl-$tRNA^{Sec}$ (Sec-$tRNA^{Sec}$), to the ribosome. Selenocysteine lacks its own aminoacyl-tRNA synthetase, so the noncanonical amino acid is instead synthesized from serine directly on $tRNA^{Sec}$ (Figure 2b).

The bacterial and mammalian selenocysteine insertion machinery has been successfully exploited to produce both natural and artificial selenoproteins. Nevertheless, because this noncanonical amino acid is encoded by a stop codon, biosynthesis of selenoproteins in good yield and homogeneous form is nontrivial. To make UGA decoding competitive with premature truncation, the biomacromolecules required for producing Sec-$tRNA^{Sec}$ and for efficient insertion must generally be overproduced [6]. Success additionally depends on having a SECIS element that is functionally compatible with the selenocysteine-specific elongation factor of the host.

To improve heterologous production of selenoproteins in *Escherichia coli*, ribosomal control of selenocysteine incorporation was recently optimized by genetic selection [7••]. Cotranslational insertion of selenocysteine was linked to cell viability using a reporter gene and an orthogonal translation system. Following random mutagenesis and selection, a single nucleotide substitution (C1100U) in the 16S rRNA was identified that boosted selenocysteine incorporation into the reporter protein 3–4 fold without affecting the efficiency or fidelity of canonical translation. Beneficial effects were also observed for the translation of an endogenous selenoprotein gene.

Efforts to rewire the genetic code have shown that the UGA stop codon is not absolutely required for selenoprotein production [8•]. Fifty-eight of the 64 codons can direct site-specific insertion of selenocysteine into proteins from $tRNA^{Sec}$ variants possessing complementary anticodons, and fifteen sense codons could be completely reassigned to selenocysteine. The accompanying tenfold increase in selenoprotein yield highlights the

Figure 1



Some well-characterized selenoenzymes and the reactions they catalyze. **(a)** Seleno-phosphate synthase (SPS). **(b)** Glutathione peroxidase (GPx). **(c)** The flavoenzyme thioredoxin reductase (TrxR or TR). **(d)** Methionine-*R*-sulfoxide reductase (MrsB1). **(e)** Deiodenases (DI1/2); deiodinase DI1 and DI3 promote subsequent loss of iodine from the inner ring of T3. **(f)** Formate dehydrogenase (FD). **(g)** Glycine reductase (GR). **(h)** Proline reductase (PR). **(i)** Selenosubtilisin. Several recent reviews summarize the properties of these and other selenoenzymes: [2,4,5].

ability of the selenocysteine machinery to outcompete abundant aminoacyl-tRNAs in decoding sense codons.

Provided that a gene can be equipped with an appropriate SECIS element, it should be possible — at least in principle — to replace any amino acid in a protein by selenocysteine. Eukaryotic expression systems are particularly forgiving in this regard since the SECIS is not in the coding region in these organisms. Bacterial expression is also possible, as illustrated by introduction of selenocysteine as a mechanistic probe into the active sites of bacterially expressed glutathione-S-transferase [9] and cytochrome P450cam [10]. In practice, programs like SECISDesign [11] can help minimize unwanted sequence changes in the target gene, though the sequence constraints of functional bacterial SECIS elements limit the generality of this approach. Specialized elongation factor SelB variants with relaxed SECIS specificity might prove useful in broadening its applicability.

To circumvent the need for SelB and the SECIS element altogether, a novel suppressor tRNA for selenocysteine ($tRNA^{UTu}$) has been engineered that is recognized by EF-Tu [12••]. As is the case for $tRNA^{Sec}$, seryl-tRNA synthetase aminoacylates $tRNA^{UTu}$ with serine and SelA converts the resulting $Ser\text{-}tRNA^{UTu}$ to $Sec\text{-}tRNA^{UTu}$. This system has been used to produce the human selenoenzyme glutathione peroxidase as well as three bacterial selenoproteins in *E. coli*. Although the