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UV light-driven prebiotic synthesis of iron-sulfur clusters

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Abstract

Iron-sulfur clusters are ancient cofactors that play a fundamental role in metabolism and may have impacted the prebiotic chemistry that led to life. However, it is unclear whether iron-sulfur clusters could have been synthesized on prebiotic Earth. Dissolved iron on early Earth was predominantly in the reduced ferrous state, but ferrous ions alone cannot form polynuclear iron-sulfur clusters. Similarly, free sulfide may not have been readily available. Here we show that UV light drives the synthesis of [2Fe-2S] and [4Fe-4S] clusters through the photooxidation of ferrous ions and the photolysis of organic thiols. Iron-sulfur clusters coordinate to and are stabilized by a wide range of cysteine containing peptides, and the assembly of iron-sulfur cluster-peptide complexes can take place within model protocells in a process that parallels extant pathways. Our experiments suggest that iron-sulfur clusters may have formed easily on early Earth, facilitating the emergence of an iron-sulfur cluster dependent metabolism.

Extant life is sustained by metabolic reactions that are dependent on iron-sulfur cluster containing proteins. This dependence may reflect a role that was played by abiotically synthesized iron-sulfur catalysts that facilitated the emergence of specific protometabolic pathways. Such a hypothesis fits well with the abundance of iron and sulfur on Earth and with the fact that iron-sulfur clusters are considered one of life's most ancient cofactors1,2. Since complex protein folds were unlikely to have been present on prebiotic Earth, it has been proposed that these early iron-sulfur catalysts were mineral surfaces3,4. Although minerals likely participated in the synthesis of organic molecules on prebiotic Earth5, it is

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Authors contributions. CB, SSc, LJ, JWS, DDS, JDS, and SSM designed the experiments. Photochemical studies, peptide synthesis, and cluster stability were performed by CB and LV. Mössbauer spectra were recorded and analyzed by SSh and DJE. The manuscript was written by CB and SSM and edited by CB, SSc, DJE, JWS, DDS, JDS, and SSM.

Data availability
The authors declare that the data supporting the findings of this study are available within the paper and supplementary information.

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unclear how iron-sulfur mineral surfaces could have led to modern day iron-sulfur proteins, and no plausible pathway for such a transition has been proposed. We suggest that a more likely alternative is that short, prebiotically formed peptides helped to assemble and stabilize catalytically active iron-sulfur clusters. Support for this theory comes from the ability of a tripeptide to coordinate a redox active [2Fe-2S] cluster in aqueous solution and that oligomerization of this tripeptide sequence to the dodecapeptide leads to a protoferredoxin with increased cluster stability and activity as a redox catalyst6. The abiological synthesis of iron-sulfur clusters in aqueous solution is typically accomplished by mixing Fe^{3+}, S^{2-}, and suitable thiolate ligands under anaerobic conditions. Iron-sulfur clusters are not normally produced if Fe^{2+} is substituted for Fe^{3+} under strictly anaerobic conditions. It is, therefore, uncertain whether similar synthetic mechanisms could have operated on prebiotic Earth where reduced iron ions (Fe^{2+}) predominated7,8.

It has been previously proposed that the reducing potential of hydrogen sulfide in the presence of iron sulfide could drive the synthesis of organic molecules9, such as pyruvate10. Recognizing the potential of hydrogen sulfide as a prebiotic reducing agent under conditions of UV irradiation, Sutherland and coworkers11 exploited the generation of hydrated electrons through photoredox chemistry with hydrogen sulfide and copper ions to synthesize sugars, nucleotides, amino acids, and lipid precursors in high yield12. Here we build upon these studies by investigating the effect of ultraviolet light and ferrous ions on the synthesis of iron-sulfur peptides. Our data support the ability of UV light from the young Sun to mediate two critically important steps in iron-sulfur cluster synthesis. First, UV light liberates the sulfide ions necessary for the synthesis of polynuclear iron-sulfur clusters from thiol containing molecules. Second, UV light leads to the photooxidation of ferrous ions, generating the ferric ions needed for cluster assembly. Both of these UV light mediated processes are analogous to protein mediated steps in the extant biological pathways for the synthesis of iron-sulfur clusters. Furthermore, our model of prebiotic iron-sulfur cluster synthesis shows little dependence on the sequence of the peptide scaffold and is fully functional within model protocells, suggesting that cellular iron-sulfur cluster synthesis could have emerged at a very early stage in the evolution of life.

Results

UV light mediated formation of polynuclear iron-sulfur clusters

Exposure of a solution containing only Fe^{3+} and a thiolate ligand under anaerobic conditions to predominantly 254 nm UV light led to the synthesis of polynuclear iron-sulfur clusters. Fe^{3+} was used instead of Fe^{2+}, because the standard protocols for the *in vitro* maturation of iron-sulfur proteins in aqueous solution utilize ferric salts. Glutathione was used as the model prebiotic thiolate, because this tripeptide (γECG) has previously been shown to coordinate an iron-sulfur cluster13, to function as a protoferredoxin6, and the formation of glutathione was calculated to be thermodynamically favored over the corresponding α-peptide14. Prior to exposure to UV light, the solution of Fe^{3+}-glutathione was violet with a UV-visible absorption spectrum similar to that of the mononuclear iron protein rubredoxin (Fig. 1a, solid line, and Supplementary Table 1). The oxidized mononuclear iron-thiolate complex was not stable and was rapidly reduced (Fig. 1a, dashed line), presumably by free

thiolates. Mössbauer spectroscopy showed an isomeric shift (IS) and quadrupole splitting (QS) of 0.68 mm/s and 3.26 mm/s, respectively, consistent with formation of a mononuclear Fe^{2+} complex (Fig. 1b and Supplementary Table 2)15. Irradiation with 254 nm light for 30 s turned the solution red and showed a UV-visible absorption spectrum similar to that of a [2Fe-2S] ferredoxin (Fig. 1c and Supplementary Table 1). Mössbauer data confirmed the presence of a [2Fe-2S] cluster. 21% of the Mössbauer spectrum, expressed as molar contribution, fit a $[2Fe\text{-}2S]^{2+}$ cluster with an IS of 0.27 mm/s and QS of 0.52 mm/s, whereas the remaining signals were due to the presence of reduced mononuclear complex (Fig. 1d and Supplementary Table 2). In the absence of UV radiation, the [2Fe-2S] cluster was not detected within 24 h (Supplementary Fig. 1). Upon further UV irradiation of the [2Fe-2S] cluster solution up to 180 s, the solution turned brown and gave a UV-visible absorption spectrum similar to [4Fe-4S] ferredoxins (Fig. 1e and Supplementary Table 1). Mössbauer spectra were fit to 10 mol% $[4Fe\text{-}4S]^{2+}$ (IS=0.48 mm/s; QS=1.06 mm/s), 64 mol% $[2Fe\text{-}2S]^{2+}$ (IS=0.31 mm/s; QS=0.57 mm/s), and 26 mol% Fe^{2+} (IS=0.68 mm/s; QS=3.32 mm/s) (Fig. 1f and Supplementary Table 2). Since the extinction coefficient of [4Fe-4S] clusters is generally higher than that of [2Fe-2S] clusters16, the UV-visible spectrum was dominated by the absorption of the [4Fe-4S] cluster.

As polynuclear iron-sulfur clusters such as [2Fe-2S] and [4Fe-4S] clusters contain inorganic sulfide within the cluster, and no inorganic sulfide was present in solution prior to irradiation with UV light, the reaction was further probed by ^1H NMR spectroscopy to determine the source of the sulfide (Supplementary Table 3–4). When a solution of glutathione was irradiated with UV light, the side-chain methylene resonance of cysteine at 2.9 ppm decreased over time and a new doublet consistent with the methyl group of an alanine at 1.3 ppm concomitantly increased (Fig. 2a). The conversion of the cysteine of glutathione (γECG, $[M+H]^+$=308.2 m/z) to an alanine (γEAG, $[M+H]^+$=276.2 m/z) was confirmed by mass spectrometry (Supplementary Fig. 2–4). The same result was obtained in the absence (Fig. 2a) and presence (Supplementary Fig. 5a) of iron ions, indicating that desulfurization was not catalyzed by iron ions. UV light also induced the formation of the oxidized form of the thiolates (*i.e.* cystines), which was then converted to alanine upon prolonged irradiation (Supplementary Fig. 5b). If the prebiotically plausible reductant hypophosphite17,18 was included in solution, glutathione was completely converted to the corresponding alanine containing tripeptide (Fig. 2a and 2b), although hypophosphite was not required for the synthesis of iron-sulfur clusters (Supplementary Fig. 4e). Therefore, longer exposure to UV light would lead to increased sulfide concentrations and thus conversion from mononuclear, to [2Fe-2S], and [4Fe-4S] clusters, since the type of cluster formed is dependent upon the sulfide to iron ion ratio19. To gain better insight into whether the observed photochemistry could have driven the synthesis of iron-sulfur clusters on prebiotic Earth, we assessed the influence of light on the desulfurization reaction more similar to that of the young Sun. Since light below 204 nm would have been largely shielded out by atmospheric CO_2 (<204 nm) and water (<168 nm) on the primordial Earth20, Fe^{3+}-glutathione was subjected to light at 205 nm, 250 nm, 300 nm, and 350 nm. We explored the wavelength dependence of photolysis with a lower intensity lamp (≤0.3 mW) than used above (ca. 35 W) so as to better mimic early Earth conditions. Irradiation at 250 nm resulted in desulfurization at a rate 3-fold, 2-fold, and 13-fold faster than at 205 nm, 300 nm, and 350 nm, respectively (Fig. 2c).

The faster desulfurization at 250 nm is consistent with the electronic transition of thiolate bonds, which have an absorbance maximum at ca. 250 nm.

Having identified the source of the inorganic sulfide, we then asked how [2Fe-2S] and [4Fe-4S] clusters that contain Fe^{3+} centers could have been synthesized prebiotically from a solution consisting of Fe^{2+}-glutathione, since mononuclear ferric complexes undergo rapid reduction to the corresponding ferrous complexes in the presence of excess thiolate ligands. In the absence of light, a [2Fe-2S] cluster cannot be synthesized from Fe^{2+}, S^{2-}, and glutathione unless the solution is subsequently exposed to air to oxidize the iron ions (Supplementary Fig. 6a). If S^{2-} was premixed with glutathione, then the addition of Fe^{3+} did lead to the formation of [2Fe-2S] clusters, because the kinetics of cluster formation were faster than the reduction of iron ions (Supplementary Fig. 6b). If, however, Fe^{3+} was incubated with glutathione prior to the addition of S^{2-}, then [2Fe-2S] clusters did not form, because the ferric ions were reduced before the sulfide ions necessary for cluster synthesis were present (Supplementary Fig. 6c). Exposure to UV light seemed to negate these reductive effects. For example, [2Fe-2S] clusters were synthesized from a solution of Fe^{2+}-glutathione when subjected to irradiation with UV light (Supplementary Fig. 6d). We confirmed previous reports of the photooxidation of ferrous ions in water21 by monitoring the increased formation of ferric thiocyanate and decreased formation of ferrous phenanthroline complexes under the same solution conditions used for cluster synthesis (Supplementary Fig. 7).

The photolysis and photooxidation reactions that we have identified (Fig. 3a and Supplementary Fig. 8-9) are analogous to the extant biological mechanisms for the synthesis of several molecules necessary to support cellular life (Fig. 3b). In biology, a highly conserved cysteine desulfurase found in all three Kingdoms of life22,23 (IscS and homologues NifS and SufS) catalyzes the β-elimination of sulfur from free cysteine and subsequent reduction to yield alanine and the sulfide necessary for the synthesis of iron-sulfur clusters, sulfur containing vitamins, and thiolated tRNA molecules24. At least in some cases, tRNA thiolation itself depends on the activity of iron-sulfur proteins25. Based on bioinformatic analysis of genetic sequences, the need for such desulfurase enzymes appears to extend back to at least the last universal common ancestor26. Since short-wavelength UV light is one of the most abundant sources of energy, similar sulfide generating mechanisms could have operated near the surface of the prebiotic Earth before desulfurase enzymes evolved. It is reasonable to expect that such photochemistry participated in the synthesis of biological molecules in the past27, because UV light continues to be exploited for the synthesis of molecules, such as vitamin D_3, in biology today. Similarly, the photooxidation chemistry may also predate modern day steps in iron-sulfur cluster biosynthesis. The *isc* operon that encodes the evolutionarily conserved protein machinery needed to synthesize iron-sulfur clusters contains a necessary redox active ferredoxin28.

Sequence dependence of iron-sulfur cluster formation

The experiments described above exploited glutathione as a model prebiotic tripeptide to stabilize the iron-sulfur clusters. Since glutathione may not have existed on prebiotic Earth,

we have explored the breadth of sequences capable of coordinating and stabilizing iron-sulfur clusters in aqueous solution. 35 different cysteine-containing tripeptides were synthesized and tested for their ability to stabilize iron-sulfur clusters upon the addition of ferric ions and sulfide (Supplementary Fig. 10-40 and Supplementary Table 5). To determine if the position of the cysteine was important for cluster coordination, the sequence of glutathione (γECG) was scrambled while retaining the γ-peptide bond through the side-chain of the glutamate. γEGC but not CγEG was capable of coordinating a [2Fe-2S] cluster. However, if the amino terminus of CγEG was acetylated, then the ability to coordinate a [2Fe-2S] cluster was recovered (Supplementary Fig. 41). Next, the importance of the γ-peptide bond was investigated. Shortening the side-chain by a single methylene unit by substituting the glutamate with an aspartate, i.e. βDCG, did not inhibit cluster coordination. Also, the introduction of a normal α-peptide bond as found in ECG and DCG had no apparent effect on cluster coordination (Supplementary Fig. 42). Next, a wide variety of cysteine containing sequences that more fully deviated from that of glutathione were tested. The presence of polar (serine, threonine, glutamine, asparagine), hydrophobic (phenylalanine, alanine, valine), and charged (lysine, aspartic acid, glutamic acid) amino acids next to the coordinating cysteine residue had no effect on the ability to coordinate a [2Fe-2S] cluster. The only additional cysteine containing tripeptides that failed to coordinate a [2Fe-2S] cluster were WCG and CGG, but we note that tryptophan is unlikely to have been a prebiotic amino acid12. Presumably the size of tryptophan interfered with cluster coordination. As observed with the other amino-terminal cysteine containing peptides, [2Fe-2S] cluster coordination was recovered by blocking the free amino group of CGG by acetylation. No [2Fe-2S] cluster was observed if the cysteine ligand was removed, e.g. GMG (Supplementary Fig. 43). It should be noted that the synthesis of the iron-sulfur cluster depended on the presence of deprotonated cysteine side-chains. Therefore, the pH of each peptide solution was adjusted to the pK_a of the thiol, which varied according to the sequence of the tripeptide. For central or carboxy-terminal cysteines, the pK_a values were in the 7.3-8.1 range (Supplementary Fig. 44 and Supplementary Table 6). Since the first pK_a of H_2S is 7.0529, the mildly alkaline pH helped retain HS^- in solution. Taken together, the data suggest that the sequence space of cysteine containing peptides is heavily predisposed towards the ability to coordinate an iron-sulfur cluster.

Having found that most tripeptides are competent for the binding of an iron-sulfur cluster, we next probed the ability of smaller thiolate ligands to coordinate a [2Fe-2S] cluster in aqueous solution. The dipeptides GC and acetylated CG were both able to coordinate a [2Fe-2S] cluster when mixed with sulfide and ferric ions. *N*-acetylated L-cysteine and *N*-acetylated L-cysteine methyl ester but not unblocked free cysteine were similarly capable of coordinating a [2Fe-2S] cluster (Supplementary Fig. 45 and Supplementary Table 5 and 7). Following previous studies on iron-sulfur cluster synthesis30,31, we next tested non-proteinaceous ligands, including dithiothreitol, cysteamine, 1,2-ethanedithiol, 2-mercaptoethanol, 3-mercaptopropionic acid, dihydrolipoic acid, and 2-mercaptopyrimidine. Of these ligands, only dithiothreitol, 2-mercaptoethanol, and 3-mercaptopropionic acid were capable of coordinating a [2Fe-2S] cluster (Supplementary Table 7). The data are consistent with the hypothesis that an amino group close to the coordinating thiolate (e.g. cysteamine and the tautomeric form of 2-mercaptopyrimidine) or improperly spaced thiolates (e.g. 1,2-

ethanedithiol and dihydrolipoic acid) interferes with [2Fe-2S] cluster coordination. Although three small, non-peptidic ligands were identified that could coordinate an iron-sulfur cluster, the concentration of the ligand necessary was higher than that for the cysteine containing tripeptides. For example, 240 mM 2-mercaptoethanol was needed to stabilize the [2Fe-2S] cluster in the presence of 0.5 mM $FeCl_3$ and 0.2 mM Na_2S, whereas only 40 mM glutathione was required under the same conditions. Larger molecules may better protect the iron-sulfur cluster from hydrolytic attack.

To confirm that the thiol containing molecules that were capable of coordinating an iron-sulfur cluster could also support photolysis mediated cluster assembly, the reactions were repeated in the presence of UV light and in the absence of an external source of inorganic sulfide. NMR and UV-visible spectroscopy showed the photolysis of all the studied thiolates and the formation of [2Fe-2S] clusters (Supplementary Fig. 46-59 and Supplementary Table 1 and 5). That is, 2-mercaptoethanol, 3-mercaptopropionic acid, *N*-acetyl cysteine, *N*-acetyl cysteine methyl ester, and 37 different di- and tripeptides all could support the synthesis of a [2Fe-2S] by the addition of iron ions in the presence UV light.

Compatibility with model protocells

Fatty acid vesicles are often used to construct model protocells, because some fatty acids are prebiotically plausible and can form vesicles that grow, divide, acquire nutrients, and house non-enzymatic, nucleic acid copying reactions[32,33]. However, fatty acid vesicle stability is sensitive to the presence of divalent cations[34]. It was previously shown that the stability of vesicles composed of oleate, myristoleate, or decanoate to Mg^{2+} could be greatly increased by the presence of citrate[35]. Since glutathione can complex iron ions, the stability of oleate and decanoate vesicles to Fe^{2+} was assessed in the presence and absence of glutathione. Oleate and 4:1:1 decanoic acid:decanol:monocaprin vesicles containing fluorophore labeled dextran were incubated for 60 min with either 5 mM Fe^{2+} or 5 mM Fe^{2+}, 200 mM glutathione. The integrity of the vesicles was then assessed by quantifying the leakage of the dextran out of the vesicles by size-exclusion chromatography and fluorescence spectroscopy. In the absence of glutathione, all of the dextran leaked out of the vesicles. Conversely, the vesicles were completely stable in the presence of Fe^{2+} when complexed with glutathione (Supplementary Fig. 60-61). Since glutathione could protect fatty acid vesicles from the effects of iron ions, we next asked whether the light driven synthesis of iron-sulfur clusters was compatible with fatty acid vesicles (Fig. 4a). First, the UV light stability of iron glutathione complexes encapsulated within oleic acid and 4:1:1 decanoic acid:decanol:monocaprin vesicles was assessed by exposing aliquots to 254 nm light for 0 or 60 min. Then the samples were fractionated by size-exclusion chromatography and subjected to a colorimetric assay with the iron sensitive chromophore tiron. No iron ions were released from either type of vesicle, with or without UV irradiation (Supplementary Fig. 62-63). To determine whether sulfide could be generated from cysteine residues inside of the vesicles (Supplementary Fig. 64) and lead to the formation of [2Fe-2S] clusters, purified vesicles containing iron-glutathione were irradiated at 254 nm and the appearance of sulfide observed with a methylene blue assay by adding *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) in hydrochloric acid to each aliquot. Since DMPD reacts with H_2S in the presence of ferric ions to form methylene blue, the appearance of methylene blue

spectral bands upon the addition of DMPD indicated the presence of inorganic sulfide and indirectly the presence of an iron-sulfur cluster (Fig. 4b and Supplementary Fig. 65). The synthesis of [2Fe-2S] glutathione from $Fe^{2+/3+}$-glutathione was confirmed by Mössbauer spectroscopy (Fig. 4c and Supplementary Table 8). Our observations show that thiolate containing peptides can not only protect model protocells from the presence of iron ions but are also compatible with light driven iron-sulfur cluster synthesis within model protocells.

Discussion

A wide variety of prebiotically plausible conditions are compatible with the formation of iron-sulfur clusters that are soluble in aqueous solution. Environments rich in Fe^{3+}, H_2S, and suitable thiolate ligands, including a broad range of cysteine containing peptides, would have led to the synthesis of [2Fe-2S] and [4Fe-4S] clusters. If only Fe^{2+} was present, then the needed Fe^{3+} would have been anaerobically generated through photooxidation in environments exposed to UV irradiation from the young Sun. Similarly, if hydrogen sulfide was not readily available in solution, perhaps due to the high volatility of hydrogen sulfide, then photolysis of thiol containing molecules would generate the needed sulfide ions for cluster synthesis. Therefore, iron-sulfur clusters could have existed in light exposed, surface conditions and dark, submerged regions. Additionally, the photolytic release of sulfide could facilitate the synthesis of nucleotides, amino acids, and lipid precursors12. Our experiments also reveal a driving force for polynuclear iron-sulfur cluster formation in the presence of sulfide: in the absence of sulfide, mononuclear iron coordination predominates, while in the presence of sulfide the formation of [2Fe-2S] and [4Fe-4S] clusters is favored. Therefore, extant metabolic pathways that solely depend on enzymes containing mononuclear metal centers, such as glycolysis, may have emerged from environments devoid of accessible sulfide, whereas metabolic reactions that exploit polynuclear iron-sulfur clusters may reflect an origin in sulfide rich environments36. This conjecture fits well with the possibility of progenitor iron peptides evolving towards the exploitation of other metal ions as the pool of soluble iron ions decreased due to the great oxidation event7,37.

Iron-sulfur clusters coordinated by cysteine containing peptides are stable to the pH and salt conditions6 needed for the formation of vesicles from fatty acids. Furthermore, the cysteine containing peptides protect the vesicles from the degradative effects of softer, thiophilic metals. Since the integrity of fatty acid vesicles is not compromised by UV light, the photolysis and photooxidation steps necessary for the synthesis of iron-sulfur clusters are not only compatible with model protocells, but also strikingly similar to extant biological mechanisms. What is still not known, however, is how such iron-sulfur peptides could have impacted the fitness of a protocell. [2Fe-2S] glutathione is redox active6, but more work is needed to determine whether such activity could be harnessed to generate a proton gradient, for example. Nevertheless, the prevalence of iron-sulfur dependent metabolism in all known living systems, the ease with which iron-sulfur peptides form under prebiotically reasonable conditions, the similarity between abiotic iron-sulfur cluster synthesis with extant cellular pathways, and the compatibility of iron-sulfur peptide synthesis with model protocells suggest that early Earth surface conditions were ripe for iron-sulfur peptides to have impacted protometabolic processes, perhaps within self-replicating protocells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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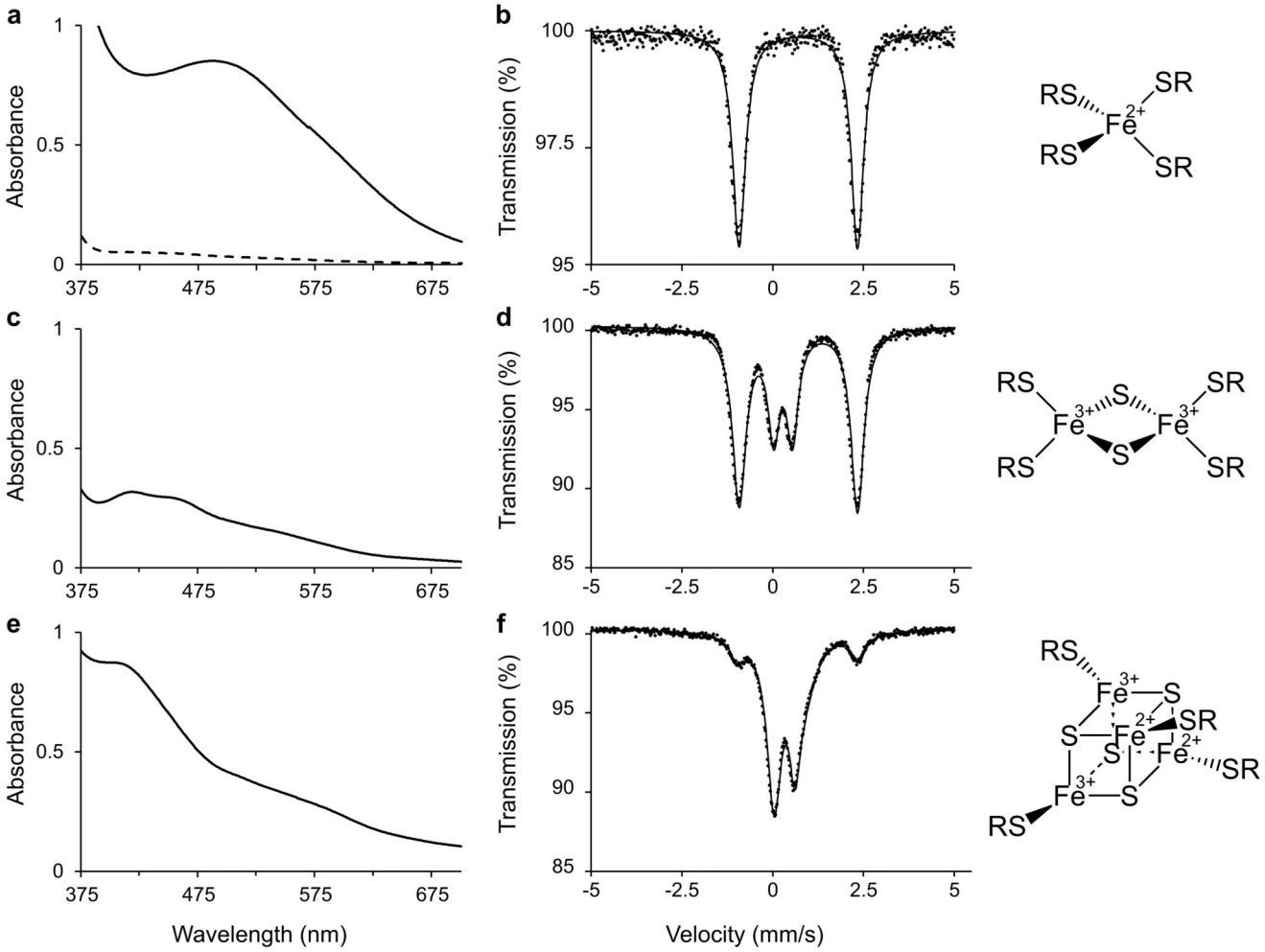

Figure 1. Light driven, prebiotic synthesis of iron-sulfur peptides.

The addition of Fe^{3+} to glutathione results in an initial UV-visible spectrum consistent with a Fe^{3+}-glutathione complex (**a**, solid line), which is then rapidly (over 180 s) reduced to a Fe^{2+}-glutathione complex (**a**, dashed line, and **b**). Irradiation at 254 nm results in the appearance of a [2Fe-2S] cluster (**c** and **d**) and then a [4Fe-4S] cluster (**e** and **f**). **a**, **c**, and **e**: UV-visible absorption spectra; **b**, **d**, and **f**: Mössbauer spectra. **d** shows a mixture of mononuclear Fe^{2+} and $[2Fe\text{-}2S]^{2+}$. **f** shows a mixture of mononuclear Fe^{2+}, $[2Fe\text{-}2S]^{2+}$, and $[4Fe\text{-}4S]^{2+}$.

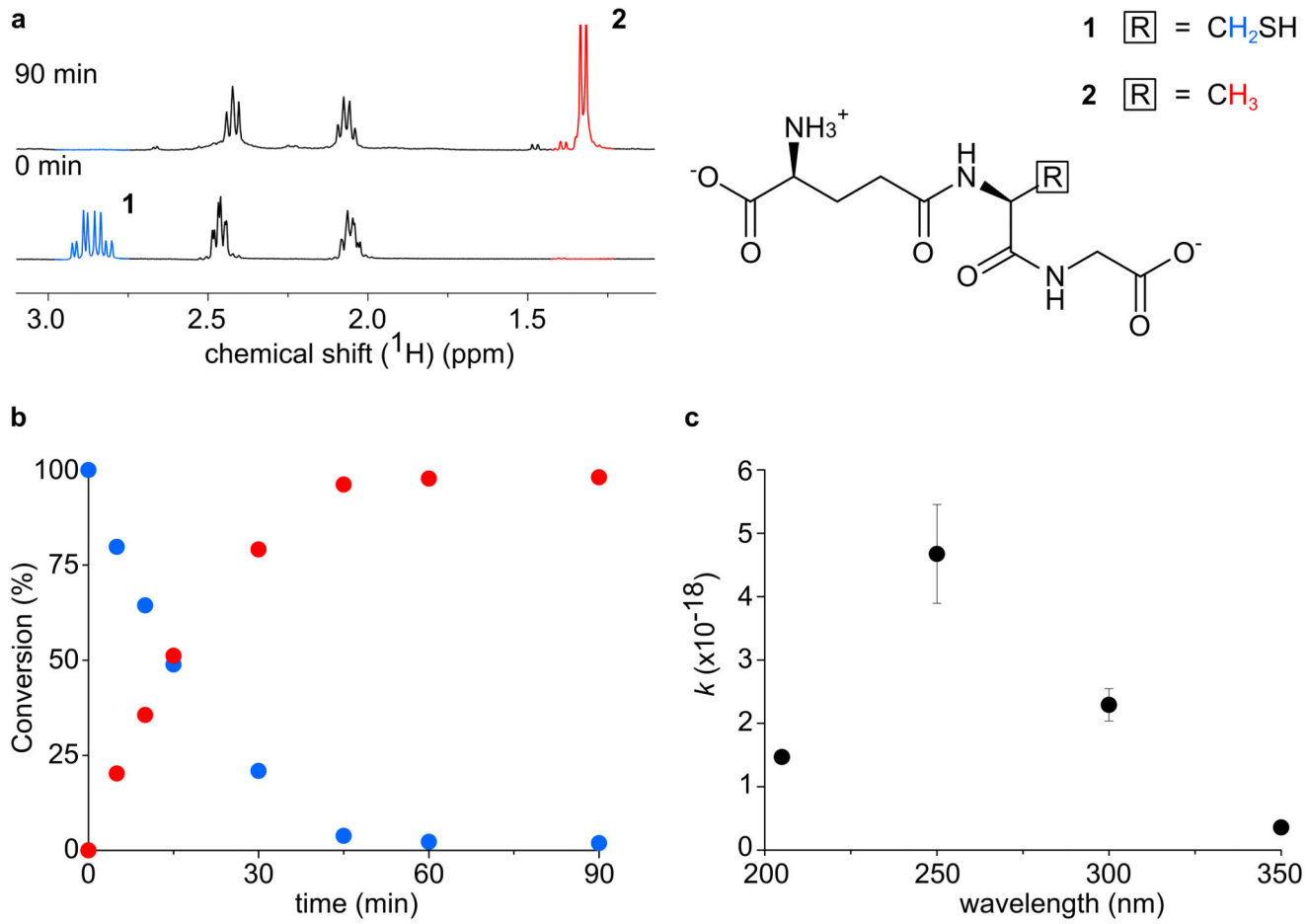

Figure 2. UV light desulfurization of the cysteine of glutathione to an alanine.

a, Conversion of the thiolate **1** (blue) to the corresponding desulfurized species **2** (red) shown by ^{1}H NMR, consistent with the release of sulfide ions. **b,** Time course of conversion as obtained by the integration of NMR peaks for the irradiated solution of glutathione in the presence of hypophosphite. Blue, glutathione; red, alanyl-analogue. **c,** Dependence of the conversion rate constant on the irradiation wavelength. The data are congruent with the electronic transitions of thiolates. The rate constant (expressed as s^{-1}) was normalized by the photon flux (expressed as s). Data represent n ≥ 3 replicates (mean and SEM).

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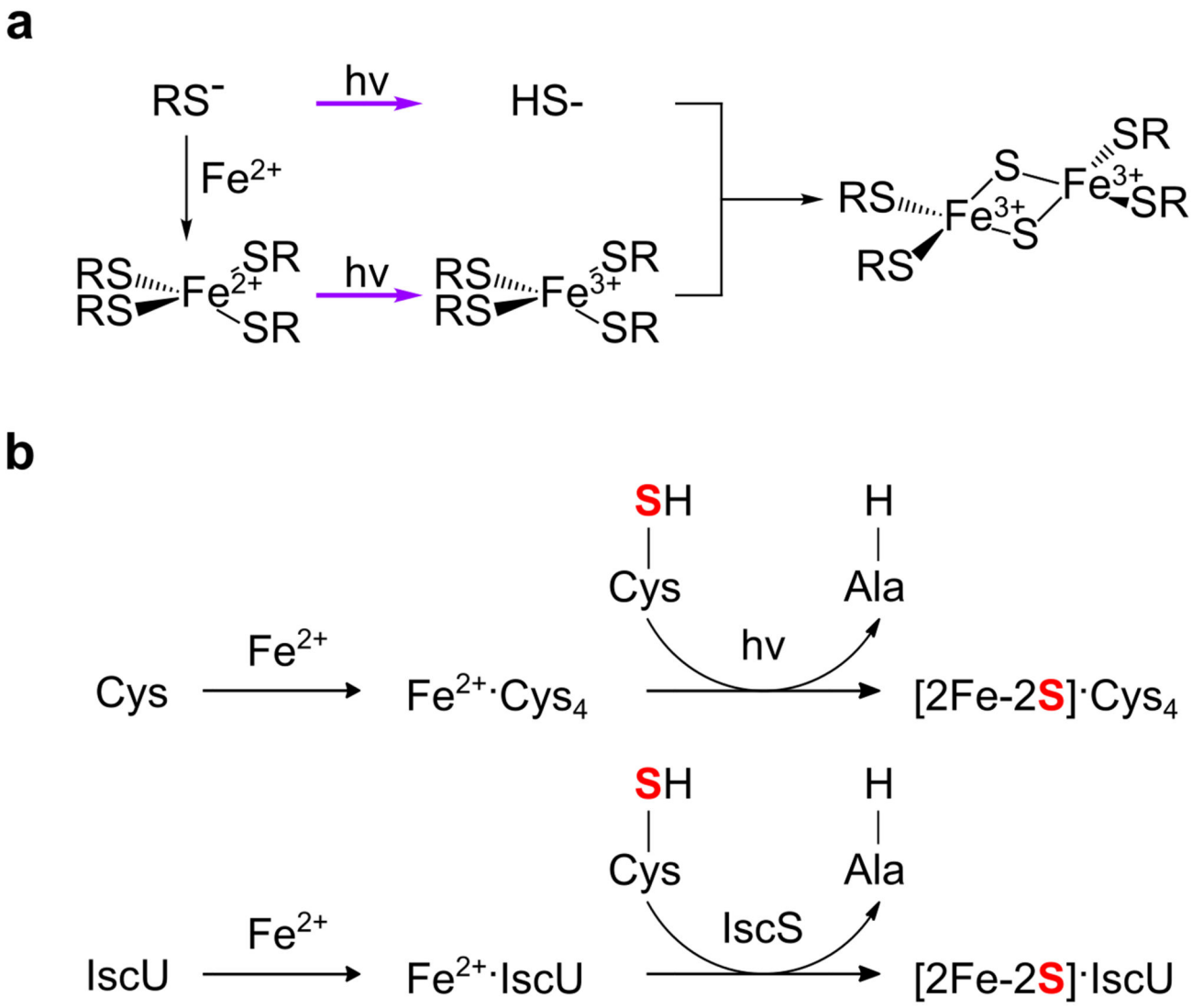

Figure 3. Prebiotic pathways for the synthesis of iron-sulfur clusters.

a, Proposed mechanism of photooxidation and photolysis for the synthesis of a [2Fe-2S] cluster from a thiolate solution in the presence of ferrous ions. **b,** A comparison between model prebiotic (top) and extant, biosynthetic (bottom) pathways for the donation of sulfide during the synthesis of an iron-sulfur cluster.

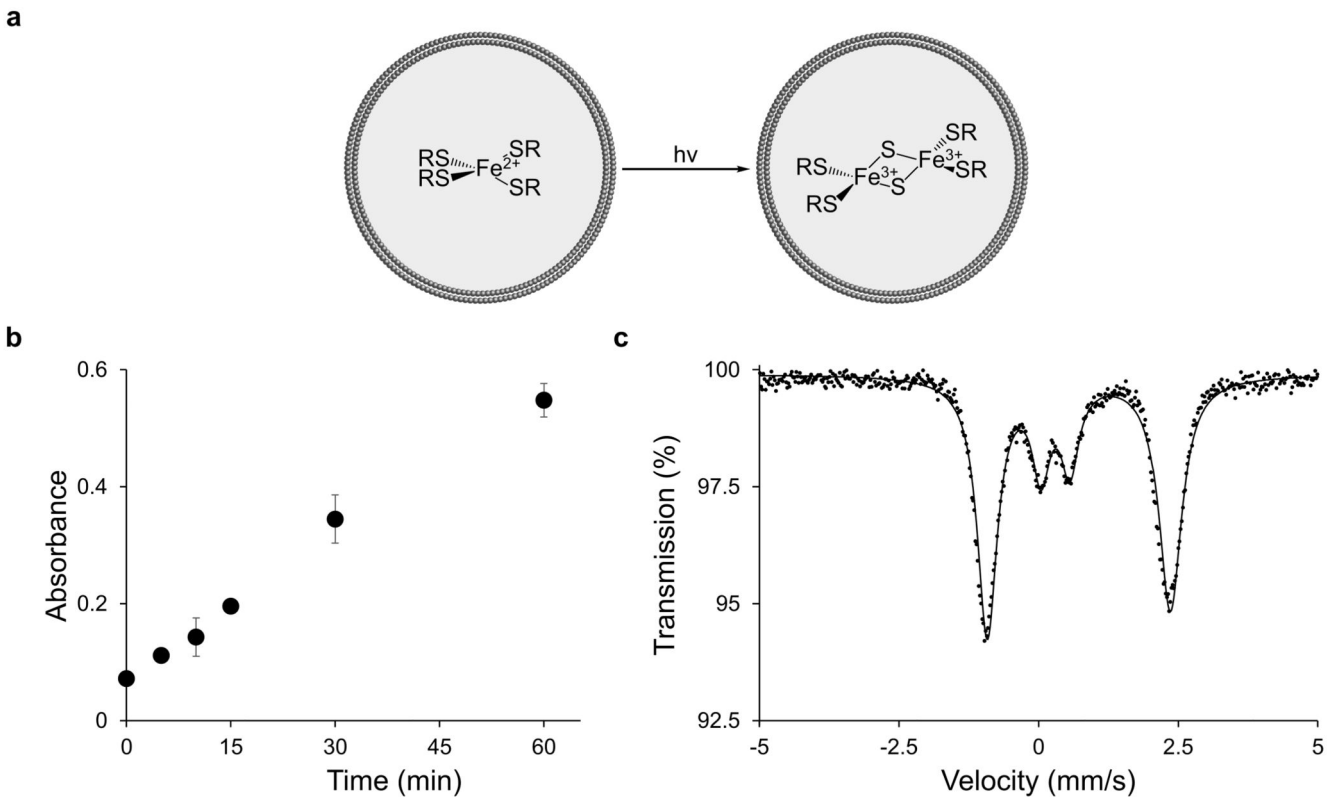

Figure 4. [2Fe-2S] cluster formation *in situ* within fatty acid vesicles.

a, Schematic representation of light-driven, [2Fe-2S] cluster synthesis within fatty acid vesicles. **b,** A methylene blue assay detected the release of sulfide within oleate vesicles induced by UV light, demonstrating the compatibility of the photolysis of entrapped thiolates with fatty acid vesicles. Colorimetric data represent mean and SEM, $n \geq 3$ replicates. **c,** Mössbauer spectrum of the $[2Fe\text{-}2S]^{2+}$ cluster, minor component, formed within oleate vesicles by UV light.

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