Using *N*-acylation as a disulfide lock simplifies chemical protein synthesis

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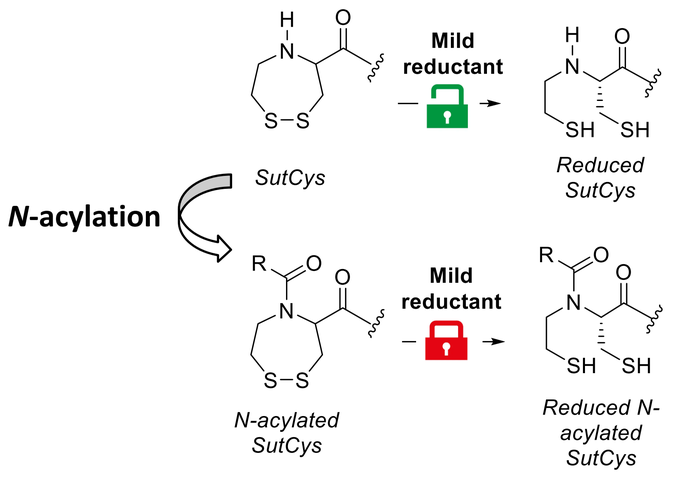
Introduction

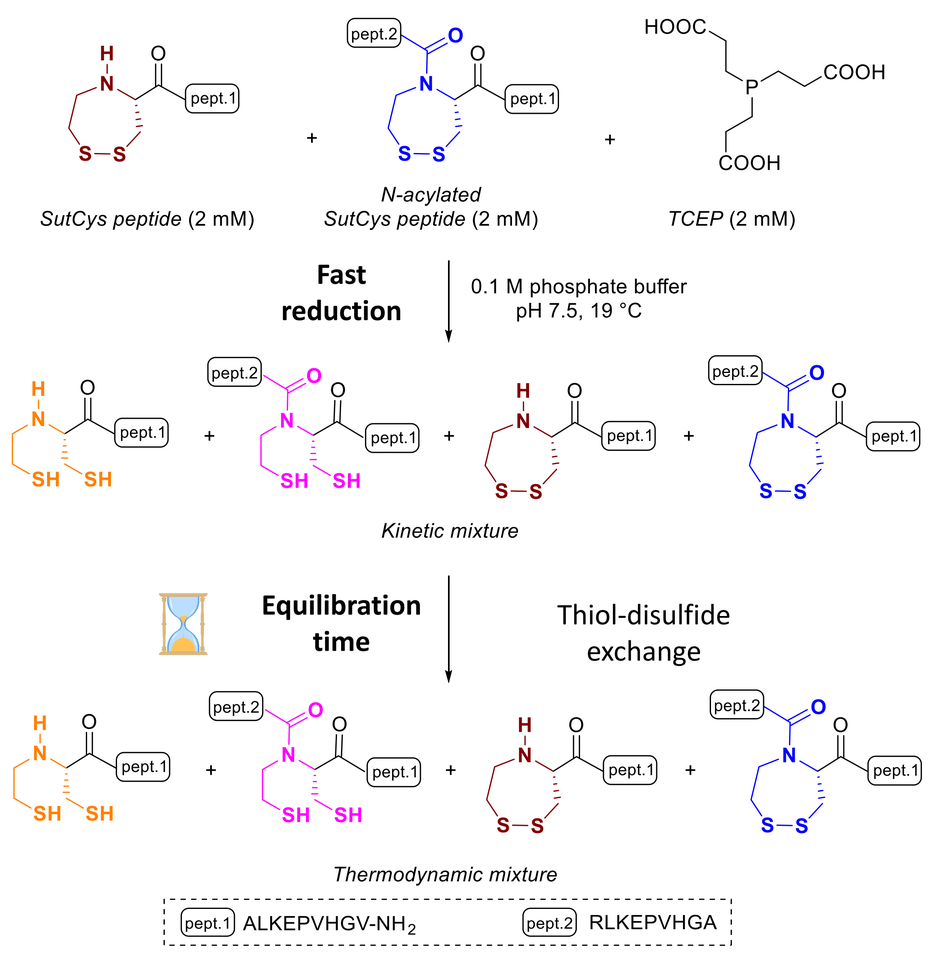
There is an increasing evidence that cells utilize some post-translational modifications to remotely control the physico-chemical properties of thiols, and hence their capacity to engage in biochemical processes. For example, recent investigations show that lysine acylation is a post-translational modification that enables to modulate the catalytic activity of the protein disulfide isomerase, a cysteine enzyme that accelerates the folding of proteins.[1] Mimicking nature and tuning thiol reactivity by a chemical event occurring remotely is an appealing strategy to create controllable chemical systems. This concept is illustrated here in the context of chemical protein synthesis.

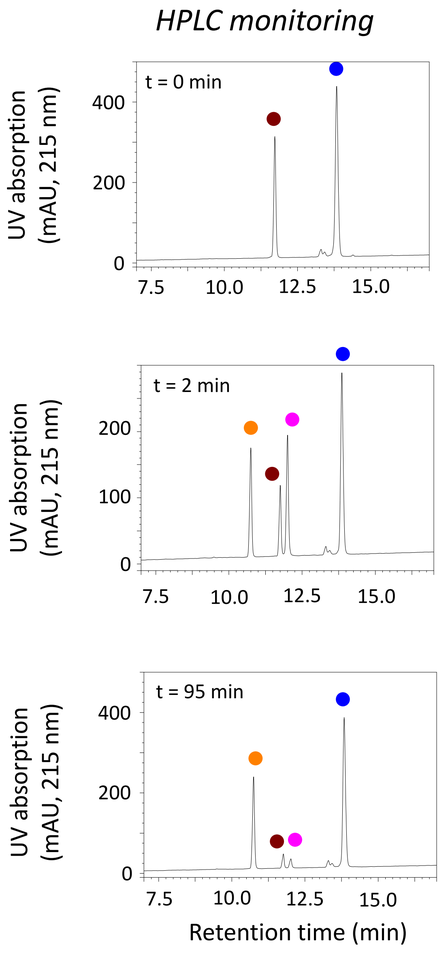
We discovered that the stability of the cyclic disulfide called SutCys towards reduction increases dramatically upon *N*-acylation, locking its opening and hence its reactivity in the presence of a mild reductant. Only the addition of a significantly stronger reductant in the reaction mixture can restore the SutCys reactivity. In this communication, we show how locking transiently SutCys ring-opening enables us to synthesize cyclic polypeptides through the concatenation of peptide segments on a water compatible solid support using native chemical ligation (NCL).

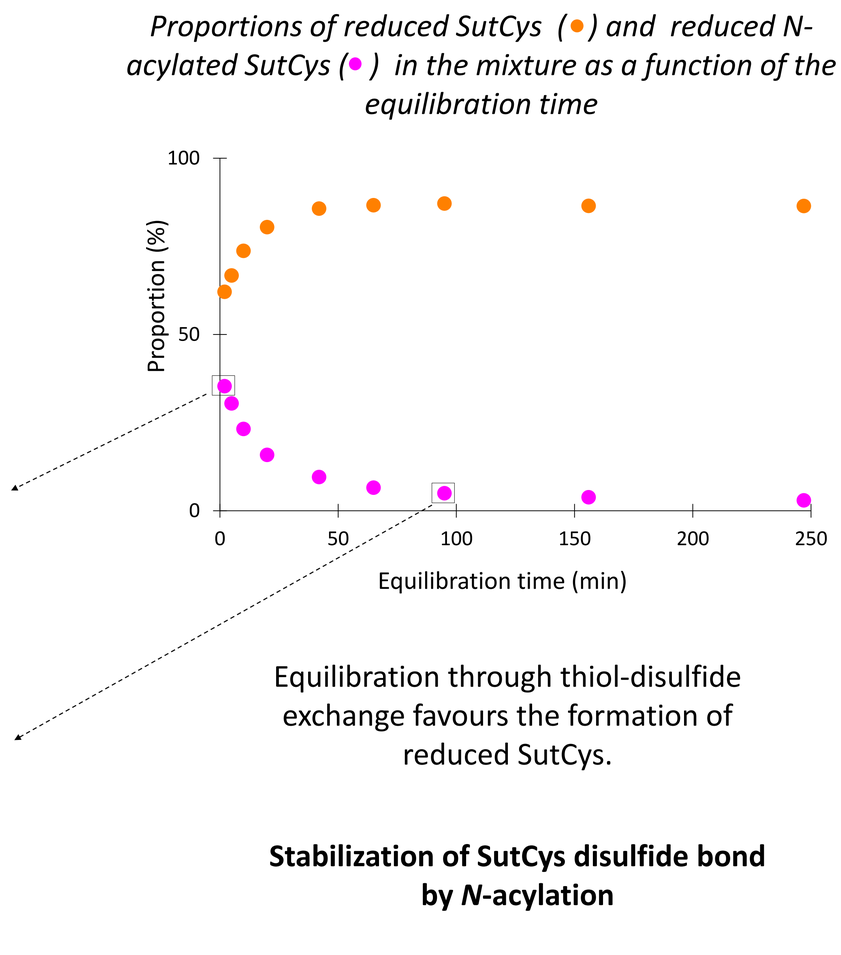


N-Acylation locks SutCys ring-opening

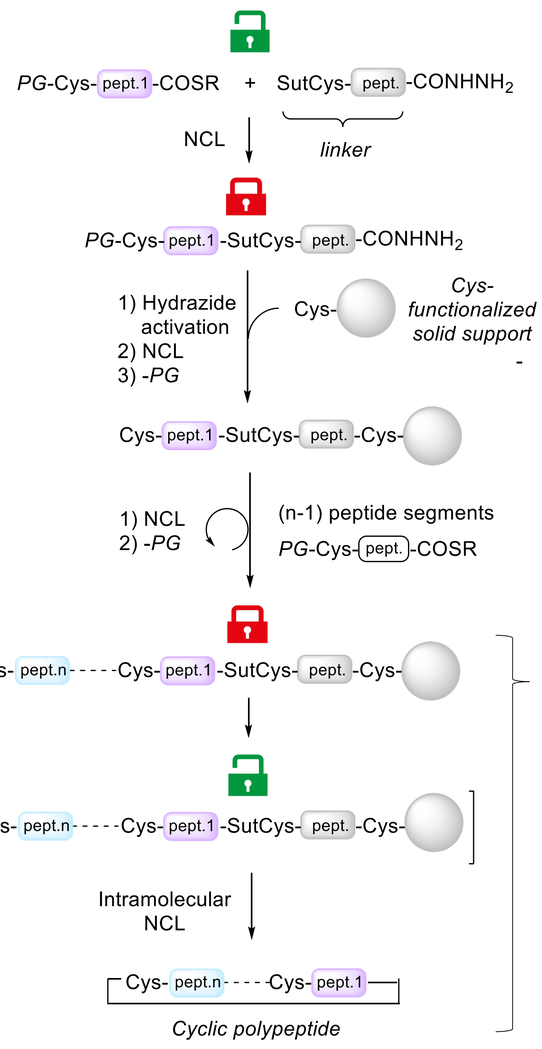
To compare the redox properties of SuCys peptides with their *N*-acylated derivatives, a 1:1 mixture of two model compounds was partially reduced using *tris*(2-carboxyethyl)phosphine (TCEP). To monitor the equilibration between the reduced and oxidised SutCys species present in solution, the reduced forms of SutCys were trapped by thiol alkylation with an excess of iodoacetamide prior to HPLC analysis.







SutCys as a novel linker for the assembly of cyclic polypeptides on solid support



① **Linker pre-assembly by *N*-acylation** locks SutCys in the presence of MPAA

NCL: MPAA, pH 7.2

② **Connection to the solid support** of the linker and first peptide segment

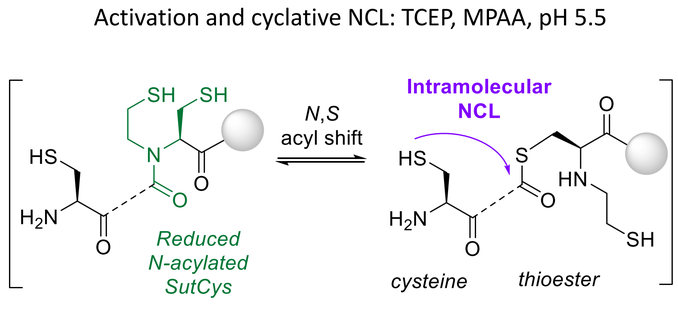
NCL: MPAA, pH 7.2

③ **Elongation**

NCL: MPAA, pH 7.2

④ **SutCys activation, cyclisation and detachment** from the solid support

Activation and cyclative NCL: TCEP, MPAA, pH 5.5



Reducing power

MPAA

mild reductant & NCL catalyst

\+

TCEP

strong reductant

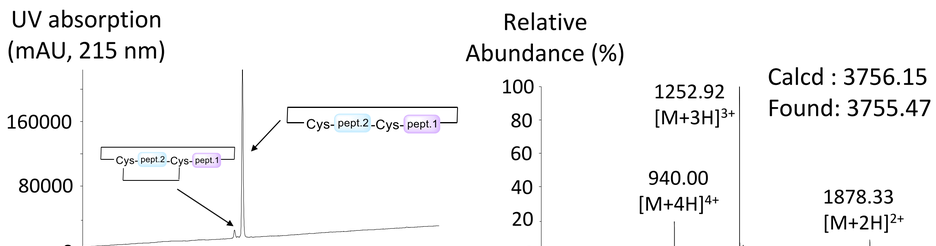
\+++

The synthesis of the target cyclic polypeptides on the solid support relies on MPAA-catalyzed NCL reactions[2-5] and uses *N*-peptidyl SutCys as a linker.

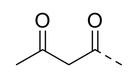
In this process:

- SutCys is acylated in solution by NCL leading to the pre-assembly of a linker that is stable under the reductive conditions imposed by the MPAA catalyst.
- *N*-peptidyl SutCys is silent during the solid phase attachment and elongation of the peptide sequence by a series of NCL reactions.
- *N*-peptidyl SutCys is activated by reduction of the S-S bond with TCEP and acts as a an acyl donor[6] during the final NCL leading to the cyclization and the detachment of the polypeptide.

LC-MS analysis of the purified cyclic polypeptide assembled from 2 peptide segments (n = 2)



16%, 5 steps (69% per step) from the solid support

PG = 

-*PG* = NH_2OH, pH 4.0

pept.1 PDTRPAPGSTAPPAHGVTA
pept.2 PDTRPAPGSTGPPAHGVTA
pept. ALKEPVHGA

Conclusion

N-Acylation of SutCys by NCL stabilizes the disulfide bond of this cysteine derivative and leads to the formation of a thioester surrogate that is latent under mild reducing conditions classically used to concatenate peptide segments by NCL. Illustrated with the synthesis of cyclic peptide sequences on solid support, this concept opens the way towards the design of novel synthetic strategies in chemical protein synthesis.[7-8]

References

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