Biochemistry

An overlooked protein crosslink

Deborah Fass & Sergey N. Semenov

Molecular crosslinks known as disulfides stabilize the 3D structures of many proteins, and sometimes regulate protein function. But disulfides are not alone — another type of regulatory protein crosslink has been discovered. **See p.460**

The characteristic shapes and functions of proteins depend on the order in which the constituent amino acids are linked into chains. However, further chemical modifications are often made after the amino acids are strung together. These modifications include crosslinks between certain amino-acid residues. The most common type of crosslink is a disulfide: two sulfur atoms connected by a covalent bond. On page 460, Wensien *et al.*[1] report an entirely different type of protein crosslink, in which an oxygen atom connects a nitrogen to a sulfur atom. Moreover, the authors present evidence that such N–O–S bridges have gone unnoticed in previously reported structural analyses of other proteins.

Wensien and colleagues were studying the enzyme transaldolase from *Neisseria gonorrhoeae*, a bacterium that causes the eponymous sexually transmitted disease. They observed that the purified enzyme was almost inactive, but that activity could be restored by using reducing agents commonly used to break disulfide bonds.

To form a disulfide, the side chains of two cysteine amino-acid residues — which can be far apart along the protein chain — must come close together in space. Noticing that the transaldolase contains a few cysteine residues, the authors reasoned that two of them might form a disulfide that inactivates the enzyme. However, when they replaced each of the cysteines individually with another amino acid, they found that only one of the resulting mutant enzymes resisted inactivation. If the enzyme really did contain an inactivating disulfide, then replacement of either of the two participating cysteines would be expected to have this effect (although there are exceptions to this simple generalization[2]). So what was going on?

Wensien *et al.* solved the puzzle by using X-ray crystallography to determine the structure of the transaldolase at atomic resolution. This analysis revealed a covalent connection between a cysteine and a lysine residue — an N–O–S bridge — instead of a cysteine–cysteine disulfide crosslink (Fig. 1). Intriguingly, the oxygen atom in the N–O–S bridge does not arise from the groups in the side chains of either of those residues (the cysteine side chain contains a thiol (SH) group, whereas the lysine side chain contains an amine (NH_2) group). However, the authors saw an oxygen molecule close to these side chains in the crystal structure of the reduced form of the protein (which lacks the crosslink). This observation supports the authors' reasonable speculation that an oxygen molecule contributes an oxygen atom to the N–O–S bridge.

The discovery of an N–O–S bridge in proteins is noteworthy because non-biological reactions that produce such a molecular motif are not known, with the possible exception of one unusual molecule (a type of cyclic aromatic compound[3]). The formation of an N–O bond in small-molecule chemistry requires strong oxidizing conditions[4], but such conditions would probably also convert sulfur atoms to higher oxidation states than that of the sulfur in the N–O–S bridge. Furthermore, small molecules containing the N–O–S motif might be in danger of undergoing disproportionation — a process in which two of the same molecules react with one another to produce two different products. In the context of a protein, favourable positioning of the cysteine's thiol, the lysine's amine and an oxygen molecule might aid the oxidation needed for N–O–S formation, whereas spatial (steric) constraints imposed by the surrounding protein structure might stabilize the crosslink and block further oxidation of the sulfur.

The authors speculate about possible mechanisms for bridge formation, favouring a reaction in which hydroxyl groups (OH) are added to both the sulfur atom of the cysteine and the amine group of the lysine side chain (see Extended Data Fig. 3b of ref. 1). Given the novelty of the N–O–S crosslink and the focus of the study on structural biology, important details of the chemical mechanism remain to be addressed. Specifically, the way in which the oxygen molecule is activated to take part in this reaction is not described. Many chemical reactions with molecular oxygen involve free radicals[5], and so radical pathways should be explicitly considered in mechanisms for generating the N–O–S bridge.

Another mechanistic issue is how the crosslink affects enzymatic activity. The structure of the catalytic site of the crosslinked transaldolase differs only slightly from that of the non-crosslinked version of the enzyme. How formation of the N–O–S bridge inhibits catalysis is thus not obvious. The authors focused their attention on these

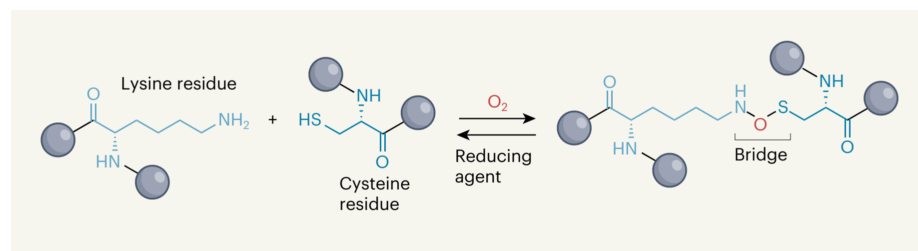

Figure 1 | Formation of a bridge between lysine and cysteine amino-acid residues. Wensien *et al.*[1] report that the transaldolase enzyme of the *Neisseria gonorrhoeae* bacterium contains a molecular crosslink in which an oxygen atom bridges a nitrogen in the side chain of a lysine amino-acid residue and a sulfur atom in the side chain of a cysteine residue. The authors propose that such N–O–S bridges are formed by the reaction of the side chains with an oxygen molecule (O_2). The N–O–S motif is ordinarily unstable, and it is presumably stabilized by the surrounding protein structure. However, it can be cleaved by reducing agents. Spheres represent other segments of the protein molecule.

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minor structural differences, but they also observed that the crosslinked transaldolase is more resistant to heat-induced unfolding than is the non-crosslinked version. This result is not surprising and implies that the crosslinked enzyme undergoes fewer conformational fluctuations that could lead to unfolding. Smaller-scale fluctuations could be required for catalytic activity, and might also be restrained by the presence of the crosslink.

There is a key conceptual difference between the use of disulfide bonds for regulating protein function and the use of an N–O–S bridge. The formation of disulfide bonds is chemically reversible, which means that, in biological systems, disulfides are frequently made and broken in 'exchange' reactions with other molecules that contain disulfides or thiols. By contrast, the N–O–S linkage is formed by a different chemistry from that by which it is cleaved – that is, molecular oxygen is used to form the bridge but is not released when the bridge is broken.

Moreover, the thermodynamics of these reactions indicate that it is difficult to form the N–O–S bridge but easy to break it. N–O–S crosslinks therefore might have evolved to enable the selective activation of an enzyme under conditions in which disulfides are preserved. The particular advantages conferred by the N–O–S bridge, and the biological scenarios in which it is more useful than a disulfide, can now be explored.

The discovery of a new protein linkage has implications beyond the specifics of the enzyme studied and of the N–O–S crosslink itself. Perhaps surprisingly, the task of generating structural models for proteins is sometimes more difficult when high-resolution X-ray data are available. Variations in protein conformation or in chemical composition might be buried in the noise of the data at low resolution, but this heterogeneity becomes visible at high resolution and must therefore be interpreted[6]. Unanticipated chemical groups might also be lurking in the data. Wensien and colleagues' study will inspire structural biologists to investigate deviations from expectation in their electron-density maps of biomolecules.

It has long been appreciated that enzymes are the world's best organic chemists, because they can promote reactions that would be almost impossible in their absence. The findings show that the covalent chemistry of enzymes themselves can also defy chemical intuition.

Deborah Fass is in the Department of Chemical and Structural Biology, Weizmann Institute of Science, Rehovot 7610001, Israel. **Sergey N. Semenov** is in the Department of Molecular Chemistry and Materials Science, Weizmann Institute of Science.
e-mails: deborah.fass@weizmann.ac.il ; sergey.semenov@weizmann.ac.il

1. Wensien, M. *et al. Nature* **593**, 460–464 (2021).
2. Mor-Cohen, R. *Antioxid. Redox. Signal.* **24**, 16–31 (2016).
3. Hassan, A., Ibrahim, Y. R. & Shawky, A. M. *J. Sulfur Chem.* **28**, 211–222 (2007).
4. Challis, B. C. & Butler, A. R. in *The Chemistry of the Amino Group* (ed. Patai, S.) Ch. 6 (Interscience, 1968).
5. Sawyer, D. T. *Oxygen Chemistry* Ch. 5–6 (Oxford Univ. Press, 1991).
6. Lang, P. T. *et al. Protein Sci.* **19**, 1420–1431 (2010).

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Chemical physics

The quantum mechanism of an ultracold reaction

Nandini Mukherjee

Quantum chemistry is challenging to model computationally. An ultracold chemical reaction has now been used to test models with great precision, providing a benchmark for future quantum-chemistry calculations. **See p.379**

Researchers have long dreamt of following chemical reactions from a single quantum state of the reactant molecules to all the possible quantum states of the products, to understand the quantum dynamics that drive chemistry at the most fundamental level. The invaluable data obtained by such an experiment could be compared directly with theoretical results to work out the quantum mechanism of reactions. On page 379, Liu *et al.*[1] report that this dream has come true for a reaction of ultracold diatomic molecules consisting of one potassium atom and one rubidium atom (KRb). By mapping the statistical distribution of all 57 of the possible quantum states of the reaction products, the authors were able to establish the validity of quantum-statistical models of reactions.

In the past few years, it has become possible to prepare dense samples of ultracold diatomic molecules of the alkali metals (those in group 1 of the periodic table) in their absolute ground states[2,3]. This means that the molecules are in their lowest electronic, vibrational and rotational energy states, and the translational kinetic energy of each molecule – the energy associated with the movement of the molecule's centre of mass through space – is vanishingly small (less than 1 microkelvin, or about 10^{-10} electronvolts).

The ability to prepare such molecules has opened up the possibility of studying chemical reactions in which the reactants start in a single internal quantum state (that is, they have identical electronic, vibrational and rotational states) and collide with a translational energy of less than 10^{-10} eV. Such reactions are an ideal test bed for validating quantum-dynamical models of reactions. Once validated and benchmarked, quantum-dynamical calculations could be used to model more-complex reactions. The reaction in which two KRb molecules exchange atoms to form K_2 and Rb_2 is particularly suitable for this purpose, because it is exoergic – the reaction releases energy. This implies that the reaction can proceed when the reactants are in their absolute ground state and have a near-zero translational kinetic energy.

The energy released when two molecules collide and react is partitioned between the various internal energy states and the translational motion of the products. The challenge in computational chemistry is to determine the reaction pathways that connect the initial reactant state to a specific product state, through a complex intermediate state. The probabilities with which product molecules in different quantum states are formed from a single reactant state must therefore be determined experimentally.

But, until the past few years, ultracold reactions were characterized only by the rate at which reactant molecules are lost from a trap as a result of scattering from reactive and non-reactive collisions[4]. The states of the product molecules were not characterized, and so the precise reaction pathways involved were obscured. Measuring the distribution of products between all the possible quantum states that can be produced in an ultracold reaction has remained an unresolved challenge.

A mass-spectrometry technique that can selectively analyse molecules in particular quantum states and map their velocities has been extensively developed for experiments with beams of molecules[5,6]. A team of researchers, including some of the authors of the current paper, previously adapted this technique to study KRb molecules in an ultracold reaction vessel[7]. However, the small sample size and large number of possible product states

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