Controlling radical reactions

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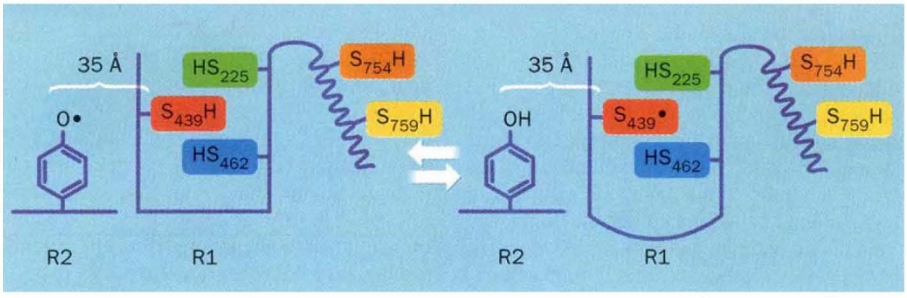
FREE radicals let loose in the cell can perpetrate all kinds of damage, including mutagenesis and molecular degradation that may manifest as disease. Could it be a coincidence that an important step in DNA biosynthesis, namely the conversion of ribonucleotides to deoxyribonucleotides by ribonucleotide reductases (RNRs), involves stable amino-acid radicals as well as transient amino-acid and nucleotide radical intermediates (ref. 1)?



Escherichia coli RNR is composed of two subunits, R1 and R2. The unusual dinuclear iron centre and tyrosyl radical cofactor reside on R2. The binding site for the ribonucleotide substrates resides on R1, as do the five cysteines that are postulated to be essential for deoxyribonucleotide formation. Communication between the two subunits is essential for catalytic activity and is thought to involve long-range electron and proton transfers.

In the RNR enzyme from *Escherichia coli*, which has two subunits R1 and R2, the subunit R2 has a stable organic radical on a tyrosine residue adjacent to a dinuclear iron centre[2] which is essential for nucleotide reduction: a one-electron reduction of the tyrosyl radical to tyrosine destroys all catalytic activity. The stability of this tyrosyl radical in subunit R2 is evidenced by its half-life of days — tyrosyl radicals in solution last only for a matter of microseconds. The actual nucleotide reduction has been proposed to occur on the R1 subunit (the 'business' end of RNR), with the R2 tyrosyl radical acting as a radical chain initiator and communication between subunits being effected by an electron transfer process (see figure)[1,3]. Now this hypothesis is given added weight by the newly determined X-ray crystallographic structure at 2.5 Å resolution of the R1 subunit from *E. coli*, described on page 533 of this issue[4].

The RNR from *E. coli* is a prototype of the mammalian and the herpes viral enzymes[5] and was the first protein found to contain a stable organic radical[2]. Experiments comparing the action of wild-type RNR enzyme on substrate analogues (mechanism-based inhibitors)[5] and using site-directed mutant protein analogues[6–9] have indicated that there are three cysteines located in the active site of R1. Two cysteines at positions 225 and 462 directly provide the reducing equivalents required for nucleoside diphosphate reduction and are themselves oxidized to a disulphide[7,10]. A third cysteine at position 439 was postulated to be transiently converted into a thiyl radical which then initiates nucleotide reduction by abstracting the 3′ hydrogen atom of the substrate[8]. This thiyl radical on R1 may be the partner in the electron transfer to the tyrosyl radical of R2.

The structure determined by Uhlin and Eklund[4] positions cysteines 225 and 462 within 6 Å of a third cysteine at residue 439. These cysteines are detected in the structure as a disulphide, which is consistent with the role just discussed, fulfilling the expectations of biochemical studies. Also, the structure reveals that glutamate 441, not previously identified, is poised to participate as a general acid/base catalyst in the mechanism of reduction.

Unfortunately, neither substrate nor allosteric effector have been co-crystallized in the reported R1 structure. The conformational flexibility of this protein, demonstrated by its ability to reduce purines and pyrimidines only in the presence of the appropriate allosteric effector, suggests that additional groups may be important in catalysis after structural reorganization.

Two additional cysteines have been proposed to play a part in nucleotide reduction, by transferring reducing equivalents provided by the protein reductant thioredoxin (or glutaredoxin) into the active-site disulphide by disulphide interchange (see figure)[6,9,11]. These cysteines (at positions 754 and 759) are not resolved in the X-ray structure because they are in the C-terminal 24 amino acids of R1, which are thermally labile. The flexibility of this tail provides a reasonable explanation for how dithiothreitol, a small organic reductant, can bypass the thioredoxin redox shuttle and provide reducing equivalents directly to the active-site cysteines at 225 and 462. The structure of R1 therefore nicely complements earlier results and offers clues through the discovery of glutamate 441 and of the flexibility of the C-terminal cysteines as to how this complex protein might function.

How has this structure affected our thinking on the nucleotide reduction process? As indicated in the figure, the tyrosyl radical on the R2 subunit of *E. coli* RNR was presumed to generate a thiyl radical at cysteine 439 on R1 as a result of long-range electron transfer. The X-ray structure, however, suggests that cysteine 439 is protonated within the active site. Its environment does not appear to have residues at a position or secondary structure appropriate to perturb its pK_a, which is necessary to generate a thiolate essential for electron transfer. The structure therefore suggests that an alternative mechanism should be considered for generating the thiyl radical: hydrogen atom abstraction (or the equivalent, a coupled electron and proton transfer). The structure of R1 and docking experiments with R2 suggested to the authors[4] that tyrosines 730 and 731 play a key role in this communication, over a distance of some 35 Å, between the active site of R1 and the tyrosyl radical on R2, a pathway that might involve electron hopping between aromatic amino-acid residues.

This structure will have an important impact in three ways: first, the confirmation that cysteine 439 resides in the active site of R1 will stimulate intense effort to demonstrate the existence of transient thiyl radicals, which are unprecedented in catalysis; second, the tentative location of one of the allosteric binding sites adjacent to the active site will encourage investigation of the basis of substrate specificity and the role of allosteric effectors; third, the structure will help clarify how the two subunits communicate. The R1 structure adds an additional piece to the complex puzzle that should eventually explain how this amazing protein can tame highly reactive radical intermediates and keep control of the substrate specificity essential to the fidelity of DNA replication. □

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