

retains its handedness in solution, although the mirror-image molecules do interconvert slowly when the solution is basic. For this reason, a base was required, as well as glass beads, to obtain single-handed crystals in the new system. But for the conversion to work, a seemingly unlikely condition must prevail: the concentration of the dissolved molecules from the minor population of crystals must be higher than that of the dissolved molecules from the major population of crystals.

The mechanism underlying this remarkable behaviour is unknown. Noorduin *et al.*¹ propose that 'Ostwald ripening' — the growth of large crystals at the expense of smaller ones — might be a crucial factor. Alternatively, a theory⁸ proposed to explain the conversion of sodium chlorate crystals to a single-handed form might also apply here. This theory concerns tiny crystal fragments chipped from the larger, growing crystals — fragments that are small enough to be subcritical (and thus expected to dissolve), but large enough to retain their handedness. Chipping off such fragments artificially accelerates the dissolution of the larger crystals. But the acceleration is reduced if the fragments are rescued from dissolving by merging with larger crystals of the same hand.

If this explanation is correct, then subcritical fragments that can be incorporated into the dominant population of crystals have more chance of being saved from dissolving than their mirror images (for which fewer rescuing crystals exist). For the dominant crystals, this selective reincorporation is particularly effective at reducing the portion of their dissolution that is induced by grinding. Abrasion therefore speeds up the net dissolution of the minor population of crystals; once dissolved, their molecules convert into their mirror-image form, thereby feeding the growth of the major population of crystals. This process also satisfies the curious requirement for the concentration of molecules dissolved from the smaller component of the solid to be greater than that of molecules from the larger component.

Crystals from the smaller population eventually convert completely into the same form as the larger population, in a process that actually accelerates as the conversion proceeds (unlike most spontaneous transformations). The conversion is entropically unfavourable, but grinding supplies the energy needed to overcome this obstacle.

Were Frank alive today, he would be delighted by these observations, because the crystallization fits his model for amplification of a single-handed entity: the major crystals catalyse their own production by capturing fragments destined for dissolution; and they catalyse the destruction of their mirror image by capturing more molecules from solution than they contribute to it.

It is impossible to say whether grinding of crystals in a prebiotic world, perhaps

by wave-tossed sand, might have supplied single-handed molecules to support early life. Nevertheless, Noorduin and colleagues¹ might have provided dramatic evidence to support the idea that substantial clusters of molecules can be incorporated intact into existing crystals. Their report suggests new opportunities for manufacturers of pigments or pharmaceuticals who must prepare solids in only one of several possible crystal forms. Furthermore, grinding is arguably the first original method for isolating single-handed crystals from a mixture of mirror-image forms since Pasteur used tweezers to effect such a separation in 1848. ■

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NITROGEN CYCLE

Out of reach

Sybil Seitzinger

Denitrifying bacteria and hungry plants do sterling work in disposing of the nitrates that we pump into rivers and streams. But as the excess influx goes up and up, the efficiency of removal goes down and down.

Thanks to human contributions — from fossil-fuel combustion, the growth of agricultural crops, and above all the fertilizers that help to keep the more than six billion humans worldwide in food¹ — nitrogen is entering Earth's soils at more than twice its natural rate. Much of the nitrogen from these sources goes on to enter streams, primarily as nitrates, and is transported downriver to coastal marine systems. There, it fuels excessive rates of plant growth and decay in the process known as eutrophication. This can result in hypoxia (shortage of oxygen) or, in extreme cases, anoxia (total loss of oxygen), creating the 'dead zones' seen in places such as Chesapeake Bay, the Gulf of Mexico and the Baltic Sea.

Fortunately, as Mulholland and colleagues detail on page 202 of this issue², not all the extra nitrates that we pile into streams make it to coastal systems; instead, some is removed and retained by biological processes. In the short term, aquatic plants and microbes take up and store nitrogen as a nutrient. But by far the greatest part in long-term nitrogen removal is played by the denitrifying bacteria that live in anoxic sediments in a stream's bed and banks. These microbes transform the nitrogen in nitrates to gaseous forms such as molecular nitrogen (N₂) and nitrous oxide (N₂O), which diffuse out of river water into the air. Such denitrification is not without environmental consequences: whereas N₂ is harmless (and makes up almost 80% of the atmosphere), N₂O, although only a small portion of the total gaseous nitrogen produced, is a

potent greenhouse gas that has also been implicated in stratospheric ozone destruction.

Because many different methods have been used to measure rates of denitrification, comparison of the many measurements that have been made is difficult. In addition, the physical and chemical characteristics of rivers that might affect nitrogen removal are extremely diverse, depending in part on the type and extent of human disturbance in the surrounding landscape. Developing predictive, widely applicable relationships linking river and watershed characteristics to nitrogen removal and retention rates is quite a challenge³.

Mulholland *et al.*² attacked these shortcomings with a study of unparalleled scale. They used a consistent method to quantify the fate of nitrate (NO₃⁻) in 72 small streams in pristine, urban and agricultural watersheds throughout the United States and Puerto Rico, adding trace amounts of nitrates labelled with the nitrogen isotope ¹⁵N to stream waters over periods of around 24 hours during spring or summer. The stable (non-radioactive) isotope ¹⁵N is rare in nature, occurring at less than 0.4% of the abundance of ¹⁴N. Using mass spectrometry, it was relatively easy to detect small decreases in ¹⁵NO₃⁻, and increases in ¹⁵N₂ and ¹⁵N₂O from denitrification. Decreases in labelled nitrates not accounted for by an increase in labelled N₂ and N₂O were attributed to uptake by plants and microbes within each stream segment, or reach. This approach maintained existing conditions in the stream, and not only permitted

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the authors to distinguish denitrification from the temporary storage of nitrogen in biomass, but also allowed the rates of denitrification and biotic uptake to be assessed for the entire reach.

The authors found that measurable rates of nitrate removal occurred through biotic uptake and denitrification in most streams, and that absolute rates of removal generally increased as nitrate concentrations increased. But the efficiency of nitrate removal — the proportion of nitrate removed relative to the total amount present — decreased exponentially as nitrate concentrations increased. This pattern held across nitrate concentrations that differed by six orders of magnitude in eight different biomes. As we use land ever more intensively, the effectiveness of the receiving streams in removing the additional nitrate pollution is likely to continue to diminish. Thus, looking at the results of this study, the pressure on policy-makers to reduce the load of anthropogenic nitrogen entering rivers from terrestrial systems will grow.

Of course, small streams are only one component of the filigree of flowing waters that drain a landscape. Small streams connect to middling channels, which connect to larger rivers, all of which propel water and its dissolved and particulate constituents towards coastal systems. The length of all the channels in a river system is often extensive — those within the 30,000 km² watershed of the Potomac River in the eastern United States amount to a length of some 25,000 km, equivalent to about five times the distance across the United States from coast to coast. Unsurprisingly, therefore, there are ample opportunities for nitrates to be removed on their journey from a river's headwaters to the coast that are not covered by Mulholland and colleagues' study.

To address this point, the researchers use a model to scale up their results to an entire river network. The outcome underscores the importance of river channels of all sizes, as well as the distribution of nitrate loading within the river network, in controlling the amount of nitrates reaching coastal waters. Nitrates not removed within a particular stream reach may be removed in the next, and so on down to the coast; but the relative importance of small, medium and large channels depends in part on where the nitrate concentration in a particular stream reach is on the curve of nitrate-removal efficiency. A small stream might remove most of the nitrates quite effectively if the concentration is low; but if the local nitrate concentration increases such that nitrate-removal efficiency decreases, the capacity of downstream reaches to remove excess nitrates becomes relatively more important. And if the nitrate concentration throughout a river network continues to increase, the network's overall capacity to remove nitrates decreases. The importance of nitrogen removal across all channel sizes in a river network underlines the need for comprehensive studies such as that of Mulholland *et*

*al.*² across a range of stream sizes and throughout the annual cycle.

Denitrification occurs not only in rivers, but in almost all environments at some time and place: nearly 80% of all reactive nitrogen is disposed of before it reaches coastal waters. Soils are generally the first receptor of the large amounts of nitrogen that we add to terrestrial systems, and the amount of denitrification in soils can substantially exceed that removed in the stream network⁴. Groundwater, lakes and coastal systems are also important sites of denitrification. For all these environments we urgently need advances in methods and models⁵ that will increase our understanding

of how large amounts of nitrogen move and are removed as they journey from soils to the sea. ■

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BIOCHEMISTRY

Radicals by reduction

Joseph T. Jarrett

Many enzymes convert their substrates into organic radicals to allow challenging reactions to occur. A microbial enzyme does so by simple electron transfer, casting fresh light on enzyme evolution.

The harmful effects of free radicals have been widely publicized over the past two decades. As a result, pharmacists are now well stocked with vitamins and nutritional supplements purported to combat the damage caused by these chemical bogeymen. Less attention has been given to the many beneficial uses of radicals in biology, where they are often generated by enzymes to overcome chemically difficult problems. On page 239, Kim *et al.*¹ report a remarkable example of this in certain anaerobic bacteria found in the human gut. These bacteria generate energy by breaking down the amino acid leucine; this is a particularly tough chemical process in the absence of oxygen. The authors show that a key enzyme for this process overcomes the reaction barriers by sequentially converting leucine into two different radicals. This previously unknown mechanism might also be used by many other enzymes.

Most stable covalent molecules, with rare exceptions, require electrons with opposite spin to be paired within each atomic and molecular orbital. But in an organic radical, one electron remains unpaired — an unstable configuration that is highly reactive. When radicals are generated within an organism and allowed to roam free, they can react rapidly with DNA, proteins and lipids, causing chemical modifications collectively known as oxidative damage. In contrast, when organic radicals are generated within an enzyme active site, their reactivity can be harnessed to promote a variety of essential biochemical transformations.

The role of organic radicals in several enzyme-catalysed reactions has been recognized only relatively recently². The first suggestion that

enzymes might use transient radicals as intermediates came with the discovery of vitamin B₁₂. The active cofactor form of this vitamin contains a weak carbon–cobalt bond that is broken to generate a carbon radical³; this radical transiently oxidizes other molecules by removing a hydrogen atom, a process that can often promote difficult chemical reactions (Fig. 1a, overleaf).

Around the same time as the discovery of vitamin B₁₂, organic radicals were shown to be essential for the activity of ribonucleotide reductase, an enzyme that controls the cellular concentrations of deoxyribonucleotides (the building-blocks of DNA)⁴. The active form of ribonucleotide reductase has a radical on the side chain of a tyrosine amino acid (Fig. 1b). This radical forms when the tyrosine side chain is oxidized, in a process that requires oxygen and two iron ions (which make the oxygen more reactive and provide a source of electrons for the reaction). Perhaps surprisingly, given that radicals usually have a fleeting existence, the tyrosyl radical is so stable that it can be observed for days.

Many more enzymes are now known to exploit radical reactions using vitamin B₁₂, S-adenosylmethionine (a common coenzyme) or various metal cofactors to turn organic compounds into radicals, usually by removing a hydrogen atom directly from the substrate. Organic radicals have thus gained acceptance as plausible reaction intermediates for biochemical reactions, with both direct and indirect evidence from dozens of enzymatic processes supporting this idea. Many of these enzymes are found in anaerobic bacteria, where they take part in the catabolic pathways