some of which are toxic, such as yellow-bellied toads (*Bombina variegata*), California newts (*Taricha torosa*) and poison dart frogs (Dendrobatidae family), the authors mapped a phylogenetic tree of species that had a range of anti-predator characteristics: fully cryptic species; fully conspicuous species; species with conspicuous coloration present as small patches on their ventral surfaces (PV); species with a fully conspicuous ventral surface (FV); and species that had both cryptic and conspicuous forms (polymorphic species). Using two data sets, a large one that lacked information on chemical defences, and a smaller one that included them, the authors uncovered a plethora of evolutionary relationships. What seems to be key to the origin of aposematism is that amphibians with hidden colour signals, specifically the chemically defended FV state, are probably the most important evolutionary precursor of the aposematic conspicuous defended state.

In turn, the FV defended state arises from the PV defended state or the undefended FV state, and these FV species themselves evolve from the undefended PV state. The authors report that around 90% of the conspicuous, FV conspicuous or polymorphic species that they analysed are chemically defended, and this is also true for a good proportion of PV conspicuous and cryptic species. This indicates that amphibians are honest signallers rather than species that 'cheat' by mimicking warning colours in the absence of defences. The authors also found evidence that aposematic species can evolve back to cryptic or polymorphic species, mirroring the surprising evolutionary flexibility seen in transitions between mimicry and crypticity in coral snakes[11], an observation that questions the idea that stable evolutionary end points exist.

Remarkably, scientists already knew about mix-and-match cryptic–conspicuous forms of protective coloration in three other contexts. One of these is deimatism, in which cryptic prey briefly flash a hidden conspicuous patch to cause the predator to hesitate — such prey might or might not have chemical defences[12]. Another is flash coloration, whereby prey expose conspicuous patches while fleeing but hide them as soon as they come to rest, causing the predator to search for an inappropriate object[13]. The third is distance-dependent camouflage, in which defended prey are cryptic far off but conspicuous up close[14].

However, none of these three examples was used to solve the aposematism paradox until now. With a new solution at hand, namely that aposematism can evolve without loss of crypsis, it is essential to examine how widespread the phenomenon is by investigating other groups of species with 'dangerous' reputations, such as sea slugs and snakes. Once again, Wallace has led the way, and we mortals simply follow on behind.

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Bioinorganic chemistry

Protein discerns between rare-earth elements

Scott Banta

A protein has been discovered that binds to the lighter members of the rare-earth family of metals more strongly than to the heavier ones — an amazing feat, given the chemical similarities of these elements. **See p.87**

The rare-earth elements (REEs) are indispensable for emerging technologies, yet their chemical similarities make them notoriously difficult to separate from each other. Some specialized microorganisms require REEs for growth, and have evolved biological machinery to process them[1]. On page 87, Mattocks *et al.*[2] report their discovery of a rare-earth-binding protein that shows unrivalled selectivity in its affinity for members of this family of elements. The finding paves the way to the development of bioseparation strategies for isolating individual REEs.

The 17 REEs consist of the lanthanide group (lanthanum to lutetium in the periodic table), as well as scandium and yttrium. The distinctive properties of these elements make them essential for many modern applications, including magnets, batteries, electronics and catalysts[3], and global demand is therefore expected to increase. The lanthanide elements predominantly form ions that carry three positive charges and have similar radii, which decrease with increasing atomic weight. The chemical similarities of the REEs cause them to co-localize in geological deposits, and also complicate their separation from each other. Industrial REE separations are challenging: organic molecules (ligands) are reacted with mixtures of REE ions in acid solution to form complexes, which are then subjected to multiple extraction steps in which the complexes of specific REEs are transferred selectively to a suitable organic solvent[4].

The term 'rare' can be a misnomer for REEs, because most of these elements (other than radioactive promethium) are more abundant in Earth's upper continental crust than is silver (see go.nature.com/3mkkhug). Indeed, lanthanum, neodymium and cerium are about as abundant as copper and nickel, which are not regarded as rare. But REEs can certainly be thought of as rare in the context of proteins — almost half of the protein structures in the Protein Data Bank contain metals, and very few of these are REEs.

However, over the past 12 years or so, REEs have been found to be essential for the biochemistry of bacteria known as methylotrophs[1]. These organisms can use organic compounds that contain just one carbon atom as carbon sources for growth — a talent that requires special metabolic capabilities. The incorporation of REEs into methylotroph proteins probably provides catalytic advantages that aid this distinctive biochemistry, to the extent that some methylotrophs are incapable of growth in the absence of REEs[5]. Investigation of the metabolic capabilities of methylotrophs led to the discovery of the protein lanmodulin in the bacterium *Methylobacterium extorquens*[6]; the protein is probably involved in regulating REE concentrations in this organism.

Lanmodulin is small, consisting of about 112 amino-acid residues, and is unstructured in the absence of REEs. It contains four EF-hand motifs — the amino-acid sequences that are responsible for the binding of calcium ions in many proteins. Workers from the

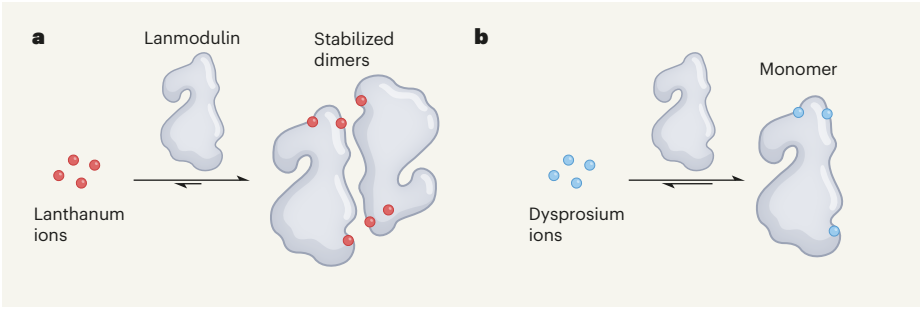

Figure 1 | Dimerization of a lanmodulin protein determines its affinity for rare-earth elements. Mattocks *et al.*[2] report a bacterial lanmodulin protein that has about 40-fold higher affinity for ions of lanthanum (La^{3+}), a light rare-earth element, than for those of dysprosium (Dy^{3+}), a heavy rare-earth element. The difference in binding affinity depends on whether the protein forms dimeric complexes with the ions. **a**, Binding of La^{3+} favours the formation of the dimeric complex, rather than the monomeric one. Dimer formation stabilizes the protein–metal binding interactions, reducing the rate at which ions are released from the dimer (the off rate, indicated by the lower arrow in the equilibrium symbol) and increasing the binding affinity of lanmodulin for the metal ions. **b**, Binding to Dy^{3+} favours the formation of a monomeric complex, which has a higher off rate than in **a**, and a lower affinity for the metal ions.

same research group as Mattocks *et al.* have previously determined[7] the 3D structure of *M. extorquens* (*Mex*) lanmodulin in complex with yttrium ions using nuclear magnetic resonance (NMR) spectroscopy. This revealed that, in the presence of REEs, lanmodulin folds into a bundle of three α-helices, with pairs of EF hands located at the ends of the bundle structure.

Calcium-binding proteins have been characterized extensively, and it is known that the calcium ion can be bound (coordinated) by seven or eight oxygen atoms in the EF hands[8]. One of the oxygen atoms is in a water molecule, and the others come from the protein (mostly from acid groups in amino-acid side chains). These interactions are generally monodentate – only one oxygen binds per side chain or molecule. By contrast, there was still some uncertainty about the precise structure and geometries of the metal-binding sites in *Mex* lanmodulin, because the previously reported NMR structure does not reveal all of the molecular details of these sites.

Mattocks and colleagues now report a crystal structure of the neodymium complex of *Mex* lanmodulin, thereby providing a detailed view of how this protein binds to REEs. In the high-affinity metal-binding sites in the structure, neodymium is coordinated by nine oxygen atoms from the protein and by two water molecules. Five of the interactions with the protein, and both of those with the water molecules, are monodentate, but one amino-acid residue makes a bidentate interaction (it binds through two oxygen atoms).

The increased number of coordinating oxygens, compared with the number observed in calcium-binding proteins, along with other structural features of *Mex* lanmodulin, lead to a remarkable REE-binding capability. The protein has an approximately 10^8-fold preference for REEs over calcium, and the binding affinities for the REEs are exceptionally high – as measured by a quantity known as the apparent dissociation constant, which ranges from about 5 picomolar for the light REEs up to about 25 pM for the heavy REEs[6]; smaller constants indicate higher affinities.

The authors also report the discovery of a lanmodulin from the methylotroph *Hansschlegelia quercus*, isolated from buds of the English oak tree. Known as *Hans* lanmodulin for short, it was identified using a bioinformatics approach in which databases of protein amino-acid sequences were searched for the characteristic clusters of sequences found in lanmodulins.

Similar to *Mex* lanmodulin, *Hans* lanmodulin folds in the presence of REEs, but it has a lower affinity for the lighter REEs (the apparent dissociation constants range from 70 to 90 pM) and a more striking difference in affinity for the heavy REEs (an almost 40-fold higher apparent affinity for the light REE lanthanum over the heavy REE dysprosium). Mattocks *et al.* find that this selectivity correlates with the protein's ability to form dimers when it binds to REEs: the propensity for dimerization decreases with decreasing ionic radius of the REE (that is, as the atomic mass of the REE increases).

The authors obtained crystal structures of the complexes of *Hans* lanmodulin with lanthanum and dysprosium ions. The structure of the dimeric lanthanum complex shows that ten oxygen atoms coordinate to the metal ion in each of the three high-affinity REE-binding sites, with all ten belonging to the protein, and that four amino-acid residues are involved in bidentate interactions. In the binding site closest to the interface formed between the monomers of the dimer, the bidentate binding of two residues from one of the monomers is enforced by the presence of an arginine residue (designated Arg 100) from the other monomer. The bonds formed at the interface increase the stability of the dimeric structure. Moreover, the stabilization of the REE binding pockets reduces the rates with which bound light REE ions are released from the complex (the off rates; Fig. 1a).

The structure of the *Hans* lanmodulin complex with the smaller dysprosium ion shows that the distance between Arg 100 and one of the glutamate residues (Glu 91) that forms a bidentate interaction in the lanthanum complex has increased, compared with the distance in the lanthanum complex. This allows Glu 91 to rotate and become monodentate, thereby reducing the number of coordinating oxygen atoms to nine. These structural changes lower the stability of the dimer, explaining why the dysprosium complex favours a monomeric structure and enabling higher off rates for heavy REE ions (Fig. 1b).

Chromatography techniques separate mixtures of molecules by taking advantage of differences in the binding affinities of the molecules for a separation medium. *Mex* lanmodulin has previously been immobilized on beads, which were then used as a medium on which to separate REEs from other metals[9]. Mattocks *et al.* now show that *Hans* lanmodulin can be used in a similar way to separate individual REEs: dysprosium and neodymium ions were separated in a single operation, yielding products with greater than 98% purity and recovering more than 99% of the material from the original mixture.

The use of protein-based reagents in chromatography is practised widely – to purify antibodies[10], for example. But although the costs associated with this approach are justified in the production of biological therapeutic agents, it remains to be seen whether it will be scalable and economically feasible for industrial REE separations[4]. Nevertheless, Mattocks and colleagues' findings provide insight into how the binding sites of proteins could have evolved to differentiate between REEs, and might inspire new biomimetic approaches to these challenging separations.

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