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BIOPHYSICS

Remote wiggling helps cold enzymes work

Mutations introduced far from the active site of an enzyme can cause local unfolding that increases enzyme activity. This finding suggests how organisms that live in the cold can speed up biochemical reactions. SEE LETTER P.324

ASHOK A. DENIZ

The biochemical sciences have tended to focus on processes that take place at 'physiological' temperatures of around 37 °C. But much of Earth's surface is covered with ocean, ice or snow, and is replete with organisms that function at much lower temperatures. Life in these environments requires suitable biological adaptations, for example in the enzymes that maintain the chemical environment of the cell. On page 324, Saavedra *et al.*[1] shed light on a biophysical mechanism for such low-temperature adaptation that operates at the molecular level. Their results show strikingly that protein modifications distant from an enzyme's active site can modulate localized unfolding of the enzyme — effectively, wiggling of parts of the enzyme's structure — that can control several facets of enzyme-reaction mechanisms.

Physical chemists have long known that the rate of chemical reactions depends on temperature, and that reaction rates generally decrease as temperatures drop. This temperature dependence also applies to enzyme-catalysed reactions, raising the intriguing question of how psychrophilic organisms (which live at low temperatures) can maintain their repertoire of enzyme-mediated functions. Related enzymes in psychrophilic organisms and in mesophilic organisms (which live at physiological temperatures) have similar activities — that is, the reactions they catalyse occur at similar rates[2]. For this to occur, the functional parameters of the cold-adapted enzymes must have been tuned to compensate for the lower temperatures.

A clue to how this tuning could occur came from previous observations[3] that psychrophilic enzymes tend to have more surface glycine mutations (in which an amino-acid residue on the protein's surface is replaced by a glycine residue) far from the active site than do similar enzymes in mesophilic organisms. However, the mechanistic details of this phenomenon were poorly understood. Saavedra *et al.* used an enzyme called adenylate kinase to test the mechanism by which such glycine mutations act from a distance to alter enzyme function.

Adenylate kinase catalyses reactions that help to maintain a balance of adenosine phosphates (molecules that act as the energy currency of cells). The authors chose this mesophilic enzyme because it has been used extensively as a model system for investigating enzyme biophysics, biochemistry and folding, including by researchers from the same laboratory as Saavedra and co-workers.

In the present work, the authors tested the previously discussed idea[2] that tuning of entropy — a measure of disorder — is a major driving force in the adaptation of enzymes to low temperatures. They sought to probe the effect of surface glycine mutations, at locations far from the active site, that might change the 'wiggling' (an entropic effect) of the protein without changing its overall folded structure.

There are three domains in adenylate kinase: the CORE domain, which contains much of the active site, and LID and AMPbd, each of which contains part of the active site. The authors studied glycine mutations in both the LID and the AMPbd domains by using a combination of biophysical and structural techniques. These studies included measuring the stability of the enzyme variants and their binding affinity to a mimic of the enzyme's substrate, and a more detailed characterization of the protein states and structural fluctuations by using nuclear magnetic resonance spectroscopy.

Taken together, Saavedra and colleagues' results demonstrate that adenylate kinase exists in at least three different states, and that the mutations change the relative occupancy (stability) of these states. Compared with the

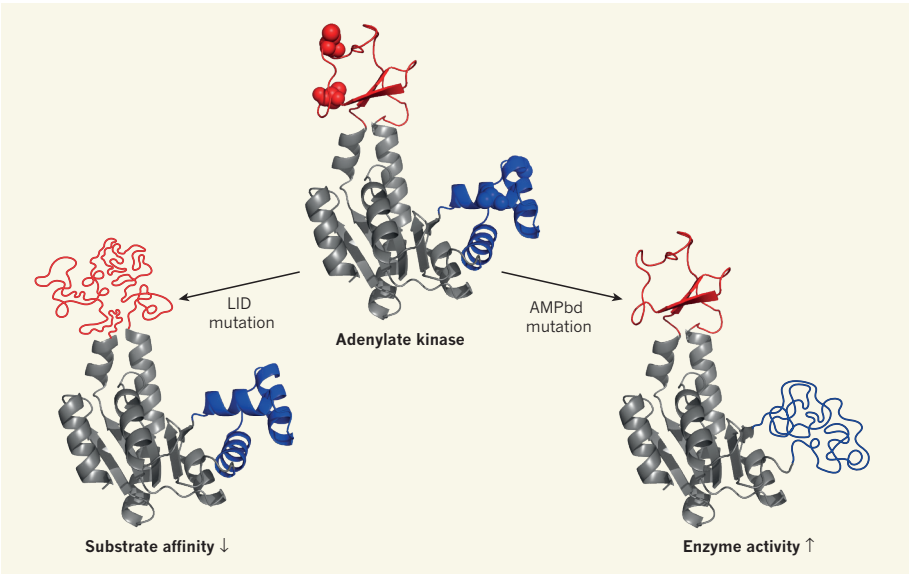

Figure 1 | Entropic tuning of enzyme function allows cold adaptation. Saavedra *et al.*[1] made mutants of the enzyme adenylate kinase, replacing non-glycine amino-acid residues with glycine residues at the surface of either the LID domain (red) or the AMPbd domain (blue). Both types of mutation caused local unfolding of the enzyme, increasing its disorder (entropy), and altered the enzyme's functional behaviour, despite being distant from the active site. The LID mutations decreased the affinity of adenylate kinase for its substrates, whereas the AMPbd mutations increased the enzyme activity. Such entropic tuning of function might be an evolutionary mechanism that allows enzymes to cope with low temperatures, which usually slow enzymatic reactions.

wild-type protein, both the LID and the AMPbd mutants decrease the occupancy of the fully folded structure, but they increase the stability of two different states in which either the LID or the AMPbd domain is locally unfolded (Fig. 1). The increased stability of these locally unfolded states stems from the fact that the footprint of a glycine amino-acid residue is smaller than that of other amino-acid residues, which means that the protein chains in the glycine mutants are more flexible than those in the wild-type protein. Remarkably, these two types of local unfolding alter different aspects of the enzyme's function: LID unfolding decreases its binding affinity, whereas AMPbd unfolding increases its activity.

A particularly interesting aspect of the authors' work is that it helps us to understand how surface glycine mutations act at a distance to support cold adaptation. This phenomenon might seem mysterious at first glance, but it is encapsulated in the idea of allosteric regulation[4,5] — a common form of enzyme regulation in which the binding of a molecular partner at a site distant from the active site affects enzyme activity. The conventional view of allosteric regulation has been that binding of the partner causes small structural changes that propagate through the protein to alter the structure of the active site. However, there is now evidence for mechanisms involving changes in the dynamics (entropy), rather than in the structure, of the unbound state for many cases of allosteric regulation[6–8]. The current work provides striking tests and examples of such entropic allosteric regulation for different enzyme properties.

Saavedra and colleagues' results have substantial implications for the evolution of enzyme function. However, their mechanistic proposal for cold adaptation was tested for just one model enzyme, so its relevance for other cold-adapted enzymes requires further testing. Deeper insight into the biophysical mechanisms of cold adaptation will also benefit from more-detailed views of the structural fluctuations of enzymes afforded by single-molecule experiments[9,10]. Nevertheless, the findings open up new avenues for exploring allosteric control of multiple modes of protein function, both in natural evolution and in rational protein engineering[11] for biotechnology. ■

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CLIMATE CHANGE

How humans and rising seas affect each other

The Paris climate agreement aims to limit global warming to no more than 2 °C. An analysis suggests that this will greatly reduce the risks of sea-level rise for coastal communities, but that it will take time to see the benefit.

AIMÉE SLANGEN

Coastal zones are among the most densely populated areas in the world[1], but they are threatened by rising sea levels caused by climate change. Writing in *Earth's Future*, Brown *et al.*[2] estimate the impact of sea-level rise in terms of the land area and the number of people exposed, for several scenarios in which global warming is limited to different temperature increases. Their study shows that the amount of exposure to sea-level rise depends on our ability to cap global temperature changes, and that the main benefits of this cap will be seen only after 2100.

Brown and colleagues began their investigation by simulating how global temperature will change in response to different scenarios of greenhouse-gas emissions, using a simple computational model of the Earth system[3]. They then calculated the global mean sea-level rise that would occur as a result of the projected temperature changes, assuming that the main causes of sea-level rise are the expansion of the volume of ocean water associated with warming, and the melting of land-based ice (that is, ice over land in glaciers, Antarctica and Greenland). To account for the fact that sea-level rise does not occur uniformly across Earth, they then scaled their time series of global mean sea levels with previously reported projections[4] of regional patterns of sea-level change for 2100.

In the scenario in which global temperature increases are capped at 1.5 °C before 2100, they find a median sea-level rise of 0.4 metres by 2100 and of 1 m by 2300. By contrast, in the scenario in which temperatures continue to increase as they are doing now, sea-level rises are 0.8 m in 2100 and a staggering 4.5 m in 2300. These results show that there will be some sea-level rise regardless of efforts to mitigate climate change, because sea levels will not immediately stop rising when the temperature targets are met. However, the effect of capping global temperatures early will be increasingly felt after 2100 and lead to significantly less sea-level rise by 2300.

In a second step, Brown *et al.* considered the area of land that has an average chance of being flooded once every 100 years (that is, a 1% chance in any given year); such flooding events can be severe (Fig. 1). To do this, they used a database of the world's coastline characteristics[5], which includes land-elevation data measured by radar. The authors combined the data with their scenarios of sea-level rises, and found that an area of 540,000 square kilometres is already at risk of 1-in-100-year coastal flooding events. For the scenario in which the global temperature rise is mitigated to 1.5 °C, this area increases to 620,000 km^2 by 2100, and to 702,000 km^2 by 2300 (values correspond to the 50th percentile of the range of predicted values for sea-level rises). In the absence of mitigation, they find that the area at risk by 2100 (708,000 km^2) is not much different from that in the mitigation scenario, but increases to 1,630,000 km^2 by 2300, which is three times the area at risk today.

In the third component of Brown and colleagues' study, the authors consider the number of people at risk from coastal flooding. If global warming is kept to 1.5 °C, they find that 1.5–2.1% of the global population will be exposed to a 1-in-100-year coastal flooding by 2100, compared with 4.3–5.4% of the global population in the non-mitigation scenario. In other words, more than half of the potential population exposure can be avoided by 2100 if global warming is capped. It is important to keep in mind, however, that population exposure does not depend only on the amount of sea-level rise — the number of people exposed could decrease if people move away from the coast, for example.

Given that sea levels will rise irrespective of future global temperature changes, Brown *et al.* stress that at least some action will need to be taken to adapt. However, their calculations of the land area exposed to sea-level rise do