CHEMICAL BIOLOGY

A pocketful of colour

S. KURIBAYASHI

One of the joys of summer evenings is watching fireflies light up the night sky. These twinkling bugs communicate with one another using flashes of light — a trick biologists have appropriated to convey readouts from their experiments. Various forms of the enzyme responsible for the fireflies' luminescence — luciferase — can produce slightly different colours, ranging from red to yellow to green. Elsewhere in this issue, Toru Nakatsu *et al.* (*Nature* **440,** 372–376; 2006) report several X-ray crystal structures of luciferase from the Japanese Genji-botaru firefly (*Luciola cruciata*, pictured) that explain how small changes in this protein can change the colour of the emitted light.

To create bioluminescence, magnesium, adenosine triphosphate and a small molecule called luciferin react with molecular oxygen. This reaction, which is catalysed by luciferase, generates an electronically excited oxyluciferin species. The yellow-green sparks we see in the night sky are emitted when oxyluciferin relaxes from its excited state to the ground state, losing energy in the form of light.

Single amino-acid changes in the active site of luciferase can alter the colour of the light emitted by the protein, but the chemical mechanism involved has been a mystery. Now Nakatsu *et al.* have obtained a series of 'snapshots' of the reaction — luciferase bound to the reactants (adenosine triphosphate and magnesium); an analogue of one of the reaction intermediates (known as DLSA); and the products (oxyluciferin and adenosine monophosphate).

The structure of luciferin bound to the reactants and the structure of the products are similar, both possessing an active site that is 'open' to the environment surrounding the protein. But when the protein is bound to DLSA, the active site closes: DLSA is tightly packed against several amino-acid side-chains, including isoleucine 288. Analysis of the three structures revealed that the side-chains of isoleucine 288 and serine 286 rotate when DLSA is present, closing the active site and forming a pocket.

The authors thought that the conformation of these amino acids might affect the colour of the light discharged by luciferase. So they solved the X-ray crystal structure of DLSA bound to a mutant luciferase known to emit red light. Their theory was borne out, as the active site of the DLSA-bound mutant protein was open.

The authors propose that the colour of the emitted light depends on the formation of this compact microenvironment during the reaction. In the normal protein, the excited oxyluciferin is held tightly in this pocket, packed against the side-chain of isoleucine, and yellow-green light is emitted. However, if this rigid pocket does not form — for example, in proteins that have mutations at isoleucine 288 or serine 286 — the excited oxyluciferin loses some energy, possibly because it can move around a little, and the protein emits red light, which is lower in energy.

In further work, Nakatsu *et al.* found that many of the other firefly luciferases that emit yellow-green light have either isoleucine or the slightly smaller amino acid valine at position 288. So it seems likely that the movement of the 288 amino acid is a common mechanism for controlling the colour of luciferase bioluminescence.

Joshua Finkelstein

brain elicited new memory deficits when injected into the brains of rats (rather than mice). The rats had learned one spatial task already (the water maze), but then had to learn another with Aβ*56 permeating their forebrain. Learning seemed to proceed rapidly but, when tested a day later, the Aβ*56-treated animals failed to show effective spatial memory. Animals injected with a control solution had no trouble remembering the second task. Aβ*56 did not, however, cause lasting cognitive problems, as both groups of rats were just as good as each other in learning a third task when trained 10 days later (there was no further injection of Aβ*56). These data imply that Aβ*56 causes a transient disruption of the physiological mechanisms responsible for memory, rather than permanent neuronal damage.

During the second decline in memory (beginning around 15 months), the Aβ*56 in the APP mouse brains did not rise. This might be viewed as inconsistent with a causal role of this Aβ assembly in memory decline. However, the dissociation could be explained by compensatory mechanisms that were able to counteract the pathogenic effects of Aβ*56 for a while but then failed as a consequence of old age. Alternatively, different stages of Aβ-induced neurological disease might involve distinct pathogenic mechanisms.

As with many discoveries, the findings by Lesne *et al.* raise questions. Can Aβ*56 be detected in other APP models with memory deficits? What is the relation between Aβ*56 and the Aβ oligomers detected in the cerebrospinal fluid of Alzheimer's patients[8]? Are these Aβ assemblies a reliable biomarker for early diagnosis, monitoring disease progression and evaluating new treatments? Might these Aβ assemblies themselves constitute targets for more effective therapeutic interventions[4,6]? And last, but not least, how do they actually derange neuronal function?

In regards to this last question, it is noteworthy that abnormalities in signalling through neuronal glutamate receptors have been identified as a likely mechanism of Aβ-induced neuronal and behavioural deficits[7,9]. This is an intriguing possibility because hippocampal glutamate receptors have a key role in spatial learning and memory[10]. It will be interesting to determine whether Aβ*56 has an effect on these receptors or related intracellular signalling pathways. Aβ*56 is certainly a star suspect, but there is more detective work to be done before it can be convicted as the real culprit. ■

Richard Morris is in the Laboratory for Cognitive Neuroscience, College of Medicine and Veterinary Medicine, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, UK.
e-mail: r.g.m.morris@ed.ac.uk
Lennart Mucke is at the Gladstone Institute of Neurological Disease and the University of California, San Francisco, 1650 Owens Street, San Francisco, California 94158, USA.
e-mail: lmucke@gladstone.ucsf.edu

1. Hebert, L., Scherr, P., Bienias, J., Bennett, D. & Evans, D. *Arch. Neurol.* **60,** 1119–1122 (2003).
2. Lesne, S. *et al. Nature* **440,** 352–357 (2006).
3. Tanzi, R. & Bertram, L. *Cell* **120,** 545–555 (2005).
4. Walsh, D. M. & Selkoe, D. J. *Neuron* **44,** 181–193 (2004).
5. Kobayashi, D. T. & Chen, K. S. *Genes Brain Behav.* **4,** 173–196 (2005).
6. Klein, W. L., Krafft, G. A. & Finch, C. E. *Trends Neurosci.* **24,** 219–224 (2001).
7. Palop, J. J. *et al. J. Neurosci.* **25,** 9686–9703 (2005).
8. Georganopoulou, D. G. *et al. Proc. Natl Acad. Sci. USA* **102,** 2273–2276 (2005).
9. Snyder, E. M. *et al. Nature Neurosci.* **8,** 1051–1058 (2005).
10. Morris, R. G. M., Anderson, E., Lynch, G. S. & Baudry, M. S. *Nature* **319,** 774–776 (1986).