Monitoring the energy status of a living organism in real time

More than 30 years ago Steve Busby — now a distinguished molecular biologist, but then a doctoral student — thought it would be interesting to put some freshly prepared rabbit muscle tissue into the sampling space of an NMR spectrometer to see what would happen. His colleagues at Oxford were sceptical, but they did the experiment anyway, and were staggered to obtain clear signals (Hoult *et al* 1974; Radda 1998). [Much earlier than that, Felix Bloch had observed a strong proton NMR signal in 1946 when he put his finger into the probe coil of his nuclear induction apparatus (Bloch *et al* 1946), but although this could be called the first biological NMR experiment it did not provide any biological information apart from confirming that human tissues are not harmed by intense magnetic fields.] It subsequently became clear that NMR allows measurement of the concentrations of metabolites, not only in fresh tissue but also in living organisms, including humans, as originally suggested by Britton Chance (Chance *et al* 1978). Initially this approach was restricted to ^{31}P NMR: this is much less sensitive than proton NMR, but nearly all ^{31}P nuclei in living cells occur in a rather small set of different environments, whereas the number of different proton environments is so large that there was little hope of resolving all of the superimposed proton signals with the instruments available 30 years ago. Moreover, the ^{31}P nuclei occur in metabolites of special biochemical interest, including ATP, ADP, AMP, glucose 6-phosphate and creatine phosphate.

It was already accepted by most biochemists that the "adenylate energy charge" (Atkinson 1968, 1975) provided a useful measure of the energy status of a cell. This is defined as

$$\frac{[\mathrm{ATP}]+\frac{1}{2}[\mathrm{ADP}]}{[\mathrm{ATP}]+[\mathrm{ADP}]+[\mathrm{AMP}]}$$

and has a value that ranges from 0 (when all the adenylate is in the form of AMP) to 1 (when it is all in the form of ATP). It is, in fact, a measure of the number of diphosphate links in the adenylate pool as a fraction of the total possible. In order to fulfil its energetic activities a healthy cell needs to have most of its adenylate present as ATP, with very little present as AMP: this implies an energy charge greater than 0.8, and values in the range 0.8–0.9 are typical.

However, it is one thing to say that the energy charge of a cell is an important indicator of its energy status, but it is quite another to measure it non-invasively in a living organism. As noted, this has been possible for more than 30 years by means of ^{31}P NMR, but that approach requires very expensive equipment and highly skilled technicians, and it is mainly limited to questions of great medical importance, and can hardly be applied to a wide range of biological problems.

In a recent paper, however, Lagido *et al* (2008) describe a new method that does not need very expensive apparatus. It is derived from a method for monitoring ATP concentrations *in vitro* with the enzyme luciferase from fireflies, together with its substrate, the protein luciferin, which becomes luminescent in the presence of ATP. This approach is well established (Stanley *et al* 1990) for measuring ATP concentrations in biological samples, but as most organisms are not bioluminescent and do not express either luciferase or luciferin it requires addition of these proteins and has therefore been restricted to measurements *in vitro*. Lagido *et al* (2008) have now constructed two mutants of the nematode worm *Caenorhabditis elegans* that express the gene for luciferase, the enzyme responsible for the luminescence of fireflies. In the presence of the protein luciferin, also needed for luminescence but not expressed in the mutant worms, the worms become highly luminescent. As the light-emitting reaction requires ATP as an energy source, one might expect that the intensity of the luminescence would provide a measure of the energy status of the living worm.

Keywords. ATP; bioluminescence; *Caenorhabditis elegans;* energy charge; NMR

To test this idea, Lagido *et al* (2008) studied the effect on bioluminescence of subjecting worms to sub-lethal doses of sodium azide (NaN_3),which inhibits mitochondrial respiration and hence results in depletion of ATP. This treatment is reversible, as the mitochondrial function is restored when the NaN_3 is washed away. As NaN_3 is toxic, care needs to be taken to show that the worms can recover and that any effects on luminescence are due solely to the depletion of ATP and not to the toxicity. What Lagido *et al* (2008) found was that inhibiting mitochondria with NaN_3 does decrease the luminescence by as much as 90% even though 100% of the worms recover normal activity after the NaN_3 is washed away. The results of this and other control experiments satisfied the authors that the degree of luminescence does supply a valid measure of the energy status of living worms.

The effect of NaN_3 in these experiments is not of interest for its own sake, of course, as it was already known that it inhibits mitochondrial function. It was necessary, however, to show that a known way of depressing the energy charge would produce the predicted effect on luminescence, and that full recovery of mitochondrial function would be accompanied by full recovery of the luminescence. In principle, therefore, we now have a method that does not require an NMR spectrometer and can be applied to any organism for which appropriate genetic methods can be used to express luciferase, and which can absorb luciferin in sufficient quantities. In the case of *C. elegans* luciferin enters the cells spontaneously (by an unknown mechanism) when it is present in the environment. The absorption is increased in the presence of agents such as DMSO that increase the permeability of the cuticle, but it still occurs in the absence of these. Moreover, studies on other organisms, such as *Drosophila*, zebra fish and Arabidopsis, indicated that diffusion and permeability of luciferin does not limit bioluminescence.

In conclusion, therefore, Lagido *et al* (2008) have provided a new method of studying the energy status of living organism that is reasonably easy to apply (for anyone who knows how to construct the necessary mutants) and does not require highly elaborate equipment. Although they refer throughout to ATP levels rather than energy status, in practice nearly all ATP-sensitive enzymes are also sensitive to the concentrations of ADP and AMP, especially AMP. It is likely, therefore, that the new method provides a way of monitoring the energy status of the organisms rather than their ATP concentration as such.

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