Hydrogenases

From chemistry to legume growth

from R. Cammack and M.G. Yates

HYDROGENASES are enzymes used by bacteria and algae either to produce hydrogen or to consume it as a fuel. They are of interest not only as natural organometallic catalysts and because of the possibility of their application to biotechnological hydrogen production but also because hydrogenase genes may be used to increase agricultural productivity by improving the efficiency of nitrogen fixation. A recent symposium in Hungary* illustrated how the study of these fascinating enzymes has progressed.

It is now clear that there are two types of hydrogenase. The first contains iron, is extremely active and occurs in anaerobic bacteria such as *Clostridium*, which produce H_2 as an electron sink during their metabolism. The second type, one of the few enzymes known to require nickel, is more widely distributed and is used principally for H_2 uptake. It is less active in H_2 production than the Fe-hydrogenase, but from the biotechnological point of view it has the advantage of being much less sensitive to oxygen.

The structural genes of the Fe-hydrogenase of *Desulfovibrio vulgaris* have been sequenced (C. Veeger, Agricultural University, Wageninen). They encode a polypeptide of relative molecular mass (M_r) 46,000 and a hitherto undetected small subunit of M_r 13,500. The sequence indicates that two of the iron–sulphur clusters in this enzyme are of the conventional [4Fe–4S] kind and similar to those in bacterial ferredoxins; the third cluster is probably involved in catalysis.

The probable catalytic site of the Fe-hydrogenase from *Clostridium pasteurianum* has been scrutinized by Mössbauer spectroscopy and magnetic circular dichroism spectroscopy (L.E. Mortenson and M.W.W. Adams, Exxon Research, New Jersey; B.H. Huynh, Emory University, Atlanta; M.K. Johnson, Louisiana State University). It seems to be a unique type of four-iron cluster in which one iron atom is in a different environment but coupled to the other three.

The mechanism of action of the Ni-hydrogenases of *Desulfovibrio* species has been studied by electron-spin resonance and other spectroscopic techniques (J.J.G. Moura, Centro de Quimica Estrutural, Lisbon; J. LeGall, CEN Cadarache, France; R. C.). The nickel centre, which from EXAFS measurements seems to be surrounded by sulphur ligands, can take up several oxidation levels including Ni (III) at an extraordinarily low redox potential. A hydride seems to be involved in the hydrogenase reaction mechanisms but its structure is not yet known.

What seems to be the commonest type of hydrogenase contains two subunits of M_rs about 30,000 and 60,000. Important examples are the membrane-bound Ni-hydrogenases, which have been isolated from the hydrogen-oxidizing bacterium *Alcaligenes eutrophus* (K. Schneider and H.G. Schlegel, University of Göttingen), the aerobic nitrogen-fixing soil bacterium *Azotobacter vinelandii* and the symbiotic nitrogen-fixing organism *Rhizobium japonicum* (D.J. Arp, University of California, Riverside). These enzymes prove to be similar in structure and antigenic characteristics, as well as in regulatory factors (O_2, H_2 and carbon substrates) and genetic determinants.

The genetics of hydrogenase has developed only recently. In *Escherichia coli* there are probably three different hydrogenases (D. Boxer, University of Dundee). Mutants in the structural genes have not been identified because a deficiency in one is masked by the presence of another, but several mutants in which the hydrogenase activity is affected have been isolated. Lee *et al.* have evidence of two classes of mutants, one of which contains *hydA* and *hydB* genes[1,2]. The *hydB* gene is probably in a single operon with a gene encoding formate dehydrogenase and another for an electron-transport protein that couples formate dehydrogenase to hydrogenase. Of two newly discovered genes, *hydC* and *hydD* (L.F. Wu and M.A. Mandran-Berthelot, CNRS, Villeurbanne), the former may be involved in Ni processing.

The *hup* genes of *R. japonicum* Ni-hydrogenase have been isolated[3]. Plasmids containing these genes complement Hup^- mutants of *Azobacter chroococcum* and the arrangements of the *hup* genes in the genomes of these two species are similar[4]. The *hup* genes of *R. japonicum* are probably contiguous and comprise 15.5–21 kilobases divided into at least three transcriptional units[5]. The *hox* (hydrogen oxidation) genes of *Alcaligenes eutrophus* are plasmid-borne, encode both the membrane-bound and a soluble, NAD-linked hydrogenase and contain the structural and regulatory genes in a 50-kilobase sequence. However, an additional chromosomal function is necessary to express the Hox activity. This function may be part of a general regulatory mechanism analogous to the *fnr* system of *E. coli* (B. Friedrich, Freie Universität, Berlin). The *hup* genes of *Rhizobium leguminosarum* are also plasmid-borne, those of *R. japonicum* may be, but those of *A. chroococcum* and one *Alcaligenes* strain are not; *hup* genes have also been isolated from the purple photosynthetic bacterium *Rhodopseudomonas capsulata* (A. Colbeau, A. Godfroy and P.M. Vignais, Centre d'Etudes Nucleaires, Grenoble).

Interest in hydrogenase genes is stimulated by the possibility that hydrogenase might improve the efficiency of biological nitrogen fixation by symbiotic rhizobia in leguminous plants, such as soybeans; many strains of rhizobia are naturally Hup^- or express low activity in legume nodules. The energy-intensive reaction of the enzyme nitrogenase produces hydrogen gas as an apparently inevitable by-product. If it builds up inside the soybean nodule, the hydrogen may inhibit the nitrogenase and it certainly represents a waste of energy resources. Hydrogenase, by recycling the hydrogen, could in principle alleviate these problems. But do *hup* genes produce bigger plants?

H.J. Evans and co-workers have investigated this question by comparing yields of soybean plants inoculated with Hup^+ or Hup^- strains of *R. japonicum*. Both naturally occurring and genetically engineered Hup^+ and Hup^- strains were used[6]. A range of responses was obtained with statistically significant higher yields in the hup^+ series in most cases. Parallel work on free-living *A. chroococcum* (M.G.Y.) shows that its hydrogenase aids growth under selective conditions; these are carbon-limited, nitrogen-fixing continuous cultures at high dilution rates, or growth intiation in batch culture under air, together with low levels of carbon substrates.

The complexity of the *hup* operon appears to be comparable with that of the nitrogen-fixing *nif* operons. The fact that they have common regulatory factors (O_2, fixed nitrogen and temperature) is probably coincidental, although Hup^- Nif^- mutants of *R. japonicum* are known[7]. Both the biochemistry and genetics of hydrogenase are important areas for continued research. □

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R. Cammack is in the Department of Biochemistry, King's College, (KQC), Campden Hill Road, London W8 7AH, UK; M.G. Yates is in the AFRC Unit of Nitrogen Fixation, University of Sussex, Brighton, BN1 9QJ, UK.

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