ambiguity that cannot be resolved easily.

Using the procedure on a 3-Å resolution map of the Bence-Jones protein Rhe, Greer has shown that it defined a main-chain line some 433 Å long, produced provisional coordinates for 113 α-carbon atoms, defined the basic β-sheet structure characteristic of Bence-Jones proteins and also found a short segment of α-helix. Even at this stage it is clear that the technique could be valuable and it seems probable that it can be developed further. Although some crystallographers will see this as a further encroachment of the computer into what they consider to be their proper province, most will note its real value in giving more time (and possibly a better start), for the important task of getting an accurate description of the molecule through which the biological problems can be solved. □

Hydrogenases and efficiency of nitrogen fixation in aerobes

from R. O. D. Dixon

WITH the high energy, and thus financial, cost of nitrogen fertilisers it is now important to rely, where possible, on microbial nitrogen fixation. The fixation of nitrogen by microorganisms occurs with varying efficiency in terms of substrate consumed for each nitrogen fixed. To use microorganisms to the best advantage it is well that we be able to identify factors which lead to greater efficiency of nitrogen fixation and thus to select the most efficient organisms.

It is known that metabolic energy in the form of ATP and reducing power is wasted, by the enzyme nitrogenase reducing protons rather than nitrogen. Evolution of hydrogen occurs with nitrogenase from all nitrogen-fixing microorganisms tested so far *in vitro*, although it has not been possible to test the extent to which this wastage occurs *in vivo* in most organisms because of the complicating presence of hydrogenases. In anaerobic organisms there is a hydrogenase which evolves hydrogen simultaneously with the nitrogenase whereas in aerobes, such as *Azotobacter*, hydrogen is taken up by a hydrogenase.

In experiments in which it was thought that the hydrogenase had been inhibited by carbon monoxide, no hydrogen evolution was detected from *Azotobacter*. It was thus suggested that, in this organism, the nitrogenase, atypically, did not reduce protons. (Postgate, in *The Chemistry and Biochemistry of Nitrogen Fixation*, 161–190, Plenum, New York, 1971). This view, which was not universally accepted, has now been shown to be erroneous by Smith, Hill and Yates (page 209 of this issue of *Nature*) who report that the *Azotobacter* nitrogenase does not in fact differ *in vivo*, from that of the other nitrogen fixers. They confirm that the lack of hydrogen evolution from intact bacteria is due to hydrogen uptake by a hydrogenase.

That the hydrogen, taken up by the hydrogenase, may be put to some use was shown some time ago by Hyndman *et al.* (*J. Bact.*, **65**, 522–531; 1953). They showed that the hydrogen taken up by *Azotobacter* was oxidised with consequent synthesis of ATP. Similar observations were made more recently with hydrogenase in *Rhizobium* bacteroids (Dixon, *Arch. Mikrobiol.*, **85**, 193–201; 1972). Possession of this hydrogenase enables these aerobic organisms to recoup some of the ATP expended on wasteful evolution of hydrogen by nitrogenase. The suggestion was made earlier (Dixon, in *Nitrogen Fixation by Free-living Microorganisms* (edit. by Stewart, W.D.P.), Cambridge University Press, 1975), and is now reiterated by Smith *et al.*, that this reutilisation of hydrogen with ATP formation is the reason for the greater efficiency which has been found for *Azotobacter* nitrogen fixation over that of anaerobes such as *Clostridium* (Hill, Drozd, and Postgate, *J. appl. Chem. Biotechnol.*, **22**, 541–558; 1972).

Although the presence of hydrogenases in free living organisms has precluded the investigation of the level of wastage by the evolution of hydrogen, the lack of hydrogenase in some root nodule associations has enabled Schubert and Evans (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 1207–1211; 1976) to estimate the degree of efficiency of the fixation of nitrogen in a number of such associations. The more efficient associations, which included all the non-legume root nodules in the study, showed evidence of a hydrogenase which takes up the hydrogen evolved. The wastage of ATP and reducing power by evolution of hydrogen is estimated to vary between 40 and 60% of the total used by nitrogenase in those associations in which hydrogenase was not a complicating factor.

Schubert and Evans calculate that with a wastage of 50%, reutilisation of hydrogen could double the amount of nitrogen fixed in cases where the energy supply is limiting. It has indeed been demonstrated in a number of cases that the supply of photosynthate from the plant limits nitrogen fixation by root nodules. Although this calculation would seem to be optimistic, if one bears in mind that the estimate of molecules of ATP utilised, per pair of electrons transferred through nitrogenase for hydrogen evolution, is three to four and the P/O ratio obtained from hydrogen uptake is not likely to be better than two, substantial savings can be made. This would be reflected, as Schubert and Evans point out, either in an increase in dry matter yield, where photosynthate is not limiting, or in an increase in nitrogen fixed. Thus selection of a strain of *Rhizobium* which possesses this hydrogenase may pay dividends in the yields of some legume crops.

Much work has been done and is being pursued on the legume–root nodule association in order to understand the infection process and nodule development, with the long term aim of transferring nodulating capability to other agricultural crops, whereas little attention has been paid to non-legume associations. But the greater efficiency of nitrogen fixation of non-legume associations shown in the data of Schubert and Evans suggests that it may be well to give them more attention than in the past. □

Irregular associations

from E. G. Richards

FOR nearly a decade it has been known that complementary trinucleotides do not associate detectably. So, we may ask, how does the anticodon on the tRNA bind with such specificity to messenger triplets on the ribosome? Part, at least, of the answer must lie in the tRNA, for trinucleotides complementary to anticodons of tRNA associate with the latter and, more dramatically, as Eisinger showed some years ago, pairs of tRNA molecules with complementary anticodons bind together with an association constant as high as 10^6 M^{-1}.

The purist may wonder what relevance the participation of tRNA molecules in these unnatural acts may have to tRNA–messenger interaction but Grosjean, Söll and Crothers have come up with some interesting suggestions (*J. molec. Biol.*, **102**, 499; 1976).

When such pairs of tRNA molecules interact, they do so with a small (0.1% of the total) absorbance change which