recognition and binding of mRNA. The other two requisitioned polypeptides are also involved in protein synthesis in the host; they are the polypeptide elongation factors (EF) Tu and Ts. Their known function in protein synthesis could be very neatly fitted with possible functions in the Qβ replicase. EF-Tu is able to bind GTP which is the only nucleotide with which Qβ replicase can initiate replication. EF-Ts can remove GDP from EF-Tu in a catalytic exchange reaction. In addition, EF-Tu is able to bind aminoacyl-tRNA and, since Qβ RNA and tRNA both have CCA at their 3′ end, the suggestion was readily made that EF-Tu in the replicase was involved in enzyme—template binding.

The method used to test these hypotheses was to alter the EF-Tu and EF-Ts subunits so that they are no longer effective in supporting protein synthesis, and then to reconstitute Qβ replicase with these modified subunits and test for enzymatic activity with synthetic and natural templates. Brown and Blumenthal have performed these experiments with unexpected results. (Brown and Blumenthal, *J. biol. Chem.*, **251**, 2749; 1976; and *Proc. natn. Acad. Sci. U.S.A.*, **73**, 1131; 1976). Using chemical modifiers, they have altered the aminoacyl tRNA binding site of the enzyme subunit which is EF-Tu and shown that, although it now no longer supports protein synthesis, Qβ replicase reconstituted with it is active —in fact its specific activity is very similar to the native enzyme. To investigate the GTP binding site on EF-Tu they used kirromycin, an antibiotic that inhibits protein synthesis by altering the GTP binding properties of EF-Tu but has no effect on the aminoacyl-tRNA binding site. Low levels of kirromycin which effectively inhibit protein synthesis do not affect the replicase activity, suggesting that in replicase EF-Tu does not function by binding GTP. Further careful experiments using reconstituted replicase revealed that kirromycin acted on the enzyme by preventing the formation of an EF-Tu·Ts complex. The authors think that it is as a complex that these two subunits function in native replicase. Support for this is provided by the experiments described in their paper in the *Proceedings of the National Academy of Sciences*. EF-Tu and EF-Ts have been chemically 'crosslinked' (joined covalently) and this complex used to reconstitute the enzyme. Although in this state the elongation factors cannot perform their function in protein synthesis, they do not at all inhibit the activity of the reconstituted enzyme.

Since the replicase is not utilising the known abilities of EF-Tu and EF-Ts perhaps they perform other functions in the host, as yet undiscovered by scientists but recognised by Qβ as useful. More likely the authors think, is that the two subunits have some structural role, perhaps holding subunit II, the one coded for by Qβ, in its active configuration. The enzyme has the ability to unfold its template RNA as it copies it and to keep the two complementary strands apart. Perhaps EF-Tu Ts could be involved. □

Zinc in enzymes

from Peter J. Sadler

WHEN cultured in normal conditions, yeast uses a zinc metalloenzyme, alcohol dehydrogenase (YADH), during the final stage of the fermentation process, for the conversion of acetaldehyde to ethanol. The role of zinc in the enzyme is intriguing. If Co^{2+} is added to the culture medium it becomes incorporated into the enzyme, and green (but still active) enzyme crystals instead of colourless (Zn) ones (Curdel and Iwatsubo, *FEBS Lett.*, **1**, 133; 1968) can be isolated. Furthermore, if Zn^{2+} is excluded from the growth medium and Mn^{2+} is added, an active Mn^{2+} enzyme is isolated (Coleman and Weiner, *Biochemistry*, **12**, 3466; 1973).

The natural yeast enzyme has a molecular weight of 140,000 and contains four zinc ions. Their role is thought to be similar to the four zinc ions in the mammalian liver enzyme (molecular weight 80,000) which converts ingested alcohol to acetaldehyde. This enzyme (LADH) has recently been the subject of X-ray crystallographic analyses at 2.4 Å resolution (Eklund *et al.*, *J. molec. Biol.*, **102**, 27; 1976) and detailed metal exchange studies in solution (Stykowski and Vallee, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 344; 1976). A close look at these results reveals the conditions in which metal ions can be swopped and activity retained.

Early attempts to remove zinc from LADH and to reconstitute it with other metal ions met with difficulty. Enzyme activity decreases as zinc is removed, but slow conformational changes occur through oxidation of formerly stable SH groups to S-S bonds (Oppenheimer, Green and McKay, *Archs Biochem. Biophys.*, **119**, 552; 1967).

But successful exchange reactions can be performed under an N_2 atmosphere. The zinc atoms behave in pairs: Zn_2 (Zn_2) representing non-catalytic (catalytic) ions. The pairs exchange at different rates. For example, Zn_2 (Zn_2) dialysed against radioactive $^{65}Zn_2$ (Zn_2) initially becomes $^{65}Zn_2$ (Zn_2) and finally $^{65}Zn_2$ ($^{65}Zn_2$). Similarly, $^{65}Zn_2$ (Zn_2) with Co^{2+} added becomes Co_2 (Zn_2) after 12 h (4 °C, *p*H 5.5), and Co_2 (Co_2) after 120 h. But Co_2 (Zn_2) has an identical activity to Zn_2 (Zn_2), whereas Co_2 (Co_2) is only 64% as active. Hence the term catalytic to describe the second pair of zinc ions.

The crystallographic analysis shows the enzyme to be a dimer of two identical subunits, each having two zinc binding sites and one coenzyme (NAD^+) site. As predicted by earlier spectroscopic studies (Foster, Hill and Williams, *Biochem. Soc. Symp.*, **31**, 187; 1970) on YADH, the zinc coordination sites are (distorted) tetrahedral and include cysteine (sulphur) ligands: four cysteines for the non-catalytic zinc, and two plus one histidine (N) and one water molecule for the catalytic zinc. The latter, which is the faster ion in exchange, is situated at the bottom of a 20 Å pocket, whereas the non-catalytic zinc is in a cleft on the enzyme's surface.

An alternative differentiation of the zinc atoms involves binding 1,10-phenanthroline (OP). OP inhibits reversibly Zn_2 (Zn_2) or Co_2 (Zn_2) by binding at catalytic zinc (H_2O displacement) without removing them. OP irreversibly inhibits Co_2 (Co_2) by removing the catalytic cobalt ions. A difference in behaviour of Co and Zn is notable here.

A near-tetrahedral zinc with a water ligand is now becoming familiar at zinc sites in metalloenzymes: carbonic anhydrase (3 His, H_2O), carboxypeptidase (2 His, Glu, H_2O), thermolysin (2 His, Glu, H_2O). Woolley (*Nature*, **258**, 677; 1975) has recently discussed the enhanced reactivity of such water ligands. This may vary with the fractional change placed on Zn by the other ligands, and may account for the exceptional catalytic efficiency of carbonic anhydrase which has one of the largest known turnover numbers, 600,000 s^{-1}.

The non-catalytic zinc or cobalt ions in LADH may simply have structural roles, but this coordination site is strikingly similar to the iron site in rubredoxin and bacterial ferredoxins, and a sequence spacing of coordinated cysteines, X, X + 3 is a regular occurrence. Substitution of the non-catalytic zinc with iron should not only produce an enzyme with interesting properties, but also perhaps with evolutionary significance! □

A hundred years ago

VIOLENT shocks of earthquake were felt again on the 5th inst. at Corinth and the surrounding district. The direction of the motion was east to west. Shortly after noon on Monday an earthquake occurred in Vienna. Three violent shocks, lasting two seconds, were felt. A panic ensued. Several houses are damaged, and a portion of the old walls has been split.
from *Nature*, **14**, July 20, 260; 1876