PEPTIDES

Closing the Ring

from our Cell Biology Correspondent

THE tyrocidines and gramicidins produced by various strains of *Bacillus brevis* are exceptionally interesting molecules; they are closely related cyclic decapeptides which contain the D form of phenylalanine and ornithine, an amino-acid, which does not, of course, occur in proteins; their synthesis does not involve any ribonucleic acids and they have antibiotic properties. Because the sequence of the ten amino-acids in these molecules is not determined by a messenger RNA, the enzymes responsible for their synthesis have, not surprisingly, attracted much attention. And as a result of recent work, notably in Lipmann's laboratory and by Stoll and his colleagues in Oslo, we now have a clear picture of how the gramicidins and tyrocidines are synthesized; there are striking differences as well as basic similarities.

It has been known for some time that two enzymes, I and II, are required for the synthesis of gramicidin S. Enzyme II activates and racemizes L-phenylalanine residues, and enzyme I, the larger of the two molecules, activates L-proline, L-valine, L-ornithine and L-leucine and catalyses the formation of the peptide bonds. Earlier studies had shown that the largest peptide precursor of gramicidin S is a pentapeptide and, because the complete molecule comprises two repeats of the sequence D-phenylalanine–L-proline–L-valine–L-ornithine–L-leucine, it had been suggested that a molecule of gramicidin S is formed by the head to tail condensation of two such pentapeptides, catalysed by enzyme I.

Stoll and his colleagues in Oslo, in a neat series of experiments, have now confirmed this idea and have shown that the condensation of the two pentapeptides occurs on a single enzyme I molecule (*FEBS Lett.*, **11**, 348; 1970). They have exploited the finding that the gramicidin synthetase accepts azetidine-2-carboxylic acid in place of proline. Enzyme I molecules charged with pentapeptides containing either proline or the analogue were mixed and synthesis of gramicidin was allowed to go to completion. If two enzyme I molecules each carrying one pentapeptide cooperate to produce the decapeptide, 50 per cent of the gramicidin produced by the mixture should be hybrid with both proline and azetidine-2-carboxylic acid residues. By contrast, if each enzyme I molecule carries two pentapeptides, which are condensed intramolecularly, no hybrid gramicidins should be formed and this turned out to be the case. The final step in gramicidin biosynthesis seems therefore to occur on a single enzyme I molecule. Stoll and his colleagues suggest that one pentapeptide sits and waits at a site on the enzyme and the second chain is synthesized attached by a thioester bond to a phosphopantetheine residue in the enzyme. The two pentapeptides once completed can then be brought close enough to allow the head to tail condensation.

Synthesis of the tyrocidines, according to Roskoski and his colleagues (*Biochemistry*, **9**, 4839; 1970), involves three enzymes with molecular weights of 100,000, 230,000 and 460,000. The light and intermediate molecules respectively activate phenylalanine and proline and the heaviest molecule activates the L forms of leucine, ornithine, valine, tyrosine, glutamine and asparagine, and is the site of peptide bond formation. As with gramicidin synthesis activation of each amino-acid involves splitting a molecule of ATP and the growing peptide chain is attached to the enzyme by a thio ester bond. Unlike gramicidin synthesis, however, the synthesis of tyrocidine involves the step by step polymerization of all ten amino-acids. Roskoski *et al.* (*ibid.*, 4846) have been able to isolate the complete series, ranging from dipeptide to decapeptide, of linear precursors of tyrocidine. And apparently once the linear decapeptide has been made, it is comparatively slowly cyclized by the formation of a peptide bond between the amino-group of the free amino-terminal residue, D-phenylalanine, and the carboxy group of the carboxy terminal leucine residue, which is both activated or held to the enzyme by the thio ester bond.

In retrospect this striking difference in the final stages of the biosynthesis of these two molecules is not all that surprising because the decapeptide sequence of tyrocidine is unique rather than a repeat of two pentapeptide sequences. More surprising is the finding reported by Roskoski *et al.* that the phenylalanine activating and racemizing enzymes in the two systems, which play a comparatively small part in the biosynthesis, are not interchangeable. The ability to determine the sequence of a peptide without the involvement of RNA clearly has its price.

Isoenzymes of Choline Acetylase

A FURTHER twist to an already complex story is added by the report by Fonnum in next Wednesday's *Nature New Biology* of the existence of multiple forms of the enzyme choline acetyl transferase (ChAc) in the brain of the rat.

Following the discovery by Whittaker and De Robertis of the existence of pinched off nerve terminals, or synaptosomes, in homogenates of mammalian brain, many studies have made use of centrifugation techniques to determine the subcellular distribution of neurotransmitters and related enzymes in neurones in the central nervous system. There has been general agreement that most of the acetylcholine in brain is localized in nerve terminals, and that a substantial portion of this acetylcholine is stored within synaptic vesicles which can be liberated from synaptosomes by mild hypo-osmotic shock (Whittaker *et al.*, *Biochem. J.*, **90**, 293; 1964). On the other hand, there has been considerable disagreement about the subcellular localization of the acetylcholine synthesizing enzyme ChAc in mammalian brain. Although this enzyme was specifically localized in synaptosomes, most of the synaptosomal ChAc seemed to be associated with synaptic vesicles in rat and rabbit brain, but in guinea-pig and pigeon the enzyme seemed to be located in the cytoplasmic fraction (McCaman *et al.*, *J. Neurochem.*, **12**, 927; 1965; Tucek, *J. Neurochem.*, **13**, 1317; 1966).

In an important series of studies Fonnum has illustrated some of the pitfalls which may befall even the wary in the interpretation of subcellular distribution results. Fonnum showed (*Biochem. J.*, **103**, 262; 1967; *Biochem. J.*, **109**, 389; 1968; *J. Neurochem.*, **17**, 1095; 1970) that the ChAc which seemed to be associated with synaptic vesicles after osmotic shock treatment of homogenates of rat and rabbit brains could be easily solubilized by increasing the *p*H and ionic strength to values similar to those likely to prevail intracellularly. Species differences existed in the ease with which ChAc non-specifically adsorbed to vesicle or other lipid membranes in media of low ionic strength. Such differences were shown to correspond to species differences in the surface charge of the ChAc molecules, those from rat and rabbit being more strongly adsorbed by cation exchangers than the enzymes from guinea-pig or pigeon.

In *Nature New Biology*, Fonnum shows that several forms of ChAc with different isoelectric points can be separated by isoelectric focusing of partially purified preparation of ChAc from rat brain. The three different forms of the enzyme, with isoelectric points in the range *p*H 7.5 to 8.3, were also demonstrated in crude extracts of rat brain.

ChAc from guinea-pig or pigeon brain, however, gave only single peaks of enzyme activity after isoelectric focusing, with isoelectric points of 6.7 and 6.6, consistent with the species differences in surface charge previously described. Further studies are in progress to determine whether the different forms of the enzyme in rat brain have the same cellular and subcellular distribution or whether they are present in morphologically or functionally distinct compartments.