Isolation of a Third Neurophysin from Bovine Pituitary Posterior Lobes

By R. RAUCH, M. D. HOLLENBERG & D. B. HOPE. (*Department of Pharmacology, University of Oxford*)

Recent work has shown that neurosecretory granules isolated from bovine pituitary posterior lobes contain two main hormone-binding proteins, neurophysin-I and -II (Hollenberg & Hope, 1968; Dean & Hope, 1968). In addition, starch-gel electrophoresis of the soluble proteins in a lysate of the same granules revealed the presence of several other minor constituents. The most conspicuous band had a mobility slightly less than that of neurophysin-I; these were eluted together by ion-exchange chromatography on CM-Sephadex C-50. We have now been able to isolate this minor component free from neurophysin-I and evidence is now reported that it has the properties of a neurophysin.

Native neurophysin was prepared from acetone-desiccated bovine pituitary posterior lobes by the method of Hollenberg & Hope (1968). The low-molecular-weight fraction obtained by gel filtration on Sephadex G-75 contained the native neurophysins. This fraction was submitted to ion-exchange chromatography on DEAE-Sephadex A-50. The proteins were eluted in a tris buffer with a sodium chloride gradient. Three protein peaks were obtained; the first and third were major peaks containing neurophysin-I and -II respectively. The protein in the intermediate peak was identified electrophoretically as the minor soluble-protein component of the neurosecretory granules. This material accounted for approximately 15% of the total protein recovered. Amino acid analysis showed that the residues present in greatest numbers were glycine, cystine, glutamic acid and proline. Histidine was entirely absent, but two residues of methionine/mol. were present assuming a molecular weight close to 20000. The new protein is thus distinct from neutophysin-I, which contains two residues of histidine/mol. The small amount of methionine previously thought to be present in neurophysin-I can now be attributed to the presence of the new protein. Equilibrium-dialysis experiments showed that the protein can bind hormone, and a protein–hormone complex has been isolated. The properties observed show that the new protein is a neurophysin.

Other work in this Laboratory indicates that oxytocin–neurophysin-I and vasopressin–neurophysin-II complexes are present in different populations of nuerosecretory granules (Dean, Hope & Kazić, 1968). The presence of a third neurophysin suggests that, in addition to oxytocin and vasopressin, there may be a third peptide associated with the third bovine neurophysin.

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Some Properties of Deoxyribonucleic Acid in Competent *Bacillus subtilis*

By W. J. HARRIS and G. C. BARR. (*Department of Biochemistry, University of Dundee*)

Transformable bacteria must be cultivated to a state known as competence before they can take up transforming (donor) DNA and insert one of the strands (Bodmer, 1965; Venema, Pritchard & Venema-Schroder, 1965) into the recipient cells' chromosomes. The mechanism by which donor DNA and recipient DNA interact is obscure. Lacks (1966) has suggested that a three-stranded intermediate may occur, of which the third strand is derived from donor DNA; the exchange of a donor strand with a recipient strand precedes final integration. In this work we have investigated the physical properties of DNA extracted from competent cells and from newly transformed cells. The evidence is taken to suggest that competent cells accumulate an unusual form of chromosomal DNA in which about 5% of the bases are present as a single-stranded form and the remainder as a double-stranded form. Likewise, transforming DNA that enters competent bacteria is quickly converted into a partially single-stranded form. There is no evidence for a fully single-stranded intermediate such as occurs in *Pneumococcus* (Lacks, Greenberg & Carlson, 1967). Later the incoming DNA is converted into a double-stranded form. It is proposed that the single-stranded regions in these molecules form the basis for the extremely specific association between donor DNA and recipient chromosome. Other evidence to support this conclusion will be presented.

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