Synthesis of the Late Proteins of Bacteriophage T₄ Amber Mutant

By S. G. DEVARE and J. D. PADAYATTY (*Department of Biochemistry, Indian Institute of Science, Bangalore-12, India*)

The head protein of bacteriophage T₄ constitutes about 90% of the structural proteins. Amber mutation in the head-protein gene causes premature termination of protein synthesis at the amber codon. The synthesis of bacteriophage proteins was studied *in vivo* and *in vitro* by following the incorporation of ³⁵S- or ¹⁴C- labelled amino acids into the proteins of bacteriophage T₄ and its amber mutant, amber B₁₇, grown on the non-permissive host *Escherichia coli* B. The incorporation of radioactivity into proteins in mutant amber B₁₇ was found to be equal to or higher than that in bacteriophage T₄. The ³⁵S-labelled proteins of bacteriophage T₄ and the amber B₁₇ mutant were dissociated with sodium dodecyl sulphate and subjected to polyacrylamide-gel electrophoresis and gel filtration on Sephadex G-150. The ³⁵S-labelled protein from bacteriophage T₄ gave a single peak of radioactivity that may constitute the head protein. In the case of ³⁵S-labelled protein from the amber B₁₇ mutant the head-protein peak was absent, though other peaks of radioactivity were present. These results may indicate that when there is no synthesis of the head protein, as in the amber B₁₇ mutant, there is an increase in the synthesis of other bacteriophage proteins.

Biosynthesis of Polymyxin, a Peptide Antibiotic

By KUNTHALA JAYARAMAN (*Department of Biological Sciences, University of Madurai, Madurai-2, Tamil Nadu, India*)

Studies with growing cultures of *Bacillus polymyxa* have shown that the biosynthesis of polymyxin D or B proceeds by a mechanism that differs from normal peptide synthesis. My studies have implicated bacterial membrane systems in the biosynthetic process. The synthesis of the antibiotic is also being studied by blocking peptide synthesis by D-leucine, which seems to interfere with the assembly of amino acids rather than the incorporation of fatty acids into the antibiotic.

Amino Acid Incorporation by Mammary Gland as Effected by Milk and Liver Tissue Extract

By AJIT SINGH and N. C. GANGULI (*National Dairy Research Institute, Karnal, India*)

Mammary gland during lactation, unlike liver, is the target tissue with respect to synthesis and secretion of milk. This tissue specificity evoked our interest in the elucidation of the factors regulating the incorporation of amino acids into proteins by mammary gland from normal (non-lactating) and lactating goats. Accordingly [¹⁴C]leucine was incubated with these tissues both individually and together *in vitro*.

Addition of milk (both from goat and from cow) inhibited the incorporation by both mammary-gland and liver tissue from either normal or lactating animals. Whey proteins of goat milk also had an inhibitory effect on the incorporation by these tissues, whereas the whey proteins of cow milk augmented the synthesis in both normal and lactating mammary glands. In normal tissue the stimulation was dramatic, in contrast with liver tissue.

Addition of boiled liver supernatant from lactating goat to homogenate of normal or lactating mammary gland stimulated the incorporation, unlike the similar liver fraction from non-lactating goat, which had an inhibitory effect. However, at higher concentration the liver fraction from lactating goat depressed the incorporation rate. On the other hand a similar boiled extract from mammary gland (normal or lactating) failed to stimulate or inhibit the protein synthesis by the liver homogenate of the animal.

Studies on the Identification of the Cadmium-Binding Protein in Rat Testis

By KARTAR SINGH and R. NATH (*Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh, India*)

Metallothionein, a protein of molecular weight 11 000 containing large amounts of cadmium and sulphur, was first recognized in mammalian kidney (Kagi & Vallee, 1961). A variant of metallothionein that contains primarily zinc has been isolated from horse and human liver (Kagi, 1970). Testis is known to be specifically damaged by very small amounts of cadmium, but the mechanism is not yet known.

Attempts have been made to characterize the site of the binding of cadmium in testis by the use of ¹⁰⁹Cd. Adult male rats were injected intraperitoneally ¹⁰⁹Cd (20 μCi of ¹⁰⁹CdCl₂/100 g body wt.), and the testes were removed after 48 h and homogenized in 0.25 M-sucrose. In the experiments *in vitro*, testes of normal adult rats were removed and a 10% homogenate in 0.25 M-sucrose was incubated at 37°C with ¹⁰⁹CdCl₂ for 1 h. Fractionation of the homogenates in the ultra-

centrifuge showed that all the radioactivity was in the supernatant fraction. Further fractionation on Sephadex G-25 and G-75 showed two peaks, one of which had a high radioactivity count and a high E_{250} value. The presence of cadmium and thiol groups was confirmed. The protein resembles metallothionein in spectral and chromatographic properties. Attempts are under way to prepare this protein in quantity, to test the homogeneity of the preparation and to measure other physicochemical properties.

Kagi, J. H. R. (1970) *Abstr. 8th. Int. Congr. Biochem., Switzerland*, p. 130

Kagi, J. H. R. & Vallee, B. L. (1961) *J. Biol. Chem.* **236**, 2435

The Denatured State of Ovalbumin

By FAIZAN AHMAD and A. SALAHUDDIN (*Department of Biochemistry, J. N. Medical College, Aligarh Muslim University, Aligarh, U.P., India*)

The denatured state of a protein obtained in 6M-guanidine hydrochloride plus 2-mercaptoethanol can be characterized by, among other things, intrinsic viscosity $[\eta]$. With increase in temperature bond rotation in the polypeptide chain will be facilitated, and therefore the chain will be shortened, which will result in a decrease in $[\eta]$. The denatured state therefore can be further characterized from measurements of $[\eta]$ as a function of temperature. A notable decrease in $[\eta]$ with temperature must be observed for the denatured protein. This diagnostic criterion of a denatured protein has been generally ignored. In the present communication we present our findings on the temperature-dependence of the intrinsic viscosity of the denatured state of ovalbumin.

The intrinsic viscosity of ovalbumin was measured in 6M-guanidine hydrochloride plus 2-mercaptoethanol at pH 4.85 and at different temperatures in a Cannon-Fenske viscometer with a flow time of 482.0 s for water at 30°C. The intrinsic viscosity of the completely unfolded ovalbumin was found to be 37.4 ml/g at 25°C, which is in agreement with the value calculated by the empirical equation of Tanford (1968). The viscosity showed little or no change in the temperature interval 25–35°C. However, as expected, at higher temperatures the intrinsic viscosity was considerably decreased, confirming our finding that in 6M-guanidine hydrochloride plus 2-mercaptoethanol ovalbumin exists as a randomly coiled protein.

Tanford, C. (1968) *Advan. Protein Chem.* **23**, 121

Studies on Ovomuroid: Hydrogen Ion Equilibrium in the Acid pH Range

By ABDUL WAHEED and A. SALAHUDDIN (*Department of Biochemistry, J. N. Medical College, Aligarh Muslim University, Aligarh, U.P., India*)

Ovomucoid is an important trypsin inhibitor and is known to interact with the enzyme. Although it is known, in a general way, that this protein-protein interaction prevents the expression of the biological activity of trypsin, very little is known about the mode and extent of this interaction. Information about the native conformation of ovomucoid and the nature of its accessible chemical groups should be helpful for a proper understanding of the trypsin-ovomuroid interaction. This prompted us to investigate the nature of the ionizable groups in ovomucoid. In the present communication we present our findings on the carboxyl groups of ovomucoid.

The H^+ ion titration curve of ovomucoid was obtained by using the cell assembly recommended by Tanford (1955). Ovomuroid was characterized on a DEAE-cellulose column. A symmetrical peak was obtained. The pI of the protein was found to be 4.4, which increased to 4.6 at I 0.15 and at 25°C. The protein concentration was 0.46–0.47%. From the H^+ ion titration curve of ovomucoid it was found that nearly 35 carboxyl groups titrate between pH 1.49 and 6.0, and the value of the electrostatic interaction factor, w , was found to be 0.033, which was similar to the calculated value ($w = 0.033$). The value of w remained unaltered throughout the pH range used in the titration. The electrostatic free energy of native ovomucoid was found to be 9.2 kJ/mol (2.2 kcal/mol) at pH 3.5.

Tanford, C. (1955) in *Electrochemistry in Biology and Medicine* (Shedlowsky, T., ed.), p. 248, John Wiley and Sons, New York

Structural Requirements of Inducer for the Formation of Penicillin Acylase in *Fusarium* sp. 7.5–5

By S. B. THADHANI, P. S. BORKAR and S. RAMACHANDRAN (*Antibiotics Research Centre, Pimpri, Poona-13, India*)

Penicillin acylase, an enzyme hydrolysing phenoxy-methyl-penicillin to 6-aminopenicillanate and phenoxyacetate, was purified 200-fold from the culture filtrate of *Fusarium* sp. and was homogeneous on polyacrylamide-disc-gel electrophoresis.

The enzyme was formed adaptatively when the organism was grown in the presence of phenoxyacetate or its derivatives. The amount of enzyme induced depended on the nature of the substitution