PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 486th Meeting of the Society was held at the University of Aberdeen on Thursday and Friday, 19 and 20 September 1968, when the following papers were presented:

COLLOQUIUM ON 'STRUCTURAL DIFFERENCES IN POLYMORPHIC PROTEINS'

Lysozymes

By PIERRE JOLLÈS. (*Laboratory of Biochemistry, Faculty of Sciences, Paris*)

Three types of lysozymes (EC 3.2.1.17), which have entirely different primary structures, but which all provoke the lysis of *Micrococcus lysodeikticus* cells, have been characterized: (1) hen egg-white lysozyme, other bird lysozymes (even the goose enzyme with its quite different behaviour), human lysozymes, etc. (mol. wt. about 15000): their main function seems to be the digestion of the bacterial cell walls; (2) phage lysozyme (mol. wt. 18000): it seems to manifest a specific activity for *E. coli* cells; (3) *Papaya latex* lysozyme (mol. wt. 25000): it is much more a chitinase than a lysozyme, and the tryptophan residues do not seem to be involved in the active site of this type.

In this communication, mainly the first (1) group of lysozymes will be studied in detail as many investigations concerning these enzymes have been performed in the author's Laboratory. Detailed formulae of two duck egg-white lysozymes and some points about the structures of goose egg-white and human milk lysozymes will be indicated. Some aspects of the relationship between structure and activity will be discussed: the above-mentioned enzymes as well as hen and guinea-hen egg-white lysozymes, two dog lysozymes, a lysozyme from an invertebrate, and ten human lysozymes (from milk, tears, saliva, placenta, spleen, normal serum and leucocytes, serum and leucocytes of patients suffering from chronic myelogenous leukemia, and urine from patients suffering from acute myeloblastic leukemia) will be included in this discussion.

Despite structural differences, lysozymes from various origins have qualitatively the same biological activity, but quantitatively the reaction velocities are quite different: to express these latter differences, affinity constants of seven different lysozymes for the bacterial substrate have been determined. The behaviour of the enzymes in the presence of inhibitors such as *N*-acetylglucosamine has been studied, and inhibition constants have been established. Some comments on the specificity of all these enzymes against high- and low-molecular-weight substrates will also be made.

Homologies between Thrombin and other Serine Proteinases

By STAFFAN MAGNUSSON. (*Medical Research Council Laboratory of Molecular Biology, Cambridge*)

The observations that fibrinogen could be coagulated by papain and prothrombin activated to thrombin by trypsin led to the postulate that thrombin is a proteolytic enzyme and also that activation of prothrombin to thrombin involves a proteolytic split of prothrombin (Eagle & Harris, 1937). That thrombin is indeed a proteolytic enzyme became clear with the demonstration that fibrinogen and fibrin have different *N*-termini (Bailey, Bettelheim, Lorand & Middlebrook, 1951), and that fibrinopeptides are released during coagulation (Lorand, 1952; Bettelheim & Bailey, 1952). The peptide bonds that are cleaved by thrombin in fibrinogen have turned out to be arginine-glycine bonds in about 30 different animal species whose fibrinopeptide sequences have been determined (Blombäck, Blombäck, Edman & Hessel, 1966). Secretin has four arginine bonds, one of which is split by thrombin (Mutt, Magnusson, Jorpes & Dahl, 1965). Arginyl (Sherry & Troll, 1954) and lysyl (Elmore & Curragh, 1963) esters with blocked α -amino groups are also substrates for thrombin. Thus the substrate specificity of thrombin resembles that of trypsin but seems to be narrower.

Inhibition of thrombin with di-isopropyl phosphorofluoridate (DFP) (Miller & van Vunakis, 1956) and the demonstration of radioactive peptides containing serine in a digest of [32 P]DFP-inhibited thrombin (Laki, Gladner, Folk & Kominz, 1958) made it appear that thrombin is a serine proteinase.

Human and bovine prothrombin both have the *N*-terminal sequence Ala-Asx-, but human thrombin has Thr- and Ile-Val-Gly-Gly- and bovine thrombin Thr- and Ile-Val-Glu-Gly-. *C*-Terminal arginine and lysine as well as *N*-terminal tyrosine appear on activation of prothrombin, indicating that this is a proteolytic process (Magnusson, 1965). The fact that nitrite both reacts with the *N*-terminal isoleucine and inactivates thrombin in the absence but not in the presence of substrate (S. Magnusson & T. Hofmann, unpublished work) supports the