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

assumption that the *N*-terminal isoleucine might be bound to Asp-194 in thrombin in much the same way as in chymotrypsin (Sigler, Blow, Matthews & Henderson, 1968). One question that is raised by the sequence homologies in chymotrypsin and trypsin (Hartley, Brown, Kauffman & Smillie, 1965; Neurath, Walsh & Winter, 1967), chymotrypsin B (Smillie, Furka, Nagabhushan, Stevenson & Parkes, 1968) and elastase (D. M. Shotton & B. S. Hartley, unpublished work) is whether the general structure of these enzymes is common only to serine proteinases synthesized in the pancreas or includes those of other tissues as well. Knowledge of the structure of thrombin, which is synthesized as prothrombin in the liver, makes it possible to begin to answer that question. The A chain has 49 amino acid residues and is bound to the B chain by a disulphide bridge. The sequence of the A chain is Thr-Ser-Glu-Asn-His-Phe-Glu-Pro-Phe-Phe-Asx-Glx-Lys-Thr-Phe-Gly-Ala-Gly-Glu-Ala-Asp-Cys-Gly-Leu-Arg-Pro-Leu-Phe-Glu-Lys-Lys-Glu-Val-Glx-Asx-Glx-Thr-Gln-Lys-Glu-Leu-Phe-Glu-Ser-Tyr-Ile-Glu-Gly-Arg (S. Magnusson, E. Merler, J. Wootton & B. S. Hartley, unpublished work). It shows no apparent sequence homology with the pancreatic proteinases. The B chain, on the other hand (S. Magnusson & B. S. Hartley, unpublished work), shows extensive sequence homology in areas that in the three-dimensional model of chymotrypsin (Sigler *et al.* 1968) can be described as 'buried' or 'internal'. Particularly noteworthy in this respect are the *N*-terminus (residues 16–32 of chymotrypsin) and residues 173–209, which include the 'methionine loop' and active-centre serine regions. However, the half-cystine at position 201 is replaced by a methionine residue in thrombin, and Cys-42 is in the sequence -Leu-Leu-Cys-Gly- rather than -His-Phe-Cys-Gly-, which seems to exclude the possibility that two histidine residues are involved with the active centre of mammalian serine proteinases, as some previous hypotheses suggested. It does not at present seem unlikely that the tertiary structure of thrombin will turn out to be similar to that of chymotrypsin.

Bovine thrombin used in this study has been and still is prepared at the Chemistry Department II, Karolinska Institutet, Stockholm, Sweden. Support from the Swedish Medical Research Council and from the U.S. National Institutes of Health is gratefully acknowledged.

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The Heterogeneity of Human Immunoglobulins

By C. MILSTEIN. (*Medical Research Council Laboratory of Molecular Biology, Cambridge*)

One of the key problems in investigations of the molecular basis of the immune response is the mechanism by which the immunological system can synthesize an indefinite number of different antibody molecules and yet retain the basic structure characteristic of all antibodies. The structural complexity of normal immunoglobulins and specific antibodies has stimulated renewed interest in the homogeneous proteins that are found in mice and in men in association with myelomatosis and certain related lymphoproliferative disorders. Sequence studies on these proteins have provided much detailed information about immunoglobulin structure, and in particular have shown that large stretches of immunoglobulin molecules have a relatively conservative structure. Such an arrangement fulfils ideally the biological functions of antibodies, which must be unique in their specific recognition of an indefinite number of antigens and yet conserve those general properties (such as the capacity to fix complement and to attach to or to traverse specific biological membranes) that are characteristic of the class of antibodies to which they belong.

The heterogeneity of immunoglobulins is the result of three types of amino acid-sequence variability: (1) the isotypic variants, which are common to all individuals of the same species and differentiate classes and types of immunoglobulins; (2) the allotypic variants, which distinguish polymorphic forms of immunoglobulins not present in all members of a given species; (3) the idiotypic variants, which characterize individual antibodies and myeloma proteins (reviewed by Cohen & Milstein, 1967). The arrangement of the disulphide bridges of the immunoglobulin chains provide a