Meeting Report

PROTEASES AND HORMONE ACTION

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Proteolytic enzymes have fascinated workers not only because of the information one gleans about the nature of proteases themselves, but also because they form important adjuncts to the arsenal of the biochemist working in almost all other areas of biological research. To the dedicated scientist worshipping his Muse, rewards have been rapidly showered. Proteases were among the first enzymes to be purified and crystallized, and this helped pave way for the eventual understanding of protein structure and of the catalytic sites on different enzymes. Molecular understanding of biological phenomenon owes much to the use of proteases and protease inhibitors used as tools to dissect cellular processes. It has become increasingly evident that partial proteolysis is of very wide occurrence and that the key lies in delineating the mechanisms whereby the scope of proteolytic action is limited in time and space.

An earlier publication [1] has already explored the role of proteases in blood coagulation, complement activation, fibrinolysis, tumorogenesis, reproduction, cell growth, and selected other cellular aspects, although this is not an exhaustive list of topics that could have been covered at that time since proteolytic control was known in several other biological systems. Since then, the list of physiological levels of regulation sensitive to partial proteolysis has lengthened considerably. Thus, activation of renin precursor [2], thrombin-stimulated cell division [3], hormone–receptor interaction [4], cyclase modulation [5,6] are more recent examples of regulation by proteases and proteinase inhibitors. The advances in the in vitro properties and isolation of acid and neutral proteinases, and of protease inhibitors of microbial origin, too, have been rapid [7]. The time was therefore ripe to analyze the issues for future conceptual and practical attack.

The workshop in Munich brought together workers in very diverse areas with a view to confronting the individual investigator with one theme comprising conceptual and technical advances in related topics, the only common point being the regulation of these systems, at one point or another, by proteinases or their inhibitors. Professor Keil (Paris) kindly reviewed the current status of proteolytic enzyme structure and function, as well as the nature of different protease inhibitors, including microbial protease inhibitors isolated just a decade or so ago and which are now on the shelves of most investigators active in this area. Although the structure and action of extracellular proteinases is known in detail in many cases, the understanding of the initiation of their synthesis in pro- and eucaryotes is relatively poor. Only four types of active sites on proteinase protein are currently known and each newly discovered proteolytic enzyme is assigned to one of these categories, thus simplifying the task of finding, by analogy, an efficient inhibitor for the proteinase in question. Furthermore, endopeptidases attack only selected amino acid residues in proteins thereby raising the problem of control during regulatory proteolysis.

Professor Hales (Cambridge) suggested that the

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very fact of specificity of proteolytic enzyme attack sites (lysine or arginine for trypsin; phenylalanine or tyrosine for chymotrypsin), further confirmed by the specificity of inhibitor action (serine residues for lima bean trypsin inhibitor), would a priori exclude the hypothesis that sensitivity to proteolytic attack is simply a question of the number of such sites on the nonprotease protein. Rather, control must exist to limit access to the sensitive site, possibly by pairs or groups of basic amino acids as sites of initiation of endoproteolytic cleavage in prohormones. The obvious question was raised (Ryan) whether the number of serine residues could be related to the extent of proteolysis but this has not yet been experimentally substantiated (Keil). A curious observation is the fact that proteolytic activation in almost all systems on record seems to be accomplished by endopeptidases, the ends of chains being protected in some manner from attack by amino- or carboxypeptidases (Agarwal).

In order to account for the genesis of polypeptide hormones from biologically inactive prepro- and proproteins, Professor Hales presented evidence that the evolutionary origin of protein hormones is by lysosomal proteolysis. In lysosomes, after macromolecular degradation, the smaller components must enter the lysosomal membrane, possibly by the intervention of transport processes, although alteration of membrane permeability by dimethylsulfoxide did not affect the facilitated diffusion analyzed under these conditions. This evolutionary argument (Hales) envisages that mother nature took advantage of the protein fragments that existed during the evolutionary sequence and converted them to hormones with utmost economy to the host. This is supported by the existence of lactation factors in fish as well as mammals that these organisms are currently not using (Keil).

Professor Heimburger (Marburg/Lahn) reviewed the current status of the control of blood coagulation by proteinase inhibitors. Approximately 20 different plasma glycoproteins are known to be involved in the cascade of blood coagulation control, most of which are synthesized in the liver; some are enzymes, others are catalysts, and still others are substrates and/or inhibitors. Again, all enzymes are endopeptidases. The protease inhibitors are: antithrombin III (equivalent to heparin); α_2-macroglobulin; α_1-antitrypsin. Coagulation can also be activated by lysosomal proteases whose plasma concentration may vary during certain types of clinical pathology. It was pointed out (Agarwal) that the influence of microbial protease inhibitors (such as leupeptins) may tell us more not only about the mechanisms of coagulation but also its modulation during bacterial and other infections.

The importance of the levels of proteolytic activity, in plasma and in several tissues, in the physiological well being of the host was discussed by Dr Soria (Paris) using prekallikrein–kallikrein system as a model. For example, low plasma kallikrein levels are responsible for clotting abnormalities (Hageman factor-dependent) at a time when blood clotting factors are in the normal range. Tissue (kidney, pancreas, salivary gland) kallikrein levels, too, in certain cases, can be correlated with the onset of altered diuresis and hypertension.

Somatomedin B is an acidic, single chain peptide (5000 mol. wt) without structural homology to growth factors or insulin, but endowed with insulin-like and growth promoting properties (Forsman). It also exhibits weak trypsin inhibitory activity and is resistant to proteolytic attack. Its structural homology with some protease inhibitors, its lack of effect in vitro, and its stimulatory influence on DNA synthesis in glial cells in vivo, would all suggest that its action is based on the antiproteolytic property, similar to other protease inhibitors [3].

Thus, human pathology may be related both with the levels of plasma or tissue proteases (Heimburger, Soria) as well as protease inhibitors (Fryklund, Heimburger), all of endogenous origin and, as suggested earlier (Agarwal), the effect of microbial protease inhibitors on mammalian physiology in vivo would be of obvious interest.

The second part of the workshop focussed attention on two recently described systems that have never been discussed in a similar forum before. Professor Fanestil (San Diego) has used a number of different cell types and tissues to suggest that the steroid recognition site on the receptor for all classes of steroid hormones contains common structural features which allow recognition by various protease inhibitors and substrates, via competitive inhibition of the hormone–receptor binding. Dr Wrange (Stockholm) demonstrated progressive fragmentation of the liver glucocorticoid receptor by a number of common

lysosomal enzymes. The glucocorticoid receptor can be cleaved into a form incapable of binding to DNA and another that accepts the steroid on a mole per mole basis. This sequence can be inhibited by gelation but not by specific protease inhibitors (leupeptins, antipain) that have been successfully used by others to demonstrate the inhibition of steroid–receptor binding; phospholipases–phosphatidylcholine systems, too, were ineffective in their study, although active in the mouse fibroblast in studies by others. A long discussion ensued and it was agreed that the variability of techniques and tissues, used by different investigators, rules out meaningful comparisons. The point was raised whether the proteases and inhibitors are directly affecting the receptor or a transformation factor in the cytoplasm that is believed to modify the receptor (Agarwal) but again no consensus could be reached.

Agarwal et al. presented their multipolar model that calls for the preferential saturation of only a part of the complex, heterogeneous, polymorphic ligand, by a given steroid in the tissue in question, leaving other sites free to concurrently accept other signals. This is in contrast to the classical model of pharmacology where all grades of agonist and antagonist activity are expressed via interaction with the one and the same protein vector. The possibility was therefore entertained that multiplicity may be an expression of regulatory proteolysis. The effectiveness of papain, trypsin and chymotrypsin, in decreasing hormone–receptor binding, is dependent upon the steroid used to saturate the vector; thus renal progesterone versus aldosterone binding did not respond in the same manner. Similarly, lima bean trypsin inhibitor and ϵ-aminocaproic acid did not affect gluco- or mineralo- receptor–steroid binding but both increased liver estrogen and decreased liver androgen binding. Leupeptin almost completely eliminated liver cortisol binding, did not influence kidney aldosterone binding, increased liver androgen binding, but decreased hepatic estrogen binding. Multiplicity of liver gluco- and renal mineralo- corticoid peaks still persisted in chromatography after inclusion of proteases or their inhibitors during cytosol preparation and all subsequent steps. Thus, multipolarity of the steroid hormone receptor would appear to be an inherent property of the cellular vector necessary for physiological activity of the signal [8,9].

Professor Ryan (Rochester) presented a particularly provoking model according to which proteases may be involved in the coupling mechanism between hormone receptors and adenylate cyclase. In their two step model, low concentrations of protease inhibitors irreversibly affect the pathway of hormonal stimulation whereas a direct influence on the initial velocity of adenylate cyclase is envisaged with higher concentrations of protease inhibitors. In the ensuing discussion (Agarwal) it was pointed out that bacterial cyclase is not influenced by bacterial protease inhibitors that have been used with such wonderful effectiveness in mammalian systems. Does this signify that bacteria have developed mechanisms of protection in physiological reactions which could otherwise be modulated by endogenous protease inhibitors? If so, what are the mechanisms of regulation in bacteria of those physiological reactions that, in the mammal, are subject to regulation by bacterial protease inhibitors? Surprisingly still, no one has studied the effect of mammalian protease inhibitors (macroglobulins, somatomedins) on those reactions (cyclases for instance) that are subject to regulation by microbial protease inhibitors. Thus, the physiological meaning of studies with microbial protease inhibitors remains in suspense.

Working along the same lines as Dr Anderson (Bethesda) who was not present, the group of Dr Hanoune (Creteil) strived to piece together concurrent evidence that rat liver or turkey erythrocyte membrane adenylate cyclase and guanylate cyclase systems could be activated by various proteolytic enzymes of mammalian origin in the in vitro assays. The authors were clearly ill prepared to handle the discussion both conceptually and from the technical angle. Thus, it was not clear whether solvent controls were used since ethanol itself can increase hormonal responsiveness of cyclases, possibly by loosening the membranes initially to facilitate receptor movement, following which inhibition may ensue (Ryan). Again, no comments could be obtained when asked about the specificity of activation by the different proteolytic enzymes, especially in view of mitotic stimulation under similar conditions. If such in vitro studies have some in vivo relevance (Agarwal), one would expect changes in cyclase activity during those conditions (stress, pancreatitis, and many others) where the lyososomal content of plasma is known to increase, coagulation alteration under these conditions being

already well documented (Heimburger). Coming to their rescue, Ryan indicated that there are a lot of proteases and protease inhibitors floating around in different tissues in varying concentrations and, despite some advance [7], one has not even touched the surface of the problem as yet.

Lack of time heralded the end of the discussion. Some thoughts for future trends would include: assessment of signals on the nonprotease, and even the protease, peptide that limit proteolysis in space and in time, especially in view of the fact that papain, the most active proteolytic enzyme, is not of animal origin; the influence of microbial protease inhibitors on various systems in the host in vivo in relation to the role of endogenous inhibitors on the very same processes; correlations between the levels of circulating or tissue proteases and the extent of activation of the protease-sensitive process; the role of amino- and carboxypeptidases and the mechanisms whereby endopeptidase-sensitive processes are resistant to exopeptidase attack, even after partial cleavage by the former.

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