Lung Metabolism

Proteolysis and Antiproteolysis

Biochemical Pharmacology

Handling of Bioactive Substances

Edited by

Alain F. Junod

Rodolphe de Haller

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Preface

The long held traditional view that the lung is solely and passively involved in gas exchanges has been profoundly altered by contemporary research, which attests to its capacity to perform various metabolic tasks. This new knowledge had accumulated gradually, and had neither been systematized nor assessed. Therefore, it was decided to organize the Fifth Davos Symposium around these recent researches on the physiological and pathological nonventilatory functions of the lung, with the object of organizing this information and reviewing and evaluating the progress achieved. Accordingly, 28 experts involved in a number of relevant specialties gathered in Davos, Switzerland, October 7–9, 1974, to discuss their experimental and clinical findings.

The early phases of the conference were devoted to considering the response of the lung involved in preserving its integrity against enzymes, both exogenous (bacterial and mycotic) and endogenous, such as those coming from leucocytes or lung tissue itself. The balance between release of proteolytic enzymes and production of antiproteolytic substances in the lung may well be an important factor in the genesis of obstructive pulmonary disease. A better understanding of the mechanisms involved is also likely to play a major role in developing meaningful therapeutic modalities.

In succeeding segments of the conference specialists in various fields emphasized the capability of the lung to metabolize exogenous substances arriving by inhalation and by digestive or parenteral absorption. Much time was devoted to discussion of the role of the lung in homeostatic processes of the whole organism, such as coagulation, production and destruction of hormones and other biologically active substances. These metabolic functions of the lung are important per se and possibly as an alternative fail-safe system, should other organs, such as liver or kidney—more regularly involved in these processes—for any reason be rendered insufficient. Experimental researches on animals all too frequently disclose major species variations; special emphasis was therefore placed on the unique potential of cardiopulmonary bypass in man as a major investigative tool completely obviating these laboratory animal uncertainties. An understanding of the range and diversity of metabolic functions of lung is certainly valuable in terms of clinical management and moreover offers distinctive approaches for therapeutic explorations.

This volume consists of the formal presentations which in many instances also review the key literature, and an edited version of the individual and general discussions. The photocopy process for reproduction was utilized as the most efficient means of facilitating early publication; consequently, the con-

tributors had no further opportunity to modify their remarks. We thank Professor M. Landy for editorial guidance, particularly in coping with the discussion transcripts. We acknowledge with thanks the expertise and devotion of our secretaries, Mrs. R. Kindschi and A. Meylan, in the complex and exacting task of preparing the camera-ready typescript. Financial support for this symposium was kindly provided by the Swiss National Science Foundation, Grant No. 3.3120.74.

These proceedings bring to clinicians and basic scientists a wealth of information and commentary on all too little known aspects of lung functions. It is our hope that this volume will provide an organized source of information on these distinctive attributes of lung and help point the way for future work by focusing on continuing major problems and issues.

A.F. Junod

R. de Haller

Contents

Section I

Proteolysis and Antiproteolysis in the Lung

PROPERTIES AND ROLE OF ANTIPROTEASES

N. Heimburger

Proteinase inhibitors (PI's) are a group of peptides and proteins characterized by their ability to block the catalytic site of proteolytic enzymes; they have been detected in all living organisms, in plants as well as in bacteria and animals. They have been found in varying concentrations in different tissues of mammals. High amounts of PI's are localized in secretory glands and in plasma. From the beginning of this century PI's became of increasing interest in regard to their wide distribution and universal function. It is now well established that PI's control proteinases involved in various biological processes: Release of proteo-hormones (insulin), activation of zymogens, digestion of food, phagocytosis, immunodefense, inflammation, fertilization, blood coagulation, fibrinolysis and growth both normal and malignant.

The earliest observations concerning the antiproteolytic activity of serum were published at the end of last century (6, 13, 16), however it took another fifty years until six PI's of human plasma were isolated and characterized chemically as well as biologically (references in 20, 21).

Identification of six inhibitors in human plasma

Human plasma can be shown to contain at least six PI's when starch gel electrophoresis and fibrin containing agar plates are combined according to the sandwich principle (39). This technique is demonstrated in Figure 1. To prove the presence of PI's, plasma is separated electrophoretically in starch gel. The gel is then cut into two discs; the upper one is stained with amido black and the lower one is used to cover agar plates containing heat-inactivated fibrin. As soon as the proteins have entered the fibrin-agar film, troughs parallel to the migration direction are cut and filled with enzyme solutions. The substrate plates are then incubated at 37° C for about 20 hours. During this time, the proteases enter the gel. Diffusion is evidenced by lysis of the fibrin. Fibrin remains intact only in the electrophoretic positions of the inhibitors. By this technique, plasma can be shown to contain inhibitors against elastase, plasmin, trypsin, chymotrypsin; five of a total of six proved to be polyvalent. The

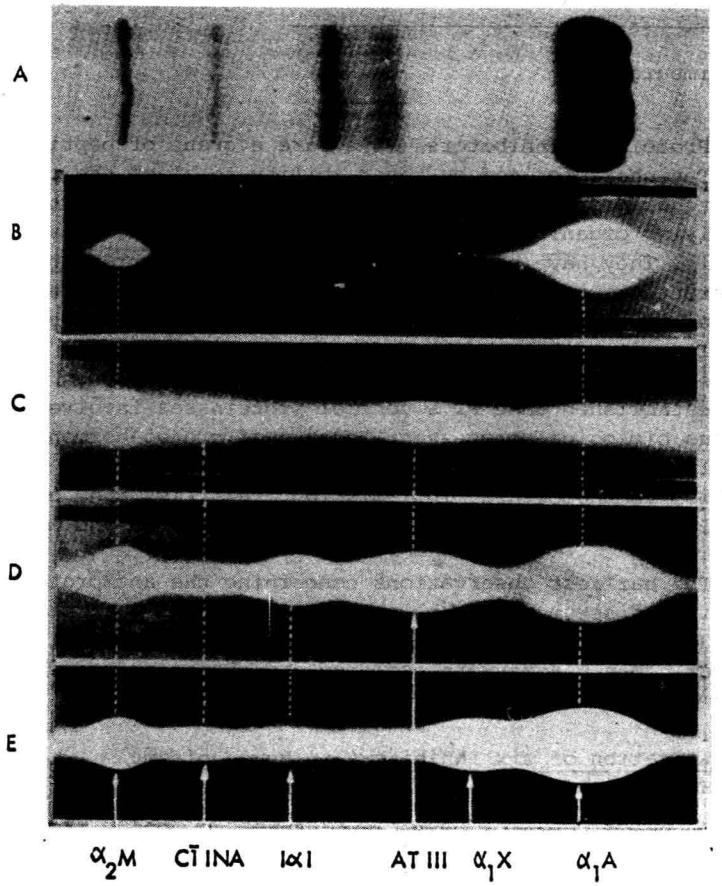

Fig. 1. Identification of six proteinase inhibitors in human plasma. Starch gel electrophoresis was used in combination with fibrin agar plates according to the sandwich technique. A. electrophoretic pattern of plasma stained with amido-black; B. fibrin plates after being covered with and lysed by elastase; C. plasmin; D. trypsin, and E. chymotrypsin.
(Heimburger et al., 1971) (Reproduced with permission of the publisher)

PI's have been designated according to their electrophoretic mobility and with respect to their most important physiological function known at the time of isolation.

Table 1 lists the names of the inhibitors together with their useful abbreviations and the normal concentration ranges in which they are found in human plasma. The inhibitors below the line (7-9) have not yet been isolated in pure and active form, however they have been traced by their function. Consequently it might be suggested that, in addition to α_2-macroglobulin (α_2M), there is a further inhibitor of thiol proteinases in plasma. There is also evidence for further inhibitors of clotting factors. It concerns the inhibition of F XIa by a protein not characterized up to now.

Figure 2 shows the main plasmatic inhibitors characterized by means of polyacrylamide gel electrophoresis. As might be expected α_1-antitrypsin (α_1A) and α_1-antichymotrypsin (α_1X) migrate in front of the plasma proteins, α_2-macroglobulin (α_2M) and Cl-inactivator (Cl INA) remain near the starting point and the inter-α-trypsin inhibitor (IαI) and antithrombin III (AT III) are found in between.

Figure 3 shows the plasmatic PI's characterized by means of immunoelectrophoresis and specific antisera. To indicate the electrophoretic positions of the PI's, human serum was separated on a plate containing heat-denatured fibrin which was developed thereafter by an antiserum to total human serum and trypsin as well (plate A in Fig. 3). It is evident that all plasmatic PI's migrate together with the α_1- and α_2-globulins. The plates B to G show electrophoretic patterns of human serum after developing with specific antisera to the six inhibitors. With the double diffusion technique of Ouchterlony we were able to demonstrate that the six PI's are individual proteins not related to each other.

All PI's are glycoproteins; the most relevant data are listed in Table 2 (20) including mean concentrations found in serum, molecular weight, peptide and carbohydrate content. As for concentration, α_1A and α_2M are predominant, while the other inhibitors are only present in traces in human serum. The concentration of all plasma inhibitors amounts up to about 700 mg/100 ml, that means 10% of the plasma proteins are carriers of inhibitor functions. The molecular weights vary from 54.000 for α_1A to 725.000 for α_2M (25). Even the carbohydrate content differs from one inhibitor to the other: α_1A and Cl INA are especially rich, containing 25% and 35%.