BASIC PROTEINS OF COHN FRACTION III OF NORMAL HUMAN PLASMA

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SUMMARY: A new class of proteins consisting of at least nine members was discovered in normal human plasma. These proteins, which were isolated from Cohn fraction III, are characterized by their basic properties. On electrophoresis at pH 8.6, they separated well from each other and formed a regular pattern comparable to that of the major plasma proteins. A nomenclature is proposed for these proteins designating them as B1 to B9. One of these basic proteins was isolated in homogeneous state and its molecular weight was calculated to be 11,000.

INTRODUCTION

Basic proteins may be defined as proteins which migrate towards the cathode at pH 8.6. The bulk of the human plasma proteins which are conventionally designated as albumin, α_1-, α_2-, β_1-, β_2- and γ-globulins, reveal negative mobilities on electrophoresis at the same pH. As to the basic proteins of human plasma, two minor components with electrophoretic mobilities slightly more positive than those of the γ_2-globulins have earlier been isolated from Cohn fraction VI (1). One of these proteins was isolated in homogeneous form and characterized in terms of its major chemical and physicochemical properties (2). Recently, two basic proteins were reported to be present in Cohn fraction III (3). The electrophoretic mobilities of these plasma constituents are considerably higher than

those of the mentioned basic proteins of fraction VI.

In the present paper, an extensive investigation of the basic proteins of Cohn fraction III of normal human plasma is briefly described. In this study, advantage was taken of the relatively specific adsorption of these proteins on Bentonite.

MATERIALS AND METHODS

Cohn fraction III (4), derived from pooled normal human plasma (1 kg), was suspended in 4 l of cold water, mixed with 30 g of bentonite prepared according to Lundblad and Hultin (5) and stirred for 30 minutes at 4°. The centrifuged bentonite was washed and then extracted several times with dilute pyri-

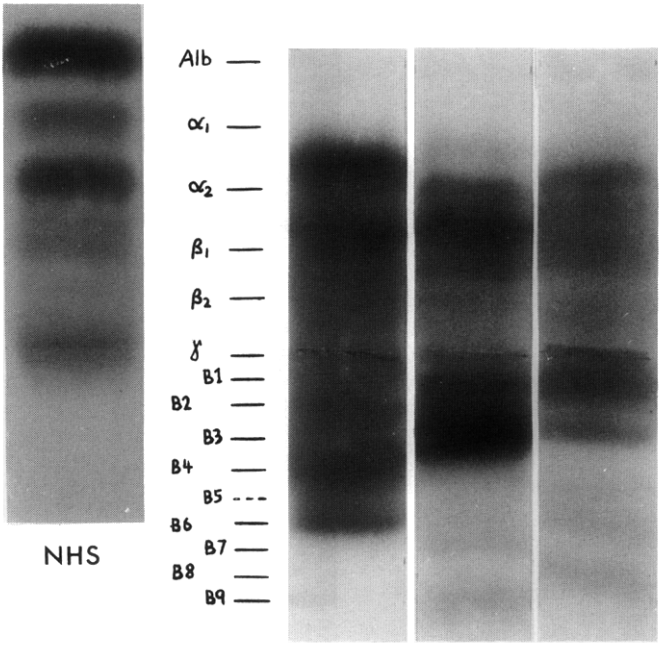

Figure 1: Paper electrophoresis of the proteins isolated from Cohn fraction III of normal human plasma. The positions of the basic proteins in the electrophoretogram are indicated by B1 to B9. These proteins migrated at different rates towards the cathode (-). For comparison, the corresponding pattern of normal human serum (NHS) with the positions of its major protein groups is included. Citrate-diethylbarbiturate, Γ/2 0.1, pH 8.6, was used as buffer.

dine-acetate buffer pH 4.5. The lyophylized extracts, when analyzed by paper electrophoresis (Fig.1), revealed several hitherto unknown basic proteins. It is of interest to note that these proteins are well separated and almost equally spaced from one another, resulting in a resolution comparable to that observed with the major plasma proteins.

RESULTS AND DISCUSSION

Fractionation of the proteins present in the mentioned bentonite extracts by gel filtration, using a Sephadex-A col-

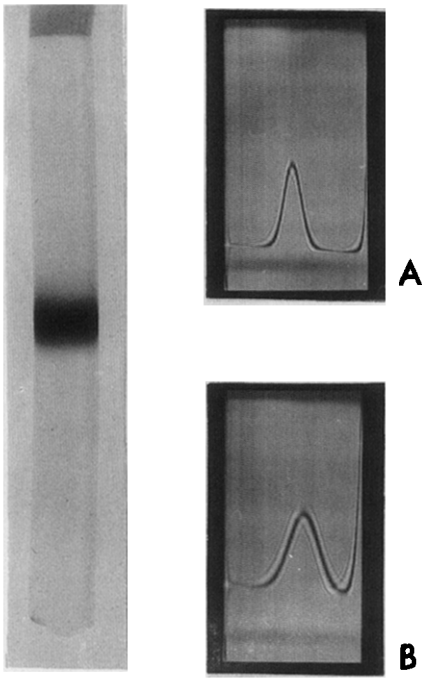

Figure 2: Disc acrylamide gel electrophoresis at pH 5 of the basic protein B6 isolated from Cohn fraction III of normal human plasma.

Figure 3: Ultracentrifugal analysis of B6. The direction of sedimentation is from left to right. A synthetic boundary cell was used for this experiment. The rotor speed was 52,000 rpm. These pictures were taken 8 (A) and 64 (B) minutes after full speed was attained. The bar angles were 45° and 60°, respectively.

umn and subsequent purification of the major component of one of the resulting subfractions by chromatography on a TEAE-cellulose and a Sephadex G-25 column, yielded, in a homogeneous state, a protein which was designated as B6.

This protein appeared homogeneous on paper electrophoresis at pH 8.6 and disc electrophoresis at pH 5.0 (Fig.2). Its electrophoretic mobility is similar to that of chicken egg white lysozyme. On ultracentrifugal analysis, B6 sedimented with a coefficent of 1.4 S at a concentration of 1%. A symmetrical refractive index gradient curve was observed (Fig.3). The molecular weight determined by the equilibrium technique of Yphantis (6) was calculated to be 11,000. Its amino acid composition (Table I) shows that, since protein B6 possesses essentially the same number of basic and acidic amino acid residues, the basic property of this protein was, therefore, achieved by amidation of a considerable number of the β- and/or γ-carboxylic groups of aspartic and glutamic acid residues. As the partially purified protein exhibited some lysozyme activity (5), the amino acid composition of B6 is compared in Table I with that of human tear lysozyme (7). It is evident that the two pure proteins are distinct from each other.

The present study reveals the presence of a series of hitherto unknown basic proteins in human plasma. These proteins form a new class of blood plasma proteins which appear to consist of at least nine members. A nomenclature is proposed for these proteins. It is suggested to designate these basic proteins with the prefixes B1 to B9. Only one of these proteins has previously been described (2). In the present investigation, another member of this class of proteins was partially characterized.

TABLE I

AMINO ACID COMPOSITION OF THE BASIC PROTEIN B6

DERIVED FROM COHN FRACTION III OF NORMAL HUMAN PLASMA

Amino Acids*	B6	Human** Lysozyme	Amino Acids	B6	Human Lysozyme
Asx	9	13	Met	1	2
Thr	6	5	Ile	3	4
Ser	5	5	Leu	8	6
Glx	11	8	Tyr	2	4
Pro	3	2	Phe	4	2
Gly	6	9	Lys	8	4
Ala	6	9	His	2	1
Val	4	6	Arg	11	9
1/2 Cys	6	5	Try	0	4
Total number of residues				95	98**
Positively charged residues				21	14
Negatively charged residues				20	21

* Expressed in moles of amino acids per mole of protein.

** The number of residues of this protein was reduced to correspond to a molecular weight of 11,000. These data were taken from Jollès and Jollès (7).

*** The number of amides are not taken into account.

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