Isolation and Characterization of a Low Molecular Weight Basic Protein of Normal Human Plasma*

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SUMMARY

A method was developed for the purification of a basic protein, designated as B1, derived from normal human plasma. Consecutive chromatography on carboxymethyl cellulose, diethylaminoethyl sephadex, and hydroxylapatite yielded a preparation that was homogeneous on starch gel electrophoresis.

The plasma protein, B1, was characterized in terms of its major physicochemical and chemical properties. The molecular weight was determined to be approximately 9000 and the \( \bar{v}_p \) value 1.3 S. The calculated shape of B1 corresponds to an ellipsoid of revolution with a frictional ratio of 1.25 and an axial ratio of 4:5. The isoionic and isoelectric points of this protein were found to be at approximately pH 10.7 and 10.1, respectively. At pH 8.6 the electrophoretic mobility of this blood constituent was \( +1.3 \times 10^{-5} \) cm\(^2\) volt\(^{-1}\) sec\(^{-1}\). B1 possesses one polypeptide chain as deduced from one amino-terminal amino acid, i.e. aspartic acid (or asparagine), and one carboxyl-terminal amino acid, i.e. glycine. It is devoid of carbohydrates and is further distinguished by the lack of histidine and tryptophan. The basic nature of this protein is due to the high amide content rather than a high content of basic amino acids. Based on optical rotatory dispersion measurements B1 has \( \alpha \)-helical structure involving about 50% of the amino acid residues.

EXPERIMENTAL PROCEDURE

Starting Material—A solution of Cohn Fraction VI which had a pH between 5.5 and 6.0 and a specific resistance between 1800 and 2100 ohm-cm was prepared according to an earlier procedure (2). Eighteen liters of this solution, derived from 300 liters of plasma, were passed over a DEAE-cellulose column (3). The proteins of the resulting effluent were chromatographed subsequently on a carboxymethyl cellulose column (3) (Fig. 1) affording 3 S and 2 S proteins with mobilities of the \( \gamma_1 \) and \( \gamma_2 \)-globulins, respectively, and a fraction, referred to in Fig. 1 as Basic, containing the mentioned basic proteins, a pink component (4), and small amounts of \( \beta \)- and \( \gamma \)-globulins (Fig. 2). For the latter separation a new type of CM-cellulose (Whatman CM 32) was utilized. Because of the homogeneous microgranular form of this ion exchange agent, the column volume could be reduced to half that of a corresponding column of conventional CM-cellulose (2, 3). The solution containing the basic components was dialyzed against cold water, and it was lyophilized yielding 250 mg. This preparation represents the starting material for the present study.

Chromatographic Materials and Analytical Methods—Hydroxylapatite was prepared according to the procedure of Tiselius, Hjerten, and Levin (5) and Levin (6). DEAE-Sephadex AK50 was purchased from Pharmacia. The other reagents were the same as those employed in earlier studies (2, 3). All chromatographic procedures were carried out at 31\(^\circ\). The methods employed for the physicochemical and chemical characterization of B1 were essentially the same as those described earlier (2, 3). The frictional coefficient was calculated according to the procedure of Edsall (7, 8) from \( M \), partial specific volume \( \bar{v} \), and \( s \) by the relation

\[
f = M (1 - \bar{v} \rho)/N\bar{s}
\]

RESULTS AND DISCUSSION

Purification of Basic Protein, B1

The starting material was dissolved in 10 ml of Tris-HCl buffer, pH 8.4, \( \Gamma/2 = 0.005 \), and applied to a Sephadel A-50
column (4.0 x 20 cm) (Fig. 3) previously equilibrated with the same buffer. The proteins not retained on the column were recovered by passing additional buffer through the column at a flow rate of 30 ml per hour (Position I in Fig. 3). This fraction designated as Basic contained, as judged by paper electrophoresis (Fig. 2A), the basic proteins, B1 and B2 (1), the mentioned pink component, not seen on the electrophoretogram after staining with the conventional Amido black technique, and trace amounts of \( \gamma \)-globulin. On starch gel electrophoresis at pH 8.6 four components were also discerned. The solution of the Basic proteins was dialyzed against cold water and lyophilized, affording 65 mg of protein. Small amounts of B1 (1) were probably lost during dialysis. The proteins (63 mg) retained on the column were displaced with 1.0 M sodium chloride in the same buffer (Position II, Fig. 3) and consisted of \( \beta \)- and \( \gamma \)-globulins. This fraction was free of basic proteins and was slightly yellowish.

Insoluble material formed during dialysis was subsequently removed, and it accounted for the difference in weight of the starting material and that of the two obtained subfractions. The recovery was approximately 50% in terms of weight of the final fractions. However, before dialysis and in terms of absorbance at 280 nm the recovery was approximately 90%.

Final purification of the basic protein, B2, was achieved by chromatography on a hydroxylapatite column (2.0 x 30 cm) at pH 6.8. The partially purified basic proteins resulting from the previous step were dissolved in 10 ml of 0.01 M sodium phosphate buffer, pH 6.8, and then applied to this hydroxylapatite column which had been equilibrated with the same buffer (Fig. 4). Fractional elution was performed by stepwise increase in the molarity of the sodium phosphate buffer from 0.01 (Position I) to 0.05 (Position II) to 0.1 (Position III). Aliquots of 5 ml of effluent were collected at a flow rate of 20 ml per hour. The chromatographic pattern (Fig. 4) showed two small peaks displaced at the two lower phosphate concentrations and a main peak eluted at the higher concentration. After dialysis and lyophilization, these fractions yielded 2.5, 2.0, and 47 mg, respectively. The pink constituent (12 mg), which will be described later, was eluted at a higher ionic strength. The total recovery was approximately 90%, corrected for salt and
moisture. Based on absorbance measurements at 280 nm, the recovery was 98%, the difference mainly being due to losses of dialyzable B₁ (1) of which the extinction coefficient at 280 nm is very small.

Paper electrophoresis indicated that the major fraction contained B₂ in essentially homogeneous form (Fig. 2H). B₂ migrated slightly slower than lysozyme, the reference protein, but faster than the other basic protein, B₁ (1). The apparent mobility of this protein on paper electrophoresis may be explained by the isoelectric point of B₁ which is between pH 9 and 10 (1) and that of lysozyme which is between pH 10.5 and 11.0 (9). Hence, the isoelectric point of B₂ was estimated to be close to pH 10. The protein of the two minor peaks was dialyzable and it migrated with the electrophoretic mobility of B₁. Sufficient quantities of B₂ from several preparations of Fraction VI were combined and used for the subsequent characterization of this protein. The content of this constituent in pooled normal human plasma was estimated to be approximately 20 mg/100 liters of plasma.

**Physicochemical Characterization**

The major physicochemical properties of the basic protein, B₂, are listed in Table I.

![Image](https://via.placeholder.com/150)

**TABLE I**

<table>
<thead>
<tr>
<th>Physicochemical properties of basic protein, B₂, of human plasma</th>
</tr>
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<tbody>
<tr>
<td><strong>Molecular weight</strong></td>
</tr>
<tr>
<td>Equilibrium procedure (pH 10.5; 7.0) Calculated (s, D, θ)</td>
</tr>
<tr>
<td>Sedimentation coefficient, s₂₀,₀</td>
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<tr>
<td>Diffusion constant, D₂₀,₀</td>
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<tr>
<td>Partial specific volume, calculated, θ</td>
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<tr>
<td>Frictional ratio</td>
</tr>
<tr>
<td>Axial ratio</td>
</tr>
<tr>
<td>Electrophoretic mobility at pH 8.6</td>
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<tr>
<td>Isoelectric point</td>
</tr>
<tr>
<td>Isoionic point</td>
</tr>
<tr>
<td>Extinction coefficient, E₁% at 278 nm</td>
</tr>
<tr>
<td>Specific optical rotation, [α]²⁰⁰</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Urea, 8 M</td>
</tr>
</tbody>
</table>

**FIG. 5.** Free moving boundary electrophoresis of B₂ in citrate-diethylbarbiturate buffer, at pH 8.6, I/2 = 0.1. The salt boundary of the ascending pattern is designated with δ, and that of the descending α. The pictures were taken after 130 min of electrophoresis with a current of 9 ma (190 volts).

**FIG. 6.** Ultracentrifugal patterns of a 0.8% (normal cell) and 0.4% (optically wedged cell) solution of B₂ in 0.10 M NaCl. The pictures were taken at 6, 134, and 198 min after full speed of 66,100 rpm had been attained. The angle of the phase plate was 45° for the first and 55° for the other pictures.

**FIG. 7.** Optical rotatory dispersion of B₂. For the calculation of the reduced mean residue optical rotation (R'), an average molecular weight of the amino acid residues of 112 was used. Correction for the refractive index of the solvent was applied.

**Free Moving Boundary Electrophoresis of B₂**—At pH 8.6 (Fig. 5) this method revealed homogeneity. The observed electrophoretic mobility is given in Table I.

**Isoelectric Point of B₂**—After passage through a Dintziis column (10), the isoelectric point was found to be between pH 10.5 and 11.

**Starch Gel Electrophoresis**—By means of this method the B₂ protein appeared homogeneous in borate buffer at pH 8.6 with an ionic strength of 0.01, and it migrated toward the cathode with a higher electrophoretic mobility than lysozyme, the reference protein. The relatively high mobility of B₂ can probably be explained by the similar electrophoretic mobilities of these two proteins observed on paper electrophoresis, modified by their different molecular weights, so that B₂ migrated faster than lysozyme.

**Ultracentrifugation**—In 0.1 M sodium chloride, B₂ revealed a single symmetrical refractive index gradient (Fig. 6) upon ultracentrifugation. The sedimentation coefficient and diffusion

1 This aspect of the study was included in a preliminary note (11).
constant (calculated by the "height-area" method) of this protein were measured within a concentration range from 0.1 to 1.0%. The obtained values, extrapolated to zero concentration, are listed in Table I. The molecular weight was derived from sedimentation equilibrium experiments (12) and further calculated from sedimentation coefficient, diffusion constant, and partial specific volume (Table I). The partial specific volume was computed from the amino acid composition (13). In addition the equilibrium runs were carried out both at pH 7.0 and 10.5, and almost identical molecular weights were obtained.

**Specific Optical Rotations and Optical Rotatory Dispersion**—The specific optical rotations of native and denatured Bz measured at 546 mp are listed in Table I. The optical rotatory dispersion (Fig. 7) carried out between 195 and 560 mp resulted in a curve which was very similar to that of a typical α-helical protein. A trough and a maximum were observed at 233 and 198 mp, respectively. The magnitudes were −6,700 for the trough and +29,000 for the peak. The crossover point was found at 228 mp and a shoulder was noted between 210 and 220 mp. A helicity of approximately 40% was calculated from the magnitude of the trough (14). From the Moffitt equation (15), a bα value of −380 was obtained, indicating an α-helical content of approximately 60%. Comparable studies of other proteins (2, 16) showed that such uncertainty in the helicity should be expected.

**Extinction Coefficient and Tyrosine-Tryptophan Contents**—The absorption curve of Bz (Fig. 8) measured at pH 6 in 0.1 m sodium chloride revealed a low maximum at 278 mp, suggesting a low magnitude of the trough (14). From the Moffitt equation (15), the magnitudes were −6,700 for the trough and +29,000 for the peak. The crossover point was found at 228 mp and a shoulder was noted between 210 and 220 mp. A helicity of approximately 40% was calculated from the magnitude of the trough (14). From the Moffitt equation (15), a bα value of −380 was obtained, indicating an α-helical content of approximately 60%. Comparable studies of other proteins (2, 16) showed that such uncertainty in the helicity should be expected.

**TABLE II**

<table>
<thead>
<tr>
<th>Chemical composition of basic protein, Bz, of human plasma</th>
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<tbody>
<tr>
<td>Amino acid residue</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Glutamic acid</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Half-cystine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>Isoleucine</td>
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<tr>
<td>Leucine</td>
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<tr>
<td>Tyrosine</td>
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<tr>
<td>Phenylalanine</td>
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<tr>
<td>Ammonia</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* Conventional corrections were applied.
* Calculated for the molecular weight of 9000.
* Calculated from cysteic acid.
* Calculated from the value of methionine sulfoxide of 24-hour hydrolysate.
* Conway.
content of tyrosine or tryptophan or both. From this value, an extinction coefficient, \( \varepsilon_{280} \), at 278 nm of 3.32 was calculated.

The absorption curve in 0.1 N sodium hydroxide showed a low maximum at 290 nm indicating the absence of tryptophan. No marked change in the absorption was observed between 1 and 24 hours of incubation at room temperature. The content of tyrosine, calculated from this curve, was 2.23% or 1.0 mole per mole of protein, agreeing with the amino acid composition of this protein (Table III). It should be noted that 1 mole of tyrosine accounts essentially for the total absorbance of Bz at 278 nm and pH 6. Hence, the extraneous absorption of this plasma constituent is very small.

**Chemical Characterization**

The major chemical properties of Bz are summarized in Table II. The polypeptide moiety accounted for the total weight of this protein. Independent measurements confirmed the absence of neutral hexoses, hexosamines, and sialic acid.

**Terminal Amino Acids**—Sanger’s procedure yielded 0.93 mole of aspartic acid (or asparagine) per mole of protein (corrected for losses of 2,4-dinitrophenyl-aspartic acid due to acid hydrolysis and paper chromatography). The carboxyl-terminal amino acid determined by hydrazinolysis proved to be glycine, and 0.54 mole (corrected) of this amino acid per mole of protein was recovered. No other amino acids were detected with or without the use of heptaldehyde. These data suggest the presence of a single polypeptide chain in this protein.

**Amino Acid Composition**—The Bz protein was hydrolyzed for 24, 48, and 72 hours and, after performic acid oxidation, for an additional 24 hours. These hydrolysates remained colorless, again pointing to the absence of tryptophan and carbohydrate in this protein. The conventional corrections were used for the calculation of the amino acid composition (Table III). The content of half-cystine was derived from the content of cysteic acid of the performic acid-oxidized protein. Spectrophotometric titration of Bz with p-hydroxymercuribenzoate revealed the absence of free sulfhydryl groups. The amino acid residues accounted for 101.3% of the total weight and for 102.0% of the total nitrogen of the protein. This basic protein consists of approximately 80 amino acid residues of which 14 are basic and 19 acidic. As there are 16 amide residues, the number of free \( \alpha-, \beta-, \) and \( \gamma \)-carboxylic groups is 4. It is of considerable interest to observe that the basic nature of this protein is primarily due to the fact that most of the carboxylic groups are present as amide. Furthermore, the lack of histidine and tryptophan; the low content of tyrosine, isoleucine, and methionine; and the relatively high content of glutamic acid or glutamine, proline, and cystine should be noted. For comparison it should be added that basic proteins (for reviews see References 17 and 18) associated with RNA and DNA of cells are usually devoid of cysteine, methionine, and tryptophan and, more important, the moir\( \alpha \) content of arginine and lysine is greater than that of the acidic amino acid residues.

Regarding the relationship between chemical composition and conformation of Bz, it is difficult to assess the exact content of \( \alpha \)-helical structure of this protein, but it appears that the helicity might be as high as 50%. The amino acid composition does not exclude the formation of helical structures. As for the disulfide bridges, it is known that in cytochrome c and insulin these bridges are either incorporated in \( \alpha \)-helices or that they stabilize such conformations (19). From the studies on hemoglobin (20) it is known that proline residues may be located within the \( \alpha \)-helical structures.

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