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ISOELECTRIC POINT OF NATIVE AND SIALIDASE-TREATED HUMAN-SERUM CHOLINESTERASE

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SUMMARY

1. The electrophoretic mobility of native and sialidase-treated human-serum cholinesterase was determined by paper electrophoresis over the pH range 2.8–9.6; the determination included corrections for electro-osmosis, evaporation, paper-structure and adsorption of protein to the paper. The performance of the method was controlled with proteins of known mobility, orosomucoid and coeruloplasmin.

2. The isoelectric point of native cholinesterase was found to be 2.9–3.0 and that of sialidase-treated cholinesterase 6.7–7.0. This is in agreement with previous findings that human-serum cholinesterase contains a large number of sialic acid residues in a terminal position.

3. At pH 8.6 the electrophoretic mobility of native cholinesterase was $-3.1 \cdot 10^{-5}$ cm²/V/sec and that of sialidase-treated cholinesterase $-0.2 \cdot 10^{-5}$ cm²/V/sec.

4. Between pH 4 and 9.6 the mobility of sialidase-treated cholinesterase was $2.9 \cdot 10^{-5}$ units above that of native cholinesterase.

5. At pH 2.8 this difference decreased to about $2 \cdot 10^{-5}$ units.

6. It is suggested that the mobility–pH curve might be of value in the differentiation between closely related types of cholinesterases.

INTRODUCTION

The decrease in electrophoretic mobility of human-serum cholinesterase by enzymic release of sialic acid indicates that serum cholinesterase is a mucoprotein with several sialic acid residues in a terminal position[1,2]. This suggests that the enzyme is a protein with a low isoelectric point. Paper electrophoresis at pH 4.4 did in fact show the acid character of the enzyme in that it migrated towards the anode faster than albumin[3].

The aim of the present investigation was to determine the isoelectric point of human-serum cholinesterase in the native and the sialic acid-free state. For this purpose the electrophoretic mobility of the enzyme was determined on paper at different pH values. The enzyme was localized on the paper by its enzymic activity. Corrections were made for evaporation, electro-osmosis, structure of the paper and adsorption of protein by comparing the migration of the enzyme with that of dextran

as an uncharged reference substance[4,5]. To control the performance of the method with acid sialoproteins, the electrophoretic mobilities of orosomucoid and coeruloplasmin on paper were compared with their free electrophoretic mobilities as reported in the literature. A preliminary report of this study has been published[6].

METHODS

Preparation of human-serum cholinesterase, orosomucoid and coeruloplasmin

General procedures: The preparation of the proteins used in this study included fractional precipitation with ammonium sulphate, chromatography on DEAE-cellulose and preparatory electrophoresis.

Chromatography on DEAE-cellulose (Whatman DE 50) was carried out at room temperature. Elution was performed with the salt and pH gradients described below; the eluates were collected in 10–25-ml fractions and their conductivity and pH were determined (conductivity meter CDM 2 and pH-meter PHM 22, Radiometer, Copenhagen).

Before electrophoresis, the eluates were desalted on a column of Sephadex G-25 (Pharmacia, Upsala, (Sweden)) or by dialysis. Concentration was performed by adsorption to a short column of DEAE-cellulose and subsequent elution with 1 M NaCl. Finally, the protein was transferred to the appropriate buffer by exchange on a column of Sephadex G-25.

Preparatory electrophoresis was carried out on a column (100 cm × 3.1 cm^2 (see ref. 7)) packed with ethanolyzed cellulose (Munktell, Sweden) in 0.1 M Tris buffer (pH 8.6) or in 0.1 M sodium acetate buffer (pH 4.65) with 3.5 V/cm for 80 h at 10°. After completion of the run, the liquid phase was displaced by means of a pump (Miniflow, LKB, Stockholm) at a rate of 25 ml/h and the eluate was collected in fractions of 10–25 ml.

The protein concentration was estimated by continuous recording (LKB Uvicord, 3-mm light path) of the transmission at 254 mμ or by measurement of the extinction coefficient at 280 mμ (Beckman spectrophotometer DB). Sialic acid was determined according to WARREN[8].

Localization of cholinesterase in the eluates was carried out with the α-naphthyl acetate-Fast Blue B method[2,9]. The cholinesterase activity in the preparations was determined titrimetrically at pH 7.0 and 25° with 5 mM of butyrylcholine iodide as substrate in pure water by means of a recording constant-pH titrator.

Human-serum cholinesterase was prepared from outdated plasma (provided by the blood bank, University Hospital, Copenhagen). To 1 l of plasma, 1 l of saturated ammonium sulphate was added. After centrifugation, the supernatant was brought to pH 4.5 with 1 N H_2SO_4 and the precipitate was removed by centrifugation. The supernatant was brought to pH 7.3 with 1 N NaOH and saturated ammonium sulphate solution was added to a saturation of 67%. These steps were carried out at 5°. The precipitate was collected by centrifugation, dissolved in distilled water and dialyzed for 18 h against running tap water. A slight precipitate was removed by centrifugation and the supernatant was then chromatographed on DEAE-cellulose (Fig. 1 A). The fraction containing cholinesterase (fraction I) was dialyzed against running tap water for 18 h and re-chromatographed on DEAE-cellulose

(Fig. 1 B) The fraction exhibiting cholinesterase activity (fraction II) was concentrated and preparatory electrophoresis performed at pH 8.6 (Fig. 1 C). After concentration of cholinesterase fraction III, electrophoresis was performed at pH 4.65 (Fig. 1 D). The cholinesterase fraction IV was finally concentrated and desalted. The purification factor was 5000 and the yield 8.1%. Another preparation (purification factor 285) was obtained by DEAE cellulose chromatography and preparatory electrophoresis at pH 8.6.

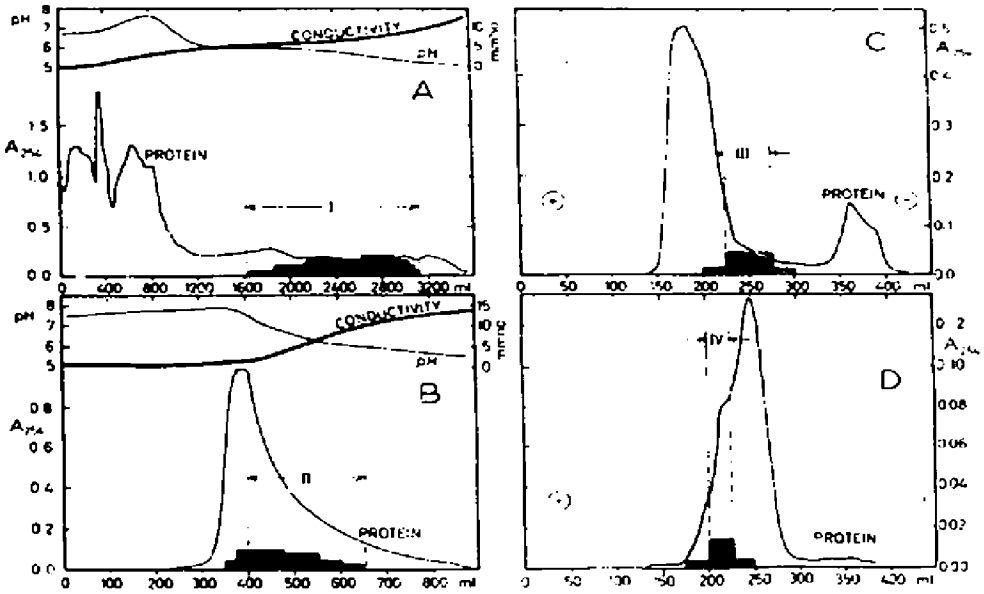

Fig. 1. Preparation of human-serum cholinesterase. (A) DEAE-cellulose chromatography of human-serum cholinesterase purified by ammonium sulphate fractionation. The column (100 cm × 5 cm²) was eluted with solutions of gradually increasing molarity from 5 mM NaH_2PO_4 (pH 7.0) to 50 mM NaH_2PO_4 + 0.3 M NaCl. The height of the black columns indicates cholinesterase activity as determined by the semi-quantitative spot assay. The upper curves show the conductivity and the pH of the effluent. (B) DEAE-cellulose chromatography of Fraction I (Fig. 1A). The column (30 cm × 5 cm²) was eluted with solutions of gradually increasing molarity from 5 mM NaH_2PO_4 (pH 7.0) to 50 mM NaH_2PO_4 + 0.2 M NaCl. (C) Preparatory electrophoresis at pH 8.6 of Fraction II. (D) Preparatory electrophoresis at pH 4.65 of Fraction III. Fraction IV was concentrated and used in the experiments.

Sialic acid-free human-serum cholinesterase was prepared from a partially purified preparation of human-serum cholinesterase (purification factor 285). 1 ml of the preparation was incubated for one week at 25° with 1 ml of buffer (0.2 M NaCl–0.1 M CH_3COONa–20 mM $CaCl_2$, adjusted to pH 5.5 with 6 N HCl) and 50 μl of a purified sialidase preparation (Neuraminidase, Behringwerke, Marburg (Germany)). One drop of toluene was added to prevent bacterial contamination. Every day paper electrophoresis was performed on a sample of the incubation mixture. After four days the migration distance of cholinesterase became constant. Desalting and separation from sialidase was carried out on a column of Sephadex G-75. By the same procedure a preparation was obtained from unpurified human-serum cholinesterase.

Human orosomucoid was prepared from 1 l of outdated plasma[10]. The preparation thus obtained was electrophoretically inhomogeneous at pH 8.6 (Fig. 2 B). Therefore, it was chromatographed on DEAE-cellulose (Fig. 2 A) and resolved into three fractions. Fractions I and II were discarded; fraction III was electrophoretically homogeneous at pH 8.6 (Fig. 2 C) and showed the same mobility as human α_1-globulin (Fig. 2 D). Fraction III was concentrated and desalted on Sephadex G 25; it contained about 10% of sialic acid, in agreement with the value of POPENOE AND DREW[11].

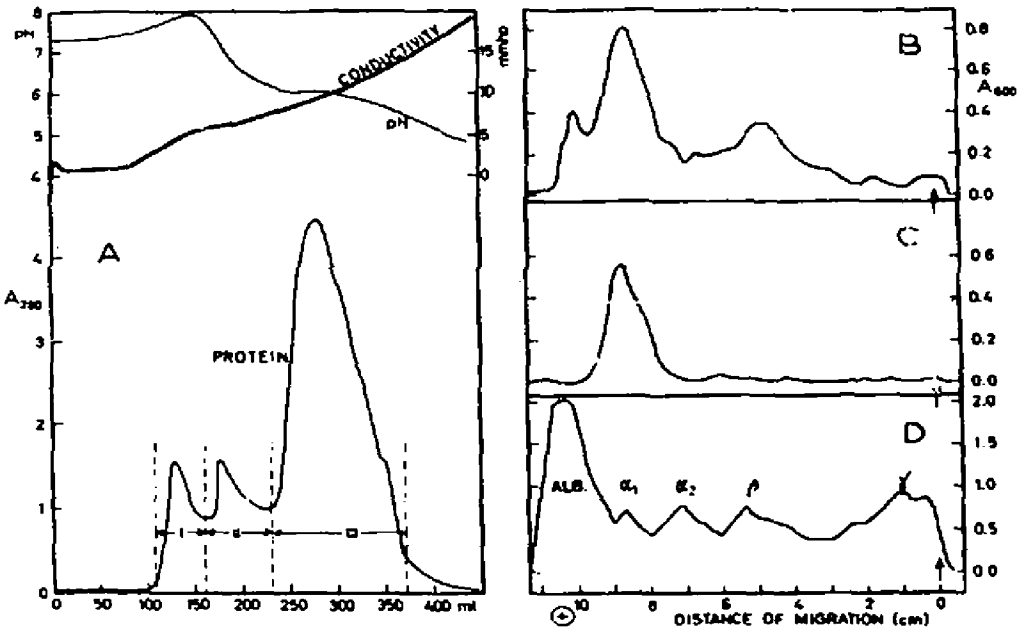

Fig. 2. Preparation of human orosomucoid. (A) DEAE-cellulose chromatography of orosomucoid prepared by fractional ammonium sulphate fractionation. The column (16 cm × 5 cm²) was eluted with solutions increasing gradually from 50 mM NaH_2PO_4 to 50 mM NaH_2PO_4 + 0.3 M NaCl. The upper curves show the conductivity and pH of the effluent. Fractions I and II were discarded; Fraction III was concentrated and used in the experiments. (B) Paper electrophoresis (barbital buffer (pH 8.6)) of the preparation before chromatography. (C) Paper electrophoresis (pH 8.6) of Fraction III. (D) Paper electrophoresis (pH 8.6) of human serum.

Human coeruloplasmin was prepared from 1 l of human plasma obtained from outdated blood. The plasma was dialyzed against running tap water for 18 h. The fibrin clot was centrifuged off and the supernatant was chromatographed on a column of DEAE-cellulose (80 cm × 21 cm²); it was eluted with solutions of gradually increasing molarity from 5 mM NaH_2PO_4 (pH 7.0) to 50 mM NaH_2PO_4 + 0.4 M NaCl. The fractions containing coeruloplasmin were easily recognized by their distinctly blue color. They were pooled, dialyzed against running tap water, and rechromatographed on DEAE-cellulose (18 cm × 5 cm²); they were eluted with solutions of increasing molarity from 50 mM NaH_2PO_4 to 50 mM NaH_2PO_4 + 0.2 M NaCl. After concentration and desalting $E^{1\%}_{1\,cm}$ at 280 mμ was 83.2 and that at 605 mμ, 3.40. The ratio of E_{605} to E_{280} indicates a purity of 60–70% (see ref. 12).

The impurities in the preparation did not affect the determination of the distance of migration, because coeruloplasmin was specifically localized on the paper by its blue color.

Determination of electrophoretic mobility

Paper electrophoresis was used in the determination of electrophoretic mobility[4,5]. Minor modifications were introduced with respect to the concentration of dextran, temperature, measurement of the potential gradient within the paper strips, and impregnation of the paper. These modifications and the application of equipment different from that used by SCHILLING AND WALDMANN-MEYER[4] necessitate a brief description of the procedure employed in this study.

The LKB electrophoresis apparatus was modified in the following way: To apply the samples without removing the lid of the cassette an extra transversal slit was made in the lid at a distance of 14.6 cm from the cathodic slit. To measure the potential gradient in the paper strips, four electrode holders were inserted through the bottom of the cassette (for location see Fig. 3). The electrodes consisted of

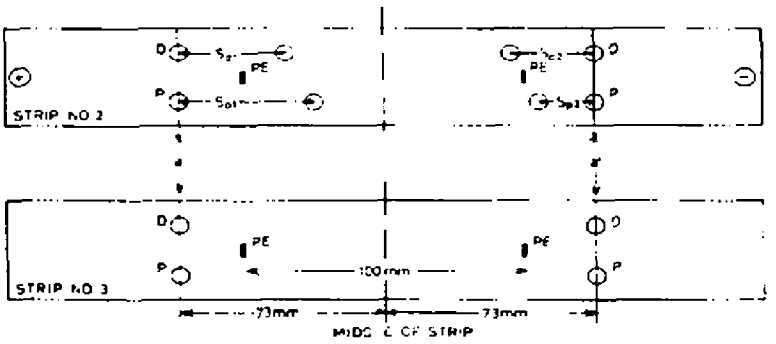

Fig. 3. Filter paper strips No. 2 and 3. Pencil lines were drawn transversally (a and a'). The application points for dextran (D) and protein (P) and the location of the platinum electrodes (PE) are indicated. Migration distances of dextran (s_{d_1} and s_{d_2}) and of protein (s_{p_1} and s_{p_2}) are shown.

0.4 mm platinum wire bent as triangles with a side length of 3 mm and placed so that one side touched the paper transversally. To avoid conduction along the moist surface of the cassette, the electrode holders were coated with silicone grease. A constant current power supply was used (output impedance at 4 mA was 15 MΩ). The potential difference between the platinum electrodes was measured with an electronic voltmeter with an input resistance of 40 MΩ and floating input. The high resistance in the circuit made the polarization negligible.

The experiments were performed in the cold room (2–7°). The temperature was measured before and after the electrophoretic run or it was recorded, during the run, by means of a thermistor thermometer. The average of the initial and the final temperatures was taken as the experimental temperature. The temperature variation during an experiment did not exceed 1°.

The protein samples were transferred to the appropriate buffers by exchange

on a Sephadex G-25 column, or by dialysis for 48 h. The proteins were used in the following concentrations: Native cholinesterase (purification factor 285) 0.06 mg/ml, native cholinesterase (purification factor 5000) 0.01 mg/ml, sialidase-treated cholinesterase (purification factor 285) 0.03 mg/ml, orosomucoid 3 mg/ml, and coeruloplasmin 10–20 mg/ml. The reference substance, Dextran 80 (Pharmacia), was diluted to a final concentration of 1% (w/v) from a 20% stock solution. When higher concentrations were used, *e.g.* 4%, as recommended by WALDMANN-MEYER AND SCHILLING[8], the migration distance of the dextran spot was reduced, probably due to the high viscosity of the sample (Table I).

TABLE I

MIGRATION DISTANCE OF DEXTRAN AS A FUNCTION OF DEXTRAN CONCENTRATION

Acetate–barbital buffer (pH 6.6) at 6° for 18 h.

Concentration of dextran 80 (%)	*Migration distance of dextran (mm)*
20.0	7
14.3	12
10.0	14
5.0	17
2.0	21
1.0	21.5
0.5	21.5
0.25	21.5

Four paper strips (Whatman 3 MM, 40 × 425 mm) were marked with pencil lines (a and a', Fig. 3). The strips were wetted in a 0.1% solution of human γ-globulin (Statens Seruminstitut, Copenhagen) in the buffer to be used in the experiment. The strips were blotted on a sheet of filter paper and placed in the cassette with the transverse pencil lines exactly below the slits in the lid and equilibrated for at least 3 h. The samples (2 μl) were applied on the two middle strips (No. 2 and No. 3, Fig. 3) and the power was switched on. After about 18 h the potential difference between the two sets of platinum electrodes was measured, and the power switched off. The duration of the experiment was determined within $\pm$ 100 sec. 8 cm of the

TABLE II

CHANGE IN pH OF THE PAPER STRIP DURING ELECTROPHORESIS IN DIFFERENT BUFFERS

Electrophoresis at 2–7° for 18 h.

Buffer	*pH*	*Number of experiments*	*Δ pH $\pm$ S.E.*
Phosphate	2.75	4	0.00
Acetate	2.9–4.9	17	+ 0.12 $\pm$ 0.01
Acetate	5.0–6.3	10	+ 0.06 $\pm$ 0.01
Acetate–barbital	6.4–8.0	12	− 0.01 $\pm$ 0.01
Acetate–barbital	8.1–9.6	10	− 0.07 $\pm$ 0.02

middle part of strip No. 1 were eluted with 10 ml of freshly deionized water and the pH of the eluate was determined and corrected for dilution[5]. The mean value of the pH of the buffer and of the corrected pH of the paper sample was taken as the experimental pH. The difference between the initial and the final pH was highest in the acid-acetate buffers (Table II). The pH of the buffer was determined in each of the two electrode compartments after the electrophoretic run; these values never deviated by more than 0.1 of a pH unit. Strip No. 4 served to maintain symmetrical evaporation in the apparatus and should, therefore, not be omitted.

Buffers: Between pH 2.8 and pH 6.0, acetate buffers were used. To 1 l of a stock solution of 0.16 M sodium acetate, 950 ml of water were added and the pH was brought to the appropriate value with 6 N HCl, and the solution was then made up to a volume of 2 l with water. From pH 6.4 to 9.6 acetate–barbital buffers were used. They were prepared from a stock solution of sodium acetate (0.08 M) and sodium diethyl barbiturate (0.08 M) in the same way as the acetate buffers. At pH 2.75 a phosphate buffer was used (0.077 M NaH_2PO_4–0.012 N HCl). The ionic strength of these buffers was 0.08.

Staining

Cholinesterase was rendered visible by the α-naphthyl acetate–Fast Blue B method[9]. Dextran and orosomucoid were stained by the periodic acid SCHIFF procedure[13]. Coeruloplasmin was directly visible on the paper. After staining, the strips were dried at room temperature.

Calculation

After staining and drying of the strips, the distance of migration from the point of application to the centre of the spot was determined. Migration distance towards the cathode was calculated as positive and that towards the anode as negative. The mobility was calculated according to WALDMANN-MEYER AND SCHILLING[5]:

$$U^T = ((s_{p_1}/\varrho) - s_{d_1})/(q\ V_t\ t)$$

where U^T, mobility at experimental temperature $T°$ (cm²/V/sec); s_{p_1}, protein migration distance (cm); s_{d_1}, dextran migration distance (cm); ϱ, $(s_{p_1} - s_{p_2})/(s_{d_1} - s_{d_2})$, adsorption factor; q, conductivity ratio (see below); V_t, voltage per cm at the conclusion of the experiment (V/cm); t, duration of the experiment (sec).

Finally, the mobility at the experimental temperature $T°$ was converted to 1° by multiplying U^T with η_T/η_1 where η_T is the viscosity of water at $T°$ and η_1 the viscosity of water at 1°.

Adsorption factor

The cholinesterase preparations were adsorbed more or less by the following untreated papers: Schleicher & Schüll 2043 bmgl, Munktell 20/150, Whatman 20, 31ET, 54 and 3MM. A decrease in the migration rate due to reversible adsorption could be avoided by impregnating Whatman 3MM paper with γ-globulin or albumin. A slight "tailing" indicates an irreversible adsorption which does not affect the

determination of the mobility. The adsorption factor was determined in each experiment (Table III).

The conductivity ratio, *i.e.*, the ratio of paper conductivity to buffer conductivity, was calculated according to:

$$q = i/(V_i \, w \, \kappa)$$

where i, current per strip (A); V_i, voltage per cm in the middle part of the strip at

TABLE III

ADSORPTION FACTOR OF OROSOMUCOID, COERULOPLASMIN AND CHOLINESTERASE PREPARATIONS ON WHATMAN 3 MM PAPER IMPREGNATED WITH HUMAN γ-GLOBULIN

Protein	*Number of experiments*	*Adsorption factor (mean ± S.E.)*
Orosomucoid	9	1.01 ± 0.01
Coeruloplasmin	7	0.86 ± 0.03
Native human-serum cholinesterase		
unpurified	8	0.97 ± 0.02
purified 285 ×	27	1.02 ± 0.01
purified 5000 ×	29	1.01 ± 0.01
Sialidase-treated human-serum cholinesterase		
unpurified	12	1.02 ± 0.01
purified 285 ×	14	1.01 ± 0.004

current i (V/cm); w, the amount of buffer per cm length of paper (cm²); κ, buffer conductivity (A/V/cm).

Determination of the conductivity ratio was carried out with paper strips impregnated with γ-globulin and equilibrated for at least 3 h in the apparatus[5]. In 16 experiments it was 0.680 (S.E. = 0.006).

The rise in temperature of the strip during electrophoresis was determined from the initial rise in paper conductivity[5]. With the small power input used in these experiments (1 mA per strip at 80–120 V) the rise in temperature averaged 0.9°, in agreement with findings in similar apparatuses[5,14].

The ionic strength in the paper strip increased during electrophoresis due to evaporation of water. The final ionic strength (μ_f) was calculated from the initial ionic strength (μ_i) using the migration of dextran (corrected for electro-osmosis) as a measure of the evaporation[5]:

$$\mu_f = \mu_i \cdot 14.6/(14.6 - (s_{d_1} - s_{d_2}))$$

where 14.6 is the distance in centimeters between the points of application. In 95 experiments the average increase of the ionic strength was 0.017 (S.E. = 0.0006).

The extension of the zone of uniform buffer concentration was determined in two experiments with barbital buffer of pH 8.6 after 18 h. The strips were cut into transverse sections of 1 cm each and eluted with water. The concentration of diethyl barbiturate was determined from the difference in absorbancy at 240 mμ when measured at pH 9 and at pH 1. The buffer content throughout the horizontal part of the strip was uniform within ± 3.5% of the mean value.

Performance of the method

The electrophoretic mobilities over the range of pH 4 to pH 9 were determined by WALDMANN-MEYER AND SCHILLING[5] for human albumin and γ-globulin; the values agreed with the determinations obtained on free electrophoresis. To control the slightly modified technique used in our experiments and the performance of the method with acid sialo-proteins, we have determined the mobilities of human orosomucoid and coeruloplasmin at different pH values; these values have than been compared with the results obtained by others on free electrophoresis (Fig. 4).

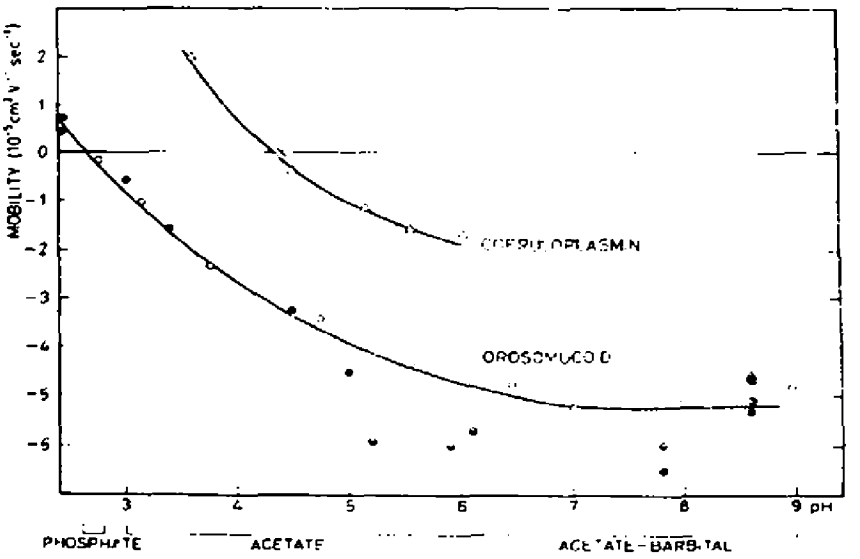

Fig. 4. Electrophoretic mobility at 1° as a function of pH for human orosomucoid (○—○) and coeruloplasmin (△ - -△) as determined by paper electrophoresis (μ = 0.080). Buffer composition is indicated on the abscissa. Each point represents a single determination. Corresponding values obtained in free electrophoresis: Orosomucoid ●, in phosphate and acetate buffers (pH 2.5–5); ◒, in phosphate buffers (pH 5–8) (SCHMID[15]); ◑, experiment of SCHULTZE *et al.*[16]. Coeruloplasmin: ×, experiment of HOLMBERG AND LAURELL[12]. ▲, experiment of SCHULTZE *et al.*[16].

The isoelectric point of orosomucoid was at pH 2.7, *i.e.*, the same as found by SCHMID[15]. The mobility at pH 8.6 was $-5.0 \cdot 10^{-5}$ cm^2/V/sec as determined by interpolation; this agrees with SCHULTZE *et al.*[16] but is $0.3 \cdot 10^{-5}$ units above the value found by SCHMID[15]. The disagreement between our determinations in acetate and acetate–barbital buffers and those of SCHMID[15] in phosphate buffers from pH 5 to pH 8 is probably due to the different buffer ions. The mobility of human coeruloplasmin was determined at pH 3.6–6 and the isoelectric point determined by interpolation to 4.3–4.4. This agrees with HOLMBERG AND LAURELL's[12] value for porcine and human coeruloplasmin (4.4). At pH 8.6 we found a mobility of $-4.5 \cdot 10^{-5}$ cm^2/V/sec as compared with $-4.6 \cdot 10^{-5}$ found by SCHULTZE *et al.*[16] on free electrophoresis.

The standard deviation of the determination was $0.08 \cdot 10^{-5}$ units as determined from the differences between duplicate determinations (60 experiments).

RESULTS

The electrophoretic mobility of native human-serum cholinesterase was determined as a function of pH at an ionic strength increasing from 0.080 to 0.097 during the experiment. At pH 5.6 the mobility was $-3.1 \cdot 10^{-5}$ cm²/V/sec. The slope of the mobility–pH curve was minimal from pH 4 to pH 9.6 and increased considerably below pH 4. By interpolation the isoelectric point was determined to be at pH 2.9 to pH 3.0 (Fig. 5, lower curve).

To investigate whether protein–protein interactions influenced the electro-

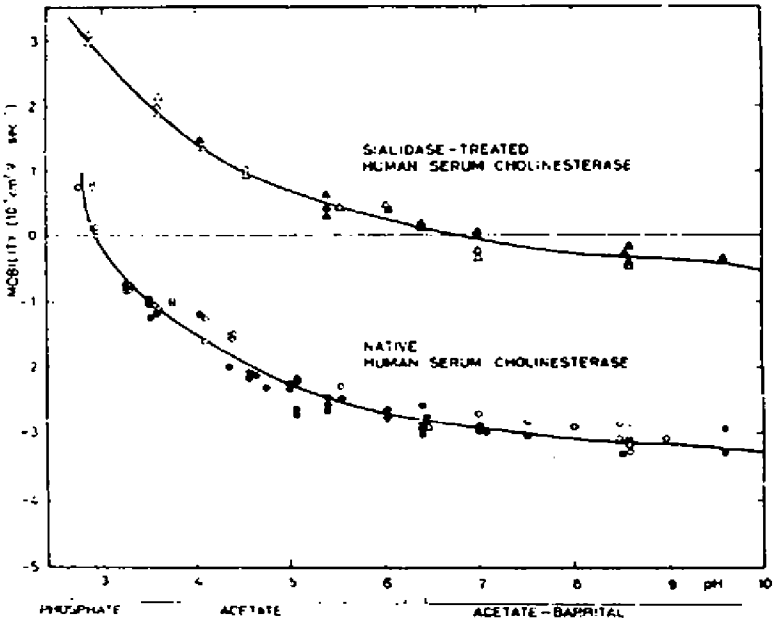

Fig. 5. Electrophoretic mobility at 1° as a function of pH for native and sialidase-treated human-serum cholinesterase (μ = 0.089). Buffer composition indicated on the abscissa. Each point represents a single determination. Native cholinesterase: ●, unpurified; ◒, purified 285 ×; ○, purified 5000 ×. Sialidase-treated cholinesterase: ▲, unpurified; △, purified 285 ×.

phoretic mobility of cholinesterase, the mobility was determined for preparations of the enzyme differing in purity: (a) unpurified human-serum cholinesterase (8 experiments); (b) a preparation of purification factor 285 (27 experiments); (c) a preparation of purification factor 5000 (29 experiments). No significant differences were observed with these preparations.

The mobility of the sialidase-treated serum cholinesterase was above that of the native enzyme. Between pH 4 and pH 9.6 this difference was nearly constant ($2.8–3.0 \cdot 10^{-5}$ units). Below pH 4 the difference decreased gradually to about $2 \cdot 10^{-5}$ units at pH 3. The isoelectric point was at pH 6.7–7.0. As with the native enzyme there was no difference in the mobility of the unpurified enzyme (12 experiments) and that of a preparation purified 285 times (14 experiments) preparation Fig. 5, upper curve).

DISCUSSION

The mobility–pH curve for native cholinesterase indicates that the negative charge of the enzyme is due mainly to sialic acid, the slope having a maximum in the vicinity of the pK of sialic acid (2.6). The course of the curve between pH 4 and pH 9.6 points to relatively few α-, β-, and γ-carboxylic groups (pK 3.5–5), α- and ε-amino groups, imidazole groups and phenolic groups (pK 7–10). The finding of identical mobilities with unpurified and with 285 times- and 5000-times purified preparations indicates that these mobilities represent true properties of cholinesterase; although it cannot be ruled out that remaining impurities might modify the mobility of the enzyme.

The enzymic removal of sialic acid from cholinesterase displaced the mobility–pH curve to higher values. Between pH 4 and pH 9.6 this displacement was constant indicating that the sialidase-treatment did not affect the polypeptide part of the molecule. At pH 2.8 the slope of the mobility–pH curve was significantly lower than with the native enzyme. The isoelectric point was displaced from pH 3 to about 7. These findings agree with the previous suggestion that native human-serum cholinesterase contains a large number of sialic acid residues present in a terminal position[2].

The mobility–pH curve of native cholinesterase did not exhibit strict similarity with the curves of other acidic serum proteins. Certain similarities with orosomucoid were, however, present: the isoelectric points were nearly identical (2.9–3.0 for cholinesterase and 2.7 for orosomucoid); the slopes of the mobility–pH curves between pH 5 and 9 were similar but the anodic mobility of orosomucoid was considerably higher than that of serum cholinesterase. Upon treatment with sialidase the mobility–pH curve of each protein was displaced in a similar way. The displacement of the isoelectric point was about 4 pH units for cholinesterase and only 2.4 pH units for orosomucoid whereas the vertical displacement of the curves was about equal at pH 5 (see ref. 11).

In addition to information as to the structure of cholinesterase the mobility–pH curve may be a valuable tool to characterize the enzyme. Kinetic properties, especially substrate and inhibitor specificity, have hitherto been the main criteria for differentiation between different types of cholinesterases from various organs and species. AUGUSTINSSON[17] used the relative electrophoretic mobility at pH 8.4 as an additional characteristic property of each enzyme, but the complete mobility–pH curve may be an even more valuable criterion.

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