AN ELECTROPHORETIC EXAMINATION OF A URINARY MUCOPROTEIN WHICH REACTS WITH VARIOUS VIRUSES

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A number of the properties of a mucoprotein which has been isolated from human urine and obtained in a highly purified state are described in the preceding paper (1). This substance is capable of reacting with certain viruses and several features of the interaction with influenza viruses are those of an enzyme-substrate system. The mucoprotein is altered by active influenza viruses; one evidence of the altered state is loss of the capacity to react with the same agent or others. The properties of the material, described in other papers (1, 2), indicate that the substance is of high molecular weight and possesses molecular asymmetry in marked degree. Because of this, it seemed desirable to investigate the electrophoretic properties of the purified mucoprotein as isolated from urine as well as after the substance had been altered by influenza virus.

The results obtained in this study show that the mucoprotein migrates in an electrical field as a single homogeneous substance; that after alteration by viral action the electrophoretic mobility of the substance is slower although the mucoprotein remains homogeneous. In addition, it is shown that inactivation by heat also results in a change in electrophoretic behavior but does not affect the homogeneity of the substance.

Materials and Methods

Mucoprotein.—The procedures used for the isolation and purification of the mucoprotein from human urine are described in detail in the preceding paper (1). The two preparations studied, A and B, were identical with those employed in a number of other experiments and represented what is designated as urinary mucoproteins (1). In most of the experiments described in this report, the dried mucoprotein was dissolved in sodium phosphate buffer of pH 6.8 and 0.05 ionic strength. The resulting viscous and slightly opalescent solution was centrifuged at 3,800 *g* for 30 minutes. The supernatant, which contained inhibitory mucoprotein in undiminished amount but had a lower viscosity than the original solution, was collected and adjusted to a concentration of 0.18 per cent, as determined spectrophotometrically. Such supernatant solutions were employed for electrophoretic studies. When solutions were held at 37°C. for 24 hours, a small amount of chloroform was added as a bacteriostatic agent.

Virus.—The Lee strain of influenza B virus was used in experiments on the effect of viral

action on the electrophoretic behavior of the mucoprotein. Infected allantoic fluid was dialyzed against sodium phosphate buffer, pH 6.8 and 0.05 ionic strength and then centrifuged at 3,800 *g* for 30 minutes. The supernatant was centrifuged at 114,000 *g* for 30 minutes and the sediment was resuspended in one-eighth volume of the same buffer.

Electrophoretic Technique.—The electrophoretic measurements were carried out at 0.5°C. in the apparatus described by Longsworth (3) with a single section cell of 11.0 ml. capacity. Prior to electrophoresis, the mucoprotein solutions were dialyzed at 5°C. for 3 days with a daily change of the buffer used in the experiments.

EXPERIMENTAL

Electrophoretic experiments were carried out with two preparations, A and B, of inhibitory mucoprotein obtained from human urine. It was found that both preparations gave but a single migrating boundary in the electrophoresis apparatus. The mobilities obtained in a number of experiments are shown in Table I. For preparation A an average value of $-9.1_6 \times 10^{-5}$ cm.^{2} · sec.$^{-1}$ ·

TABLE I

Electrophoretic Mobility of Inhibitory Mucoprotein

Mucoprotein preparation	Electrophoretic mobility $\times 10^5$
A	-9.2_9
"	-9.0_8
"	-9.1_0
B	-8.3_9
"	-8.3_5

volt^{-1} was found in three experiments; for preparation B an average value of $-8.3_7 \times 10^{-5}$ was found in two experiments. These experiments were carried out in sodium phosphate buffer of pH 6.8 and ionic strength 0.05 at a potential gradient of 6 volts per cm. The difference in the mobility values of the two preparations may be attributable to small variations in a few charged groups. As was indicated in the preceding paper (1), preparations A and B appeared to vary slightly in chemical composition.

Typical electrophoretic patterns obtained with preparation A are reproduced in Fig. 1. It is apparent that at pH 6.8 the mucoprotein was homogeneous in that the ascending boundary did not spread unduly on moving through a distance of 4 cm. (Fig. 1, *e*). Furthermore, it is not resolved into discrete refractive index gradient maxima except for the tendency to the "saw-tooth" type of patterns so frequently encountered in work with viscous solutions, as for instance in the case of nucleic and hyaluronic acids. The descending patterns are a record of the migration of the mucoprotein in the other side of the channel of the electrophoresis cell, where the mucoprotein adhering to the wall must "drain away" as the boundary descends. Consequently, the gradients therein

are more irregular. In view of these facts, the mobilities were computed from the ascending pattern with the aid of the first moment of the entire gradient curve. The same preparation, when analyzed in a sodium diethylbarbiturate buffer of pH 8.6 and 0.05 ionic strength, also moved as a single component. The

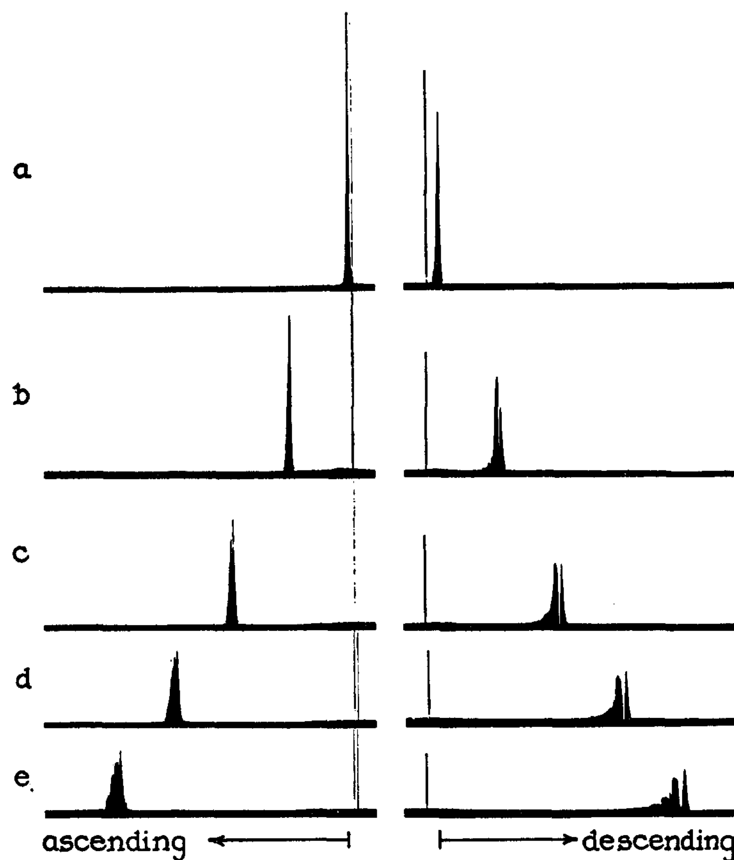

FIG. 1. Electrophoretic patterns of mucoprotein, preparation A. Electrophoresis was carried out in sodium phosphate buffer of pH 6.8 and ionic strength 0.05 at a potential gradient of 6 volts per cm. The patterns shown in line *a* represent the initial boundaries, the others were obtained at intervals of 30 minutes.

electrophoretic patterns obtained with preparation B were similar in appearance to those shown in Fig. 1. The present electrophoretic examination was not extended to include mobility measurements over a wide range of pH and, as a consequence, the isoelectric pH values of the mucoprotein preparations were not determined.

Effect of Heat.—It has been shown (1) that the inhibitory capacity of the

urinary mucoprotein is greatly reduced after heating at 70°C. Electrophoretic experiments with mucoprotein heated either in distilled water or in phosphate buffer of pH 6.8 and ionic strength 0.05 for 30 minutes at 70°C. demonstrated a moderate reduction in mobility as is shown in Table II. The heated mucoprotein also gave a single homogeneous boundary. However, as is shown in the

FIG. 2. Electrophoretic patterns of a mixture of active and virus-inactivated mucoprotein, preparation B. Electrophoresis was carried out in sodium phosphate buffer of pH 6.8 and ionic strength 0.05 at a potential gradient of 6 volts per cm. for 5400 seconds. The stationary boundaries represent chloroform which had been added as a bacteriostatic agent.

TABLE II

Effect of Heat or Active Lee Virus on the Electrophoretic Mobility of Inhibitory Mucoprotein

Mucoprotein preparation	Treatment	Electrophoretic mobility × 10^5
A	None (average of 3 experiments)	-9.1_6
"	70°C., 30 min. in H_2O	-8.1_5
"	" " " " phosphate buffer	-8.2_4
B	None (average of 2 experiments)	-8.3_7
"	37°C., 24 hrs.	-8.3_6
"	" " " + heated Lee virus	-8.4_4
"	(*c*) " " " + active " "	-6.7_7
"	(*d*) " " " + " " "	-6.6_1
B mixed with (*c*)	{37°C., 24 hrs. {" " " + active Lee virus	{-8.3_6 {-6.6_5
B mixed with (*d*)	{37°C., 24 hrs. {" " " + active Lee virus	{-8.5_4 {-6.7_5

succeeding paper (2), mucoprotein heated under identical conditions gave evidence of two components in the ultracentrifuge.

Effect of Active Lee Virus.—The electrophoretic behavior of inhibitory mucoprotein which had been altered by active influenza virus, Lee strain, was studied. The preparations, containing 0.2 per cent of the substance and 160 hemagglutinating units of virus, were held for 24 hours at 37°C. Controls consisting of similar solutions to which an equivalent amount of heat-inactivated (65°C. for 30 minutes) virus or phosphate buffer was added were treated identically.

Mucoprotein after treatment with active virus had no inhibitory activity when tested with heated (56°C. for 30 minutes) Lee virus. The control solutions showed no demonstrable reduction in inhibitory activity.

The results of a number of experiments are shown in Table II. In each case mucoprotein altered by active Lee virus gave a single boundary on electrophoretic analysis. However, the mobilities of the preparations treated with active virus were -6.7_7 and -6.6_1, respectively, whereas those of the control preparations were -8.3_6 and -8.4_4, respectively. Since the mobility change which accompanied inactivation by active virus was considerable, a separation of a mixture of the virus-treated and untreated mucoprotein into two distinct components was to be expected. That such a resolution was achieved is shown by the electrophoretic patterns presented in Fig. 2. In the experiment illustrated by these patterns, 0.2 per cent solutions of the active and virus-inactivated mucoprotein were dialyzed in separate flasks against buffer of the same composition and equal volumes were mixed prior to the start of the electrophoretic experiment. The stationary boundaries were caused by chloroform which had been added to the solutions. The mobilities of each of the two components of such a mixture obtained in two separate experiments are given in Table II. It is evident that they are in close agreement with the mobilities found for the respective individual components in separate experiments.

DISCUSSION

Previous investigations on mucoproteins capable of reacting with influenza viruses, but derived from sources other than human urine, have not revealed an electrophoretically homogeneous substance. Lanni *et al.* (4, 5) studied a purified preparation isolated from egg white and found it to consist of at least two, sometimes three, distinct electrophoretic components with different mobilities. The biologically active components of their best preparations showed electrophoretic mobilities which appear to be somewhat different from that of the inhibitory mucoprotein isolated from human urine.

It is emphasized that, without exception, each experiment in the present study yielded only one electrophoretic boundary with either of the active mucoprotein preparations employed. This finding and the evidence that the substance is also homogeneous on ultracentrifugation, as described in the succeeding paper (2), indicate that a single substance capable of reacting with certain viruses has been separated from human urine. That the mucoprotein examined in this investigation is in fact the component which possesses biological activity is indicated by the following: (*a*) the extraordinarily high activity of the substance (1); (*b*) the evidence obtained on ultracentrifugation (2); and (*c*) the change in electrophoretic mobility of the mucoprotein which accompanies loss of activity as a result of viral action.

That inactivation of the mucoprotein by influenza virus is of an enzymatic

nature is supported by the kinetic studies reported in the preceding paper (1) as well as by the investigations of others (6, 7). Gottschalk (7) isolated an end-product of the reaction between urinary mucoprotein and influenza virus and has reported that the substance is an isoglucosamine amino acid complex. This end-product represented only about 1 per cent of the total mucoprotein and its loss would hardly be expected to lead to a drastic change in the substance. As was shown recently by one of us (8, 9), the removal by enzymatic action of one or two charged groups from strategic points of a protein molecule can cause changes in the electrophoretic mobility of the same order of magnitude as those found after alteration of the mucoprotein by influenza virus. Thus, the change in the electrophoretic behavior of the mucoprotein after treatment with virus may be considered as additional support for the enzymatic nature of the reaction.

SUMMARY

A mucoprotein isolated from human urine and possessing the capacity to react with a number of viruses is electrophoretically homogeneous at pH 6.8 and 8.6. After treatment with influenza virus and elimination of its biological activity, the substance remains homogeneous and its electrophoretic mobility is decreased by approximately 20 per cent.

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BIBLIOGRAPHY

1. Tamm, I., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1952, **95,** 71.
2. Bugher, J. C., Tamm, I., and Horsfall, F. L., Jr., in preparation.
3. Longsworth, L. G., *Chem. Rev.*, 1942, **30,** 323; *Ind. and Eng. Chem., Analytical Edition*, 1946, **18,** 219.
4. Lanni, F., Sharp, D. G., Eckert, E. A., Dillon, E. S., Beard, D., and Beard, J. W., *J. Biol. Chem.*, 1949, **179,** 1275.
5. Sharp, D. G., Lanni, F., Lanni, Y. T., Csáky, T. Z., and Beard, J. W., *Arch. Biochem.*, 1951, **30,** 251.
6. Gottschalk, A., and Lind, P. E., *Nature*, 1949, **164,** 232.
7. Gottschalk, A., *Nature*, 1951, **167,** 845.
8. Perlmann, G. E., *J. Am. Chem. Soc.*, 1949, **71,** 1146.
9. Perlmann, G. E., *Nature*, 1950, **166,** 870.