THE ISOELECTRIC POINT OF ADSORBED HEMOGLOBIN*

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It is well established that various proteins, as gelatin and albumin, can be adsorbed on various adsorbents so that the protein-coated particle of adsorbent behaves electrophoretically as a particle of protein (Loeb, 1923; Freundlich and Abramson, 1928). Dummett and Bowden (1933) have recently reported, however, that the behavior of adsorbed hemoglobin varies with the adsorbent surface. Thus, when ox hemoglobin was added in sufficient concentration to coat completely particles of quartz, evacuated blood charcoal, and colloidal copper, respectively, the isoelectric point on quartz was 5.82, on charcoal 5.83, and on copper 6.72. They postulate a binding of some of the ionized groups of hemoglobin by the adsorbent surface.

While this interpretation of their results is reasonable, it is not the only one possible. The other possibility is that other substances of lower isoelectric points than hemoglobin might have been present and preferentially adsorbed. Since no one can guarantee that any sample of hemoglobin is 100 per cent pure, a decision between the two possibilities may be reached by observing whether or not the isoelectric point of adsorbed hemoglobin approaches that obtained by the moving boundary method as the purity of the sample of adsorbed hemoglobin is increased. This we have done and find that the isoelectric point of adsorbed hemoglobin does approach that of freely dissolved hemoglobin with increasing purity. This indicates that Dummett and Bowden’s results were due to impurities of lower isoelectric point than hemoglobin. Since the impurities may be preferentially adsorbed, a

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small percentage of contaminant might suffice to shift the isoelectric point significantly.

EXPERIMENTAL

Electrophoresis determinations were made in a cylindrical cell of a modified Mattson type (1933) with the technique previously described (White and Monaghan, 1935). Observations were at a level of 0.147 cell diameter below the cell roof, at which level there is no electroosmotic movement. The visual axis was the vertical diameter of the cell. Glass or quartz particles of 1 to 2 μ diameter were suspended in the hemoglobin solutions and their movements observed. A field strength of 9.1 volts per cm. was used; five observations of particle movement for each direction of current were made and the average velocity computed. In the first series of experiments horse hemoglobin was used in three stages of purification with respect to non-hemoglobin protein. A stock solution was prepared as follows. The cells from fresh oxalated horse blood were washed five times with 10 times their volume of 0.9 per cent NaCl solution to free them from plasma proteins, the washed cells laked by adding 10 volumes of distilled water, and the white cells centrifuged down.

A sample of this stock, which contained stroma material, was diluted 100 times with HCl-KCl or NaOH-KCl mixtures of varying pH, the pH being varied by varying the proportion. The concentration was kept at 0.02 M; pH was measured in all cases with the glass electrode after addition of the hemoglobin. The final dilution was thus about 0.03 per cent hemoglobin; this concentration is more than enough to coat glass particles completely, as is shown by the fact that the isoelectric point and mobilities of the particles do not change further in higher concentrations. From the mobilities observed at different values of pH a curve was constructed which crossed 0 mobility at pH 5.50; i.e., the isoelectric point is at pH 5.50.

The above procedure was repeated except that the stock solution was first passed through a Seitz filter, removing part of the stroma material, as evidenced by the finding that ghosts were no longer found in the filtrate. Glass particles suspended in this product were isoelectric at pH 6.26.

The stock solution was first brought to about pH 5.5 by slow
addition of 5 cc. of 0.1 N HCl to 50 cc. of laked cell stock. A precipitate brought about by the mutual aggregation of hemoglobin and stroma material is formed, since at pH 5.5 hemoglobin is on the acid side of its isoelectric point (6.8) and stroma material is on the alkaline side of its isoelectric point (about 3.0) (cf. White and Monaghan, 1936). The material is then passed through a Seitz filter, with a resultant more nearly complete removal of stroma material than is effected by filtration without acidification. Glass particles suspended in this filtrate are isoelectric at pH 6.73.

Similar observations were carried out with beef hemoglobin adsorbed on quartz particles. Here the isoelectric point of the preparation filtered without acidification was at pH 6.65 and that of the preparation filtered after acidification (3 cc. of 0.1 N HCl to 50 cc. of 1:10 laked cell stock), i.e. the one most nearly free from non-hemoglobin protein, was at pH 6.77.

**DISCUSSION**

It is seen that as steps are taken to remove more completely the stroma material from a fresh hemoglobin solution, the isoelectric point of the hemoglobin preparation approaches 6.8, that shown by the moving boundary method. It is evident that such contamination with stroma material would not affect determinations based on observations of the movement of the colored hemoglobin column. With the microscopic method, however, it is essential that no protein other than the one under investigation be present, since the contaminant may be adsorbed on the particle with the result that the observed isoelectric point is the resultant of those of the protein under investigation and of contaminant and will vary with the proportion of these adsorbed. This difficulty may also exist with microscopic observation of hemoglobin crystals, since the crystal surface may adsorb non-hemoglobin protein. This presumably accounts for Abramson's (1934) observation that the isoelectric point of hemoglobin crystals is usually less than 6.8.

It follows that the properties of the hemoglobin are not altered by adsorption on quartz or glass, as Dummett and Bowden supposed. Thus the microscopic method, so far as concerns any case at present known, gives the true isoelectric point of a pure
protein. It is granted that cases may subsequently be observed where the electrical behavior of the adsorbed protein may be different from that of the unadsorbed. Practical deductions from this work are that the microscopic method may be used to test a hemoglobin sample for freedom from non-hemoglobin protein, and that in the preparation of pure hemoglobin crystals it should be advantageous to pass the laked blood through an asbestos filter to reduce greatly the content of stroma material.

SUMMARY

The more nearly free a preparation of hemoglobin is from non-hemoglobin protein the more nearly does the isoelectric point approach 6.8, that seen with the moving boundary method. With the microscopic method, where a surface is involved, a protein must be pure in order to get correct values of mobilities and isoelectric point, but the electrical properties of the protein are not changed by adsorption on quartz or glass. These remarks apply to all proteins so far investigated, including hemoglobin, although exceptions may later be discovered.

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