ELECTROPHORETIC STUDIES OF GLOBINS PREPARED FROM NORMAL ADULT AND SICKLE CELL HEMOGLOBINS*

BY E. HAVINGA† AND H. A. ITANO‡

THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY,§ CALIFORNIA INSTITUTE OF TECHNOLOGY

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When the electrophoretic difference between normal adult hemoglobin and sickle cell hemoglobin was first detected, investigations were undertaken to determine whether the basis of this difference resides in the heme or the globin portions of these molecules. Dimethyl esters of the protoporphyrins were prepared from the hemes of each of these hemoglobins. X-ray diffraction photographs indicated that these protoporphyrin esters are probably identical.[1] Denatured globins were prepared from the hemoglobins of normal individuals and individuals with sickle cell trait and sickle cell anemia. Electrophoretic analyses of these denatured globins in acidic buffers failed to disclose any significant differences in composition or mobility.[2] The present study was undertaken in order to compare the electrophoretic properties of the native globins, which, unlike the denatured globins, are soluble in their isoelectric region.

Experimental Methods and Results.—The separation of heme from globin was accomplished by use of the acid acetone method of Anson and Mirsky.[3] The globin thus prepared was dialyzed against 0.01 *M* Na_2HPO_4 to effect reversal of the denaturation caused by the acid acetone. The loss in insoluble protein approximated 10%. The native globins were then examined in the Tiselius apparatus in 0.01 *M* Na_2HPO_4 and in cacodylate buffer of pH 7.0 at potential gradients of 16.6 and 5.0 volts per centimeter, respectively. The cacodylate buffer was 0.08 *M* in NaCl, and its ionic strength was 0.1.

Although 0.01 *M* Na_2HPO_4 is a useful solution in which to detect small mobility differences between components in mixtures of hemoglobins,[4] its buffering capacity is poor. Hence, reproducible values of absolute mobilities are difficult to obtain in this solution, and the results are best discussed in terms of differences in mobilities. Figure 1, (*a*) and (*b*), illustrates the scanning diagrams obtained following electrophoresis of native globins prepared from normal adult and sickle cell trait hemoglobins. These globins were dialyzed simultaneously against the same solution of 0.01 *M* Na_2HPO_4 and were examined under identical conditions. Only one major component is evident in the preparation of normal adult globin. In the sickle cell trait globin two components are present, one of which has the same mobility as the normal adult globin. The absolute mobility of the other component in sickle cell trait globin is lower than that of the normal adult

globin, by 0.6×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}. Each of these globins migrated as a negative ion in this system.

Figure 1, (*c*) and (*d*), shows two different globin preparations examined in 0.01 *M* Na_2HPO_4 on different days. A large fraction of the globin prepared from sickle cell anemia globin appears to be homogeneous. The small component of lower mobility may represent a denaturation product. The native globin prepared from a mixture containing normal adult and sickle cell anemia hemoglobins in approximately equal amounts shows considerable heterogeneity. The mobility difference between the two major peaks is the same as that between the two components in sickle cell trait globin. The sickle cell anemia hemoglobin in these experiments contained less than 5% fetal hemoglobin.

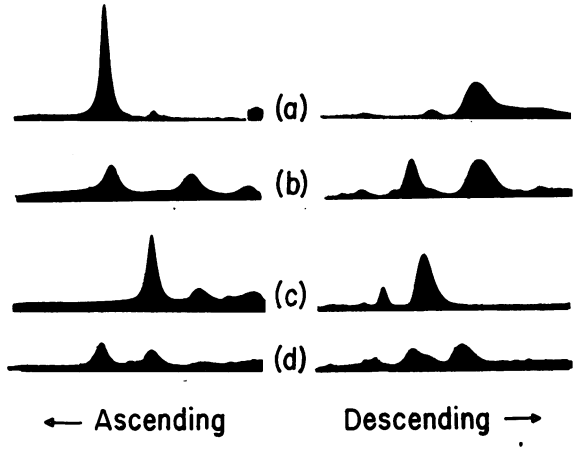

FIGURE 1

Longsworth-scanning diagrams of globins prepared from human hemoglobin. (*a*) Normal adult globin, 22,860 sec.; (*b*) sickle cell trait globin, 22,860 sec.; (*c*) sickle cell anemia globin, 14,400 sec.; (*d*) globin prepared from a mixture of normal adult and sickle cell anemia hemoglobins, 14,400 sec. Electrophoresis in 0.01 *M* Na_2HPO_4 at 16.6 volts per centimeter and 1.5° C.

On the acid side of the isoelectric point, in cacodylate buffer of pH 7.0, the mobilities were 2.6×10^{-5} and 3.4×10^{-5} cm.^{2} sec.$^{-1}$ volt,$^{-1}$ respectively, for normal adult and sickle cell anemia globins.

Normal and sickle cell anemia globins were treated with 4 *M* guanidinium chloride for 1 hour at 4°C. Following removal of the guanidinium chloride by dialysis, the globins were no longer soluble in 0.01 *M* Na_2HPO_4. Examination in the cacodylate buffer of pH 7.0 revealed a marked increase in heterogeneity. The mean mobilities of the denatured specimens in this buffer were nearly the same, 2.8×10^{-5} and 2.9×10^{-5} cm.^{2} sec.$^{-1}$ volt,$^{-1}$ respectively, for normal adult and sickle cell anemia globins.

Discussion.—The presence of only one major component in the native globins prepared from normal adult and sickle cell anemia hemoglobins but of two in the native globins prepared from sickle cell trait hemoglobin and a mixture containing both normal adult and sickle cell anemia hemoglobins suggests that normal adult and sickle cell globins differ in their globin portions. The mobility relationship between the globins is similar to that between the corresponding hemoglobins in that the normal protein has a greater mobility on the alkaline side of the isoelectric point. The mobility difference between the native globins is slightly higher than that observed between the hemoglobins.[1]

Since the amino acid compositions of normal adult and sickle cell hemoglobins are nearly the same,[5] and since the acid denatured globins do not differ significantly in their electrophoretic behavior, it has been suggested that the two hemoglobins might have identical polypeptide chains, and that their electrophoretic difference results from a difference in the way in which these identical chains are folded in the native molecule.[5] It was found in the present investigation that the dialysis of acid-acetone treated globin against an alkaline buffer resulted in soluble globins which corresponded in composition and relative mobility to the hemoglobins and hemoglobin mixtures from which they were prepared. Treatment of the soluble globins with guanidinium chloride resulted in heterogeneous mixtures which did not differ significantly from each other in their mean mobilities. These observations suggest that, if the polypeptide chains are identical, the denaturation which takes place when heme is removed by treatment of hemoglobin with acid acetone does not completely unfold the chains. Each globin retains a sufficient part of its specific configuration to enable it to return to its native configuration upon treatment with a weakly alkaline phosphate solution. The denaturation due to guanidinium chloride apparently was not reversed upon removal of the denaturing agent.

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† Present address, Laboratory of Organic Chemistry, University of Leiden.

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