Pure native globin from human hemoglobin: preparation and some physico-chemical properties

In the course of research on hemoglobin resynthesis, we became interested in obtaining a suitable preparation of pure globin from human hemoglobin and in studying some of its molecular characteristics. In fact, very little information has so far been available about the properties of this protein; GRALÉN[1] has shown that globin preparations obtained by the method of ANSON AND MIRSKY[2] from horse hemoglobin did not behave in the ultracentrifuge and in diffusion experiments as a monodisperse component. Electrophoretic studies with human globin have also been described[3,4]. However, in all these cases no evidence has been presented that the globin was in the true native state; the recombination products obtained with these globin preparations often showed differences from the native hemoglobin. We believe that the best criterion for judging the native state of hemoglobin is the study of the oxygen equilibrium. It must be pointed out that the only data on oxygen equilibrium available in the literature are those given by DAVIES[5] who has shown for reconstituted hemoglobin a loss of the Bohr effect and of heme-heme interaction.

In our experiments, globin was prepared as follows: a salt-free solution of human oxyhemoglobin (crystallized from ammonium sulphate) was treated with 20–25 vol. of acid acetone at −20°; after 15 min the suspension was centrifuged at same temperature. The colourless precipitate was dissolved in cold distilled water, dialyzed against distilled water for a few hours and then against 0.0015 M $NaHCO_3$ for about 30 h.

The electrophoretic pattern of globin prepared by this method shows one single homogeneous and symmetric component which migrates at pH 7 with mobility $U = 0.2 \cdot 10^{-5}$.

Fig. 1 shows the sedimentation diagrams of 0.6% solution of human globin; the globin behaves as a monodisperse system and one single homogeneous and symmetric boundary is present.

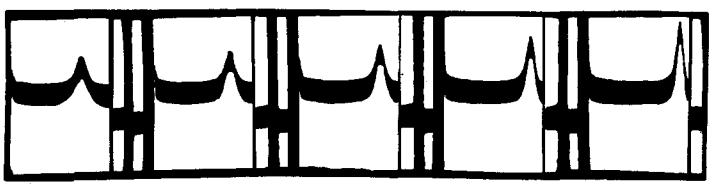

Fig. 1. Sedimentation diagrams of 0.6% human globin, dissolved in 0.0015 M $NaHCO_3$. Exposure 30 min after reaching full speed (59,780 r.p.m.) and thereafter at 8 min intervals.

The sedimentation coefficient, extrapolated to zero protein concentration was $S^0_{20} = 2.55 \cdot 10^{-13}$.

In diffusion experiments the globin preparations showed a high degree of homogeneity and the average diffusion coefficient was found to be $D_{20} = 5.49 \cdot 10^{-7}$.

On the basis of sedimentation, diffusion and partial specific volume ($\bar{v} = 0.75$) the molecular weight was calculated to be 42,000 and the frictional ratio f/f_0, 1.75. The coupling capacity of these globin preparations for protohematin was 3.7 g heme/100 g globin, *i.e.* 4 moles of protohematin for each 66,000 g globin. Hemoglobin reconstituted from this globin is equal to the native pigments as regards its physical, physico-chemical and physiological properties (oxygen equilibrium).

The data reported above demonstrate that the protein thus obtained is to be considered as pure native globin, which offers excellent possibilities for studies on the structure of hemoglobin.

Further results on the characteristics of the human globin, on its recombination with hemes and on the properties of reconstituted hemoglobin will be reported in detail.

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