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Preparation of Globin from Human Hemoglobin. (21184)

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Considerable interest has been shown in the preparation of plasma expanders from human globin(2,4,5). For this purpose it is not necessary to utilize so-called "undenatured" globin, but it is desirable to use a colorless globin protein which can then be modified as desired. The usual methods of preparing globin protein from hemoglobin are those of Anson and Mirsky(1), Strumia and Chornock(2) and Strumia and Sample(3). These methods utilize the precipitation of a globin hydrochloride from a reaction mixture obtained by mixing an aqueous hemoglobin solution with acidified acetone. The globin obtained by these procedures is unfortunately contaminated with considerable quantities of heme or heme complexes. The purpose of this communication is to describe a new method of preparing a globin protein which is almost devoid of colored impurities.

This new procedure is based on two findings: 1, a true solution of dissociated human hemoglobin can be obtained in certain mixtures of water, water-miscible solvents and strong acids; and 2, activated carbon will adsorb the heme from such solutions. Thus, the method consists of dissolving hemoglobin in a suitable acid-water-solvent solution, treating the solution with activated carbon, filtering off the carbon, and recovering the globin from the filtrate. One suitable set of conditions is described below.

Materials and methods. Fresh, packed human red blood cells at 10°C were filtered through cheese cloth to remove clots. The filtered cells were lysed by the addition of 2 volumes of distilled water at room temperature and the solution adjusted to pH 5.8 ± 0.1 with 1 N HCl. The precipitated stroma was removed by centrifugation. To 100 ml of stroma-free lysate, 300 ml of acetone containing 5 ml of 37% hydrochloric acid were added slowly with stirring. As soon as a clear solution was obtained, 15 g of Darco G-60* were added and the mixture stirred gently for 10 minutes. Seventy-five ml of distilled water were added to the slurry which was stirred for an additional 20 minutes. The slurry was then filtered with vacuum through a Buchner funnel. One gram of Hyflo Super Cell† was added to the carbon slurry to speed filtration.

The filtrate was adjusted to pH 7.0 with 5% sodium hydroxide. One hundred and fifty ml of acetone were added with stirring to precipitate the globin which was then collected by filtration. The globin can also be obtained

* Obtained from Darco Department, Atlas Powder Co.

† Obtained from Johns Mansville.

in the acid form by precipitation from the Darco G-60 filtrate by the addition of 500 ml of acetone. The globin, or acid globin, was dried by slurrying twice in 150 ml volumes of dry acetone and air-drying after each suspending operation.

Results and discussion. Sixty-five to 70% of the nitrogen in the original lysate was obtained in the globin. $E^{1\%}_{1\,cm}$ at 403 mμ was 0.032 for an aqueous solution of acid globin prepared by the method described. This is compared to a value of 0.134 for a solution of acid globin prepared by the method of Strumia and Chornock(2). The solubilities of the 2 products are similar.

Many modifications of the above procedure can be made. Other solvents such as ethanol, dioxane, and mixtures of acetone and ether or acetone and methyl ethyl ketone have been used with varying degrees of success. The addition of water to the carbon slurry may be eliminated with a slight decrease in globin recovery. The efficiency of heme removal increases with the hydrochloric acid concentration up to 0.25 N, with a concurrent decrease in globin recovery. Hydrochloric acid was the most efficient acid tested; sulfuric acid and citric acid produced only fair heme removal. The concentration and type of activated carbon have a large effect on the quality of the globin produced. Concentrations of Darco G-60 greater than 15% produced globin with still less color, but the yields were considerably smaller. Darco G-60 was the most efficient of 11 brands of activated carbon tested.

The temperature at which the adsorption is carried out (from 0° to 30°C) has only a slight effect on the color quality of the globin produced. The yield of globin is less at lower temperatures (0° to 10°C), probably due to the decreased solubility of the globin at the lower temperatures.

Care must be exercised during the process to keep down oxidative reactions. Such precautions as the use of fresh red cells and only very gentle stirring during all mixing operations are essential if a product low in color is to be obtained.

Summary. A method is described of preparing nearly colorless globin protein from human hemoglobin. The method consists of adsorbing the heme on carbon from acidic solution of dissociated hemoglobin and then precipitating the globin protein from the solution after filtration to remove the carbon.

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