

Separation of myoglobin and haemoglobin on a column of dextran gel

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Myoglobin and haemoglobin may frequently be found together in the urine of patients who have suffered from crush injuries. The separation of these two haemoproteins by differential solubility in salt solutions and their spectroscopic characterization are technically difficult. With this in mind, it appeared worth while to test whether the considerable difference in molecular size of the proteins (M.W. 18,000 and 67,000) could form the basis of a more satisfactory procedure. Gel filtration exploits the differing rates of diffusion of molecules within the interstices of a cross-linked gel to separate the molecules roughly according to their size. With the appropriate low cross-linked dextran gel, efficient separation was achieved, as the following experiment shows.

Human psoas muscle obtained after death was finely minced, and approximately 0.2 ml. of distilled water was added. The muscle was then crushed, frozen by adding solid carbon dioxide and then allowed to thaw. The juice was pressed out through cheese cloth and immediately saturated with carbon monoxide in the dark. The process was twice repeated to give, in all, a concentrated crude extract of muscle containing carbonyl myoglobin, carbonyl haemoglobin, and other soluble muscle constituents including proteins. Then 2 ml. of the extract was applied to a column (80 × 2 cm.) of low cross-linked dextran gel (Sephadex G-75, 100 to 200 mesh) which was developed with sodium chloride solution (0.05 M) saturated with carbon monoxide. Two red-coloured zones migrated down the column, well separated from each other at outflow, and, on passing from the column, were collected in 5 ml. fractions. Each of these was diluted with water, treated with sodium hydrosulphite (Na₂S₂O₄) and resaturated with CO, and the absorption maxima measured in a Beckman D.U. spectrophotometer. The wavelengths of maximum absorption for zone 1 were: α band 569 mμ, Soret region 420 mμ (human carbonyl haemoglobin gives 570, Lemberg and Legge, 1949), and 418 mμ (Hicks and Holden, 1929);

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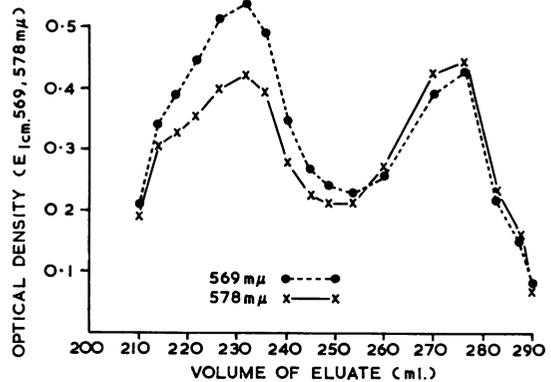


FIG. 1. Separation of carbonyl myoglobin and carbonyl haemoglobin contained in 2 ml. crude human muscle extract. Zone 1: HbCO. Zone 2: MbCO.

for zone 2: α band 578 mμ, Soret region 424 mμ (carbonyl myoglobin (human) α band 577 mμ (Theorell and de Duve, 1947), Soret region (horse) 422 mμ (Colpa-Boonstra and Minnaert, 1959).

When the optical density readings at the absorption maxima, 569 and 578 mμ for HbCO and MbCO respectively, are plotted against the corresponding volumes of eluate (Fig. 1), the high degree of separation becomes apparent. Doubtless the residual slight overlapping could be eliminated by use of a longer column packed with a gel of finer mesh. No other coloured zones, as of cytochromes, were detected.

This simple procedure could be used as a rapid diagnostic manoeuvre in suspected myoglobinuria. If haemoglobin is added as a marker it will migrate ahead of myoglobin which can, thus, be positively identified.

SUMMARY

The carbonyl derivatives of myoglobin and haemoglobin contained in human psoas muscle were separated on a column of low cross-linked dextran gel. This simple procedure could facilitate the recognition of myoglobinuria.

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