PRO EXPERIMENTIS

An Improved Technique for Rapid Identification of Hemoglobin Chains by Starch Gel Electrophoresis

The identification of hemoglobin chains is very important in the study of vertebrate evolution and in clinical diagnosis. The methods of identification are, however, usually laborious and time-consuming.

The action of *p*-hydroxymercurybenzoate on disulfide bridges in tertiary structure of proteins is already known[1]. BUCCI and FRONTICELLI[2] described a method for preparation of α- and β-chains of hemoglobin using *p*-chloromercurybenzoate. This method is generally used for preparation of hemoglobin chains with its heme groups[3,4].

We report a modification of BUCCI and FRONTICELLI's method but simplified for comparative purposes, to be used instead of the urea gel method.

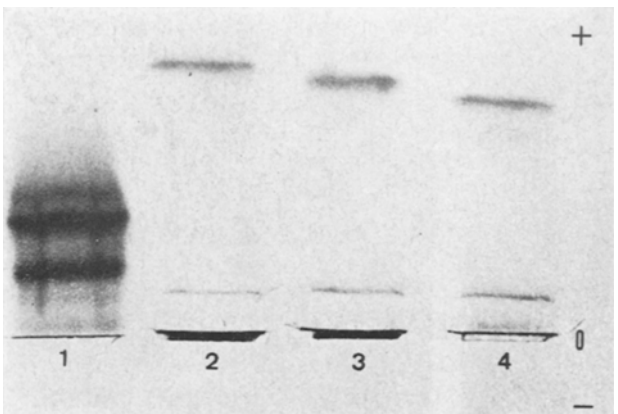
A piece of rectangular Whatman 3-mm filter paper is soaked in a saturated solution (0.5 *M*) of sodium *p*-hydroxymercurybenzoate (HMB). Another rectangular but smaller piece of filter paper is soaked in the hemoglobin solution (1 g/100 ml) to be analyzed. Both pieces, the HMB one in front, are inserted in the gel slot. On running the hemoglobin passes through the cleavage agent and is split into chains.

Horizontal starch gel electrophoresis was carried out using *tris*-borate-acid EDTA buffer, 0.036 *M*, pH 8.1, in gel and borate buffer 0.35 *M*, pH 9.0 in the bridge. A strength field of 6.5 V/cm for 4 h was used.

In case of multiple bands, as in heterozigous human hemoglobin, it is possible to identify the chains of each band by a bidimensional system. The first dimension can be made in the usual electrophoretic conditions and the second one as described above but inserting a narrow piece of the first gel containing the band to be analyzed instead of the filter paper.

The electrophoretic pattern obtained is comparable to the one included in BUCCI and FRONTICELLI's paper. The faster band was identified as β- and the slower one as α-chain[2] (Figure).

Our method substitutes the urea gel with the following advantages: 1. cleavage is made with heme groups, therefore it is not necessary to have a previous separation of globin; 2. the identification of hemoglobin chain bands can be made by peroxidasic activity; 3. results can be obtained in about 3–5 h; 4. only normal equipment for starch gel electrophoresis is required; 5. by a bidimensional system it is possible to determine the types of chains of each hemoglobin band without laborious separation and purification procedures; and 6. it can be used in population screening[5].



Bidimensional starch gel final electrophoresis showing the differences between β-chains of Hb A, Hb S, Hb C. A piece of gel containing Hb A (2), Hb S (3) and Hb C (4) bands was inserted into the gel with HMB (origin, O). Slower band = α-chain, faster band = β-chain. In (1) Hb AS for comparison showing (+ → −) Hb A_3, Hb A, Hb S, non-heme protein and Hb A_2 bands. Amido black 10 B stain.

Resumen. Los autores describan un método simple de identificación rápida de cadenas de hemoglobina usando *p*-hidroximercuribenzoato de sódio como agente de clivaje, durante la electroforesis en gel de almidón.

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[1] A. WHITE, P. HANDLER and E. L. SMITH, in *Principles of Biochemistry*, 3rd edn. (McGraw-Hill Book Co., New York 1964), pp. 237 and 894.
[2] E. BUCCI and C. FRONTICELLI, J. Biol. Chem. *240*, PC 551 (1965).
[3] R. L. NAGEL and Q. H. GIBSON, J. Biol. Chem. *242*, 3428 (1967).
[4] R. L. NAGEL and Q. H. GIBSON, J. Mol. Biol. *22*, 249 (1966).
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CONGRESSUS

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Österreichische Gesellschaft für Biophysik. President: Prof. Dr. E. Broda.
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