PN 1129

Isolation of a homogeneous lysine-rich histone from calf thymus

Various procedures have been described for the isolation of the total mixture of histones present in calf thymus and for separating them into two fractions, the arginine-rich and the lysine-rich fraction. Both fractions are far from homogeneous. The purpose of our work has been the isolation of a homogeneous component of the lysine-rich fraction. We have started from the observation made in this laboratory by BIJVOET that, in contrast to the arginine-rich fraction, the lysine-rich fraction is not precipitated by 5 % trichloroacetic acid. This suggested that the latter histone fraction, uncontaminated with other proteins, might be extracted directly from a thymus homogenate by adding to it trichloroacetic acid to a final concentration of 5 %. Indeed our surmise that in the presence of 5 % trichloroacetic acid the dissociation of the nucleohistone and the precipitation of DNA and of all proteins, with the exception of the lysine-rich histone fraction, would be achieved simultaneously has appeared to be correct*.

A typical preparation of lysine-rich fraction was obtained as follows (all manipulations were carried out at 2–4°):

100 g thymus (removed immediately after the death of the animal and frozen in solid CO_2) was homogenized in the Waring Blendor in 300 ml 0.14 *M* NaCl (pH 4.0). The homogenate was centrifuged at 2000 × *g* for 30 min. The supernatant was discarded and the sediment washed twice with 300 ml 0.14 *M* NaCl (pH 4.0). (If these washings are omitted an opalescent trichloroacetic acid extract will be obtained.) The washed sediment was then blended for 2 min in 100 ml distilled water, after which 20 % trichloroacetic acid was added to a final concentration of 5 %. The precipitate was spun down (2000 × *g*, 15 min), resuspended in 5 % trichloroacetic acid in the Waring Blendor and centrifuged again. The combined supernatants were dialysed against distilled water and lyophilized. The yield was about 500 mg.

A diagram of free-boundary electrophoresis in Tris–HCl buffer** at *I* 0.10 and pH 8.2 of the lyophilized material is given in Fig. 1. It shows that all protein present migrates towards the cathode, thus demonstrating the exclusive presence of histones

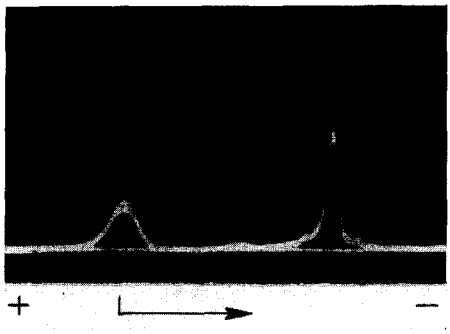

Fig. 1. Free-boundary electrophoresis at pH 8.2 of 5 % trichloroacetic acid extract of calf thymus; ascending diagram.

* The principle of this method has already been communicated on our behalf by BIGWOOD[1] at the 11th Solvay Conference on Chemistry in Brussels (June 1959). As shown by JOHNS AND BUTLER[2] 5 % $HClO_4$ can be used instead of 5 % trichloroacetic acid.

** The advantage of the use of this buffer is described by DE NOOIJ AND NIEMEIJER[3].

(isoelectric point about 12). The main peak represents about 90 % of the total lysine-rich fraction*.

Starch-gel electrophoresis in sodium acetate–acetic acid buffer, I 0.10 and pH 4.9 also indicated the presence of a similar major fraction besides five minor fractions (see Fig. 2a).

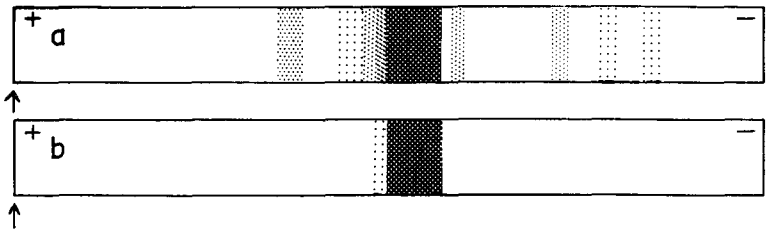

Fig. 2. Starch-gel electrophoresis. Starting point indicated by arrow. a, total lysine-rich histone. b, main component of lysine-rich histone after isolation.

For further investigation ample amounts of this fraction were obtained by means of preparative starch-gel electrophoresis. A starch-gel electrophoresis diagram of the eluted major fraction is shown in Fig. 2b. No indications of heterogeneity were obtained by starch-gel electrophoresis in the pH region of 1.2–8.2. The fraction appeared to contain 42.0 % of the total N as lysine-N. With the DNP-method an α-amino terminal amino acid could not be detected, while the phenylthiohydantoin method showed the presence of only trace amounts of α-amino terminal glycine, serine and alanine. The virtual absence of an α-amino terminal group is in accordance with Phillips' suggestion that in the lysine-rich histone fraction the terminal amino groups are blocked by acetyl groups[5].

Laboratory for Physiological Chemistry,
The University, Utrecht (The Netherlands)

E. H. DE NOOIJ
H. G. K. WESTENBRINK

[1] E. J. BIGWOOD, *Inst. Intern. de Chimie Solvay, 11ième Conseil de Chimie*, Brussels, 1959.
[2] E. W. JOHNS AND J. A. V. BUTLER, *Biochem. J.*, 82 (1962) 15.
[3] E. H. DE NOOIJ AND J. A. NIEMEIJER, *Biochim. Biophys. Acta*, in the press.
[4] P. BIJVOET, *Biochim. Biophys. Acta*, 25 (1957) 502.
[5] D. M. PHILLIPS, *Biochem. J.*, 80 (1961) 40 P.

Received July 4th, 1962

* This diagram is identical with the diagram of lysine-rich calf-thymus histone, prepared according to BIJVOET[4], in which the nucleohistone is prepared first, followed by dissociation in HCl and separation of the arginine-rich and lysine-rich fractions of the total histone by precipitation with ethanol.