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STUDIES ON HETEROGENEITY OF THE LYSINE-RICH HISTONE FROM OX PANCREAS

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1. Histone H1 from ox pancreas has been isolated by preparative electrophoresis at pH 2.7 in polyacrylamide slab gel, using the fraction F1 of Oliver et al. (1972, Biochem. J., 129, 349-353) as starting material.

2. The isolated histone H1 showed higher heterogeneity on isoelectric focusing than on polyacrylamide electrophoresis in long gel. The isoelectric points of the main subfractions of histone H1 were at pH 8.0-8.4.

Histone H1\(^1\), unlike histones H3, H4, H2B and H2A, is not required for formation of the nucleosome substructure of chromatin (Fredericq, 1976), and may be involved in the higher-order structure of the chromosome (Bradbury et al., 1975).

Histones in general show conservatism of structure over a wide range of species and tissues, except histone H1 which is both species- and tissue-specific (Subirana et al., 1970; Panyim et al., 1971; Johnson et al., 1973; Franco et al., 1977; Vanhoutte-Durand et al., 1977). The proportions of particular subfractions of histone H1 vary from tissue to tissue of the same species (Bustin & Cole, 1968; Kinkade, 1969; Panyim & Chalkley, 1969b); this may imply that they are functionally not identical.

Smith & Stocken (1973) demonstrated that \"crude histone F1\" extracted with 5% perchloric acid from nuclei of rat liver and ascites-hepatoma cells contains, in addition to histone H1, 5-8% of non-histone proteins.

\(^1\) The new nomenclature proposed for histones is: H1 (F1, I, KAP); H2A (F2a2, IIB1, ALK); H2B (F2b, IIB2, KAS); H3 (F3, III, ARK); H4 (F2a1, IV, GRK) [Ciba Symposium on the Structure and Function of Chromatin (1975) vol. 28 (new series), pp. 1-4. Elsevier, Excerpta Medica, North Holland, Amsterdam].
In our previous work (Lipińska & Kłyszejko-Stefanowicz, 1975), an additional histone component F1* was found in the ox pancreas histone F1 extracted by the method of Oliver et al. (1972). In the present work, heterogeneity of histone H1 was further studied, and its amino acid composition was determined.

MATERIAL AND METHODS

Material. Ox pancreas was obtained directly after killing of the animal and transported in cold 0.01 M-citric acid to the Laboratory. The fat and connective tissue were removed, and nuclei were isolated as described previously (Lipińska & Kłyszejko-Stefanowicz, 1975). From the nuclei, chromatin was isolated according to Speksberg & Hnilica (1971) at 4°C. The preparation of nuclei and the isolation of chromatin were carried out in the presence of 1 mM-phenylmethylsulphonyl fluoride in dioxane as inhibitor of proteolytic activity. Whole histone was obtained by three-fold extraction of chromatin with 0.25 M-HCl. After centrifugation at 12,000 g for 15 min, the proteins were precipitated with 10 vol. of cold acetone. From whole histone, the very lysine-rich histone F1 was extracted with 0.5 M-HClO₄ according to Oliver et al., (1972).

Preparative gel electrophoresis. This was performed as described by Martinage et al. (1976). The gel (17% acrylamide) was prepared in 0.9 M-acetic acid/2.5 M-urea, pH 2.7, the sample (10 mg of protein dissolved in 2 ml of 0.01 M-HCl/8 M-urea) was applied, and the electrophoresis was run at 25 V per gel for 18 h at room temperature. Two lateral guide strips were stained for 5 min with 1% Naphthalene Black in 7% acetic acid/20% ethanol, and destained electrophoretically. From the unstained gel, the area corresponding to histone H1 was cut off and ground in 100 ml of 0.01 M-HCl; the mixture was stirred for 2 h and centrifuged. This treatment was repeated twice more. The pooled extracts were passed through a Millipore filter (0.45 µm), dialysed against water, and freeze-dried.

Analytical electrophoresis in polyacrylamide gel (10×0.6 cm) was carried out at pH 2.7 in 0.9 M-acetic acid/2.5 M-urea according to Panyim & Chalkley (1969a) at 20°C and 1.5 mA/gel. Electrophoresis in long gels (25×0.6 cm) was performed at 4°C. The gels were pre-run for 24 h at 1 mA/gel, then histone H1 samples (25 µg/gel) were applied in 0.01 M-HCl/8 M-urea/0.5 M-2-mercaptoethanol. The electrophoresis was run at 110 V per gel for 24 h. The proteins were stained with 1% Naphthalene Black 10B in 7% acetic acid/20% ethanol, and scanned at 560 nm in an ERJ 65 densitograph (Carl Zeiss, Jena).

Isoelectric focusing in polyacrylamide gel. This was performed essentially as described by Kopelowich et al. (1976) in 5% acrylamide (15×0.6 cm), with ampholytes at a concentration of 1.1% (a mixture of ampholytes of pH 3-10 and pH 9-11, 1 : 9 v/v). pH was determined in blank gels. The gels were removed from the tubes and immediately frozen for 5-10 min in solid CO₂, then cut into 2-mm slices. The slices were suspended in 2 ml of degassed 0.01 M-KCl, and left standing overnight at room temperature; then the pH of each solution was measured. It should be noted that the pH distribution in the gel was the same after
freezing in solid CO₂ as after freezing at -18°C (Kiliańska & Kłyszejko-Stefanowicz, 1976)

The protein-containing gels were stained as described by Spencer & King (1971) except that the Coomassie Blue concentration was raised to 0.05% (w/v). The gels were destained in acetic acid/water/methanol (5:70:25, by vol.) for 48 h.

Amino acid analysis. Samples of purified histone H1 were hydrolysed in 6 M-HCl at 110°C for 24 h, and analysed with the Jeol JLC-6AH amino acid analyser. No correction was made for losses during hydrolysis.

Reagents. Phenylmethylsulphonyl fluoride was from Calbiochem (San Diego, Calif., U.S.A.); perchloric acid from Hopkin & Williams (Chadwell Heath, Essex, England); acrylamide from Koch-Light Lab. (Colnbrook, Bucks., England); N,N'-bisacrylamide and 1,4-tetramethyleylenediamine from Fluka A.G. (Buchs S.G., Switzerland), Naphthalene Black 10B from George T. Gurr (High Wycombe, Bucks., England), and Coomassie Brilliant Blue R, from Sigma Chem. Co. (St. Louis, Mo., U.S.A.) Ampholines, 40% (w/v) pH 3 - 10 and 20% (w/v) pH 9 - 11, were purchased from LKB-Producer AB (Bromma, Sweden). Other reagents were analytical grade products supplied by POCh (Gliwice, Poland).

RESULTS AND DISCUSSION

The histone fraction F1 from ox pancreas was contaminated with high-molecular-weight material, which was removed by preparative electrophoresis (Fig. 1). The purity of the H1 preparation, assessed by analytical gel electrophoresis (Fig. 2a), demonstrated that the electrophoresis on slab gel at pH 2.7 in the presence of 2.5 M-urea according to Panyim & Chalkley (1969a) is a rapid and very effective

![Fig. 1 Preparative gel electrophoresis of histone H1 obtained from fraction F1. The lateral guide strip from the slab gel was stained for 5 min with 1% Naphthalene Black in 7% acetic acid and 20% ethanol.](image-url)
procedure for obtaining pure histone H1. The amino acid composition of the purified ox pancreas histone H1 (Table 1) was found to be similar to that of the corresponding calf thymus histone (Johns, 1971).

Analytical electrophoresis of histone H1 from ox pancreas in 25-cm polyacrylamide gels (Fig. 2b) demonstrated a higher degree of heterogeneity than was observed in the 10-cm gels, and subfraction H1" was resolved into two bands.

![Graphs showing electrophoresis results](image)

Fig. 2. Analytical polyacrylamide-gel electrophoresis at pH 2.7 and densitometric tracing of purified histone H1: a, on a short gel (10×0.6 cm) and b, on a long gel (25×0.6 cm). The amount of protein applied was 40 µg in a and 25 µg in b. For details see Methods.

![Graph showing isoelectric focusing](image)

Fig. 3. Isoelectric focusing of histone H1 from ox pancreas in the system of Kopelovich et al. (1976). ●, pH gradient; ——, densitometer tracing. Histone H1, 200 µg of protein in 6.25 M-urea, was applied.
On isoelectric focusing, histone H1 showed higher heterogeneity (Fig. 3) than in analytical polyacrylamide-gel electrophoresis. The main subfractions had isoelectric points at pH 8.0-8.4. The high heterogeneity at alkaline and neutral pH values could be thought to be due to aggregation. However, according to Kopelovich et al. (1976), the presence of urea both in the gel and in the protein solution applied precludes histone aggregation.

**Table 1**

*Amino acid composition of histone H1 from ox pancreas*

Samples, containing 1 mg of protein/ml, were hydrolysed for 24 h in 6 M-HCl at 110°C. The results, expressed as moles/100 moles, are mean values from 4 determinations. Amino acid composition of the homologous calf thymus histone, taken from Johns (1971), is included for comparison.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>H1, ox pancreas</th>
<th>H1, calf thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.0</td>
<td>5.6</td>
</tr>
<tr>
<td>Serine</td>
<td>8.2</td>
<td>5.6</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>4.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Proline</td>
<td>8.5</td>
<td>9.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>7.5</td>
<td>7.2</td>
</tr>
<tr>
<td>Alanine</td>
<td>23.8</td>
<td>24.3</td>
</tr>
<tr>
<td>1/2 Cystine</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Valine</td>
<td>6.0</td>
<td>5.4</td>
</tr>
<tr>
<td>Methionine</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Leucine</td>
<td>3.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Histidine</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Lysine</td>
<td>25.8</td>
<td>26.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Lysine to arginine ratio</td>
<td>14.3</td>
<td>14.9</td>
</tr>
</tbody>
</table>

Rall & Cole (1971) demonstrated that in non-dividing cells the heterogeneity of fraction F1 was due mainly to differences in the primary structure of lysine-rich histones. On the other hand, the observed electrophoretic resolution could reflect charge differences, e.g. due to acetylation.

We wish to express our sincere appreciation to Dr. Pierre Sautière and Dr. Arlette Martinage (Institut de Recherches sur le Cancer, Lille, France) for training one of us (A.L.) in the method of preparative electrophoresis in polyacrylamide slab gel, and to W. Michalak, M.Sc. (Laboratory of Biological Sciences, University of Łódź) for the amino acid analysis.
REFERENCES


BADANIA NIEJEDNORODNOŚCI SILNIE LIZYNOWEGO HISTONU TRZUSTKI WOŁU

Streszczenie


2. Wyższy homolog histonu H1, analizowany metodą izoelektrycznego ogniskowania, wykazał niższą niejednorodność niż podczas elektroforezy w długich żelach. Punkty izoelektryczne głównych podfrakcji histonu H1 odpowiadały wartościom pH 8,0-8,4.

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