CHARACTERIZATION OF LOW MOLECULAR WEIGHT NONHISTONE CHROMOSOMAL PROTEIN FROM DOG LIVER

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1. Introduction

The nonhistone chromosomal proteins (NHC-proteins) are viewed as the possible positive effectors of the transcription control in eukaryotic cells [1]. The reproducible isolation and characterization of individual NHC-proteins are essential in order to explore their function in the control of gene activity. In this communication we describe the isolation and characterization of an electrophoretically homogeneous low molecular weight NHC-protein from dog liver.

2. Methods

2.1. *Preparation and fractionation of NHC-proteins*

The NHC-proteins from dog liver nuclei were prepared and separated on Sephadex G-200 column (2.5 X 45.0 cm) into three fractions (I, II and III) according to the methods described earlier [2]. All the extraction solutions and buffers used for fractionation of NHC-proteins contained 0.1 mM phenylmethylsulphonyl fluoride (PMSF), a 'serine type' protease inhibitor [3]. The NHC-proteins were solubilized overnight in TUBES buffer, pH 8.5 (0.02 M Tris–HCl, 4 M urea, 0.005 M 2-mercaptoethanol, 0.002 M disodium ethylenediaminetetraacetate, 0.1% sodium dodecylsulphate (SDS) and 0.1 mM PMSF) at room temperature followed by elution from Sephadex column with the same buffer in the cold room at 0–4°C.

Further separation of fraction III components was carried out on DEAE-Sephadex A 25 columns (0.9 X 60 cm). The dialyzed and lyophilized fraction III, dissolved in 0.01 M Tris–HCl buffer (pH 8.0) containing 5 M urea and 0.1 mM PMSF was loaded on the column followed by elution with 200 ml of a NaCl gradient from 0 to 1 M in the same Tris–urea–PMSF buffer.

2.2. *SDS polyacrylamide gel electrophoresis*

The electrophoretic analysis of the total NHC-proteins and the fractions was carried out according to Laemmli [4]. The 15% separation gels (0.5 X 6 cm) in 0.375 M Tris–HCl buffer (pH 8.8) containing 0.1% SDS were overlaid with 5% stacking gels (0.5 cm) in 0.125 M Tris–HCl buffer (pH 6.8) with 0.1% SDS. The electrode buffer consisted of 0.025 M Tris, 0.192 M glycine and 0.1% SDS, pH 8.3 The electrophoresis was carried out at 1.5 mA/gel for 5 to 6 hours till the marker (Bromophenol Blue) moved 5.5 cm in the separation gels. The gels were fixed in 15% trichloroacetic acid for 60 min., stained in 0.25% Coomassie Brilliant Blue in 7% acetic acid –50% methanol mixture for 3 hr followed by destaining by diffusion in 10% acetic acid –5% methanol mixture.

The samples for electrophoresis were prepared according to Van den Broek, et al. [5] by dissolving 1 mg of lyophilized fractions in 200 μl of solubilization buffer (0.0625 M Tris–HCl, pH 6,8, containing 0.1% SDS, 0.1% 2-mercaptoethanol and 0.1 mM PMSF) for 24 hr and then dialyzing against 40 vol of the same buffer containing 10% glycerol overnight at room temprature.

For molecular weight determination, the longer gels (0.5 X 10 cm) were used under similar conditions. The marker proteins employed were bovine serum albumin (mol. wt. 68 000, Pentex), equine heart cytochrome *c* (monomer mol. wt. 12 500, dimer mol. wt. 25 000 and trimer mol. wt. 37 500, Calbiochem.), bo-

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vine pancreas insulin (mol. wt. 5700, Calbiochem.), and bovine pancreas glucagon (mol. wt. 3 485, Calbiochem.).

2.3. *Analytical methods*

The proteins were determined by Lowry's method [6], RNA by orcinol method [7] and DNA by diphenylamine method [8]. To determine NH_2-terminal amino acid, the isolated protein was dansylated according to Gray [9] and identified by thin-layer chromatography employing two different solvent systems, 1) benzene: pyridine: acetic acid (16:4:1, v/v) and 2) toluene: 2-chloroethanol: NH_4OH (100:80:6, v/v). The amino acid analysis was carried out on a single column system in Beckman Model 110 autoanalyser after hydrolysing 60 to 100 μg protein in 500 μl of 6 N HCl at 110°C for 18 hr.

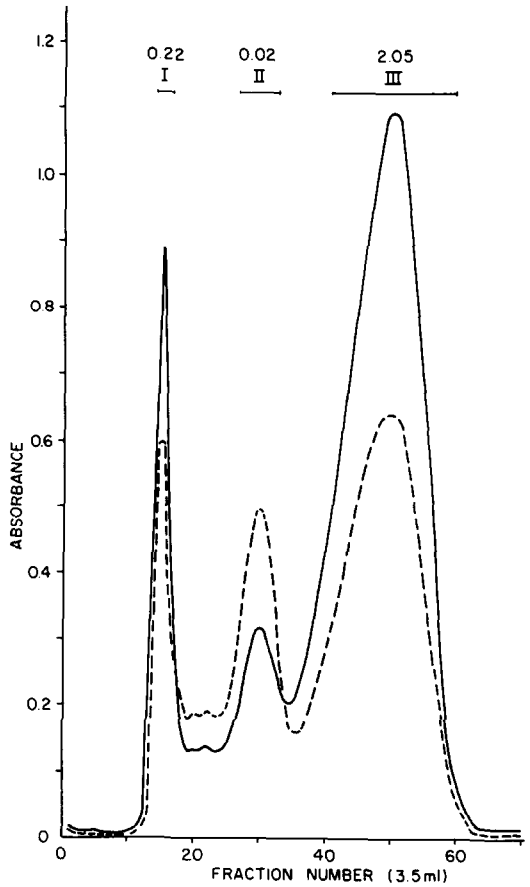

Fig. 1. Separation of dog liver NHC-proteins on Sephadex G-200 column. Numbers above each peak represent RNA/protein mass ratio for pooled fractions. (- - - -) Absorbance at 280 nm; (——) absorbance at 260 nm.

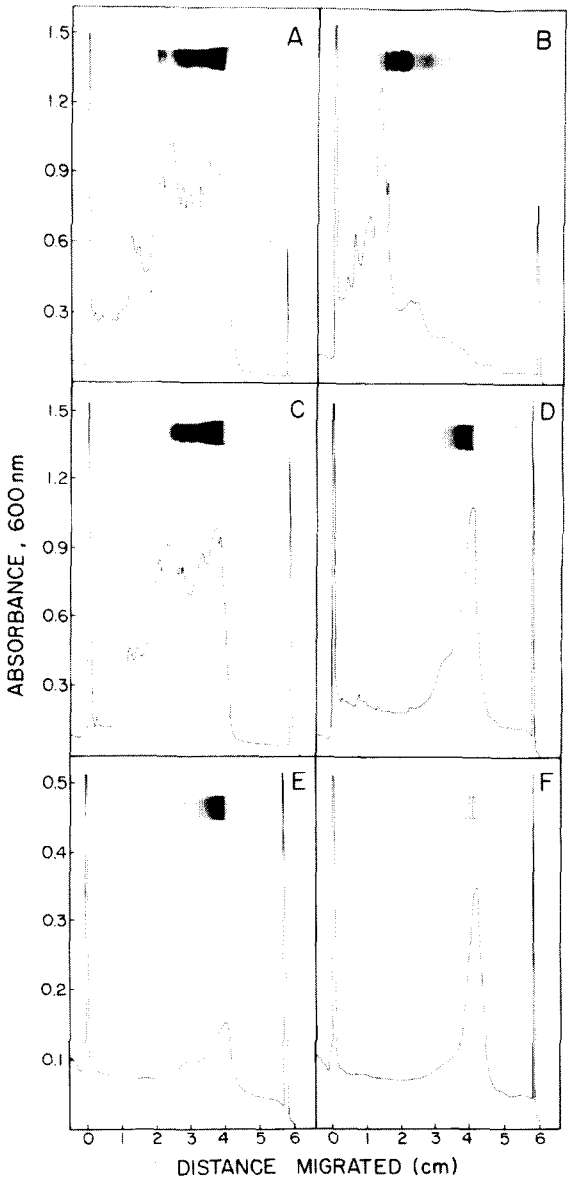

Fig. 2. Polyacrylamide Gel Electrophoresis of (a) Total NHC proteins, (b) Sephadex G-200 Fr. I, (c) Sephadex G-200 Fr. II, (d) Sephadex G-200 Fr. III, (e) DEAE-Sephadex Fr. B., and (f) DEAE-Sephadex Fr. C. The stained gels were scanned at 600 nm in a Gilford spectrophotometer.

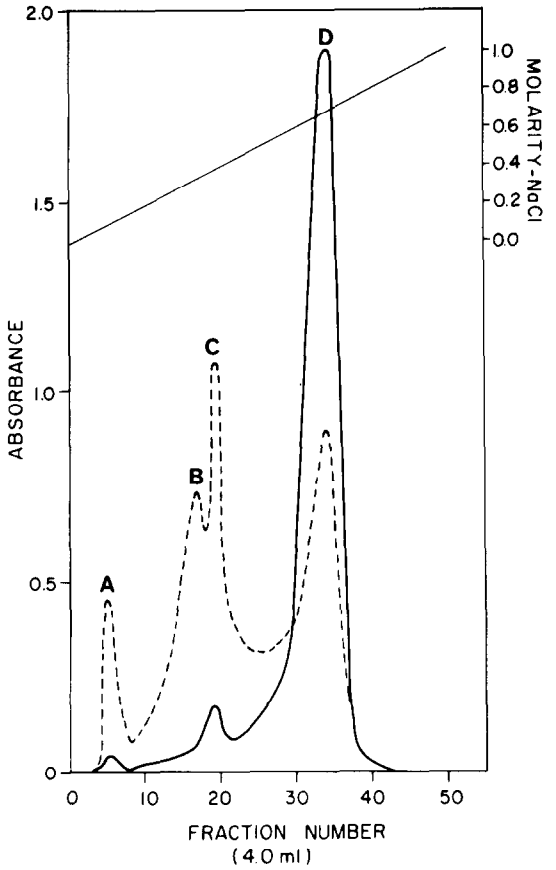

Fig. 3. Elution pattern of Sephadex G-200 Fr. III on DEAE Sephadex A 25 column. (- - -) Absorbance at 230 nm and (——) absorbance at 260 nm.

3. Results

The NHC-proteins prepared from dog liver nuclei which contained 6% RNA, no DNA and traces of histones [2] separated into three fractions (I, II, and III) on Sephadex G-200 column chromatography (fig. 1). The analysis of these fractions has shown that fraction I contained firmly bound RNA (RNA/protein mass ratio 0.22) and fraction III contained loosely associated RNA (mass ratio 2.0). Fraction II contained very little RNA (the mass ratio 0.02). The profiles of electrophoretic separation in SDS-polyacrylamide gels of total NHC-proteins and the three fractions are presented in fig. 2. The results indicate that the three Sephadex fractions are approximately separated according to the molecular size of the protein components in each fraction. All three fractions are heterogeneous mixtures of proteins, the least heterogeneous being fraction III. The protein and RNA components of the Sephadex G-200 fraction III were further separated on DEAE-Sephadex A 25 column employing NaCl gradient of 0 to 1 M in 0.01 M Tris–HCl buffer, pH 8.0 containing 5 M urea for elution. The elution curve (fig. 3) shows that fraction III separated into three protein components (A, B and C) and a low molecular weight RNA (D). Among the three protein components, C was eluted with 0.42 M NaCl and was electrophoretically homogeneous in SDS-polyacrylamide gel (fig. 2). Component B was eluted with 0.32 M NaCl and was separated into two bands moving very closely to one another and the faster moving band corresponded to that of C. Component A was not identified because of the limited amounts obtained.

The electrophoretically homogeneous protein component C has only serine as the NH_2-terminal amino acid as determined by dansyl method and a mol. wt. of 7900 as determined by SDS-polyacrylamide gel electrophoresis. Its amino acid composition is presented in table 1. The ratio of acidic to basic amino acids is 2.6.

Table 1
Amino acid composition of a small molecular weight NHC-protein isolated from dog liver

	Prep. 1	Prep. 2
Asp	11.27	11.17
Thr	5.05	5.07
Ser	13.54	13.30
Glu	15.51	16.43
Pro	4.93	4.54
Gly	10.66	11.02
Ala	8.51	8.59
Half Cys	ab.	ab.
Val	3.54	3.47
Met	1.48	1.68
Ile	2.29	2.28
Leu	6.36	6.36
Tyr	2.60	2.94
Phe	3.23	3.03
Lys	5.58	6.26
His	1.52	1.44
Arg	3.97	2.48
$\frac{Asp + Glu}{Lys + His + Arg}$	2.42	2.71

All values expressed as mole %.

Aspartic (11 mole %) and glutamic (16 mole %) acids together amount to 27% of the total amino acids present. It also contains high amounts of serine (13 mole %) and glycine (11 mole %). It does not contain cystine. The NH_2-terminal amino acid analysis of fraction B confirms its heterogeneity. It shows glycine, alanine, glutamic acid and serine as the main dansyl amino acid spots on the thin-layer chromatogram.

4. Discussion

An electrophoretically homogeneous protein has been isolated from dog liver. This protein accounts for approximately 1.4% of the total NHC proteins. Serine is its NH_2-terminal amino acid and mol. wt. is 7900. These results indicate that it is a single peptide chain. The ratio of acid to basic amino acid (2.6) is comparable to those reported in the range from 1.7 to 2.7 for most fractions of NHC-proteins [1]. Thus the isolated protein seems to be acidic in nature. Amino acid composition is determined without the consideration of possible amides. We have not investigated whether it is a phosphoprotein and whether it contains trytophan.

The isolated protein is probably the lowest molecular weight component of the NHC-proteins; however, it is improbable that it is a degradation product. A potential inhibitor of proteolytic activity (PMSF) was present in all the solutions used for isolation and fractionation of NHC-proteins. All operations were performed in the cold room at a temperature from 0–4°C. And at last, but not least the results of all separate isolations and characterizations of this protein are highly reproducible and independent of variations in the length of isolation which would require a rapid and highly specific degradation at a specific linkage.

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