FRACTIONATION OF CALF THYMUS DEOXYRIBONUCLEOPROTEIN ON ION EXCHANGE COLUMNS

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Various studies have suggested that histones are mixtures of proteins which upon separation from DNA are subject to dissociative and self-associative reactions (Davison et al. 1954; Cruft et al. 1954; Ui 1957; and Bijvoet 1957). As an alternative approach to the study of histones we have investigated the fractionation of whole calf thymus deoxyribonucleoprotein (TNP) on columns of DEAE cellulose.

Materials and procedures. - TNP was prepared and purified as described (Bakay et al. 1955). The DEAE cellulose used (Brown Co., Berlin, N.H.) had an adsorption capacity in 0.01 M pH 7 sodium phosphate of about 150 mg. TNP per g. dry DEAE. 8 x 34 cm. columns packed with 250 g. DEAE cellulose (previously washed with NaOH, water and alcohol) were equilibrated with 0.01 M pH 7 sodium phosphate buffer of 0.1 M pH 7 TRIS-HCl buffer, cooled to 2° and charged with 3-4 g. TNP dissolved in 2000 ml. water. Elution (2°, about 200 ml. per hour) was either stepwise with 2 liter portions of 0.1, 0.2, 0.3 .... 1.2 M NaCl in 0.01 M pH 7 sodium phosphate, or by linear gradient from 0.1 to 1.2 M NaCl in 0.01 M pH 7 sodium phosphate (18 liters) or from 0.3 to 0.6 M pH 7 TRIS-HCl (20 to 36 liters), fractions being collected every six hours.

Analytical. - Determinations of conductivity, pH and E_{260}/E_{280} gave information about acidity and salt content of the fractions and the nature of the eluted material. Analytical difficulties arising from the high salt/nucleoprotein ratios (up to 3000) of the fractions were overcome by lanthanum precipitation

(Chargaff and Saidell 1949). In the presence of 0.01 M $LaCl_3$, in the cold over night, precipitation was complete in all fractions, in terms of nucleic acid (according to E_{260}) and protein (according to TCA precipitation). The alcohol-washed and dried precipitates served for N (Kjeldahl-Nessler), nucleic acid and amino acid analyses.

Nucleic acid nitrogen was estimated from the UV absorption of formic acid hydrolysates (Wyatt 1951), and protein nitrogen was obtained from the difference between total nitrogen and nucleic acid nitrogen. Purines and pyrimidines were determined by paper chromatography of the formic acid hydrolysates (Wyatt 1951), and amino acids were determined on acid hydrolysates (24 hours with 6 N HCl at 120° in evacuated sealed tubes) according to the procedure of Moore et al. (1958).

Results. - Tables I and II show typical experimental data and Fig. 1 summarizes the recurring trends seen under different conditions of fractionation. In elutions with NaCl the pH of the fractions varied between 7 and 9.2, while in the elutions with TRIS buffer the pH remained at 7. We have shown experimentally that the release of fractions is a function of Cl^- concentration and that between 7 and 9 pH has little or no effect on release. However, the constant pH system seems less likely to produce artifacts. On rechromatography of primary fractions under identical conditions they generally appeared again at the eluent concentrations at which they had been collected and there was little evidence of further resolution in terms of nucleobase ratio or amino acid composition. Only the very early (high protein) fractions tend to spread and shift toward higher salt concentration, suggesting instability.

Discussion. - In successive fractions (a) the lysine/arginine ratio decreases and then levels off, (b) the arginine content of the protein moiety increases and then (while the lysine/arginine ratio remains stable) decreases again, (c) the DNA/protein and the (A+T)/(G+C) ratios also increase and then decrease, and (d) the maxima of the four parameters do not coincide (Fig. 1).

The amino acid data of some of the large middle fractions closely resemble those of pure "arginine-rich histone" (see Busch and Davis 1958). The small early fractions show an approach toward the values of "lysine-rich

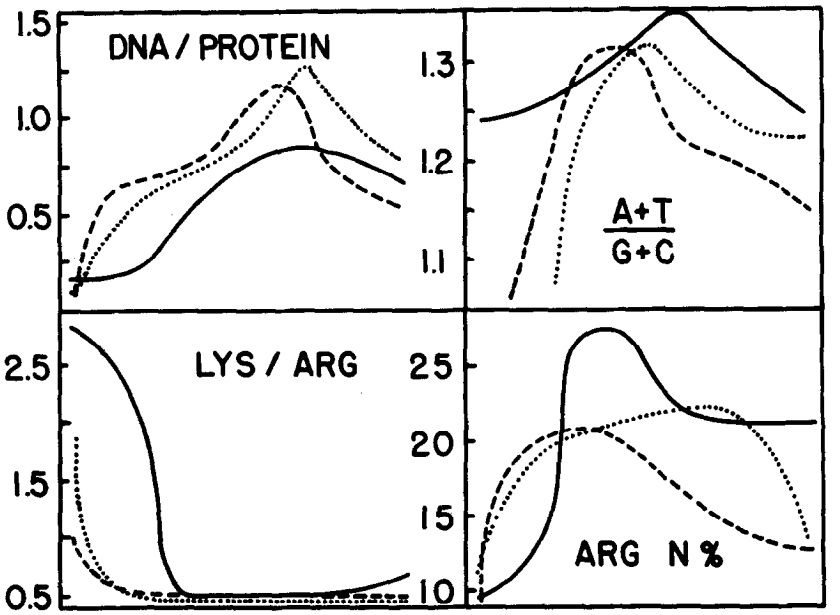

Fig. 1. Comparison of the resolution patterns obtained by elution with NaCl (———, Table I) and by gradient elution with TRIS-HCl buffer (-----, 20 liters, and ·····, 36 liters). The distances on the abscissae are based on percentages of total eluted volume.

TABLE I

Elution of TNP from DEAE columns with NaCl in 0.01 M pH 7 sodium phosphate buffer (averages of several runs).

M NaCl (influent)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.2	
Share of total E_{260}, %	1.5	0.2	0.9	1.8	4.6	31	23	10	5.3	3.5	5.9	2.5	TNP
E_{260}/E_{280}	1.11	1.14	1.02	0.99	1.21	1.56	1.56	1.45	1.30	1.22	1.11		1.41
Protein N as share of N, %			87		83	67	60	55	54		60		62.5
Share of totl protein %	8		8		12	34	19	6	11			2	
LYS N : ARG N			2.8		2.1	0.51	0.52	0.51	0.52	0.57	0.62		0.71
ARG N[a] %			9.6		14.1	27.6	26.7	22.4			21.3		
(A+T):(G+C)			1.04-1.24		1.26	1.29	1.32	1.35	1.30		1.25		1.27
pH of eluate	7.0	7.1	7.2	7.8	8.9	9.2	9.1	8.5	8.0	7.7	7.5	7.2	

[a]as share of amino acid and ammonia N.

TABLE II

Amino acid composition of early, middle and terminal fractions eluted with NaCl in 0.01 M pH 7 sodium phosphate[a]

M NaCl (influent)	Lys	Arg	Ala	Glu	Pro	Val	Leu	Ser	Thr	His	Asp	Iso	Phe	Tyr	Met	Cys
0.2 + 0.3 M	29.1	10.5	16.1	5.7	7.8	5.7	4.3	5.3	4.0	3.8	3.5	1.7	0.7	0.6	0.6	0.5
0.5 M	16.8	30.4	7.8	6.1	5.2	5.2	5.8	3.3	3.6	4.2	3.3	3.6	1.1	2.1	1.2	0.3
1.0 M	14.9	24.0	8.6	7.1	4.3	5.2	7.2	4.5	4.4	5.6	4.6	4.1	1.8	2.5	0.9	0.3

[a]as percentages of the sum of the 16 amino acids shown.

histone". Analysis of smaller sub-fractions, which may have disclosed the presence of lysine-rich histone in greater purity, was precluded by lack of material. In all cases the terminal fractions show decreases in both lysine and arginine, together with increases in dicarboxylic and aromatic amino acids, which are not compatible with simple mixtures of "lysine-rich" and "arginine-rich" histones. Apparently in these fractions at least one additional histone type occurs besides the two mentioned. Results partly resembling ours have been obtained (Lucy and Butler 1955) by successive extractions of octanol-$CHCl_3$ gels of TNP with 0.6 M NaCl.

The fractions may represent fragments of the native TNP macromolecule of mol. wt. 1.8×10^7 (Zubay and Doty 1959), since preparation of the starting material presumably involves disaggregation to mol. wts. below 10^6 (Allgen 1950). This work is being continued in an effort to establish whether the nucleoprotein species contained in the starting material remain unchanged during fractionation or whether artifacts may result from interchanges between their nucleic acids and histones.

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