ISOLATION OF HISTONES FROM VIRUS-INDUCED TUMORS

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1. Introduction

The surge of interest taken in the chemical study of the histones – the basic proteins associated with DNA – is partly due to the generally received opinion that these proteins have a major role in gene expression. This hypothesis of gene control by histones [1] cannot yet be supported by the results of many investigations. Recent comparative chemical studies of histones from various tissues [2–6] have shown a lack of specificity in these proteins. Moreover, no significant differences have been found between the compositions of histones from tumor and normal tissues [5,7,8].

However, since histone specificity has been reported in some differentiated cells as erythrocytes [9–11], silkgland cells [12], or baker's yeast cells [13], it was tempting to determine whether the histones extracted from tumors induced by oncogenic viruses presented any feature of specificity.

The considerable progress made in the preparation and fractionation procedure of histones [14,15] allowed us to isolate and compare the now well-defined five main histone fractions of three types of virus-induced tumor cells: cells from tumors induced in newborn hamsters by human adenovirus 12 [16], cells from hamster tumors induced by the hybrid virus human adenovirus 2 – simian virus 40 [17,18] and rat embryo cells transformed *in vitro* by human adenovirus 2 [19].

The chemical study of tumor histones can contribute in some way to the understanding of the cellular processes involved in DNA replication and oncogenesis.

2. Experimental procedures

The hamster tumors and the rat transformed cells have been kindly supplied by Pr. J.Samaille (Department of Virology of the Institut Pasteur of Lille).

2.1. *Tumors*

Adenovirus 12 and Ad2-SV40-induced tumors were obtained by injecting subcutaneously 8–10 day old hamsters with 0.1 ml of a hamster tumor cells suspension titering 2 to 3 × 10^7 cells/ml. When the tumor mass weighed approximately 1 to 2 g, and before necrosis occurred, the tumors were excised, minced, dispersed in a Waring-Blendor at low speed for 2 min in 4–5 volumes of phosphate-buffered saline, and filtered through three layers of gauze. The homogenate was washed three times in 0.25 M sucrose – 0.01 M Tris – HCl – 3.3 mM $CaCl_2$ buffer pH 7.0 and the tumor cells were obtained by centrifuging at 600 × *g* for 10 min at 4°C.

2.2. *Transformed cells*

The continuous cell line of Ad2-transformed rat embryo cells was grown in suspension in EAGLE's basal medium supplemented with 5% horse serum. The cells were harvested by mild centrifugation at 600 × *g* for 10 min at 4°C, resuspended in 0.25 M sucrose – 0.01 M Tris – HCl – 3.3 mM $CaCl_2$ and successively centrifuged and washed three to four times in this buffer in order to eliminate all traces of culture medium.

2.3. *Preparation of nuclei*

All fractionation procedures were carried out at 4°C.

The cells pellets were homogenized in a Potter-Elvehjem homogenizer with a teflon pestle at 3,500 rev/min for $2\frac{1}{2}$ min in 10–15 ml of the 0.25 M sucrose – Tris – HCl – $CaCl_2$ buffer. The suspension was centrifuged at 1,000 × *g* for 10 min and the nuclei thus obtained were then washed and centrifuged three times in the sucrose solution.

2.4. *Preparation of nucleohistones*

The nuclear pellets were resuspended in 0.14 M NaCl containing 0.01 M sodium citrate pH 7.0 and one drop of a 1% diisopropylfluorophosphate solution (DFP) was added per ml of this nuclear suspension, as enzyme inactivator. The suspension was then homogenized by using an Ultra-Turrax homogenizer successively three times for 1 min and the homogenate centrifuged at 3,000 × *g* for 15 min. The sediment was washed five times with the NaCl – Na citrate buffer, without DFP.

2.5. *Preparation of whole histone*

Whole histone was prepared by the method of Johns et al. [20] using 0.25 N HCl.

2.6. *Preparation of histone fractions*

The selective extraction of histone fractions F2(a)1 and F2(a)2 was carried out by using a mixture of ethanol and guanidinium chloride at pH 7.0 [21]. Each of the F2(a) subfractions was greatly contaminated by the other [8]. They were therefore further purified by another method, using acetone precipitation at low pH value [14]. The other histone fractions were removed according to the method of Johns [22].

2.7. *Analyses*

Purity of the fractions was checked by analytical disc electrophoresis in 15% acrylamide gels containing 6.25 M urea at pH 4.3, according to a modification [23] of the method of Reisfeld et al. [24]. Amino-acid analyses were performed after 24 hr hydrolysis in 5.6 N HCl at 110°C under vacuum.

3. Results and discussion

Polyacrylamide gel electrophoresis of the whole histone from Ad2-SV40 tumor cells (fig. 1) shows a pattern of four bands corresponding, successively from cathode to anode, to fractions F2(a)1, the faster-moving band, F2(a)2, the most strongly stained band, F2b and F3 together as a single weakly stained band behind F2(a)2, and F1, the slowest migrating band.

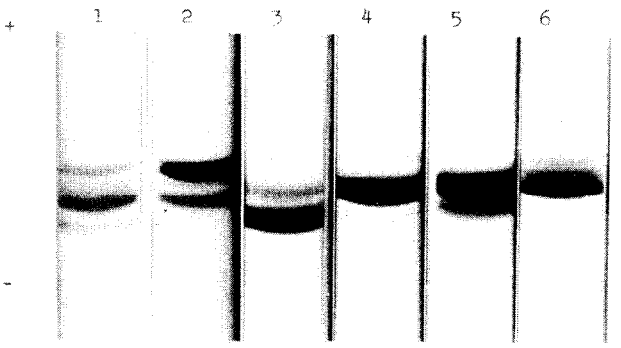

Fig. 1. Polyacrylamide gel electrophoresis of histones from Ad2-SV40-induced tumor. 1: whole histone; 2: F1; 3: Fs(a)1; 4: F2(a)2; 5: F2b; 6: F3. Similar patterns were obtained with corresponding fractions from other tumor cells.

The electrophoretic patterns of the histone fractions (fig. 1) show that each one is slightly contaminated by the others: fractionation by selective extraction and selective precipitation of the histones does not yeld pure fractions. Another step, such as gel filtration chromatography, is therefore necessary to purify them. Nevertheless, the purity of each fraction obtained by the selective extraction procedure is generally accepted as sufficient for amino-acid analysis comparison.

The amino-acid compositions are presented in table 1 The general characteristics of each fraction is preserved: F1 is lysine-alanine rich, F2(a)1 glycine-arginine rich, F2(a)2, F2b and F3 are arginine-lysine rich. The most remarkable point is that all corresponding fractions from the three types of tumor have the same amino acid composition. But some differences, notably a higher content of acidic amino-acid, are observed with whole histone and histone fractions from calf thymus.

Fractions F1 show the most significant differences with F1 from calf thymus and from other animal tissues, i.e. a higher content of acidic amino-acid and

Table 1

Amino acid analysis of histone fractions from adenovirus-induced tumors and from adenovirus-transformed cells. The amino acid contents are expressed as moles/100 moles of all amino acids found. No correction has been made for hydrolytic losses. ND: not detected; A: acidic; B: basic.

	Ad 12 Tumor					Ad2-SV40 Tumor					Ad2-Transformed Cells					
Histone fractions	F1	F2(a)1	F2(a)2	F2b	F3	F1	F2(a)1	F2(a)2	F2b	F3	F1	F2(a)1	F2(a)2	F2b	F3	Whole histone
Amino acid																
Asp	5.2	6.2	5.8	5.6	7.4	5.1	5.9	6.8	5.4	7.8	5.3	6.3	5.7	5.8	6.4	7.5
Thr	5.2	5.4	5.5	6.4	5.3	5.6	6.3	4.3	5.3	5.3	5.5	5.7	4.7	5.4	5.0	5.3
Ser	7.1	4.5	4.4	8.9	5.8	7.1	3.0	4.0	7.2	6.0	7.1	4.3	4.7	7.0	6.5	6.2
Glu	7.7	10.5	10.7	8.9	10.7	7.9	8.4	10.9	8.7	11.7	8.1	10.3	10.0	9.5	11.3	11.2
Pro	7.9	4.2	4.3	5.3	5.4	7.5	3.1	3.6	5.4	4.9	8.6	3.7	5.7	5.4	5.2	6.5
Gly	9.0	11.0	9.7	7.6	9.7	8.2	14.1	11.9	9.3	10.1	10.9	11.1	10.3	10.5	9.6	8.7
Ala	16.3	9.6	10.5	10.3	10.6	15.4	8.7	12.0	10.0	9.8	13.6	10.2	10.8	10.7	11.5	8.9
Cys	0.3	0.6	0.3	0	0	0	0	0	0	0.7	0.5	0	0.1	0	0.2	0.5
Val	4.8	5.8	5.7	5.2	5.4	5.3	6.3	5.1	5.9	4.9	5.0	5.9	5.1	5.8	5.0	5.9
Met	1.0	1.4	0.8	0.5	0.1	traces	0.8	0.2	1.3	1.6	0.9	1.0	0.8	1.2	1.0	1.3
Ile	2.5	4.7	4.6	4.3	3.8	2.7	4.8	4.3	4.5	4.2	2.7	4.7	3.7	4.0	3.5	4.2
Leu	5.6	10.2	10.4	4.9	8.1	6.3	9.4	11.3	6.3	8.7	5.1	9.3	9.9	6.5	7.7	8.1
Tyr	1.7	3.0	2.6	3.1	2.4	1.7	3.6	2.6	3.3	2.9	1.7	2.8	2.2	2.8	2.6	2.6
Phe	3.0	2.6	2.0	4.5	2.5	1.7	2.0	1.3	2.0	3.3	1.9	1.9	1.8	1.5	2.2	2.6
Lys	17.3	8.8	9.8	15.3	12.6	19.9	9.9	11.0	14.1	8.7	17.2	9.5	12.5	14.2	12.6	11.0
His	1.0	1.8	2.2	2.1	1.9	1.2	1.8	2.4	2.2	1.7	1.1	2.0	2.3	2.0	2.0	2.0
Arg	4.3	9.6	10.7	7.0	8.2	4.4	11.9	8.3	9.1	7.6	4.7	11.1	9.6	7.6	7.6	7.4
A	12.9	16.7	16.5	14.5	18.1	13.0	14.3	17.7	14.1	19.5	13.4	16.6	15.7	15.3	17.7	18.7
B	22.6	20.2	22.7	24.4	22.7	25.5	23.6	21.7	25.4	18.0	23.0	22.5	24.4	23.8	22.2	20.4
B/A	1.8	1.2	1.3	1.7	1.2	1.9	1.6	1.2	1.8	0.9	1.7	1.3	1.5	1.5	1.2	1.1
Lys/Arg	4.0	0.9	0.9	2.2	1.5	4.5	0.8	1.3	1.5	1.1	3.6	0.8	1.3	1.8	1.6	1.5

a lower content of lysine and alanine. Such differences concerning glutamic and aspartic acids and lysine in fraction F1 have been previously reported for histones from hepatopancreas of *Mytilus edulis* [4]. Moreover, as reported by Palau et al. [25], most of the nuclear proteins from metabolically acitve cells are more acid than histones from inactive cells: this is the case for our tumor cells. It is also interesting to note that an acidic contaminant of histone fraction F2a has been recently described [26]. A high amount of this contaminant in tumor histones could perhaps explain the differences observed in amino-acid composition of whole histone and of histone fractions.

In summary, the amino-acid compositions generally do not show a specificity of the histone fractions isolated from virus-induced tumors. However, some particularities concerning fraction F1 deserve further investigation.

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