Journal of Chromatography, 128 (1976) 244–248

CHROM. 9705

Note

Identification of histones F2b and F1 in stained gels

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(Received February 16tn, 1976)

Histones from a wide variety of plants and animals have generally proven divisible into five main fractions[1,2]. Although no definitive biological role has ever been demonstrated for any of the histones, it is assumed that in the various organisms there are corresponding fractions which have corresponding functions. Identification of these corresponding fractions, especially F2b and F2a2, presents a problem. Some authors have questioned the existence of F2b and F2a2 fractions in higher plants[3,4].

The two arginine-rich histones, F2a1 and F3, are easily identified since their sequences have been highly conserved in evolution[5] and the proteins display characteristic electrophoretic mobilities[1,2]. Histone F1 is also easily identified because of its low mobility in acetic acid–urea polyacrylamide gels, its high lysine content and solubility in 5% perchloric acid[6].

Identification of the remaining fractions (F2b and F2a2) has proven more difficult. Electrophoretic mobility in acetic acid–urea polyacrylamide gels is not a reliable criterion. For example, relative positions of F2b and F2a2 are not the same in all vertebrates[1]. Also, these fractions migrate faster than F3 in all animal systems studied[1], while fractions of supposedly comparable functions migrate slower than F3 in plant systems[2] and have been simply designated as "plant histones" by Nadeau *et al.*[3].

Distinctions on the basis of amino acid content are not sufficiently clear to be completely satisfactory and separation techniques of animal histones do not yield entirely comparable fractions when applied to plant histones[7–9].

Until distinct biological functions have been demonstrated for all histones, identification of comparable fractions in various organisms and any nomenclature system for histones will not be completely satisfactory. However, any aid to identification of the fractions, especially if applicable to extremely small amounts of materials, should prove valuable in comparative histone studies. Additionally, a simple way of identifying histone fractions would be particularly useful in studies of the role of histones in chromatin structure in various organisms.

Recently I have found a way of identifying histone F2b in gels stained with amido black. When gels destained by standard means [either electrophoretically or by diffusion in 7% (v/v) acetic acid–20% (v/v) ethanol are further destained in 0.1 *M* $FeCl_3$, all detectable stain is lost from F1 (of calf thymus and pea), and from

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F2b (from calf thymus) and from what has previously been designated[2] the "F2b-like" histone (from pea). Other pea and calf thymus histones retain from 13–54% of the originally bound stain. The technique has been used to identify "F2b-like" histones from several grasses and from yeast[10].

MATERIALS AND METHODS

Histones

Histones of pea were isolated as previously described[11]. Histones of calf thymus were kindly supplied by Dr. Roger Chalkley.

Electrophoresis and staining

Approximately 20–30 μg of pea or calf histones were applied to acetic acid–urea polyacrylamide gels (0.45 × 10 cm) as described by Panyim and Chalkley[12] and subjected to electrophoresis for 3 h. Immediately after electrophoresis they were stained for 3–12 h in 0.1% (w/v) amido black–7% (v/v) acetic acid–20% (v/v) ethanol. The amido black was from Calbiochem, Los Angeles, Calif., U.S.A.

Destaining and quantitation

After staining was completed, preliminary destaining was carried out by diffusion at room temperature against 7% (v/v) acetic acid–20% (v/v) ethanol with a diffusion destainer (Hoefer) until a clear background in the gel was first obtained (*ca.* 20 h). Care was taken not to over-destain. At this time the gels were placed in a Gilford 2412 cuvette (path length 0.6 cm) and densitometer tracings were made utilizing light of 600 nm in a Gilford 2400 spectrophotometer equipped with a linear transport and operated in the absorbance mode.

Control gels (all individually identified) were placed in a large excess of the acetic acid–ethanol mixture and stirred gently for 4 days at room temperature.

Remaining gels (also individually identified) were placed in a large excess of 0.1 *M* $FeCl_3$–20% (v/v) ethanol, stirred gently at room temperature for either 48 or 72 h, then removed to the acetic acid–ethanol mixture (for an additional 24–48 h) to remove the $FeCl_3$. Densitometer tracings of control and $FeCl_3$-destained gels were then made as mentioned above.

Areas under the peaks corresponding to the various histone fractions were then determined and compared to those areas obtained after preliminary destaining. This operation was carried out with the aid of a DuPont 310 curve resolver as described previously[11].

For accuracy of presentation, densitometer tracings were made on the back (unlined) side of chart paper and photographed directly. Gels were photographed using the method of Oliver and Chalkley[13].

RESULTS AND DISCUSSION

The complete disappearance of histone bands F1 and F2b of calf thymus on $FeCl_3$ destaining is illustrated in Fig. 1. Histone bands F3, F2a2 and F2a1 remain. Pea histone F1 also disappears on $FeCl_3$ destaining as does the fraction which has been previously designated "F2b-like". This fraction has been designated "F2b-like"

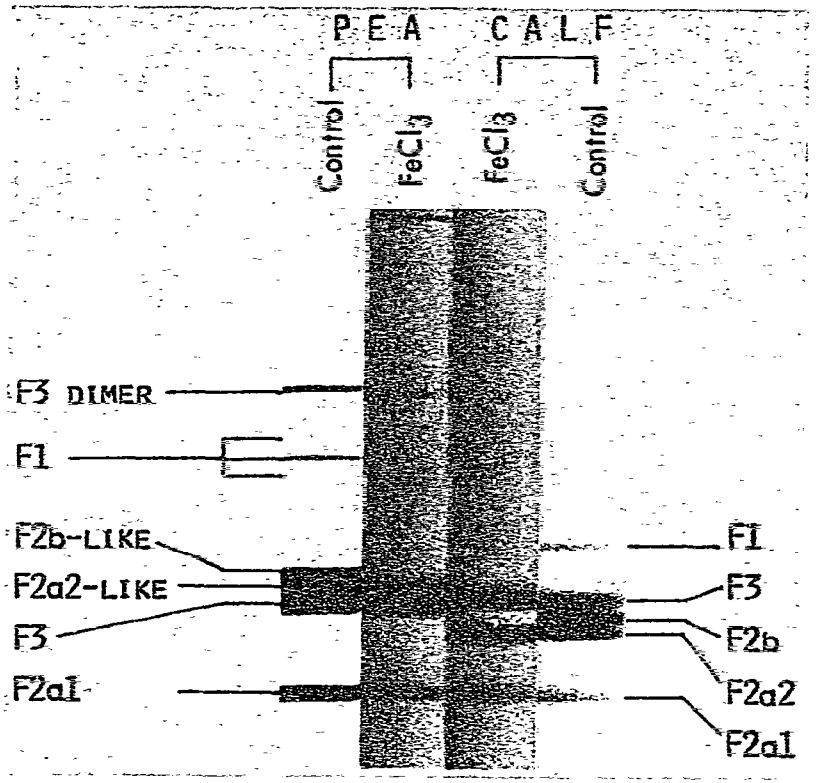

Fig. 1. Polyacrylamide gels of histones of calf and pea stained in amido black and destained in either 7% (v/v) acetic acid–20% (v/v) ethanol (control), or 0.1 *M* $FeCl_3$–20% (v/v) ethanol ($FeCl_3$) as described in the text. Ethanol is included in the $FeCl_3$ destain only to prevent excessive swelling of the gels. $FeCl_3$-destained gels have been over-exposed in printing to emphasize the remaining bands. $FeCl_3$ has been removed by diffusion leaving a clear background. Calf thymus histones have been labeled by the nomenclature of Johns (see ref. 6) as correlated with bands in acetic acid–urea polyacrylamide gels by Panyim *et al.*[1]. Pea histones F1, F3 and F2a1 meet the criteria to be thus labeled[7], but the remaining plant histones do not. However, by a variety of criteria outlined in the text, they are clearly similar to vertebrate F2b and F2a2 histones and thus have been labeled "F2b-like" and "F2a2-like". Migration in the gels is from the top toward the bottom.

because it is the most abundant histone, has electrophoretic mobility close to that of F3 and stains purple with amido black as compared to the blue of F3 (ref. 2). Pea F3, F2a1 and "F2a2-like" fractions remain visible in $FeCl_3$-destained gels.

Figs. 2 and 3 show densitometer tracings of control gels of calf thymus and pea (full scale absorbance 3.0) and of $FeCl_3$-destained gels (full scale absorbance 1.0).

Table I indicates the amount of stain retained by all fractions of control gels and by $FeCl_3$-destained gels. F1 loses stain in control gels more rapidly than do other fractions. Also F2a1 appears to be more sensitive to $FeCl_3$ destaining than do F3 and F2a2. Gels which have been destained in $FeCl_3$ cannot be restained with either amido black or coomassie blue.

The rate of destaining with $FeCl_3$ can be highly increased by elevating the temperature, but this will abolish the selectivity. At 70° all bands lose all detectable stain within 20 min.

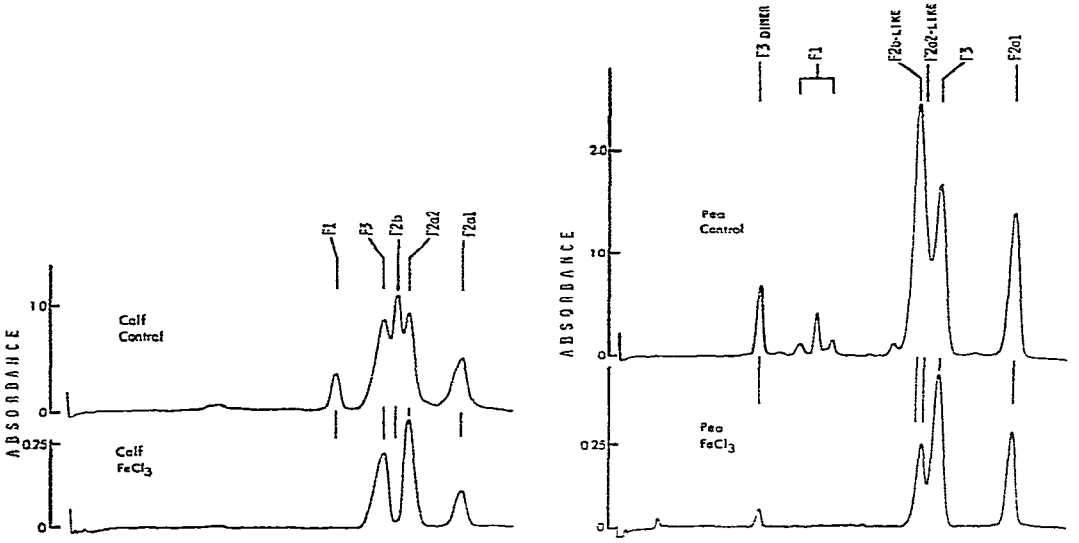

Fig. 2. Densitometer tracings of the calf thymus histone gels from Fig. 1. Note that the scales of absorbance at 600 nm are different. The control gels were scanned at full scale absorbance of 3.0 while the $FeCl_3$-destained gels were scanned at full scale absorbance of 1.0. Migration in the gels represented by the densitometer tracings is from the left toward the right. Note that peaks representing bands of F1 and F2b are missing in the densitometer tracings of the $FeCl_3$-destained gels.

Fig. 3. Densitometer tracings of the pea histone gels from Fig. 1. As in Fig. 2, the control gels were scanned at full scale absorbance (600 nm) of 3.0 while $FeCl_3$-destained gels were scanned at full scale absorbance of 1.0. Migration in the gels represented by the densitometer tracings is from the left toward the right. Note that in the tracing of the control gel the peak representing the "F2b-like" histone is the major peak while the "F2a2-like" histone is barely detectable as a shoulder on the "F2b-like" peak. In the $FeCl_3$-destained gel, the "F2b-like" peak is completely missing making conspicuous the F2a2 peak of slightly greater electrophoretic mobility. F1 histones are also completely destained by $FeCl_3$ leaving the oxidized disulfide dimer of F3 as the only peak in the low mobility portion of the gel.

TABLE I

PERCENT STAIN REMAINING IN HISTONE FRACTIONS IN CONTROL AND $FeCl_3$-DESTAINED GELS

Values are averages of three experiments.

Fraction	*Control*		*$FeCl_3$ destain*		
	Calf	*Pea*	*48 h*		*72 h*
			Calf	*Pea*	*Pea*
F1	64	72	0	0	0
F3	85	90	34	35	24
F2b*	91	93	0	0	0
F2a2**	72	89	31	54	36
F2a1	81	90	13	23	15
Whole histone	80	89	16	19	12

* "F2b-like" from the plant source.

** "F2a2-like" from the plant source.

The method described above in conjunction with other methods will allow for the identification of all histone fractions in stained gels. The method of Barrett and Johns[14] allows identification of arginine-rich histones which are also easily identified by their characteristic electrophoretic mobilities. F1 is easily identified because of its low electrophoretic mobility in acetic acid–urea polyacrylamide gels and is unlikely to be confused with F2b even though both of these histones lose all stain when destained in $FeCl_3$.

Histone F2a2 can be identified by elimination as the band which loses all stain by the method of Barrett and Johns[14] but retains approximately half of its stain when destained in $FeCl_3$.

Lysine-rich and arginine-rich histones of calf thymus can be distinguished by the differential color staining method of Barrett and Johns[15] but there is no indication of the applicability of this method to other organisms. The "F2b-like" histones of rye, triticale, wheat[16] and from yeast[10] have been identified using this procedure. Hopefully sensitivity to $FeCl_3$ destaining will also prove useful in resolving the problem of whether some cryptogamous plants have "F2b-like" histones[2] and in identifying possible "F2b-like" histones in unicellular eukaryotes and fungi.

ACKNOWLEDGEMENTS

Thanks are due to my colleagues in the laboratory for their sincere and helpful comments during the progress of this investigation. The work was supported by grants from ERDA, NSF and from Public Health Service Research grant no. CA-11624 from The National Cancer Institute.

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