SPECIFICITY OF THE ENZYMATIC METHYLATION OF PEA HISTONE

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Some lysyl residues of animal histones can be modified in vitro by the enzymatic methylation of ε-amino groups (Comb, Sarker and Pinzino, 1966). Although ε-N-methyllysine has recently been shown to be present in an arginine rich histone from peas (Fambrough and Bonner, 1968) a corresponding methylation in vitro has not been previously described in plants. This communication reports on the enzymatic transfer of methyl groups from S-adenosyl methionine to the ε-N of lysine in histones IIb and III in isolated pea chromatin.

METHODS

Pea seeds (Pisum sativum var Meteor) were surface sterilised with a suspension of bleaching powder in water, washed, and grown for 7 days in the dark at 25° in moist vermiculite. Pea shoots were cut 2 cm from the apex, and the nuclear chromatin isolated by the method of Fambrough and Bonner (1966). The methylation reaction mixture (1ml) comprising chromatin from 450 g pea shoots, 0.05 M tris - HCl pH 8.0, 0.001 M $MgCl_2$, and 100 μC/ml (^{3}H-methyl)S-adenosylmethionine, was incubated for 2 hr at 25°. In a control reaction, chromatin was heated at 100° for 5 minutes before

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addition of labelled S-adenosyl methionine. After the incubation, the chromatin was separated from the reaction mixture by centrifugation through 1.7 M sucrose. The labelled histone was extracted and fractionated by electrophoresis on polyacrylamide gels containing 6 M urea (Fambrough and Bonner, 1966). After staining overnight in 0.5% naphthalene black in 1 M acetic acid the gels were electrophoretically destained. To fractionate the gels, they were frozen in a groove in a block of dry ice, allowed to warm to about -15°, and transverse sections of 0.5 mm made with a gel cutter. Successive pairs of slices were combined in scintillation vials to give fractions which corresponded to 1 mm of gel. The protein bands were located by extracting each fraction overnight with 0.3 ml formamide at 25° on a shaker and measuring the extinction of the extracts at 620 nm in 1 cm microcells. The formamide was returned to the scintillation vials, together with 15 ml ethanol : toluene scintillator (Lagerstedt and Langston, 1966). ^{3}H activity was measured in a Packard Tricarb scintillation spectrometer, with a channel setting of 50-1000, and 50% gain. Fractions scraped from thin layer chromatograms were shaken with 0.2 ml water and 0.2 ml NCS solubilizer (Nuclear Chicago Corp.), and ^{3}H activity counted after adding 15 ml dioxane-based scintillator (Bruno and Christian, 1961). ε-N-methyllysine was synthesised by the method of Benoiton (1964) for use as a chromatographic standard. To identify the methylated residue, the labelled histone was hydrolysed for 20 hr at 105° with 6 N HCl in a sealed tube, after flushing with nitrogen. The HCl was removed by repeated vacuum evaporation over sodium hydroxide, and the hydrolysate separated into acidic, neutral and basic fractions by electrophoresis on Whatman 3 MM paper, using pyridine-acetic acid buffer, at pH 6.3. The basic fraction, which contained nearly all the ^{3}H label, was eluted and freeze-dried. Portions were dissolved in water and streaked on Whatman No. 1 paper, and developed with butanol: acetic acid:water (60:20:20 v/v) or tert-butanol:formic acid:water (70:15:15 v/v) (Benoiton, 1964). In both systems the ^{3}H label travelled with

-N-methyllysine. After development with the tert-butanol solvent, the chromatogram was cut transversely into 1 cm strips, which were eluted with water. The concentrated eluates which contained ^{3}H activity were applied as spots to a cellulose thin layer (Eastman Chromogram Sheet 6064), and developed with buffered phenol: m-cresol (25 g phenol: 25 g cresol: 7 ml borate buffer pH 9.3) (Levy and Chung, 1953).

RESULTS AND DISCUSSION

The distribution of ^{3}H label in the different histone fractions is shown in Fig. 1. The nomenclature of Fambrough and Bonner (1966), adapted from that for calf-thymus histone (Rasmussen, Murray and Luck, 1962) has been used. Apart from minor incorporation into histone II, all the incorporation has been into the arginine-rich histone III and the moderately lysine-rich histone IIb. There was no incorporation into histone when the pea chromatin

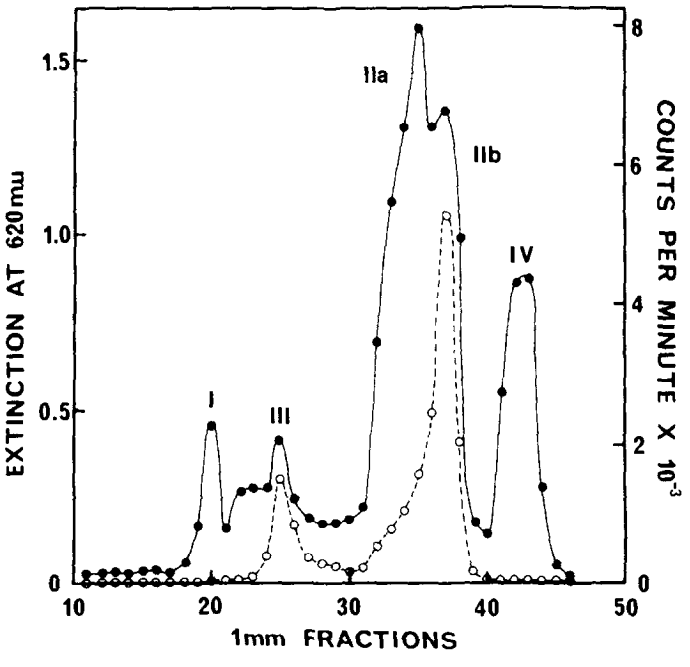

Fig. 1. Distribution of ^{3}H label in pea histones after separation by gel electrophoresis. —●— , extinction at 620 nm of amido black dye; -- O -- ^{3}H activity in counts/min.

had been heated to 100° before addition of S-adenosyl methionine. After the labelled material in the histone hydrolysate had been purified as described in the Methods section, and separated using the buffered phenol-cresol solvent (Levy and Chung, 1953) the ^{3}H label was confined to a single spot which stained with ninhydrin, and ran in the same position as ε-N-methyllysine. A spot corresponding to ε-N-dimethyllysine was not detected.

Recently ε-N-methyllysine has been reported to be present in arginine-rich histone III, but not in histone IV from peas (Fambrough and Bonner, 1968). The reaction described here also results in the methylation of histone III, but not histone IV, suggesting that the specificity of the reaction is conserved in vitro. Whether ε-N-methyllysine is a normal constituent of histone IIb will be of interest, as in isolated chromatin this histone is methylated to a similar specific activity to histone III.

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