ELECTROPHORETIC INVESTIGATION OF INTERACTION BETWEEN CHROMATIN PROTEINS AND NITROGEN MUSTARD (HN2)

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Unlike histones, which are resistant to the action of nitrogen mustard, the nonhistone proteins of chromatin are readily alkylated, with the formation of simple protein-protein bonds for the particular class of chromatin proteins.

KEY WORDS: Chromatin proteins; histones; action of nitrogen mustard.

The effect of the classical bifunctional alkylating mutagen nitrogen mustard (mustine, HN2) on the electrophoretic mobility of histones and of nonhistone proteins of chromatin and also of certain other proteins which, in their amino acid composition and molecular weight, are closely similar to certain chromatin proteins, was studied.

EXPERIMENTAL METHOD

Histones and nonhistone proteins were isolated from calf thymus which had been frozen immediately after decapitation of the animal and kept for more than 24 h at −70°C. Total histone was isolated by the method of Johns [7]. Nonhistone proteins were obtained by chromatography on calcium hydroxyapatite [8]. Ribonuclease (RNase) and bovine serum albumin (BSA) were from Reanal (Hungary) and glutamate dehydrogenase from Serva (Czechoslovakia).

The samples were incubated (1 mg protein to 1 ml, 50 mM Tris-HCl, pH 7.5, 1-10 mM HN2) at 37°C for 1 h. Unreacted HN2 was removed by dialysis in the cold.

Electrophoresis at pH 2.8 was carried out by Panyim's method [9]. Gels containing 10% acrylamide, 0.1 M Tris-HCl buffer, 0.1% sodium dodecylsulfate (SDS), and 5 M urea were used for electrophoresis at pH 7.5.

EXPERIMENTAL RESULTS AND DISCUSSION

As Fig. 1 shows, treatment of histones and RNase with the HN2 preparation in a concentration of 10^{-2} M did not change the electrophoretic mobility of the histone fractions or RNase. However, the mobility of the BSA was more than doubled after similar treatment; this could be evidence primarily of an increase in the total positive charge on the molecules of this protein, due to addition of the alkyl radical to the negatively charged carboxyl groups of the albumin, i.e., to alkylation of the albumin. At the same time, although this is less probable, the increase in mobility of the BSA could have been due to a decrease in its molecular weight, i.e., to rupture of the peptide chain of the protein as, for example, was described in a paper by Alexander [1].

Since HN2 is bifunctional it might be expected that besides the depolymerization of the protein, protein-protein cross-linkages could be formed. This could be shown by a change in the molecular weight of the protein when the conditions in the medium were such as to completely inhibit nonspecific aggrega-

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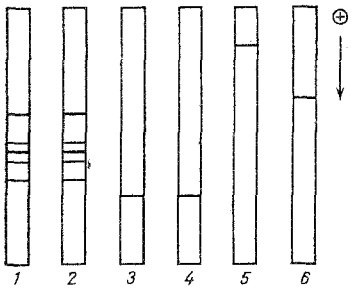

Fig. 1. Electrophoresis at pH 2.8: 1) total histone; 2) total histone + 10^{-2} M HN2; 3) RNase; 4) RNase + 10^{-2} M HN2; 5) BSA; 6) BSA + 10^{-2} M HN2.

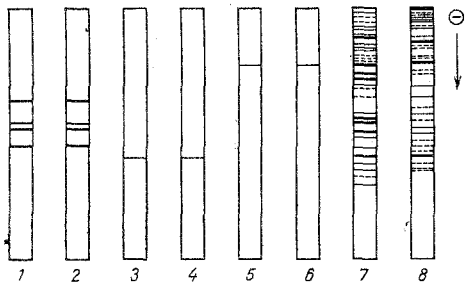

Fig. 2. Electrophoresis at pH 7.5 in the presence of SDS: 1-6) as in Fig. 1; 7) nonhistone proteins; 8) nonhistone proteins + 10^{-2} M HN2.

tion of the protein through electrostatic or hydrophobic interaction and formation of S-S bonds. Such conditions are provided by a solution consisting of 1% SDS, 5 M urea, 0.01% β-mercaptoethanol in 0.1 M Tris-HCl buffer, pH 7.5. It has also been shown that the presence of SDS in a homogeneous electrophoretic system can enable protein fractions to be separated on the basis of their molecular weight, for the effect of the intrinsic charge on the protein molecules is almost entirely suppressed by the great excess of molecules of SDS associated with one protein molecule by means of hydrophobic bonds [10].

Comparison of the results of electrophoresis (Fig. 2) in this system shows that only in the case of the nonhistone proteins of chromatin does HN2 induce any significant decrease in electrophoretic mobility of many of the fractions of these proteins, whereas the mobility of the histones, RNase, and BSA was unchanged. Consequently, the view that BSA is depolymerized by HN2 is unlikely to be correct. By using proteins of known molecular weight – glutamate dehydrogenase (150,000), BSA (60,000), and RNase (12,000), subfractions with molecular weights of 150,000, 150,000-60,000, 60,000-12,000 and under 12,000, respectively, were isolated from the whole electrophoretic spectrum of nonhistone proteins. After treatment with HN2 a redistribution of the nonhistone fractions was observed, with an increase in the content of those with highest molecular weight. For instance, the content of nonhistone proteins with a molecular weight of over 150,000 and of those with molecular weights of 60,000-12,000 was 10 and 47% respectively, compared with 45 and 23% respectively after treatment with nitrogen mustard.

It can accordingly be concluded that under the conditions used, HN2 interacted only with proteins whose isoelectric point lay in the weakly acid region (BSA, nonhistone chromatin proteins), whereas proteins with well-marked basic properties (histones, RNase) do not interact with HN2. The results of electrophoretic investigation of interaction between BSA and HN2 indicate alkylation of the carboxyl groups of this protein without change in molecular weight under the conditions of treatment used; this is contrary to the results obtained earlier by Alexander et al. [4]. The reason could be that the workers cited observed nonspecific aggregation of the alkylated BSA molecules.

It was only in the case of nonhistone chromatin proteins that interaction with HN2 was found to lead to the formation of stable protein-protein bonds resistant in the presence of a high concentration of urea, sodium dodecylsulfate, and β-mercaptoethanol. It may be expected that not only stable DNA-protein bonds were formed by the action of HN2 [3, 5, 6] (and, in particular, between DNA and the nonhistone fraction of chromatin proteins), but stable bonds were also formed between the nonhistone proteins themselves.

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