Two-dimensional Gel Electrophoretic Separation of the Proteins Present in Chromatin of *Escherichia coli*

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The polypeptides present in ^{35}S -labelled chromatin prepared from *Escherichia coli* cells, and polypeptides present in the DNA and RNA complexes obtained by micrococcal nuclease digestion of the chromatin, were analysed by two-dimensional non-equilibrium polyacrylamide gel electrophoresis. Three hundred and thirty-five ^{35}S -labelled polypeptides were detected in the chromatin whereas the DNA- and RNA-containing fractions of the micrococcal nuclease digest contained 126 and 183 polypeptides respectively. The major basic low-molecular-weight polypeptides were found in the DNA-containing fractions.

INTRODUCTION

In a previous study, chromatin from *Escherichia coli* was isolated by a new method and subsequently characterized (Sjåstad *et al.*, 1982). Briefly, the *E. coli* cells were lysed by treatment with T4 lysozyme followed by freezing and thawing once. The lysate was incubated on ice with a solution containing the non-ionic detergent Nonidet P-40 and the insoluble fraction was collected by centrifugation. The washing with the detergent solution was repeated three times and the resulting viscous material was termed bacterial chromatin. The chromatin contained DNA and RNA in a ratio of approximately 1:1 and the amount of protein present was approximately three times that of the nucleic acids. The proteins characterized by one-dimensional gel electrophoresis consisted of a number of high- and low-molecular-weight components.

In the present report we describe the analysis by two-dimensional gel electrophoresis of the polypeptides of protein present in chromatin and in the DNA- and RNA-containing fractions separated by sucrose gradient centrifugation of micrococcal nuclease digested chromatin.

METHODS

Chromatin from *E. coli* K12 DG75⁻ *thy leu* was prepared essentially as described previously (Sjåstad *et al.*, 1982). The polypeptides were labelled by adding $^{35}\text{SO}_4^{2-}$ to the M9 medium. One-dimensional gel electrophoresis was carried out according to the method of Laemmli & Favre (1973) and two-dimensional gel electrophoresis as previously described (O'Farrell, 1975; O'Farrell *et al.*, 1977; Sjåstad *et al.*, 1982). Ribosomes were prepared as described by Kaji (1968). Outer and inner membranes of *E. coli* were prepared by the method of Sato *et al.* (1977).

For ease of comparison of the different gels, the polypeptides have been given reference numbers based on coordinates as indicated in Fig. 1. The position of polypeptides in the basic, low-molecular-weight region (abscissa < 50, ordinate < 50) was largely dependent on the duration of electrophoresis. The coordinates assigned to polypeptides in this region were derived from the electrophoresis of samples of cytoplasmic proteins from *E. coli* which bound to a DNA-cellulose column and were eluted by a salt gradient (Sjåstad *et al.*, 1983).

RESULTS AND DISCUSSION

The two-dimensional gel electrophoretic patterns of the polypeptides present in total chromatin, and in the 24S RNA fraction and the 10S DNA fraction obtained after sucrose gradient separation of the micrococcal nuclease digested chromatin, are shown in Figs 1, 2 and 3

Fig. 1. Fluorograph of the two-dimensional electrophoretic separation of ^{35}S -labelled proteins present in total chromatin of *E. coli* K12. The pI markers used were the same as described by Sjöstad *et al.* (1983).

respectively. The total numbers of labelled polypeptides detected in these samples were 335 (Fig. 1), 126 (Fig. 2) and 183 (Fig. 3). The polypeptide patterns of the DNA- and RNA-containing fractions were different. Several polypeptides co-sedimented with both DNA and RNA, suggesting that some of the proteins may form complexes with both kinds of nucleic acid or, alternatively, that some RNA was present in the DNA fraction and vice versa. The major polypeptides associated exclusively with the 24S RNA are indicated by coordinate numbers in Fig. 2 and their molecular weights and charge characteristics are shown in Table 1.

The majority of the low-molecular-weight basic polypeptides co-sedimented with the DNA peak whereas the acidic low-molecular-weight polypeptides were found either in the top fractions of the sucrose gradient or in the 30S to 40S fractions, which also contained fragments of spheroplast envelopes (results not shown). The results suggested that the low-molecular-weight basic proteins were bound to the DNA, whereas the presence of acidic low-molecular-weight polypeptides was due to solubilized or unremoved membrane fragments.

The polypeptide patterns shown in Figs 1, 2 and 3 were compared with the pattern obtained after electrophoresis of the total cytoplasmic DNA-binding proteins of *E. coli* (Sjöstad *et al.*, 1983). Only the major polypeptides were analysed in this way. A total of 26 of the major polypeptides shown in Fig. 3 had identical mobilities to polypeptides present in the different salt eluates of the DNA-cellulose column. These are indicated by their coordinate numbers in Fig. 3 and their identities are presented in Table 2. Four of these polypeptides (39,29; 94,130; 100,132; 112,113) were also present as major polypeptides in the outer membrane fractions prepared according to Sato *et al.* (1977) whereas two (30,37 and 112,113) were present in ribosome preparations. The polypeptides 69,22 and 69,25, not detected among the total cytoplasmic DNA-

Fig. 2. Fluorograph of the two-dimensional electrophoretic separation of ^{35}S -labelled proteins present in the 24S RNA fraction isolated from *E. coli* K12 chromatin after nuclease digestion and sucrose gradient centrifugation.

Fig. 3. Fluorograph of the two-dimensional electrophoretic separation of ^{35}S -labelled proteins in the 10S DNA fraction isolated from *E. coli* K12 chromatin after nuclease digestion and sucrose gradient centrifugation.

Table 1. *Major polypeptides co-sedimenting exclusively with the 24S RNA fraction isolated from E. coli K12 chromatin after nuclease digestion and sucrose gradient centrifugation*

Polypeptide coordinates*	$10^{-3} \times$ Mol. wt	Migration relative to pI markers†
48,23	15	8.5
73,91	47	7.2
78,133	98	6.8
100,92	47	5.6
105,97	50	5.3
110,109–118	60–70	5.0
113,103	55	4.9
115,110	62	4.8
119,25	16	4.5

* Coordinates are the abscissa and ordinate values (in arbitrary units) shown in Fig. 1.

† Estimated pI of peptide derived by comparison of migration with that of peptides of known pI.

Table 2. *Identification of major polypeptides present both in the 10S DNA fraction and among the cytoplasmic DNA-cellulose binding proteins of E. coli K12*

10S DNA coordinates*	Cytoplasmic DNA-cellulose binding polypeptides†		$10^{-3} \times$ Mol. wt	Migration relative to pI markers‡
	No.	NaCl concn		
16,30	160	0.4	18	10.5
18,36	148	0.4	21	10.1
26,24	77	0.15–0.6	16	9.7
28,28	73	0.15–0.6	17	9.6
29,34	202	0.6–2.0	20	9.6
30,37	145	0.4–2.0	22	9.5
32,20	–	0.15	14	9.3
39,29	159	0.4	18	8.9
70,122	86	0.4	77	7.2
73,52	188	0.4–2.0	29	7.1
82,88	20	0.15	44	6.6
82,101	11	0.15–0.4	53	6.6
82,136	170	0.6–2.0	105	6.6
89,87	179	0.6–2.0	43	6.2
93,84	23	0.15	42	5.9
94,130	4	0.15–0.4	92	5.8
97,59	122	0.4	32	5.7
99,117	88	0.15–0.4	70	5.6
100,89	177	0.6–2.0	45	5.6
100,132	169	0.4–0.6	95	5.6
100,134	2	0.15–2.0	100	5.6
103,19	79	0.15–2.0	13	5.3
103,124	5	0.15–0.6	80	5.3
105,151	166/67	0.4–2.0	160	5.2
110,80	180/181	0.15–2.0	40	5.0
112,113	95	0.15–2.0	65	4.9

* Coordinates are the abscissa and ordinate values (in arbitrary units) shown in Fig. 1.

† Figures are the polypeptide number and the NaCl concentration (M) at which eluted from DNA-cellulose column (Sjåstad *et al.*, 1983).

‡ Estimated pI of peptide derived by comparison of migration with that of peptides of known pI.

cellulose binding proteins, did bind to double-stranded DNA-Sepharose. The 69,22 polypeptide also bound to single-stranded DNA-Sepharose (A. Holck, unpublished observation).

The results suggested that protein species in the chromatin can be divided into several classes, those specifically associated with RNA, those bound to DNA, and a group of proteins only

loosely associated with the chromatin and found at the top of the sucrose gradient after sedimentation of the micrococcal nuclease digested chromatin. Also, there may be a class of proteins which can bind equally well to both RNA and DNA.

Less than half of the major proteins co-sedimenting with the 10S DNA were found among the cytoplasmic proteins known to bind to DNA-cellulose. The reason for this is probably related to the way the protein samples were prepared. For preparation of cytoplasmic proteins, the cells were disrupted by sonication and cell walls and membranes sedimented by high-speed centrifugation prior to nuclease digestion of the supernatant solution (Sjåstad *et al.*, 1983). For preparation of chromatin, the membranes were lysed by the non-ionic detergent Nonidet P-40. In this process DNA-binding proteins present in the membrane may have been solubilized and then become associated with chromatin. It is therefore suggested that some of the high-molecular-weight proteins of the 10S fraction may be membrane proteins. Recently a number of membrane proteins have been shown to have affinity for DNA (Jacq & Kohiyama, 1980; Kohiyama *et al.*, 1977; Hendrickson *et al.*, 1982; Nicolaidis & Holland, 1978; Portalier & Worcel, 1976; Wolf-Watz & Norqvist, 1979).

An alternative explanation for the differences in polypeptide patterns may be the occurrence of post-translational modification (phosphorylation, acetylation, etc.) of protein, causing the forms predominantly present in the cytoplasm to differ from those which are bound to the DNA.

Studies are now in progress to determine which of the polypeptides can be considered to be true DNA-binding proteins.

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