ON THE CHEMICAL NATURE OF ALTERATION AND MODIFICATION OF DNA DEPENDENT RNA POLYMERASE OF *E. COLI* AFTER T4 INFECTION

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1. Introduction

After infection of *E. coli* with bacteriophage T4 the host DNA dependent RNA polymerase is changed by two consecutive processes [1, 2]. The *first* of them, termed *alteration* is very rapid and coincides with the shut off of host transcription [3]. It requires neither protein synthesis nor the phage genome [2] and therefore might reflect a dormant capacity of the host which is triggered by phage adsorption. The *second* process, termed *modification*, leads to a structural change of subunits β' and β, a further change of α and the absence of σ factor in the purified enzyme [1, 2, 4, 5, 6, 7]. It requires protein synthesis and might be phage gene dependent. The work reported here aims at the understanding of the chemical nature of alteration and modification.

2. Experimental

Unlabeled host-, T4 altered and T4 modified RNA polymerase were prepared as reported previously [2, 8]. A microprocedure specifically developed for the preparation of labeled polymerases will be described elsewhere [9]. Subunits were obtained in pure state by *preparative electrophoresis* on cellogel blocks under the conditions described in the legend of fig. 1.

They were removed from the blocks as previously described [12], precipitated by ammonium sulfate (60% saturation), redissolved in 0.01 M tris-HCl, pH 8, 0.02 M β-mercaptoethanol, dialysed, reprecipitated by ammonium sulfate, dissolved in and thoroughly dialysed against 0.01 M tris-HCl, pH8.

For the estimation of the nucleotide content in α_A and α_M the concentration of α_N was determined using the micromethod previously described [12] and that of α_A and α_M assumed to be equal when the extinction values at 295 nm were identical with that of α_N.

Radioautographs were prepared by contact exposure of X-ray film Agfa Structurix D7 or D10 with cellogel pherograms or thin layer plates for several days applying a total radioactivity of a few thousand dpm.

3. Results

3.1. *Subunit patterns of unchanged host-, T4 altered and T4 modified enzymes*

In *polyacrylamide gel electrophoresis* (fig. 1a) subunits α, β and β' of E_A* show the same mobilities and relative concentrations as those of E_N. σ Factor is still present though in reduced amount. In addition to the normal subunits, the *cellogel electrophoresis* (fig. 1b) pattern of E_A exhibits three or even more other bands one of which migrates between σ_N and α_N and others ahead of σ_N. The band between σ_N and α_N migrates like α_N in disc electrophoresis and therefore appears to be an α with increased negative charge (α_A). In many independently pre-

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* *Abbreviations*: E_N, E_A and E_M = DNA dependent RNA polymerase from uninfected *E. coli* (holoenzyme), T4 altered and T4 modified enzyme, respectively. The same subscripts are used for the differentiation of the subunits of the different enzymes.

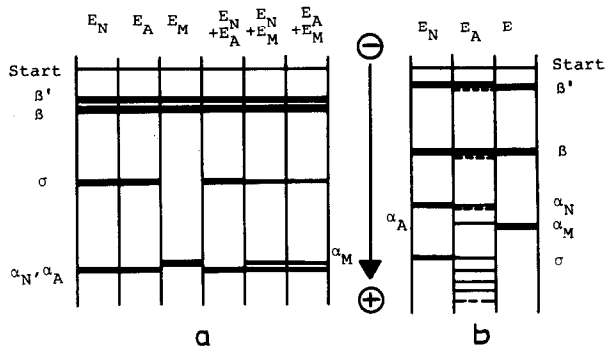

Fig. 1. Schematic representation of (a) polyacrylamide gel ('disc') electrophoresis of E_N, E_A, E_M and mixtures of these enzymes in the presence of 0.1% SDS and 6 M urea at pH 9 [10]. (b) Electrophoresis of E_N, E_A and E_M on cellogel (cellulose acetate sheet) in 0.6 M ammonium borate (pH 9) 0.01 M EDTA, 0.02 M β-mercaptoethanol and 6 M urea [4, 11].

pared batches of E_A obtained from cells harvested far after the completion of the alteration reaction the relative amount of α_A approaches but never exceeds that of (unchanged) α_N. Since on the other hand the enzymological properties of E_A, for example the ratio of activities on T4 and calf thymus DNA in excess of template, are drastically different from those of E_N [13] and cannot be accounted for by assuming an incomplete alteration, it appears probable that the composition of the core of E_A is $\beta'\beta\alpha_N\alpha_A$ and not $\beta'\beta(\alpha_N)_2 + \beta'\beta(\alpha_A)_2$. Further evidence for the asymmetry of the core in respect to the α subunits (which is reflected by the alteration of only one of them) comes from the existence of an incomplete core $\beta\alpha_2$, isolated by phosphocellulose chromatography of E_N and from the supernatant of a centrifuged DNA-enzyme complex [5]. Its two α subunits must be bound to structurally different sites on the β chain.

Subunits β'_M and β_M show mobilities not significantly different from those of β'_N and β_N in disc- and cellogel electrophoresis. It has, however, been demonstrated immunochemically [4] and by tryptic fingerprint maps [5], that they are indeed modified as compared to β'_N and β_N. The disc electrophoretic mobility of α_M is lower than that of α_N (which is identical with that of α_A) indicating an apparent increase in molecular weight. Other interpretations are also possible.

The mobility of α_M in cellogel electrophoresis is considerably higher than that of α_N and slightly higher than that of α_A indicating an even stronger increase of negative charge than that seen in α_A.

3.2. *Characterization of changed subunits in T4 altered and modified enzyme*

The *difference spectra* of α_A and α_M versus α_N exhibit maxima at 260 nm. The extinction ratios, for example $E_{260\,nm}/E_{280\,nm}$, closely correspond to those of adenylic acid. Assuming that the molar extinction of bound adenosine residues corresponds to that of free adenylic acid (15×10^3 moles^{-1}l^{-1}) very close to one adenine residue would be present per peptide chain in both α_A (0.97) and α_M (0.89). A summary of spectral data of the different α subunits is given in table 1.

When E_A *and* E_M *isolated from bacteria grown in a low phosphate medium containing* ^{32}P-*phosphate* of known specific activity for about 6 generations before infection (a time period sufficient for the saturation of pools) both enzymes contained ^{32}P. E_N from bacteria labeled for the same time period but uninfected was not significantly labeled. As demonstrated by direct counting of sections, by scanning of label with a thin window counter and by radioautography after cellogel electrophoretic separation of subunits, the label in E_A appeared directly under α_A and slightly ahead of σ_N. In E_M it was confined to α_M (fig. 2).

After disc electrophoretic separation of subunits of E_A both the α and the σ section exhibited radioactivity when gel slices were directly counted. The radioactivity of E_M was again confined to the α section. Quantitatively, not less than two phosphate residues per peptide chain were estimated for both α_A and α_M.

In radioautograms of *fingerprint maps* of tryptic digests of α_A the bulk of the label appeared in one spot corresponding to a weakly ninhydrin positive,

Table 1

Spectral data of the α subunits from normal, T4 altered and T4 modified *E. coli* RNA polymerase.

	α_N	α_A	α_M
$\epsilon_{280\,nm}$ nm^{-1}ml^{-1}	0.917	1.527	1.313
$\epsilon_{280\,nm} : \epsilon_{260\,nm}$	1.64	1.10	1.15
ϵmax	277 nm	272 nm	273 nm
ϵmin	251 nm	249 nm	249 nm

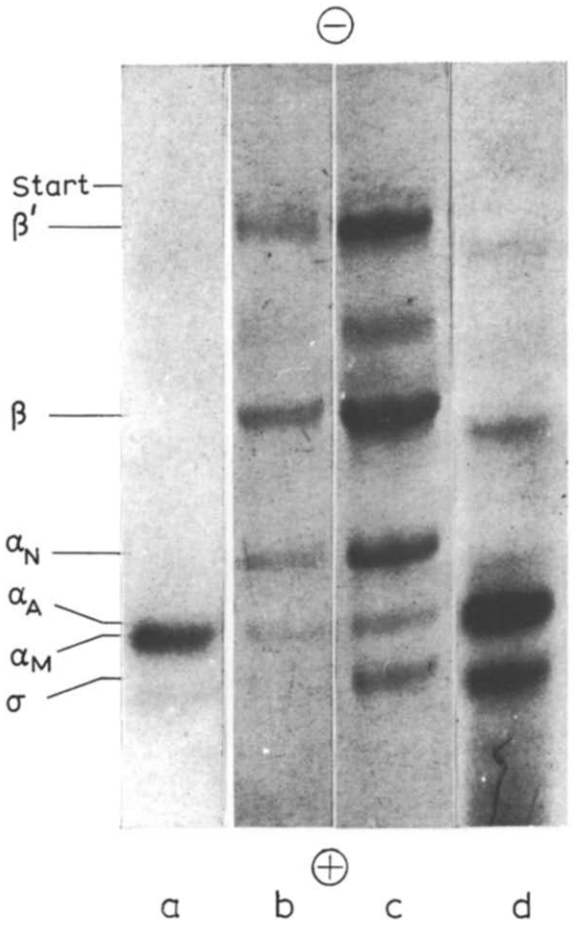

Fig. 2. Cellogel electrophoresis of ^{32}P labeled E_A and E_M with E_N as carrier. b and c: Amido Black staining of the protein bands of $E_N + E_M$ (b) and $E_N + E_A$ (c) after cellogel electrophoresis. c: radioautogram of b. d: radioautogram of c.

hydrophilic and acidic peptide (fig. 3). Two minor spots, possibly corresponding to derivatives, were visible. α_M by the same procedure yielded only one labeled spot which was different from all three radioactive spots obtained from α_A (more acidic and hydrophobic).

4. Discussion

The two consecutive changes of subunit α of *E. coli* RNA polymerase induced by infection with bacteriophage T4 can be differentiated on the basis of three independent observations: (1) α_M is slightly faster (more acidic) than α_A in cellogel electrophoresis, (2) α_M is slightly slower (apparently slightly heavier) than α_A and α_N in disc electrophoresis in the presence of SDS, (3) in α_M the ^{32}P label appears in a different peptide than in α_A.

The high velocity of alteration and the fact that both phosphates which probably are present per α_A chain appear to be bound to one peptide support the assumption that adenine and phosphate residues are introduced simultaneously and linked to each other. Since alteration neither requires protein synthesis nor phage genes it is possibly a process also occurring in the uninfected cell under certain conditions. The adenylylation of *E. coli* RNA polymerase observed by Chelala et al. [14] might also be a reflection of this potential.

It is clear from our results that the pA residues postulated by Goff and Weber [7] in T4 modified α are not introduced during modification but already by the alteration reaction. Modification apparently occurs at the altered site (or close to it) as indicated by the different positions of labeled peptides in the tryptic fingerprints of α_A and α_M. The elucidation of the structural changes in altered and modified polymerases is in progess.

It is not clear yet whether the ^{32}P label slightly ahead of σ_N observed in the cellogel subunit pattern of E_A corresponds to a phosphorylated (or adenylylated) σ, perhaps comparable to that demonstrated by Orlando et al. [15]. The finding of label in the σ region of SDS polyacrylamide gels of E_A appears to support this assumption. A conclusion, however, cannot be drawn until direct evidence, for example from tryptic fingerprints, is obtained. The label in front of σ does not coincide with one of the two (or even more) stained bands seen in the cellogel pattern ahead of σ which appear as constituents of highly purified enzyme and might be altered σ or σ-like factors. In spite of its content of at least some normal σ (which is known to act catalytically) E_A behaves like normal core enzyme on a number of templates [13]. This then must be due to its content of α_A and is a first demonstration of the role of α in initiation, probably in the σ catalyzed step.

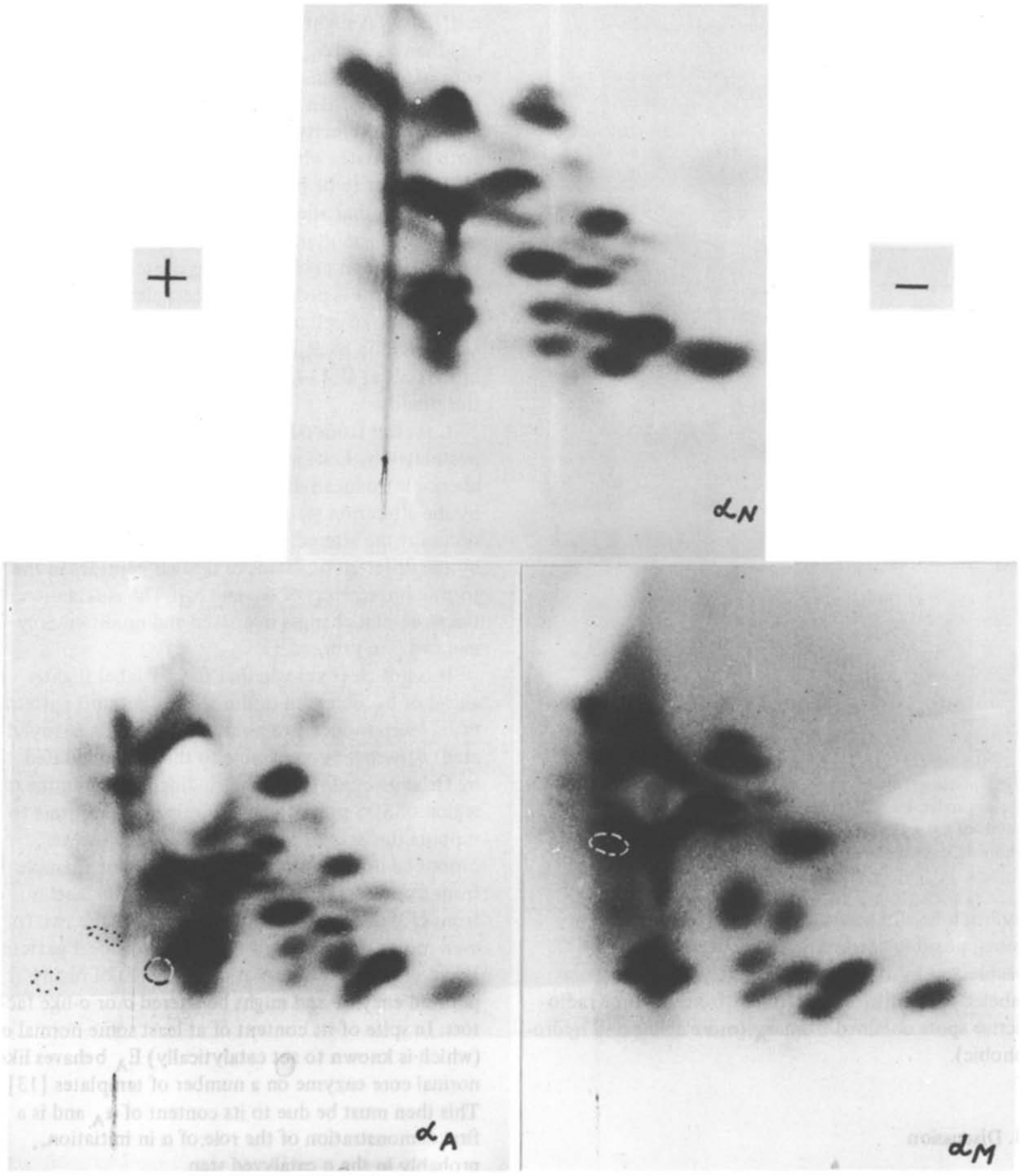

Fig. 3. Fingerprint maps of α_N, α_A and α_M stained with ninhydrin [5]. Strong radioactive spots are encircled with dashes, weak radioactive spots with points. The dash in the lower left inferior corner represents the start. Electrophoresis was from left to right, chromatography from bottom to top.

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