Chemical modifications of the sigma subunit of the *E. coli* RNA polymerase*Chittampalli S.Narayanan[§] and Joseph S.Krakov⁺

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ABSTRACT

The function of arginine, cysteine and carboxylic amino acid (glutamic and aspartic) residues of sigma was studied using chemical modification by group specific reagents. Following modification of 3 arginine residues with phenylglyoxal or 3 cysteine residues with N-ethylmaleimide (NEM) sigma activity was lost. Analysis of the kinetic data for inactivation indicated that one arginine or cysteine residue is essential for sigma activity. At low NEM concentration alkylation was limited to a non-critical cysteine which was identified as cysteine-132. Modification of arginine or cysteine residues had no observable effect on the binding of the inactivated sigma to the core polymerase. Modification of aspartic and/or glutamic acid residues with the water-soluble carbodiimides 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDC) or 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-p-toluene sulfonate (CMC) resulted in loss of sigma activity. The inactivation data indicated that one carboxylic amino acid residue is essential for sigma activity. Sigma modified with EDC, CMC or EDC in the presence of glycine was inactive in supporting promoter binding and initiation by core polymerase. Reaction with EDC plus (³H)glycine resulted in the incorporation of glycine into sigma. The (³H)glycine-sigma was unable to form a stable holoenzyme complex.

INTRODUCTION

The DNA-dependent RNA polymerase of *Escherichia coli* is composed of a catalytically competent core unit containing three subunits (α_2 , β , β') and the dissociable subunit, sigma. The form of the RNA polymerase involved in promoter recognition and initiation is termed the holoenzyme ($\alpha_2\beta\beta'\sigma$). The detailed mechanism by which sigma participates in the initial steps in transcription remains to be clarified. Two general, but not necessarily mutually exclusive, mechanisms have been proposed for sigma action. The binding of sigma to the core polymerase may effect a conformation in the holoenzyme essential for promoter binding¹. Alternatively, sigma may, as a component of the holoenzyme, directly participate in promoter recognition by binding to sites in the promoter²⁻⁴.

Relatively little is known regarding the relationship between sigma

structure and function. The isolation and characterization of sigma mutants⁵⁻⁷ provides one approach toward a correlation of site specific alterations in amino acid sequence with sigma activity. By using reagents which modify particular amino acids it should be possible to assess their role in sigma function. In a previous study⁸ we found that modification of sigma lysine residues with trinitrobenzenesulfonic acid resulted in a loss of sigma activity without impairing the ability of the modified sigma to form a holo-enzyme complex. The present study concerns the effects resulting from the modification of arginine, cysteine, glutamic acid and aspartic acid residues on the activity of sigma.

MATERIALS AND METHODS

Materials. *E. coli* K12 cells (3/4 log phase) were purchased from Grain Processing Corporation. Ribonucleoside triphosphates and d(A-T)_n were obtained from P-L Biochemicals. Restriction endonuclease HindIII was purchased from Bethesda Research Laboratories. N-ethylmaleimide and CMC were obtained from Sigma Chemical Co. and phenylglyoxal from Aldrich Chemical Co. EDC was purchased from Pierce Chemical Co. Liquifluor and (³H)NEM were products of New England Nuclear Corp. Specific activity of the (³H)NEM was determined by first forming the cysteine-NEM complex using 100-fold molar excess of cysteine and drying an aliquot on a GFC filter disc and counting in Liquifluor. (³H)glycine and (¹⁴C)acetophenone were purchased from ICN; the latter was used to prepare (¹⁴C)phenylglyoxal (specific activity, 5000 cpm/nmol) by the method of Riley and Gray⁹.

Buffers. Binding buffer: 20 mM Tris-HCl, pH 8.0, 40 mM KCl, 10 mM MgCl₂, 0.1 mM EDTA, 0.1 mM dithiothreitol, 500 µg/ml bovine serum albumin, and 5% (w/v) glycerol. Tris-borate buffer: 80 mM Tris base, 80 mM borate and 2.5 mM EDTA, final pH 8.3. TMS: 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 10 mM MgCl₂ and 2 mM 2-mercaptoethanol.

RNA polymerase and the sigma subunit were purified from *E. coli* cells by modifications⁸ of the methods of Burgess and Jendrisak¹¹ and Lowe et al.¹². Protein was determined by the method of Schaffner and Weissmann¹³ using bovine serum albumin as a standard. Protein content was determined for all fractions following dialysis. T5 DNA was prepared by the method of Thomas and Abelson¹⁴. DNA concentration, unless otherwise indicated, is expressed as the nucleotide content ($\epsilon_{260} = 6750 \text{ M}^{-1} \text{ cm}^{-1}$).

The activity of the sigma subunit was assayed using T5 DNA and core polymerase at a ratio of 2 to 1 by weight. The reaction mix (250 µl) con-

tained: 80 mM Tris-HCl, pH 7.6, 20 mM MgCl_2 , 40 mM mercaptoethylamine, 1 mM each of ATP, CTP, GTP and (^3H)UTP (5000 cpm/nmol). A molar ratio of sigma to core of one was used. After incubation for 10 minutes at 37°C the trichloroacetic acid precipitable radioactivity was collected on a glass fiber filter, dried and counted in 5 ml Liquifluor-toluene. The sigma-dependent single step synthesis of pApU was carried out as described by Hansen and McClure¹⁰. Promoter recognition assays using HindIII fragments of T5 DNA were carried out by the method of Gabain and Bujard¹⁵ as previously described⁸. T5 DNA fragments were analyzed by electrophoresis in 0.7% agarose gels using Tris-borate buffer. Sigma association with core polymerase was determined by centrifugation in a 15 to 35% glycerol gradient in TMS buffer. The samples were centrifuged in a Spinco SW 50 rotor at 45,000 rpm for 20 hours at 4°C . Fractions of 0.4 ml were collected and analyzed by SDS polyacrylamide gel electrophoresis¹⁶.

RESULTS

Arginine modification. Incorporation of (^{14}C)phenylglyoxal into sigma shows a linear time course for at least 180 minutes at 37°C at phenylglyoxal concentrations of up to 5 mM (Figure 1). The calculated number of arginine

Figure 1. Rate of modification of sigma arginine groups as a function of (^{14}C)phenylglyoxal concentration. The reaction mix (20 μl) containing 10 μg sigma, 50 mM potassium phosphate, pH 8.0, and (^{14}C)phenylglyoxal (5000 cpm/nmol) at the indicated concentration was incubated at 37°C . At the indicated time points, 50 μl of 0.5 M arginine was added and incubated for an additional 5 minutes followed by dialysis overnight at 5°C against 40 mM potassium phosphate, pH 7.0.

Figure 2. Effect of sigma modification on the d(A-T)_n-directed synthesis of pApU. Core polymerase (10 pmol) with varying amounts of sigma, NEM-sigma (3 cysteines modified/sigma) or PG-sigma (5 arginines modified/sigma) were incubated with 40 mM Tris-HCl (pH 8.0), 80 mM KCl, 10 mM MgCl₂, 1 mM DTT and 10 nmol d(A-T)_n for 10 minutes at 37°C. The mix (final volume, 50 μl) was brought to 2 mM AMP and 200 μM (³H)UTP (150 cpm/pmol) and incubation continued for 10 minutes at 37°C. The reaction was stopped and chromatographed as described in Materials and Methods.

residues modified is based on the assumption that two molecules of phenylglyoxal are incorporated per arginine^{17,18}. In the reactions run with 5 mM (¹⁴C)phenylglyoxal, 17 of the 46 arginine residues of sigma¹⁹ have reacted and are accessible to the reagent.

Hansen and McClure¹⁰ have developed a sensitive assay for sigma which takes advantage of the minimal activity of core polymerase in synthesizing pApU in a reaction containing d(A-T)_n plus AMP and UTP. Addition of sigma to core polymerase results in a pronounced stimulation of dinucleotide synthesis (Figure 2). When sigma reacted with phenylglyoxal (5 arginines modified per sigma) is added no stimulation of pApU synthesis by core polymerase results. The data presented in Figure 3 indicate that loss of sigma activity in stimulating core polymerase in the T5 DNA-directed reaction occurs when only three arginine residues have been modified by phenylglyoxal.

The rate of sigma inactivation as a function of phenylglyoxal concentration is shown in Figure 4. Since the reaction follows pseudo-first order kinetics, the equation $k' = k''(I)^n$ (where k' is the pseudo-first order rate

Figure 3. Relationship of arginine modification to loss of sigma activity. Reactions (20 μ l) were carried out with 10 μ g sigma, 50 mM potassium phosphate, pH 8.0, and varying concentrations of unlabelled or (14 C)phenylglyoxal at 37°C for 60 minutes. After dialysis overnight against 40 mM potassium phosphate, pH 7.0, aliquots containing 2.5 μ g sigma were taken from the unlabelled phenylglyoxal reaction for the determination of sigma activity using the T5 DNA-directed reaction. Aliquots from the (14 C)phenylglyoxal reaction were taken for determination of the number of arginine residues modified.

constant, k'' the apparent second order rate constant, I the inhibitor concentration and n the reaction order) can be used to determine the number of molecules of phenylglyoxal required to inactivate sigma. The values for n as a function of the pH at which the modification of sigma by phenylglyoxal was carried out range between 1 and 2.5 mol phenylglyoxal per mol sigma. Since two molecules of phenylglyoxal react per arginine residue^{17,18}, it would appear that the modification of a single critical arginine residue is sufficient to inactivate sigma.

The effect of modification of sigma by phenylglyoxal at different pH's on the apparent second order rate constant, k'' , is shown in Figure 5. The

Figure 4. Inactivation of sigma by phenylglyoxal. In a 30 μ l reaction mix, 12 μ g sigma in 50 mM potassium phosphate, pH 8.0, was incubated with the indicated concentrations of phenylglyoxal. Following incubation at 37°C for the times indicated, 5 μ l aliquots were removed for assay of sigma activity using the T5 DNA-directed reaction.

pK of the critical arginine residue was estimated to be approximately 8, a value which is considerably lower than the pK of 12 for free arginine.

Although functionally inactive, the phenylglyoxal-modified sigma (PG-sigma, with 5 arginines modified per sigma) can still form a complex with core polymerase. The results shown in Figure 6 indicate that the modified sigma binds to core polymerase with the expected stoichiometry of one PG-sigma to one core polymerase.

Gabain et al.²⁰ and Gabain and Bujard¹⁵ have shown that digestion of T5 DNA with HindIII endonuclease produces 16 fragments of which only fragments ILMO lack promoters. The remaining 12 DNA fragments contain from one to four promoters and consequently can form tight complexes with the RNA polymerase holoenzyme which are resistant to displacement by added competitor DNA (Figure 7). Holoenzyme reconstituted with PG-sigma in which one or two arginine residues have been modified can still form tight complexes with the promoter-containing fragments derived from T5 DNA. Upon modification of 4 arginine residues per sigma, the PG-sigma-core enzyme is unable to form the

Figure 5. Inactivation of sigma by phenylglyoxal as a function of pH. Reactions were carried out as described in the legend to Figure 4 with the following buffers: pH 6.5 to pH 8.0, 50 mM potassium phosphate; pH 9.0, 40 mM Bis-Tris propane; pH 10, 100 mM glycine-NaOH. The second order rate constant, k'' , for each of the pH values was calculated from the equation, $k'' = k'/(I)^n$.

high affinity complexes with the promoter-containing DNA fragments.

Cysteine modification. The sigma subunit contains three cysteine residues¹⁹ of which two are accessible to modification by sulphydryl reagents such as sodium tetrathionate²¹ and N-(1-pyrene)maleimide¹. Wu, Yarbrough and Wu¹ have reported that sigma containing about 2 mol of N-(1-pyrene)maleimide retained about 80% of native sigma activity in stimulating transcription of T7 DNA by core polymerase. The data presented in Figure 8 demonstrate that all three cysteine residues of sigma react within 40 minutes at 37°C with N-ethylmaleimide at 15 mM or above. Sigma is inactivated when the three cysteine residues have been modified by N-ethylmaleimide when assayed for stimulation of core polymerase using the T5 DNA-directed synthesis of RNA (Fig. 9) or the d(A-T)_n-directed synthesis of pApU (Fig. 2). Modification of the most reactive cysteine had no apparent effect on sigma activity which was progressively lost as the remaining two cysteine residues were alkylated (Fig. 9). Based on the amino acid sequence of sigma¹⁹ the three cysteine

Figure 6. Reconstitution of PG-sigma with core polymerase. In 50 μ l TMS buffer, 200 μ g core polymerase was mixed with varying amounts of (14 C)PG-sigma (5 PG/sigma), incubated for 5 minutes at 37°C, layered on the gradient and centrifuged as described in Materials and Methods. Fractions of 0.4 ml were collected and analyzed for radioactivity. The core polymerase to (14 C) PG-sigma ratios are: A, 0:1; B, 0.5:1; C, 1:1. Fraction 1 is at the top of the gradient.

residues are located at positions 132, 291 and 295. Cysteine 132 is located within a large cyanogen bromide fragment (gly106-cys132-met273) while the other cysteine residues are located in a small cyanogen bromide fragment (lys289-cys291-cys295-met297). After modification of the most reactive cysteine residue with (3 H)N-ethylmaleimide the labeled sigma was cleaved with cyanogen bromide. Following resolution by SDS-polyacrylamide gel electrophoresis the radioactivity was recovered in the large cyanogen bromide polypeptide (data not shown). The nonessential cysteine residue is cys132.

Reconstitution experiments similar to those with (14 C)phenylglyoxal-modified sigma were carried out using sigma with the three sulfhydryl groups modified with (3 H)NEM. The fractions were analyzed by SDS polyacrylamide gel electrophoresis. The results demonstrated that NEM-sigma is able to reconstitute with core polymerase (data not shown). Promoter recognition studies indicated that NEM-sigma (3 NEM per sigma) is unable to form tight complexes with promoter-containing fragments of T5 DNA.

Carboxyl group modification. It is possible to modify carboxyl groups

Figure 7. Formation of T5 DNA promoter-polymerase complex. T5 DNA (25 nmol, equivalent to 2 pmol of promoters) restricted with HindIII and 1 μ g (2.5 pmol) of core polymerase + 0.25 μ g of sigma or sigma with 1, 2, or 4 arginine residues modified with phenylglyoxal were preincubated in 50 μ l binding buffer at 37°C for 2 minutes and competed with denatured calf thymus DNA (15 μ g); the incubation was continued for 10 minutes (B,E,H,L,N), 1 hour (C,F,I) or 4 hours (D,G,J,M,O), filtered and electrophoresed as described in Materials and Methods. A, 1 μ g of T5 DNA fragments; B,C,D, core polymerase + sigma; E,F,G, core polymerase + sigma with 1 modified arginine; H,I,J, core polymerase + sigma with 2 modified arginines; L,M, core polymerase + sigma with 4 modified arginines; N,O, core polymerase. Lane K was not used.

of proteins under mild conditions using water-soluble carbodiimides²²⁻²⁵. The data obtained following modification of sigma with EDC and CMC are summarized as follows. The rate of inactivation of sigma follows pseudo-first order kinetics (data not shown). The reaction order for inactivation using EDC is 1 and for CMC it is 0.83, indicating that the incorporation of one mol EDC or CMC per mol sigma results in loss of activity. The essentially identical second order rate constants for EDC ($k'' = 10 \text{ M}^{-1} \text{ min}^{-1}$) and CMC ($k'' = 11 \text{ M}^{-1} \text{ min}^{-1}$) suggest that the reactivity of the critical glutamic acid or aspartic acid residue with the water-soluble carbodiimide is the same. Sigma

Figure 8. Rate of modification of sigma cysteine groups as a function of (^3H)NEM concentration. The reaction mix (20 μl) containing 10 μg sigma, 50 mM TEA- H_2SO_4 , pH 7.0, and (^3H)NEM (9600 cpm/nmol) at the indicated concentration was incubated at 37°C . At the times indicated, 5 μl of 1 M 2-mercaptoethanol was added and the incubation continued for 5 minutes. The acid precipitable radioactivity was determined.

modified with EDC, CMC or EDC in the presence of glycine is inactive in supporting promoter binding or initiation by the core polymerase. Reaction of sigma with EDC in the presence of (^3H)glycine results in the incorporation of (^3H)glycine into the protein. The incorporation of glycine and loss of sigma activity are linearly related. Complete loss of sigma activity occurs when 1.3 mol glycine are incorporated per mol sigma (Fig. 10). The data presented in Figure 11 show that following reaction with EDC in the presence of (^3H)glycine the (^3H)glycine-sigma is unable to form a stable holoenzyme complex.

Over 90% of the (^3H)glycine incorporated is stable following incubation of the (^3H)glycine-sigma (5 glycine per sigma) in 0.5 M hydroxylamine (pH 7.0) at 25°C for up to 5 hours; sigma activity is also not recovered after this treatment. This rules out the possibility that tyrosine modification²⁵ could be responsible for the loss of binding to core polymerase. After modification of sigma with EDC plus glycine (5 glycine per sigma) the three cysteine residues were still available for reaction with (^3H)NEM. The results indicate that the reaction of sigma with EDC plus glycine is specific for carboxylic

Figure 9. Relationship of cysteine modification to loss of sigma activity. The reaction mix (20 μ l) containing 12 μ g sigma, 50 mM TEA-H₂SO₄, pH 7.0, and varying concentrations of NEM was incubated at 37°C. After varying times of incubation, 5 μ l of the mix was assayed for residual sigma activity using stoichiometric amounts of core polymerase in the T5 DNA-directed system. The corresponding values for mol cysteine modified/mol sigma were obtained from the data presented in Figure 8.

amino acid side chains and that modification of aspartic and/or glutamic residues results in loss of binding to core polymerase. Following modification of sigma with EDC plus (³H)glycine the labelled sigmas (1, 2, 3 and 5 (³H)glycines per sigma) were cleaved with cyanogen bromide. Autoradiography of the fragments resolved by SDS polyacrylamide gel electrophoresis showed that the (³H)glycine was located in the large fragment: gly106-met273.

DISCUSSION

In a previous study⁸ we had shown that modification of lysyl groups resulted in the loss of sigma function while still allowing binding of the modified sigma to the core polymerase. The critical lysine residue was one of a class of lysyl residues which reacted most rapidly with trinitrobenzene-sulfonic acid. The present study contributes further information on the role

Figure 10. Relationship of carboxyl group modification to loss of sigma activity. The reaction mix (40 μ l) containing 20 μ g sigma, 100 mM TEA-H₂SO₄ (pH 7.0), 100 mM (³H)glycine or 100 mM glycine and varying concentrations of EDC was incubated at 25°C for 60 minutes. The reaction was stopped by bringing the reaction mix to 0.5 M sodium acetate and then dialyzing overnight against Tris-HCl, pH 7.0. For each concentration of EDC the mol (³H)glycine incorporated per mol sigma was determined and the residual sigma activity (using the preparation modified in the presence of the unlabelled glycine) was determined using the T5 DNA-directed assay.

of amino acid residues in the function of sigma. The result of modification of arginine or cysteine residues seems to parallel the effects found after lysine modification. The critical arginine residue reacts more rapidly with phenylglyoxal than the remainder of the 46 arginines present in sigma. After only three arginine residues have been modified, sigma activity is lost. Kantrowitz and Lipscomb¹⁸ found that the arginine residue which reacted most rapidly with phenylglyoxal was also essential for the activity of aspartate transcarbamylase. They suggested that the enhanced reactivity toward phenylglyoxal may be a consequence of the special environment of the enzyme active site. The possible effect of the environment on the critical arginine residue of sigma is indicated by its apparent pK for reaction with phenylglyoxal which is substantially below the pK of 12 expected for free arginine. The reaction of phenylglyoxal with ribonuclease¹⁷, aspartate transcarbamylase¹⁸,

Figure 11. Reconstitution of (^3H)gly-sigma with core polymerase. (^3H)gly-sigma was prepared as follows: the reaction mix (40 μl) contained 100 μg sigma, 5, 10, 20 or 40 mM EDC, 100 mM (^3H)glycine (20,000 cpm/nmol) and 100 mM TEA- H_2SO_4 , pH 7.0. After incubation at 25°C for 60 minutes the reaction was stopped by bringing the reaction mix to 0.5 M sodium acetate and dialyzing the mix against 20 mM Tris-HCl, pH 7.0. At the indicated concentrations of EDC approximately 1, 2, 3 and 5 mol (^3H)glycine were incorporated per mol sigma.

Reconstitution was assayed as follows: in 100 μl TMS buffer, 30 μg (^3H)gly-sigma and 250 μg core polymerase were incubated at 37°C for 5 minutes, layered on the gradient and centrifuged as described in Materials and Methods. Fractions of 0.4 ml were collected and analyzed for radioactivity. The mol (^3H)glycine per mol sigma are: A, 1; B, 2; C, 3; D, 5. The arrow indicates the position of core polymerase.

bovine ornithine decarboxylase²⁶, creatine kinase²⁷ and *E. coli* elongation factor, EF-G²⁸, show comparable pH behaviors. While sigma does not possess an active site analogous to that of an enzyme, its role in conferring on RNA polymerase the special conformation required for promoter binding and initiation may involve unique sites in this subunit. The critical arginine residue in sigma may be involved in a step following the binding of sigma to the core polymerase. The work of MarSchel and Bodley²⁹ demonstrated the significant role of arginine residues in protein-protein interactions. Based on their study of the inactivation of elongation factor-Ts by butanedione, MarSchel and Bodley concluded that there are two reactive arginine residues in EF-Ts of which one is protected by and essential for its interaction with EF-Tu. It is also possible that sigma may, as a component of the holoenzyme, directly interact with promoter nucleotides. This might involve the positively charged arginine

and lysine³⁵ residues of sigma. Photochemical crosslinking of RNA polymerase promoter complexes showed that both the β and σ subunits are crosslinked to the DNA³. Complexes of supertwisted plasmid DNA and sigma can be demonstrated⁴. Several template directed polymerases are inhibited by phenylglyoxal³⁰. These include the AMV and RLV reverse transcriptases, calf thymus DNA polymerase β , *E. coli* DNA polymerase I and the *E. coli* RNA polymerase. The calf thymus terminal deoxynucleotidyl transferase which does not require a template is relatively resistant to inhibition by phenylglyoxal at concentrations as high as 500 μ M. Srivastava and Modak³⁰ suggest that the template-requiring polymerases contain arginine groups essential for template binding.

Of the three cysteine residues of sigma¹⁹, the one which reacts most rapidly with NEM is not required for sigma activity. As the remaining two sulfhydryl groups are modified sigma activity is progressively lost. As is also the case following modification of critical arginine and lysine residues, loss of sigma activity does not affect the ability of the NEM-modified sigma to bind to the core polymerase. The possible role of the critical cysteine residues in sigma function is less obviously apparent than that which may be proposed for lysine, arginine, glutamic acid or aspartic acid residues. The critical cysteine residue(s) is (are) less accessible to attack by NEM; this presumably is a reflection of the environment in which they reside in sigma. Modification of the critical cysteine residue(s) with NEM may prevent sigma from effecting the necessary conformational change in the core polymerase after formation of the holoenzyme. Wu, Yarbrough and Wu¹ found that after modification of two cysteine groups with N-(1-pyrene)maleimide the labelled sigma retained 80% of its activity in stimulating T7 DNA-directed transcription by core polymerase. Their studies using sigma covalently tagged with this fluorescent probe showed that the modified sigma bound to the core polymerase with an affinity comparable to that of unmodified sigma. Wu, Yarbrough and Wu¹ proposed that the binding of sigma to core involves at least two steps, a rapid association followed by a relatively slow induced conformational transition in the holoenzyme. The binding of sigma to the core polymerase involves contacts with regions in each of the core subunits. This has been demonstrated by chemical crosslinking³¹ and is compatible with a model for the holoenzyme structure determined by small-angle neutron scattering³². The results we have obtained indicate that modification of lysine, arginine or cysteine residues of sigma does not prevent the binding of sigma to the core polymerase. Binding of sigma to core polymerase occurs at temperatures below that at which a conformational change in the holoenzyme occurs^{1,33}

and this change in conformation is thought to be essential for promoter activation. Thus it is possible that the modified sigma can bind to the core polymerase but is unable to induce the necessary conformational change. The alternative possibility is that with the side chain modifications which allow formation of a holoenzyme complex the inactivation of sigma affects the putative interaction(s) with nucleotide moieties in the promoter required for formation of the binary, open and/or closed promoter complexes with the holoenzyme.

Modification of sigma with reagents such as CMC, EDC and EDC plus glycine which react with the carboxyl groups of aspartic and glutamic acid residues results in a modified sigma which does not form a stable complex with the core polymerase. Of the RNA polymerase subunits, sigma is the most acidic. The amino acid sequence of the sigma subunit determined by Burton et al.¹⁹ shows a preponderance of acid residues clustered within the first 215 residues from the N-terminus. One possible role of the acidic amino acid clusters may be in association with positively charged residues in the sigma binding domain in the core polymerase. Such electrostatic interactions cannot be the sole mechanism involved in formation of the holoenzyme since it is known that sigma does not dissociate from the core polymerase in high ionic strength buffers. Thus, other interactions must also play a role in the binding of sigma to the core polymerase. It has been suggested³⁴ that the binding of sigma in the holoenzyme may involve negatively charged amino acids in sigma since copolymers containing glutamic acid residues plus tyrosine bind to and inhibit RNA polymerase. The effect of modification by EDC and CMC in inhibiting binding of the sigma suggests a role for glutamic and/or aspartic acid residues in interaction with the core polymerase.

Abbreviations used: NEM, N-ethylmaleimide; DTT, dithiothreitol; TEA, triethanolamine; KP, potassium phosphate buffer; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; CMC, 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-p-toluene sulfonate; SDS, sodium dodecyl sulfate; PG, phenylglyoxal.

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