Cysteinyl Residues of *Escherichia coli* recA Protein†

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ABSTRACT: The *Escherichia coli* recA protein has three cysteinyl residues at positions 90, 116, and 129. All of them are reactive with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). In the presence of ATP or ADP, only one cysteinyl residue reacts with DTNB. The residue was also reactive with *N*-[7-(dimethylamino)-4-methylcoumarinyl]maleimide (DACM) in the presence of ATP. The results on an analysis of the DACM-modified protein cleaved at the nonmodified cysteinyl residues after cyanation with 2-nitro-5-(thiocyano)benzoic acid show that two cysteinyl residues protected in the presence of ATP or ADP are identified as Cys-90 and Cys-129. When the ionic strength is higher than 1, one cysteinyl residue does not react with DTNB. This residue is Cys-90 or Cys-129, because one of the two cysteinyl residues, which are not modified with DACM in the presence of ATP, does not react with DTNB at high ionic strength. The binding of single-stranded DNA to the recA protein does not change the reactivity of the cysteinyl residues with DTNB.

The recA protein of *Escherichia coli* is essential for recombination between homologous DNA molecules and for induction of SOS functions (Little & Mount, 1982; Radding, 1982). The purified recA protein shows many activities (Ogawa et al., 1978; McEntee & Weinstock, 1982): (1) the binding activity to single- and/or double-stranded DNA, (2) ATPase activity, (3) stimulation activity for cleavage of some repressors, and (4) the ability for self-aggregation. As a multifunctional protein, the recA protein is probably composed of functional domains. It is, therefore, important to identify the domain in the protein which is responsible for each function. A simple method of identifying the region involved in each function is to characterize the properties of the protein modified at a certain amino acid. Previously, we found that all three cysteinyl residues of the recA protein [at positions 90, 116, and 129 (Horii et al., 1980)] can be modified with DTNB[1] and the binding of ATP or ADP protected two cysteinyl residues against the reaction with DTNB (Kuramitsu et al., 1981). In the present report, we describe the positions of the two cysteinyl residues protected from the modification with DTNB in the presence of ATP.

Experimental Procedures

Materials

[γ-^{32}P]ATP (3000 Ci/mmol) was obtained from Amersham. Calf thymus DNA (type I) from Sigma Chemical Co. was further purified by phenol treatment and ethanol precipitation. DACM and EDTA were products of Wako Pure Chemicals Co., and HEPES, dithioerythritol, dithiothreitol, DTNB, Gdn·HCl, and SDS were obtained from Nakarai Pure Chemicals Co.

Methods

Preparation of the recA Protein. The previous method (Kuramitsu et al., 1981) was slightly modified. After the ammonium sulfate fractionation, the recA protein was dialyzed against 50 mM Tris-HCl (pH 7.5), 5 mM mercaptoethanol, 10% (v/v) glycerol, and 20 mM $MgCl_2$. The resulting precipitate in a dialysis tube was dissolved and applied to a column of Sephacryl S-300 (Cotterill et al., 1982). The phosphocellulose chromatography was carried out as described except that a linear gradient of 20–350 mM phosphate buffer (pH 6.5) was used instead of a gradient of KCl. DEAE-cellulose chromatography was as described (Kuramitsu et al., 1981). The recA protein was stored in a buffer containing 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 5 mM mercaptoethanol, 0.15 M NaCl, and 50% (v/v) glycerol at −20 °C.

Modification of the recA Protein with DACM. The recA protein was used after further purification by a column of Sephadex G-25 that had been equilibrated with a 10 mM phosphate buffer (pH 6.5) containing 0.1 mM EDTA and 0.15 M KCl. All the buffers used for the modification of cysteinyl residue(s) had been degassed and subsequently saturated with nitrogen gas. The protein was modified with DACM under the following conditions: 3×10^{-5} M recA protein, 3.1×10^{-5} M DACM, 50 mM HEPES, 3 mM potassium phosphate, 0.15 M KCl, 10 mM ATP, and 10 mM $MgCl_2$ at pH 7.9 and 25 °C for 2 h. The reaction mixture was concentrated by dialysis against 1 L of buffer (TEM buffer), which contained 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 5 mM mercaptoethanol with $(NH_4)_2SO_4$ added to 80% saturation at 0 °C. The precipitates formed were suspended in TEM buffer and dialyzed against the same buffer supplemented with 0.15 M KCl. The protein solution was diluted 5-fold with TEM buffer and applied to a column (2.1 × 15 cm) of DEAE-cellulose (Brown) that had been equilibrated with TEM buffer. The recA protein was eluted with a linear gradient of NaCl from 0 to 0.35 M in a total volume of 300 mL of TEM buffer (Figure 1). The recA protein modified with DACM was eluted at around 0.2 M NaCl. The cyanation and the cleavage reactions for the DACM-modified recA protein were performed according to the method of Stark (1977).

Titration of Cysteinyl Residues with DTNB. The number of cysteinyl residues in the recA protein were titrated with DTNB as previously described (Kuramitsu et al., 1981).

Amino Acid Analysis. Peptide samples obtained from the DACM-modified recA protein were hydrolyzed with 6 N HCl

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[1] Abbreviations: ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); CD, circular dichroism; DACM, *N*-[7-(dimethylamino)-4-methylcoumarinyl]maleimide; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Gdn·HCl, guanidine hydrochloride; HEPES, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid; NTCB, 2-nitro-5-(thiocyano)benzoic acid; SDS, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

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in evacuated, sealed tubes for 20, 40, and 70 h at 110 °C. The HCl was removed by evaporation using a Savant Speed Vac concentrator. Amino acid compositions were analyzed by using an LKB 4400 amino acid analyzer in the Scientific Instrument Center of Kobe University.

Polyacrylamide Gel Electrophoresis. Electrophoresis of the peptide fragments was carried out with an SDS–Tris system using 12.5% polyacrylamide gel according to the procedure of Laemmli (1970) as modified (Kuramitsu et al., 1981). The gel was fluorometrically scanned with a Hitachi fluorescence spectrophotometer, Model MPF-4.

Measurement of Aggregation State. The state of the aggregation of the DACM-modified recA protein was measured by Sephacryl S-300 chromatography with 50 mM Tris, 0.1 mM EDTA, 1 mM dithioerythritol, and 0.15 M NaCl at pH 7.5 and 25 °C (Kuramitsu et al., 1981).

Spectroscopy. The buffer used for spectrophotometric measurements contained 50 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithioerythritol, and 0.15 M KCl at pH 7.5.

The concentration of the intact recA protein was determined spectrophotometrically by using an extinction of $A^{1\%}_{1cm} = 5.7$ at 278 nm (Kuramitsu et al., 1981). The concentration of calf thymus double-stranded DNA was determined by using an extinction of $A^{1\%}_{1cm} = 200$ at 260 nm (Marmur, 1961). The single-stranded DNA was obtained by heat denaturation of the double-stranded DNA. The DNA concentration was expressed as concentration of nucleotide.

The circular dichroic (CD) spectra of the recA protein were recorded with a Jasco spectropolarimeter, Model J-500A, and absorption spectra were measured as previously described (Kuramitsu et al., 1981).

Assay of ATPase Activity. The ATPase activity of the recA protein in the presence of single-stranded DNA was measured as previously described (Kuramitsu et al., 1981).

Assay of DNA Binding Activity. An alkali-treated nitrocellulose filter (McEntee et al., 1980) binds the recA protein but does not bind single-stranded DNA. Binding of the recA protein to ϕX174 single-stranded [^{3}H]DNA was measured by the retention of DNA on the alkali-treated nitrocellulose filter. The reaction mixture for the measurement of the DNA binding activity of the recA protein (50 μL) contained 1–2 μM recA protein, 6 μM ϕX174 single-stranded [^{3}H]DNA (about 5000 cpm), 0.5 mM ATPγS, 23 mM Tris-HCl, 10 mM $MgCl_2$, 1.8 mM dithiothreitol, 6 mM NaCl, and 2% (w/v) glycerol at pH 7.5. The reaction was performed at 37 °C for 15 min followed by rapid chilling on ice. The reaction mixture was immediately applied to an alkali-treated nitrocellulose filter, washed with 2.2 mL of buffer containing 23 mM Tris-HCl, 10 mM $MgCl_2$, and 0.2 M NaCl (pH 7.5), dried, and assayed for radioactivity. In the absence of recA protein, less than 0.2% of the labeled single-stranded DNA was retained by the filter.

Results

Modification of the recA Protein in the Presence of ATP. We have shown that the three cysteinyl residues of the recA protein at positions 90, 116, and 129 react slowly with DTNB in the absence of ATP or ADP and that in the presence of ATP or ADP, one of the three cysteinyl residues readily reacts with DTNB and two other cysteinyl residues fail to react (Kuramitsu et al., 1981). The reactivities of the three cysteinyl residues with DTNB are unaffected in the presence of 100 μM single-stranded DNA and 10 mM $MgCl_2$ at 0.05 ionic strength both in the presence and in the absence of ATP or ADP (not shown).

The product of cysteinyl residue modified with DTNB is unstable. Our effort to modify cysteinyl residues with iodoacetic acid or 2-nitro-5-(thiocyano)benzoic acid was unsuccessful. We, therefore, used DACM, a reagent with more hydrophobic nature than DTNB and with fluorescent chromophore, for modification of cysteinyl residues. The recA protein was modified with DACM in the presence of ATP, and the modified protein was purified by DEAE-cellulose column chromatography (Figure 1). In order to determine the fractions that contained the DACM-modified protein, the ratio of the absorbance at 390 nm to that at 280 nm of each fraction was measured. The ratio around the fractions shown by the bar was 0.97, indicating the presence of the modified protein. A small amount of the intact recA protein emerged before the modified recA protein. Fractions shown by the bar were used for further experiments.

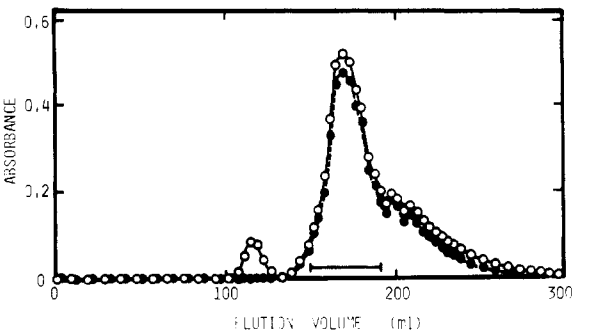

FIGURE 1: Chromatography of recA protein treated with DACM on a DEAE-cellulose column (2 × 15 cm) at 4 °C. (○) The absorbance at 280 nm; (●) the absorbance at 390 nm. The protein eluted with a salt gradient from TEM buffer (150 mL) to TEM buffer containing 0.35 M NaCl (150 mL). Fractions indicated by the bar were used for further experiments.

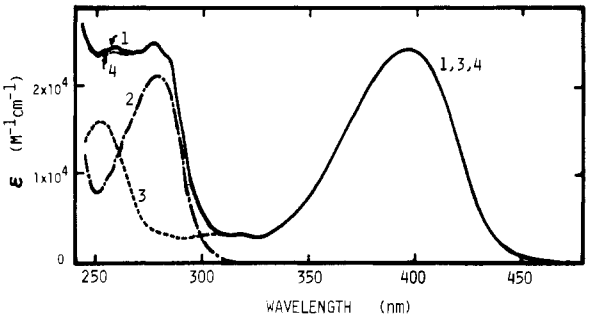

FIGURE 2: Absorption spectrum of DACM–recA protein in 50 mM Tris, 0.1 mM EDTA, 1 mM dithioerythritol, and 0.15 M KCl at pH 7.5, 25 °C (1). Molar extinction coefficients for DACM–recA protein (1), for intact recA protein (Kuramitsu et al., 1981) (2), and for DACM residue (Machida et al., 1977) (3). Curve 4 indicates the sum between curves 2 and 3. See text for details.

Properties of the DACM-Modified recA Protein. The DACM-modified recA protein had an absorption maximum at 400 nm in the region above 350 nm (Figure 2, curve 1). This indicates that the DACM attached to the recA protein is "closed type adduct" with an extinction of $A^{1\%}_{1cm} = 6.4$ at 400 nm (Machida et al., 1977; Yamamoto et al., 1977). The curve of the molar extinction coefficient of the modified recA protein in the region above 240 nm (curve 1) is almost identical with curve 4, which represents the sum of the molar extinction coefficient of the intact recA protein [curve 2 (Kuramitsu et al., 1981)] and that of the DACM group [curve 3 (Machida et al., 1977; Yamamoto et al., 1977)]. This result indicates that 1 mol of DACM is attached to 1 mol of the recA protein.

The CD spectrum of the DACM-modified recA protein in the region between 200 and 250 nm was identical with that of the intact protein (not shown). This indicates that the gross conformation of the recA protein is not altered by the modification with DACM. The aggregation state of the modified

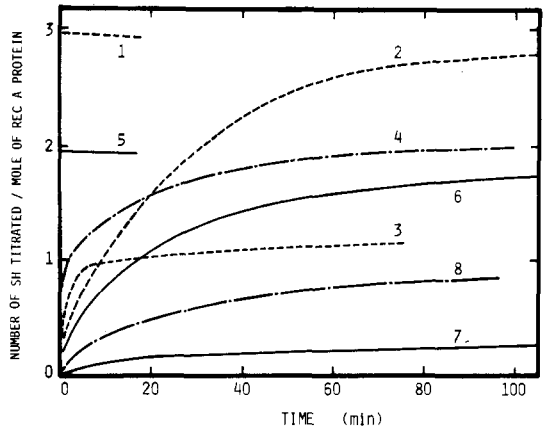

FIGURE 3: Titration of SH groups of intact (1–4) and DACM-treated (5–8) recA proteins with DTNB at pH 7.9, 25 °C. Curves 1 and 5, in the presence of 3 M Gdn·HCl; curves 2 and 6, in the absence of Gdn·HCl; curves 3 and 7, in the presence of 10 mM ATP and 10 mM $MgCl_2$; curves 4 and 8, in the presence of 1.25 M KCl. The protein concentration was 6×10^{-6} M. The concentration of DTNB was 0.5 mM. The buffer solution contained 50 mM HEPES, 4 mM phosphate, 0.04 mM EDTA, and 0.15 M KCl.

protein was the same as that of the intact protein (not shown). When the DACM-modified recA protein was mixed with ϕX174 single-stranded [^{3}H]DNA in the presence of ATPγS and the mixture was passed through an alkali-treated nitrocellulose filter, 87 ± 2% of the DNA was retained on a filter. When a similar experiment was carried out with the intact recA protein, 74 ± 3% of the DNA was retained on the filter (probable error was obtained from the experiments in five times). We conclude that the modification does not change the affinity of the recA protein to the single-stranded DNA. On the other hand, the ATPase activity of the DACM-modified recA protein was less than 1% of the activity of the intact protein (data not shown). These results might suggest that while the modification of the cysteinyl residue does not affect the gross conformation of the recA protein, the modification causes some changes in a local structure of the protein around the cysteinyl residue.

Reaction of the Cysteinyl Residues with DTNB. In order to know the number and environment of the unmodified cysteinyl residues in the DACM-modified recA protein, the reactivity of the cysteinyl residues with DTNB was measured for the modified and unmodified protein. All the cysteinyl residues of the recA protein were titrated with DTNB in the presence of 3 M guanidine hydrochloride where the recA protein is completely denatured (Figure 3, curve 1; Kuramitsu et al., 1981). In the case of the DACM-modified recA protein in 3 M guanidine hydrochloride, two cysteinyl residues were titrated with DTNB (Figure 3, curve 5). This indicates that only one of the three cysteinyl residues was modified with DACM (Figure 2). In the absence of ATP, three cysteinyl residues of the intact recA protein were slowly reacted with DTNB (Figure 3, curve 2). In the presence of ATP, however, only one of these cysteinyl residues is titrated with DTNB, and the reaction is accelerated; the other two cysteinyl residues are protected against the titration with DTNB (Figure 3, curve 3). The two cysteinyl residues of the DACM-modified recA protein were also reacted with DTNB in the absence of ATP and were protected from modification in the presence of ATP (Figure 3, curves 6 and 7). These results suggest that the residue modified with DTNB in the presence of ATP is identical with the residue that can be modified with DACM.

Determination of the Positions of the Cysteinyl Residues Protected from Modification in the Presence of ATP. In order to determine the position of the cysteinyl residue modified with

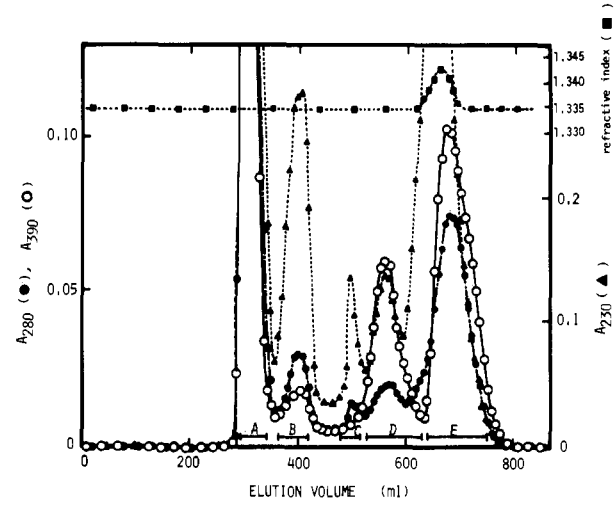

FIGURE 4: Chromatography of DACM-recA protein treated with NTCB on a Sephadex G-100 superfine column (2.5 × 110 cm) equilibrated with 0.15 M ammonium acetate. The left ordinate represents the absorbance at 280 (●) and 390 nm (○), and the right ordinate represents the absorbance at 230 nm (▲) and the refractive index at 25 °C (■).

Table I: Amino Acid Compositions of the Peptides Obtained by Cleavage with NTCB[a]

amino acid	fraction B	residues[b] 1–89	fraction D	residues[b] 90–128
Asx	5.6	5	6.1	6
Thr	5.4[c]	6	1.0[c]	1
Ser	6.3[c]	7	1.0[c]	1
Glx	13.0	12	5.0	5
Pro	2.0	2	1.9	2
Gly	11.7	12	2.2	2
Ala	9.9	10	5.0	5
Val	3.2	3	1.2	1
Met	2.9	3	0.1	0
Ile	7.8[d]	8	3.4[d]	4
Leu	9.3[d]	9	5.0[d]	5
Tyr	1.0	1	0.9	1
Phe	1.1	1	1.0	1
His	0.1	0	1.0	1
Lys	6.1	6	1.2	1
Arg	4.0	4	1.1	1
Trp	ND[e]	0	ND[e]	0

[a] Average of 20-, 40-, and 70-h values except for the data footnoted. Original composition data, expressed in nanomoles for each amino acid residue, were normalized in such a way that the total number of residues in the peptide was equal to that estimated from the DNA sequence data (Horii et al., 1980). [b] From the DNA sequence data (Horii et al., 1980). [c] Extrapolated to zero time. [d] Values from 70-h hydrolysates. [e] Not determined.

DACM in the presence of ATP, the remaining two cysteinyl residues were modified to cyanide with 2-nitro-5-(thiocyano)benzoic acid (NTCB), followed by the cleavage at the amino group of the cysteinyl residue modified with NTCB. The reaction products were separated by a Sephadex G-100 superfine column (2.5 × 110 cm) and eluted with 0.15 M ammonium acetate (Figure 4). The peptide fractions A, D, and E had absorption at 390 nm. Fraction A was turbid. Fraction E contained the substances with low molecular weight such as salt and degraded DACM. Fractions A, B, C, and D contained 8.9, 3.1, less than 0.1, and 0.59 mg of peptides, respectively. The amino acid compositions of the peptides in fractions B and D agree with those of the peptide from 1 to 89 and the peptide from 90 to 128 of the recA protein, respectively (Table I). The composition of fraction C agrees neither with that of peptide 90–115 nor with that of peptide

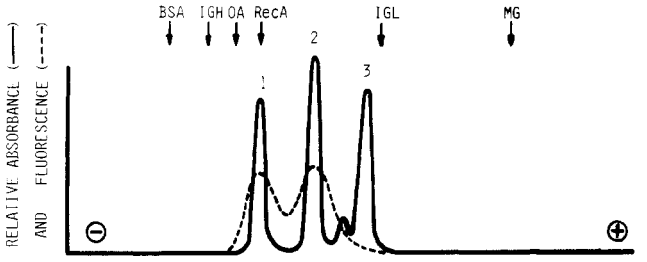

FIGURE 5: SDS–polyacrylamide gel electrophoretic analysis of fraction A in Figure 4. The broken line represents the fluorometric scanning at 460 nm excited at 390 nm of the gel without staining, and the solid line represents the densitometric scanning at 565 nm of the gel stained with Coomassie brilliant blue R250. The mobility of bovine serum albumin (BSA), heavy (IGH) and light (IGL) chains of human immunoglobulin G, ovalbumin (OA), recA protein, or myoglobin (MG) is also shown.

116–128 (data not shown) that is expected if the cleavages by NTCB occur at Cys-90 and Cys-116 or at Cys-116 and Cys-129, respectively. This fraction may contain minor products formed by a side reaction. Fraction A contained at least three kinds of peptides which were separated by SDS–polyacrylamide gel electrophoresis. As shown by the results on the densitometric scanning at 565 nm of the gel after staining with Coomassie brilliant blue R250 (solid line in Figure 5) and the fluorometric scanning at 460 nm on excitation at 390 nm of light without staining (broken line in Figure 5), the peptides in peaks 1 and 2 were fluorescent, and that in peak 3 was not. Molecular weights of peptides in peaks 1, 2, and 3 were determined as 37 800, 28 000, and 24 000, respectively. The mobility of the peptide in peak 1 corresponds to the uncleaved recA protein. The mobilities of peptides in peaks 2 and 3 correspond to those of the peptide containing the amino acid residues from 90 to 352 and of the peptide from 129 to 352, respectively. No peptide cleaved at Cys-116 residue was obtained from the DACM-modified recA protein, indicating that the residue modified with DACM was Cys-116. The relative area of peaks 1, 2, and 3 were 1.00, 1.54, and 1.37, respectively. This means that the cleavage reactions at Cys-90 and Cys-129 are incomplete. We could not detect the product of incomplete digestion that corresponds to the amino acid residues from 1 to 128 in the analysis of the fractions of Sephadex G-100 column chromatography. This result reflects the difference in the efficiency of cleavage at Cys-90 or Cys-129. The cleavage efficiency at Cys-90 or Cys-129 is calculated to be 67 or 41% (100% represents the complete cleavage), respectively. The relative yields of various peptides can be calculated from the values of relative efficiency of cleavage; a peptide containing the amino acid residues 1–352 is 19%, 90–352 is 30%, 129–352 is 26%, 1–128 is 5%, 1–89 is 17%, or 90–128 is 3%. The calculated yields of these peptides agree quite well with the experimental values (Figure 4). These results show that the residues protected by ATP are Cys-90 and Cys-129.

Titration of Cysteinyl Residues with DTNB at Various Ionic Strengths. The state of aggregation of the recA protein depends on the ionic strength (Kuramitsu et al., 1981). In order to obtain further information on the conformational change of the recA protein with an increase in ionic strength, the titration of the cysteinyl residues of the recA protein with DTNB was carried out at various ionic strengths. As shown in Figure 6, the increase in the ionic strength accelerated the reaction of two SH groups but retarded the reaction of the other SH group.

Two cysteinyl residues were titrated after 10 h at ionic strengths higher than 1.0 (Figure 6, insert) instead of three

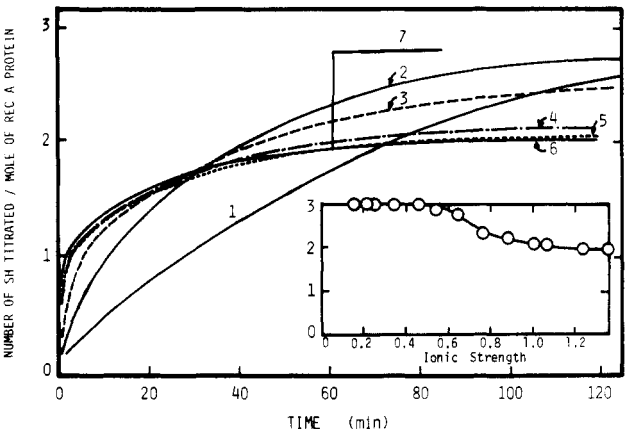

FIGURE 6: Titration of SH groups of intact recA protein at various ionic strengths and at pH 7.9, 25 °C. The protein concentration was 6×10^{-6} M. The concentration of DTNB was 0.5 mM. The buffer solution contained 50 mM HEPES, 4 mM phosphate, and 0.04 mM EDTA with KCl added to the ionic strength 0.16 (1), 0.34 (2), 0.52 (3), 0.88 (4), 1.00 (5), and 1.24 (6). For curve 7, Gdn·HCl was added to 3 M at ionic strength 1.24 and 60 min after addition of DTNB. Insert shows the number of SH groups titrated after 10 h at various ionic strengths.

at a low ionic strength. But all residues reacted in the presence of 3 M Gdn·HCl (curve 7 in Figure 6) where the recA protein is completely denatured (Kuramitsu et al., 1981). On the other hand, the CD spectra in the region between 200 and 250 nm and the fluorescence spectra excited at 295 nm of the recA protein were unaltered with an increase in ionic strength (not shown). These results suggest that an increase in ionic strength induces a conformational change in the region around cysteinyl residues, while gross conformation and the conformation around tryptophyl residues near the C-terminal end are not altered with an increase in ionic strength (not shown). The one cysteinyl residue of the Cys-116–DACM recA protein was protected against DTNB at high ionic strength as observed for the intact recA protein (Figure 3, curves 4 and 8). This finding indicates that the protected amino acid residue at high ionic strength is either Cys-90 or Cys-129.

Discussion

Among three cysteinyl residues only Cys-116 is modified with DACM in the presence of ATP. Cys-90 and Cys-129 are protected in the presence of ATP against the modification. The reactivity of Cys-116 with DTNB was accelerated in the presence of ATP. The Cys-116–DACM recA protein binds to single-stranded DNA and ATP but shows no ATPase activity in the presence of single-stranded DNA. These results suggest that the region which contained all three cysteinyl residues is in the proximity of the ATP binding site and that Cys-90 and Cys-129 are concealed by the bound ATP, while Cys-116 is exposed. Alternatively, a possibility might exist that ATP binds to the other region of the protein apart from these cysteinyl residues, but the binding of ATP induces a large conformational change in the region around these cysteinyl residues. Cotterill et al. (1982) have found that about 1 mol of ATP bound per mol of the recA protein at pH 8.1 and 25 °C. Weinstock et al. (1981b) showed that the affinity of ATPγS, which is a nonhydrolyzable analogue of ATP, for the recA protein treated with 10 mM *N*-ethylmaleimide was almost the same as that for the intact recA protein. The ATPase activity of the *N*-ethylmaleimide-modified protein, however, was lost (Weinstock et al., 1979; McEntee et al., 1979). These data suggest the catalytic role of the cysteinyl residue(s) and support our results that ATP binds near the region including

Cys-90 and Cys-129. The sequence from residues 60 to 80 of the recA protein is very similar to the sequence of the ATP binding protein (Walker et al., 1982).

The reduced reactivity of cysteinyl residues at a high ionic strength (Figure 6) showed a conformational change around the cysteinyl residues; nevertheless, the gross conformation of the recA protein is not altered. The decrease in the extent of aggregation of the recA proten was observed with an increase in ionic strength (Kuramitsu et al., 1981). These results suggest that the change in the aggregation state of the recA protein at high ionic strength affects the reactivity of the cysteinyl residues with DTNB, the details of which will be reported elsewhere.

Acknowledgments

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Registry No. ATP, 56-65-5; ADP, 58-64-0; ATPase, 9000-83-3; L-cysteine, 52-90-4.

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Molecular Mechanisms of Chemical Mutagenesis: 9-Aminoacridine Inhibits DNA Replication in Vitro by Destabilizing the DNA Growing Point and Interacting with the DNA Polymerase†

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ABSTRACT: 9-Aminoacridine was found to inhibit dNTP incorporation into DNA homopolymer duplexes by phage T4 DNA polymerase in vitro. Systematic variation of the molar ratio of 9-aminoacridine to DNA, to DNA polymerase, and to DNA precursors demonstrated that this inhibition at 9-aminoacridine concentrations below 10 μM was mainly due to interaction of 9-aminoacridine with the DNA and suggested that the basis for the preferential inhibition of incorrect precursor incorporation was destabilization of the DNA growing point. Consistent with destabilization, 9-aminoacridine stimulated the hydrolysis of correctly base paired DNA by the 3′–5′ exonuclease activity of phage T4 DNA polymerase. This is the first indication to my knowledge that an intercalating dye destabilizes the DNA growing point, whereas it raises the overall T_m of the DNA. At 9-aminoacridine concentrations above 10 μM overall incorporation of dNTPs was inhibited by 9-aminoacridine interaction with the DNA polymerase. A possible explanation for the induction of both deletion and addition frameshift mutations by 9-aminoacridine during DNA biosynthesis is discussed in light of growing-point destabilization.

Planar aromatic dyes such as 9-aminoacridine (9-AA)[1] have been shown to inhibit DNA replication in vitro (Goodman et al., 1974) and to enhance the occurrence of substitution mutations (Goodman et al., 1974; Shearman et al., 1983) as well as frameshift mutations (Crick et al., 1961; McCann et al., 1975). The aromatic dyes intercalate into double-strand DNA (Lerman, 1961, 1963; Sakore et al., 1977; Hogan et al., 1979), and this action has been suggested as the basis for both their

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[1] Abbreviations: 9-AA, 9-aminoacridine; Tris, tris(hydroxymethyl)-aminomethane; HPLC, high-pressure liquid chromatography.

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