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The Physical and Enzymatic Properties of *Escherichia coli* recA Protein Display Anion-specific Inhibition*

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The enzymatic activities of *Escherichia coli* recA protein are sensitive to ionic composition. Here we report that sodium glutamate (NaGlu) is much less inhibitory to the DNA strand exchange, DNA-dependent ATPase, and DNA binding activities of the recA protein than is NaCl. Both joint molecule formation and complete exchange of DNA strands occur (albeit at reduced rates) at NaGlu concentrations as high as 0.5 M whereas concentrations of NaCl greater than 0.2 M are sufficient for complete inhibition. The single-stranded DNA (ssDNA)-dependent ATPase activity is even less sensitive to inhibition by NaGlu; ATP hydrolysis stimulated by M13 ssDNA is unaffected by 0.5 M NaGlu and is further stimulated by *E. coli* ssDNA binding protein ~2-fold. Finally, NaGlu has essentially no effect on the stability of recA protein-εM13 DNA complexes, with concentrations of NaGlu as high as 1.5 M failing to dissociate the complexes. Surprisingly, NaGlu also has little effect on the concentration of NaCl required to disrupt the recA protein-εM13 DNA complex, demonstrating that destabilization is dependent on both the concentration and type of anionic rather than cationic species. Quantitative analysis of DNA binding isotherms establishes that the intrinsic binding affinity of recA protein is affected by the anionic species present and that the cooperativity parameter is relatively unaffected. Consequently, the sensitivity of recA protein-ssDNA complexes to disruption by NaCl does not result from the competitive effects associated with cation displacement from the ssDNA upon protein binding but rather results from anion displacement upon complex formation. The magnitude of this anion-specific effect on ssDNA binding is large relative to that of other nucleic acid binding proteins.

The product of the *recA* gene is required for genetic recombination in *Escherichia coli* (Clark, 1973). *In vitro*, this protein binds cooperatively to single-stranded DNA (ssDNA)[1] (Menetski and Kowalczykowski, 1985), resulting in an activation of ATPase activity (Weinstock *et al.*, 1981). recA protein also promotes both the renaturation of complementary ssDNA (Weinstock *et al.*, 1979) and the reciprocal exchange of DNA strands between ssDNA and complementary dsDNA (Cox and Lehman, 1981). As for nearly all protein-nucleic acid interactions, these DNA binding-dependent activities are sensitive to increasing salt concentration, and the magnitude of inhibition is greater for NaCl than for sodium acetate (NaOAc) (Menetski and Kowalczykowski, 1985; Roman and Kowalczykowski, 1986; Kowalczykowski and Krupp, 1987). The presence of 200 mM NaCl, for example, is sufficient to totally inhibit DNA strand exchange, and 200 mM NaOAc inhibits the reaction by 89% (Roman and Kowalczykowski, 1986).

The sensitivity of recA protein-promoted DNA strand exchange *in vitro* is somewhat difficult to reconcile with the observation that the intracellular ionic environment of *E. coli* changes dramatically with external osmolarity (Epstein and Schultz, 1965; Munro *et al.*, 1972; Measures, 1975). As external osmolarity increases from 0.1 to 1.1 osmolal, the internal concentration of potassium ion increases from 0.23 to 0.93 molal and that of glutamate ion increases from 0.03 to 0.26 molal (Richey *et al.*, 1987). These *in vivo* ion concentrations are sufficiently high to raise concern of the efficacy of the recA protein-promoted DNA strand exchange reaction *in vivo*. However, this apparent dilemma is potentiated by a growing appreciation that the anionic component of a salt has a large impact on protein-nucleic acid stability (see Kowalczykowski (1990) for summary). In particular, the glutamate anion is the least inhibitory toward both nucleic acid enzyme activity and nucleic acid binding affinity (Leirmo *et al.*, 1987; Overman *et al.*, 1988; Griep and McHenry, 1989; Prince and Villarejo, 1990). For these reasons, we examined the effects of sodium and potassium glutamate on the *in vitro* activities of recA protein. As might have been anticipated based on these precedents, both the ssDNA-dependent ATPase and DNA strand exchange activities are much less sensitive to inhibition by NaGlu (or KGlu) than by NaCl. Furthermore, the stability of recA protein-ssDNA (εM13 DNA) complexes is not affected by the presence of up to 1.5 M NaGlu. These results suggest that most, if not all, of the sensitivity of recA protein to salt concentration is due to the anionic component of the salt. Therefore, recA protein function should be relatively insensitive to changes in the intracellular potassium glutamate concentration.

MATERIALS AND METHODS

Chemicals—All chemicals were reagent grade, and solutions were made in glass-distilled H_2O. ATP was purchased from Boehringer Mannheim and was dissolved as a concentrated stock solution at pH 7.5. The concentration of ATP was determined using an extinction coefficient of 1.54×10^4 cm^{-1} M^{-1} at 260 nm.

Proteins—RecA protein was purified from *E. coli* strain JC12772

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[1] The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB protein, *E. coli* single-stranded DNA binding protein; G32P, T4-coded gene 32 protein; εM13 DNA, single-stranded M13 DNA containing 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine residues; poly(εrA), poly(1,N^6-ethenoadenylic acid)NaGlu, sodium glutamate; KGlu, potassium glutamate.

(Uhlin and Clark, 1981) using a preparative protocol[2] based on spermidine acetate precipitation (Griffith and Shores, 1985). The concentration of recA protein was determined using an extinction coefficient of 2.7×10^4 cm^{-1} M^{-1}at 280 nm. SSB protein was purified from *E. coli* strain RLM 727 (LeBowitz, 1985). The concentration of SSB protein was determined using an extinction coefficient at 280 nm of 3×10^4 cm^{-1} M^{-1}. The T4 bacteriophage gene 32 protein (G32P) was purified as described by Kowalczykowski *et al.* (1981).

DNA—Bacteriophage M13 ssDNA and replicative form dsDNA were isolated as described by Messing (1983). The replicative form was linearized using *Eco*RI restriction endonuclease. The concentrations of ssDNA and M13 dsDNA were determined using extinction coefficients of 8784 and 6500 cm^{-1} M^{-1} at 260 nm, respectively. ϵM13 DNA was made as described by Menetski and Kowalczykowski (1985), and its concentration was determined using an extinction coefficient of 7.0×10^3 cm^{-1} M^{-1} at 260 nm (Menetski and Kowalczykowski, 1987). Poly-1,N^6-ethenoadenylic acid (poly(ϵrA)) was purchased from P-L Biochemicals, and its concentration was determined using an extinction coefficient of 5.11×10^3 cm^{-1} M^{-1} at 260 nm (Kowalczykowski *et al.*, 1981).

DNA Binding Assay—The binding of recA protein to ϵM13 DNA was measured in 20 mM Tris-HCl (pH 7.5), 0.1 mM dithiothreitol, and added salts at the concentration indicated. Titrations and their analysis were performed as described (Menetski and Kowalczykowski, 1985).

The binding of G32P to poly(ϵrA) (1.0 μM) was measured in 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM dithiothreitol, and added NaGlu at the concentration indicated. Titrations and their analysis were performed as described (Kowalczykowski *et al.*, 1981; Kowalczykowski *et al.*, 1986).

ATP Hydrolysis Assay—ATP hydrolysis was measured by either of two methods which yielded equivalent results. Assays were conducted at 37 °C and contained 3 μM of the indicated ssDNA, 1 μM recA protein, and 0.6 μM SSB, when present, in buffer consisting of 20 mM Tris-HCl (pH 7.5), 0.1 mM dithiothreitol, 10 mM $MgCl_2$, 0.5 mM ATP, and salt as indicated. The first method measured inorganic phosphate production, using the method of Lanzetta *et al.* (1979). At early time points (*i.e.* less than 10 min), the production of inorganic phosphate was linear and was used to determine the initial rate of ATP hydrolysis. The alternate procedure was to use an enzyme-linked spectrophotometric assay which measures the oxidation of NADH (Kreuzer and Jongeneel, 1983; Kowalczykowski and Krupp, 1987). The components of the assay mixture did not limit the observed rate of ATP hydrolysis by recA protein at concentrations of NaGlu up to 1.5 M.

DNA Strand Exchange Assay—DNA strand exchange was assayed as described previously (Cox and Lehman, 1981). Reactions contained 10 μM M13 ssDNA, 20 μM M13 linear dsDNA, 6 μM recA protein, and 0.9 μM SSB protein (when included). Incubation was at 37 °C in buffer containing 25 mM Tris acetate (pH 7.5), 1 mM dithiothreitol, 8 mM magnesium acetate, 3.5% glycerol, 1 mM ATP, and NaGlu as indicated. An ATP regenerating system, consisting of 25 units/ml pyruvate kinase and 5.0 mM phosphoenolpyruvate, was included in all reactions. Aliquots were removed at 10, 20, 30, 60, and 90 min and were microdialyzed on Millipore "V" membranes to remove the salt (Marusyk and Sergeant, 1990). After electrophoresis in 0.8% agarose gels, the gels were stained with ethidium bromide, photographed using Polaroid type 55 positive/negative film, and then scanned using a Zeineh soft laser scanning densitometer.

RESULTS

DNA Strand Exchange Occurs at Elevated Sodium Glutamate Concentrations—DNA strand exchange promoted by recA protein is sensitive to increasing NaCl concentration (with complete inhibition occurring at 200 mM NaCl), but it is somewhat less sensitive to increasing NaOAc concentration (the rate of product formation is inhibited 89% at 200 mM NaOAc (Roman and Kowalczykowski, 1986)). In comparison, recA protein-promoted DNA strand exchange is more tolerant of increasing NaGlu concentrations (Fig. 1); at 250 mM NaGlu, the rate of product formation is inhibited only 75%, and at 500 mM NaGlu, formation of joint molecules and gapped circular dsDNA product is still detectable.

[2] S. C. Kowalczykowski, manuscript in preparation.

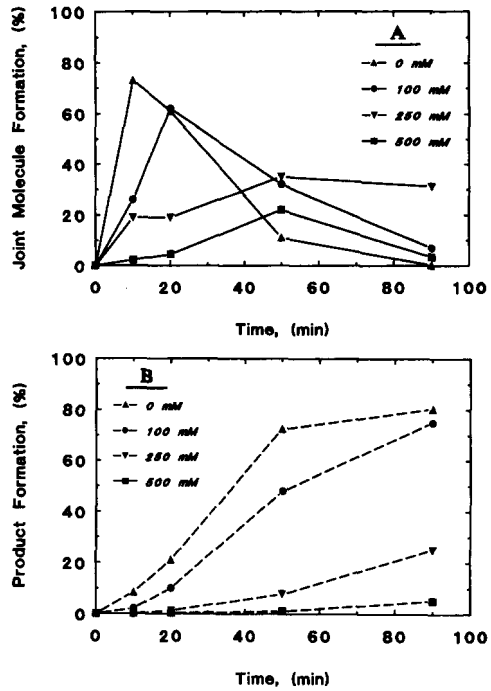

FIG. 1. **Effect of NaGlu on DNA strand exchange.** The DNA strand exchange reaction was conducted as described under "Materials and Methods." *Panel A* represents joint molecule formation, and *panel B* represents gapped circular dsDNA product formation: *triangles*, 0 mM NaGlu; *circles*, 100 mM NaGlu; *inverted triangles*, 250 mM NaGlu; and *squares*, 500 mM NaGlu.

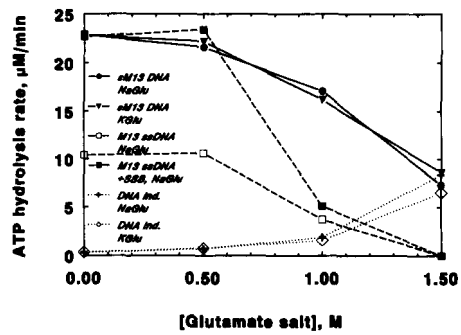

FIG. 2. **Effect of glutamate salts on ssDNA-dependent ATPase activity.** Reactions were conducted using standard conditions and contained the following: *circles*, ϵM13 DNA and NaGlu; *inverted triangles*, ϵM13 DNA and KGlu; *open squares*, M13 ssDNA and NaGlu; *filled squares*, M13 ssDNA, SSB protein, and NaGlu; *pluses*, NaGlu but DNA omitted; and *diamonds*, KGlu but DNA omitted. In the presence of DNA, the ATP hydrolysis given is the net rate obtained upon subtraction of the DNA-independent component.

ssDNA-dependent ATP Hydrolysis Occurs at 1.5 M NaGlu—The effect of NaGlu on M13 ssDNA-dependent ATPase activity is shown in Fig. 2. In the absence of SSB protein, the ATP hydrolysis rate is unaffected by 0.5 M NaGlu and then decreases to zero at the highest concentration of NaGlu examined (1.5 M). At 1.0 and 1.5 M NaGlu, a portion of the observed ATP hydrolysis is DNA-independent (Pugh and Cox, 1988) and is substrated from the observed rate to yield the net DNA-dependent value. SSB protein stimulates ATP hydrolysis at 0 and 0.5 M NaGlu but has a smaller effect at 1.0 M. In comparison, M13 ssDNA-dependent ATP hydrolysis is inhibited approximately 80–90% at 400 mM NaCl or at 850 mM NaOAc, and SSB protein-stimulated ATP hydrolysis is inhibited approximately 50% at 400 mM NaCl (Roman and Kowalczykowski, 1986; Kowalczykowski and Krupp, 1987;

Lavery and Kowalczykowski, 1988). Fig. 2 also shows the behavior of ATP hydrolysis dependent on ϵM13 DNA, which is a fluorescent derivative of M13 DNA that is devoid of secondary structure. The rate of ϵM13 DNA-dependent ATP hydrolysis is essentially unaffected by 0.5 M NaGlu but is reduced by approximately 25 and 70% at 1.0 and 1.5 M NaGlu. This trend is independent of the cation present, since KGlu elicits the same effects (Fig. 2). As for M13 ssDNA-dependent ATP hydrolysis, NaCl is a more potent inhibitor of the ϵM13 DNA-dependent reaction (a 40% reduction at 400 mM NaCl (Kowalczykowski and Krupp, 1987)). Thus, ssDNA-dependent ATP hydrolysis, as well as DNA strand exchange, is inhibited less by NaGlu than by either NaCl or NaOAc.

The recA Protein-ϵM13 DNA Complex Is Not Dissociated by Sodium Glutamate—Since the enzymatic properties of recA protein are less inhibited by NaGlu, its effect on ssDNA binding affinity was examined. A measure of the relative stability of protein-DNA complexes is the concentration of salt required to disrupt the protein-DNA complex (Kowalczykowski *et al.*, 1981; Kowalczykowski *et al.*, 1986). In the absence of nucleotide cofactor, the recA protein-ϵM13 DNA complex is half-dissociated by approximately either 250 mM NaCl or 840 mM NaOAc (Menetski and Kowalczykowski, 1985). This same complex is completely stable to concentrations of sodium glutamate as high as 1.5 M (data not shown), demonstrating that the apparent affinity of recA protein for ϵM13 DNA is at least 10^7 M^{-1} at this concentration of NaGlu.

To determine whether high concentrations of NaGlu affect the apparent binding affinity, NaCl titrations were performed in the presence of either 0.5 or 1.0 M NaGlu. The NaCl titration midpoints are given in Table I. Surprisingly, the presence of 1 M NaGlu decreases the NaCl concentration required to dissociate 50% of the complex by only 24 mM NaCl, relative to the concentration required when NaGlu is absent. These data suggest that the 1.0 M sodium ion in the form of NaGlu has little effect on the apparent binding affinity of recA protein for single-stranded DNA and that the complex instability caused by NaCl is almost entirely due to the chloride anion rather than the sodium cation. Thus, the salt concentration dependence of the apparent ssDNA binding affinity of recA protein must be due almost entirely to the anionic species present.

Although anion-specific effects on the stability of protein-nucleic acid complexes have been reported, the complete absence of dissociation by 1.5 M NaGlu is atypical (see Kowalczykowski (1990)). For nonspecific ssDNA binding proteins, the effects of NaGlu have been reported only for *E. coli* SSB protein. The binding affinity of SSB protein for ssDNA is approximately 1,000-fold greater in the presence of glutamate than in chloride and 11-fold greater than in acetate (Overman *et al.*, 1988). To determine whether the lack of dissociation of recA protein-ssDNA complex by NaGlu was unique, the effect of NaGlu on the stability of complexes between bacteriophage T4-coded gene 32 protein (G32P) and poly(ϵrA) was examined. Complex formation was found to be non-stoichiometric at NaGlu concentrations of about 500 mM or greater, with the protein binding isotherms being characteristically sigmoidal (data not shown). The monomer cooperativity parameter, ω, was determined to be 1,000 ± 500 and independent of NaGlu concentration; these observations are in agreement with the values of 1,000–2,000 determined previously in NaCl (McGhee and von Hippel, 1974; Kowalczykowski *et al.*, 1986; Kowalczykowski *et al.*, 1981). A plot of log *Kω versus* log[NaGlu] derived from the protein titrations yields a slope of approximately −4.1 (Fig. 3), which is intermediate to that published for NaOAc (−5.1) and for NaF (−3.5) (Kowalczykowski *et al.*, 1981); at 0.35 M salt, *Kω* is approximately 11-fold greater in NaGlu than in NaCl. Thus, the effect of NaGlu on the binding of gene 32 protein to ssDNA is within the range of anion effects previously noted but is in sharp contrast to the effects observed for recA protein. Therefore, it appears that the anionic contribution to the salt concentration dependence of recA protein binding affinity is unusually large.

The Intrinsic Binding Constant, but Not the Cooperativity Parameter, of recA Protein Is Affected by the Anionic Species—Because recA protein binds to ssDNA cooperatively, an increase in the apparent affinity of recA protein for ssDNA could be due to an increase in either the intrinsic binding affinity or the cooperativity of binding. These parameters can be determined by conducting protein titrations under non-stoichiometric binding conditions (*i.e.* see Menetski and Kowalczykowski (1985) and Kowalczykowski *et al.* (1986)). Unfortunately, because the binding of recA protein to ϵM13 DNA is stoichiometric even at 1.5 M NaGlu, these parameters cannot be determined in the presence of this salt. However, binding in the presence of elevated NaOAc concentrations is non-stoichiometric (Menetski and Kowalczykowski, 1985). Therefore, the thermodynamic binding parameters can be determined in the presence of this salt and compared with those determined in NaCl to see which thermodynamic parameter is anion-sensitive. The binding isotherms were found to be sigmoidal (data not shown), and *Kω* was determined (Menetski and Kowalczykowski, 1985); a plot of log *Kω versus* log[NaOAc] is shown in Fig. 3. In the absence of nucleotide

TABLE I

Effect of sodium glutamate on the stability of recA protein-ssDNA complexes

Salt titrations were conducted at 37 °C in buffer containing 5.4 μM ϵM13 DNA, 1.6 μM recA protein, 10 mM $MgCl_2$, and the NaGlu concentration indicated.

[NaGlu]	Salt titration midpoint
M	*mM NaCl*
0.0	274
0.5	260
1.0	250

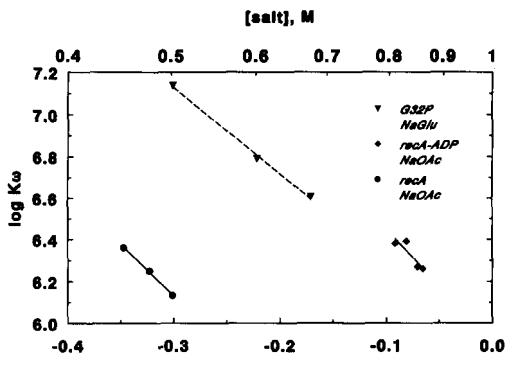

FIG. 3. **The effect of salt concentration on the ssDNA binding affinity.** The values of *Kω* were determined from protein titrations for either recA protein (*solid line*) or G32P (*dashed line*). recA protein titrations were carried out at 25 °C in DNA binding assay buffer containing 1.6 μM ϵM13 DNA, 4 mM magnesium acetate, and NaOAc at the indicated concentration; G32P titrations were carried out at 37 °C in DNA binding buffer containing 1.0 μM poly(ϵrA) and NaGlu at the concentration indicated. The units of *Kω* and salt concentration are M^{-1} and M, respectively. The data are: *circles*, recA protein in the absence of nucleotide cofactor ($y = -5.4x + 5.9$); *diamonds*, recA protein in the presence of 100 mM ADP ($y = -5.0x + 4.6$); *triangles*, G32P ($y = -4.1x + 5.9$).

cofactor, the apparent affinity of recA protein for ssDNA is substantially less sensitive to NaOAc concentration than to NaCl concentration (dlog $K\omega$/dlog[NaOAc] = −5.4 *versus* dlog $K\omega$/dlog[NaCl] = −11.1; Menetski and Kowalczykowski (1985)). The severe difference in the salt concentration sensitivities makes a comparison of relative affinities highly dependent on the salt concentration chosen, *e.g.* the affinity at 220 mM NaOAc is approximately 200-fold higher than in NaCl but is as large as 10^7-fold difference at 800 mM salt. In the presence of ADP, the NaOAc concentration dependence of affinity is comparable with that for NaCl (dlog $K\omega$/dlog[salt] = −5.05 and −4.7, respectively; Menetski and Kowalczykowski (1985)); the binding affinity is approximately 100-fold greater at 220 mM NaOAc than in NaCl. Over the range of 450–860 mM NaOAc, the apparent monomer cooperativity parameter, ω, is approximately 125 ± 25 (data not shown), which is 2.5-fold greater than that observed in the presence of NaCl (ω = 50; Menetski and Kowalczykowski (1985)). This 2.5-fold increase in cooperativity is not sufficient to account for the much greater increase in the apparent binding affinity when NaOAc is substituted for NaCl. Therefore, the increase in the apparent ssDNA binding affinity observed in the presence of acetate is largely due to an increase in the intrinsic binding constant, K, and not in the cooperativity of binding. We presume that a similar explanation accounts for the increased stability observed in the presence of NaGlu.

DISCUSSION

Glutamate is a major anionic species in *E. coli*, and its intracellular concentration varies widely with the osmolarity of the external environment (Record *et al.*, 1985; Richey *et al.*, 1987). For these reasons, we determined the effect of NaGlu on the biochemical properties of the recA protein. We find that both the DNA strand exchange and ssDNA-dependent ATPase activities are inhibited less by NaGlu than by any other sodium salt tested. Similarly, ssDNA binding studies confirm that the affinity of recA protein for ssDNA is affected least by NaGlu. These observations confirm and extend a number of reports demonstrating that glutamate salts are the least inhibitory to either enzyme activity (Leirmo *et al.*, 1987; Griep and McHenry, 1989; Prince and Villarejo, 1990) or to DNA binding affinity (Overman *et al.*, 1988), when compared to the more typically used chloride or acetate salts.

The inhibition of recA protein function by increasing salt concentration is greatly influenced by the anionic species and follows the series: chloride > acetate > glutamate. In contrast, neither the ssDNA-dependent nor the DNA-independent ATPase activity (Pugh and Cox, 1988) shows a differential sensitivity to sodium *versus* potassium ion. For each salt, the DNA strand exchange activity of recA protein is the most sensitive to inhibition, followed by ssDNA-dependent ATPase activity, and finally by ssDNA binding activity. Since DNA strand exchange activity is inhibited at salt concentrations significantly lower than those needed to inhibit either ssDNA-dependent ATPase activity or ssDNA binding, neither of these properties can be limiting at salt concentrations that inhibit DNA strand exchange. This suggests that a different activity, such as dsDNA binding or an essential protein-protein interaction, becomes limiting. The ability of recA protein to displace SSB protein from ssDNA is not compromised at elevated NaGlu concentrations, as evidenced by the ~2-fold stimulation of M13 ssDNA-dependent ATPase activity by SSB protein at up to 0.5 M NaGlu. Similarly, this observation implies that SSB protein also can bind M13 ssDNA under these conditions. Consistent with this inference, we find that SSB protein binds to M13 ssDNA in 1.0 M NaGlu with an apparent stoichiometry of 64 nucleotides/tetramer, although stoichiometric binding does not occur in 2 M NaGlu.[3]

The most unanticipated finding is that not only is the recA protein-ϵM13 DNA complex stable in 1.5 M NaGlu but that the presence of 1.0 M NaGlu has a negligible effect on the NaCl concentration required to disrupt the complex. This suggests that essentially all of the observed NaCl concentration dependence of binding is an effect of the chloride, and not the sodium, ion concentration. Quantitative analysis shows that the primary effect of anion substitution is on the intrinsic affinity of recA protein for ssDNA and not on the cooperativity of binding. The cooperativity parameter, ω, is approximately 2.5-fold higher in the presence of NaOAc than that obtained in NaCl, a difference which is insufficient to account for the much greater alteration in apparent affinity observed in the presence of these two salts. By analogy to this effect of acetate, we assume that the major effect of glutamate substitution also involves the intrinsic ssDNA binding affinity of recA protein. This result is surprising because the affinity of a protein for DNA is generally thought to be affected more by cation concentration than anion concentration, although reports of anion effects are becoming more frequent (see Kowalczykowski (1990)). Since the cationic component of the salt sensitivity of DNA binding affinity reflects the number of ionic interactions between the protein and the DNA phosphate backbone (Record *et al.*, 1976), our data suggest that recA protein makes no direct ionic bridges with the phosphate backbone of DNA, even though these residues may be occluded within the complex (Leahy and Radding, 1986). As a consequence, variations in intracellular KGlu concentrations due to changes in external osmolarity will have essentially no effect on recA protein binding to ssDNA and will have relatively little effect on recA protein enzymatic activity.

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REFERENCES

Clark, A. J. (1973) *Annu. Rev. Genet.* **7,** 67–85
Cox M. M., and Lehman, I. R. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 3433–3437
Epstein, W., and Schultz, S. G. (1965) *J. Gen. Physiol.* **49,** 221–239
Griep, M. A., and McHenry, C. S. (1989) *J. Biol. Chem.* **264,** 11294–11301
Griffith, J., and Shores, C. G. (1985) *Biochemistry* **24,** 158–162
Kowalczykowski, S. C. (1990) in *Landolt-Bornstein: Numerical Data and Functional Relationships in Science and Technology (New Series) Group VII: Biophysics, Nucleic Acids* (Saenger, W., ed) Vol. 1d, pp. 244–263, Springer-Verlag, Berlin
Kowalczykowski, S. C., and Krupp, R. A. (1987) *J. Mol. Biol.* **193,** 97–113
Kowalczykowski, S. C., Lonberg, N., Newport, J. W., and von Hippel, P. H. (1981) *J. Mol. Biol.* **145,** 75–104
Kowalczykowski, S. C., Paul, L. S., Lonberg, N., Newport, J. W., McSwiggen, J. A., and von Hippel, P. H. (1986) *Biochemistry* **25,** 1226–1240
Kreuzer, K. N., and Jongeneel, C. V. (1983) *Methods Enzymol.* **100,** 144–160
Lanzetta, P. A., Alvarez, L. J., Reinach, P. S., and Candia, O. A. (1979) *Anal. Biochem.* **100,** 95–97
Lavery, P. E., and Kowalczykowski, S. C. (1988) *J. Mol. Biol.* **203,** 861–874
Leahy, M. C., and Radding, C. M. (1986) *J. Biol. Chem.* **261,** 6954–6960
LeBowitz, J. (1985) *Biochemical Mechanism of Strand Initiation in*

[3] J. P. Menetski, A. Varghese, and S. C. Kowalczykowski, unpublished observations.

Bacteriophage λDNA Replication. Ph.D. thesis, The Johns Hopkins University
Leirmo, S., Harrison, C., Cayley, D. S., Burgess, R. R., and Record, M. T., Jr. (1987) *Biochemistry* **26,** 2095–2101
Marusyk, R., and Sergeant, A. (1980) *Anal. Biochem.* **105,** 403–404
McGhee, J. D., and von Hippel, P. H. (1974) *J. Mol. Biol.* **86,** 469–489
Measures, J. C. (1975) *Nature* **257,** 398–400
Menetski, J. P., and Kowalczykowski, S. C. (1985) *J. Mol. Biol.* **181,** 281–295
Menetski, J. P., and Kowalczykowski, S. C. (1987) *J. Biol. Chem.* **262,** 2085–2092
Messing, J. (1983) *Methods Enzymol.* **101,** 20–78
Munro, G. F., Hercules, K., Morgan, J., and Sauerbier, W. (1972) *J. Biol. Chem.* **247,** 1272–1280
Overman, L. B., Bujalowski, W., and Lohman, T. M. (1988) *Biochemistry* **27,** 456–471
Prince, W. S., and Villarejo, M. R. (1990) *J. Biol. Chem.* **265,** 17673–17679
Pugh, B. F., and Cox, M. M. (1988) *J. Biol. Chem.* **263,** 76–83
Record, M. T., Jr., Lohman, T. M., and deHaseth, P. (1976) *J. Mol. Biol.* **107,** 145–158
Record, M. T., Jr., Anderson, C. F., Mills, P., Mossing, M., and Roe, J. (1985) *Adv. Biophys.* **20,** 109–135
Richey, B., Cayley, D. S., Mossing, M. C., Kolka, C., Anderson, C. F., Farrar, T. C., and Record, M. T., Jr. (1987) *J. Biol. Chem.* **262,** 7157–7164
Roman, L. J., and Kowalczykowski, S. C. (1986) *Biochemistry* **25,** 7375–7385
Uhlin, B. E., and Clark, A. J. (1981) *J. Bacteriol.* **148,** 386–390
Weinstock, G. M., McEntee, K., and Lehman, I. R. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 126–130
Weinstock, G. M., McEntee, K., and Lehman, I. R. (1981) *J. Biol. Chem.* **256,** 8829–8834