drastic increase of transcriptional activity due to supercoiling as reported in Figure 2B. These results can be explained by assuming that a fraction of RNA polymerase molecules bound to DNA in initiation complexes is lost during the relaxation process. As a consequence, the number of active RNA polymerase II molecules considered in the calculation of the DNA unwinding angle is overestimated.

## Conclusions

When the DNA double helix is subjected to the torsional strength induced by supercoiling, its conformational may vary in a discontinous fashion; melting of A-T-rich sequences, induction of hairpin-loop structures, and transition of DNA from the B to the Z form can occur at critical values of supercoiling. Here we have described the transcriptional behavior of yeast RNA polymerase II on circular DNA at various degrees of supercoiling and demonstrated that, in vitro the eukaryotic enzyme is sensitive to conformational variation of the template. The pattern of the RNA products analyzed in Figure 2 indicates that a critical value of supercoiling is required by RNA polymerase II for initiation and termination of in vitro transcription. Further increases of the supercoiling strongly affects the size and the amount of the RNA products.

We have set up a method for studying the binding of the enzyme to DNA through the measurement of the DNA unwinding angle. The values obtained must be considered relative ones due to the presence of systematic undetermined errors in their determination. Nevertheless, they have enabled us to show that the binding of RNA polymerase to DNA is enhanced by supercoiling and that the enzyme itself can lower the supercoiling degree of the template by melting the DNA double helix.

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**Registry No.** RNA polymerase, 9014-24-8.

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# Complexation and Phase Transfer of Nucleic Acids by Gramicidin S[†]

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ABSTRACT: A novel interaction between gramicidin S (GrS) and nucleic acids is characterized which, like that between GrS and nucleotides, exploits both the dicationic and amphiphilic properties of the peptide. Complex formation between calf thymus DNA and GrS is demonstrated by (i) phase transfer to $CHCl_3$ of ultrasonically irradiated DNA and (ii) inhibition of phase transfer to $CHCl_3$ of adenosine 5′-triphosphate by either native or ultrasonically irradiated DNA. The stoichiometry of the interaction is 2:1 (DNA-P:GrS), which is consistent with a predominantly electrostatic mode of binding. The apparent affinity of GrS for DNA is considerably higher than it is for free nucleotides. The interaction of the monocationic derivative [2-$N^\delta$-acetylornithyl]gramicidin S with calf thymus DNA is considerably weaker. DNA binding by GrS provides a rationale for the lag between germination and RNA synthesis exhibited by wild-type spores of producer strains of *Bacillus brevis* but not by GrS-negative mutants. On the basis of these results in vitro, a protective role is proposed for GrS in the dormant spore.

Very little is known of the physiological role of gramicidin S [GrS,[1] *cyclo*-(Val$^1$-Orn$^2$-Leu$^3$-D-Phe$^4$-Pro$^5$)$_2$] in the producer strains of *Bacillus brevis*, even though the mode of biosynthesis (Katz & Demain, 1977) and metabolic fate (Egorov et al., 1970) of the peptide are relatively well characterized. GrS is synthesized enzymatically at the end of logarithmic growth [at which time a GrS-dependent decrease in energy charge (Silaeva et al., 1965; Vostroknutova et al., 1981) and nucleotide efflux are observed (Glazer et al., 1966)] and is incorporated into the spore (Egorov et al., 1970; Nandi & Seddon, 1978). During germination and early vegetative growth, the GrS content of the culture decreases, and products of GrS hydrolysis are detected in the medium. It has also been

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[1] Abbreviations: GrS, gramicidin S; AcGrS, [2-$N^\delta$-acetylornithyl]-gramicidin S; $Me_6$GrS, [2,2′-$N^\delta$-trimethylornithyl]gramicidin S; ATP, adenosine 5′-triphosphate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; NAS, *N*-acetoxysuccinimide; Orn, ornithine; TLC, thin-layer chromatography; $Me_2$SO, dimethyl sulfoxide; UV, ultraviolet.

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established that GrS, while retained in the spore, is not released by the young vegetative culture as a bactericidal agent (Egorov et al., 1970). Despite the temporal relation of synthesis to sporulation, GrS-deficient mutants produce normal quantities of spores which are morphologically unremarkable (Kambe et al., 1974; Nandi & Seddon, 1978; Marahiel et al., 1979). However, GrS-deficient spores initiate RNA synthesis rapidly after germination, unlike wild-type spores (Nandi & Seddon, 1978; Seddon & Nandi, 1978). The molecular basis of this difference in outgrowth rates has not been elucidated.

We recently showed (Krauss & Chan, 1983) that GrS forms complexes with nucleotides which undergo phase transfer to organic solvents. Complexation in water is directed by coordination of the positively charged Orn side chains of the peptide with the negatively charged phosphoryl groups of the nucleotide. The contribution of the nucleoside moiety to the stabilization of the complexes appears insignificant. This suggests that complementary interanionic spacing might be a sufficient condition for GrS binding to polynucleotides as well. Indeed, the interanionic distance between adjacent residues in B DNA is estimated from the Corey–Pauling–Koltun model to be ~7 Å, which is comparable to the intercationic distance of 6–8 Å in the peptide in solution (Krauss & Chan, 1982).

In the present paper, a nucleic acid–GrS interaction is demonstrated by the direct phase transfer of nucleic acids to $CHCl_3$ in the presence of GrS and by the nucleic acid dependent inhibition of GrS-mediated ATP phase transfer. The interaction is primarily ionic, as was the case with free nucleotides. The stoichiometry of DNA–GrS binding is established; modeling of the complex suggests a close topochemical complementarity between GrS and double-helical nucleic acids. Finally, the possible implications of these results for the physiological role of GrS are discussed in light of earlier microbiological studies.

## Experimental Procedures

*Materials.* GrS dihydrochloride (Sigma) was filtered and lyophilized prior to use. [2,2′-$N^\delta$-Trimethylornithyl]gramicidin S ($Me_6GrS$) was synthesized as previously described (Krauss & Chan, 1982). Adenosine 5′-triphosphate, disodium salt (Sigma), was used without further purification.

*Preparation of Nucleic Acids.* The procedure was similar to that employed by Anderson et al. (1978). Ultrasonic irradiation is reported to decrease the molecular weight of calf thymus DNA toward a limiting value of $5 \times 10^5$ (Cohen & Eisenberg, 1966).

(*A*) *DNA Preparation I.* Calf thymus DNA (Sigma), 4 mg/mL in a buffer containing 0.1 M NaCl and 0.01 M tris(hydroxymethyl)aminomethane (Tris), pH 7.0, was irradiated in an ice bath for 15 min on a Heat Systems-Ultrasonics W225R probe ultrasonicator operating at 20 kHz, 200-W maximum output [microtip, power setting = 5 (maximum = 10), 50% duty cycle, total immersion time = 30 min]. The slightly turbid solution was diluted 4-fold with buffer and dialyzed at 4 °C against (1) 0.01 M EDTA and (2) 0.02 M NaCl + 0.01 M Tris, pH 7.0.

(*B*) *DNA Preparation II.* Calf thymus DNA, 0.4 mg/mL in a buffer containing 0.08 M NaCl and 0.04 M Tris, pH 7.0, was sonicated as described previously for between 0 and 5 min. Dialysis was performed as in preparation I. DNA phosphate (DNA-P) concentration was determined from the absorbance at 258 nm, by using $\epsilon_{max}$(monomer) = $6.6 \times 10^3$. The increase in absorbance with sonication was less than 5%.

Soluble RNA, type III, from bakers' yeast (Sigma), 0.8 mg $mL^{-1}$ in a buffer containing 0.08 M NaCl and 0.04 M Tris, pH 7.0, was dialyzed at 4 °C against (1) 0.005 M EDTA and (2) 0.02 M NaCl + 0.01 M Tris, pH 7.0, and centrifuged briefly to remove particulate contaminants.

*Synthesis of [2-$N^\delta$-Acetylornithyl]gramicidin S (AcGrS).* Synthesis of AcGrS was performed by the *N*-acetoxysuccinimide (NAS) method (Lindsay & Shall, 1971), using equimolar amounts of peptide and acylating agent. NAS was prepared from $Ac_2O$ and *N*-hydroxysuccinimide via dicyclohexylcarbodiimide coupling (Lindsay & Shall, 1971) and recrystallized from EtOAc. An aqueous solution of GrS (220 mL, 5 mg $mL^{-1}$) was adjusted to pH 8.5 with NaOH and 1.9 mL of a 75 mg $mL^{-1}$ solution of NAS in dioxane added over a period of 1.5 h at 25 °C with vigorous stirring. The pH was readjusted to 8.5 after addition of each aliquot of NAS. A white powder was obtained after silicic acid column chromatography, gel filtration (Sephadex G-15, 60% aqueous EtOH), and lyophilization which yielded a single ninhydrin-positive spot on TLC [$R_f = 0.56$ in $CHCl_3/MeOH/H_2O$ (65:25:2) compared with $R_f = 0.21$ for GrS]. $^1H$ NMR spectra of the purified material at 500.13 MHz were similar to those of native GrS but contained new resonance at $\delta$ 7.8 ($Me_2SO$-$d_6$, t, 1, N*H*COCH$_3$) and 2.0 (MeOH-$d_4$, s, 3, NHCOC*H*$_3$) and a decrease in intensity of the broad Orn $N^\delta H_3^+$ resonance from six to three protons. In $Me_2SO$-$d_6$, many of the $^1H$ resonances were doubled, indicating a loss of $C_2$ symmetry in the peptide. This was not observed in methanol. The amino acid composition of the dansylated (Gray, 1972) acetyl derivative was found to be the following: Val, 1.80; Orn, 1.06; Leu, 2.16; Phe, 1.96; Pro, 2.10. The composition, NMR spectrum, and TLC mobility were thus all consistent with acetylation of the terminal amino group of a single Orn residue as the sole modification in the peptide. The overall yield was 15% based on the initial quantity of GrS.

*Extraction Procedure.* Equilibration of the various aqueous solutions with $CHCl_3$ was carried out at as previously described (Krauss & Chan, 1983). There were two differences of consequence: (i) The aqueous phase in DNA-containing extractions contained 0.01 M NaCl and 0.02 M Tris, pH 7.0. The presence of salts lowered the maximum extraction efficiency ($E_{max}$) for the extraction of ATP by GrS at zero nucleic acid concentration ($[ATP]_i = 10^{-5}$ M, $[GrS]_i = 2 \times 10^{-5}$ M) to 0.57 from 0.83, the value obtained previously in the absence of added salts. The absolute magnitude of $E_{max}$ was of no significance in the interpretation of the extraction–competition data. (ii) Phase transfer was generally monitored by the increase in absorbance at 270 nm of the organic layer, which is assumed proportional to the concentration of nucleotide or nucleic acid transferred; absorbance by the peptide at this wavelength is negligible. Absorbance measurements were made on a Beckman Instruments ACTA CIII spectrophotometer by using 1-cm path-length cells at 25 °C. The molar extinction coefficient of ATP is virtually unchanged by complexation and phase transfer.

When buffered solutions of DNA or RNA alone were agitated with $CHCl_3$, there was no interfacial precipitation and no absorbance change in the aqueous layer, except below 245 nm where an increase in absorbance attributable to dissolved $CHCl_3$ was detected.

## Results

*Interaction of GrS with Sonicated DNA.* Figure 1 presents the results of an extraction–competition experiment in which variable quantities of sonicated calf thymus DNA were added to the aqueous phase in a standard ATP–GrS extraction at pH 7.0. The initial ATP and GrS concentrations were $10^{-5}$ and $2 \times 10^{-5}$ M, respectively; the stoichiometry of the ATP–

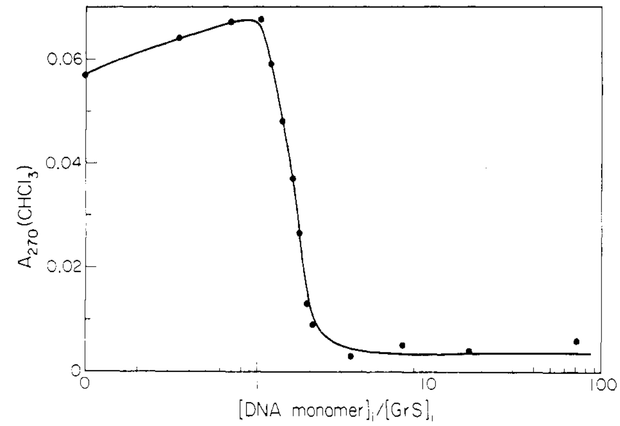

FIGURE 1: Extraction–competition employing ultrasonically irradiated calf thymus DNA. The aqueous phase prior to extraction contained $10^{-5}$ M ATP, $2 \times 10^{-5}$ M GrS, and DNA, pH 7.

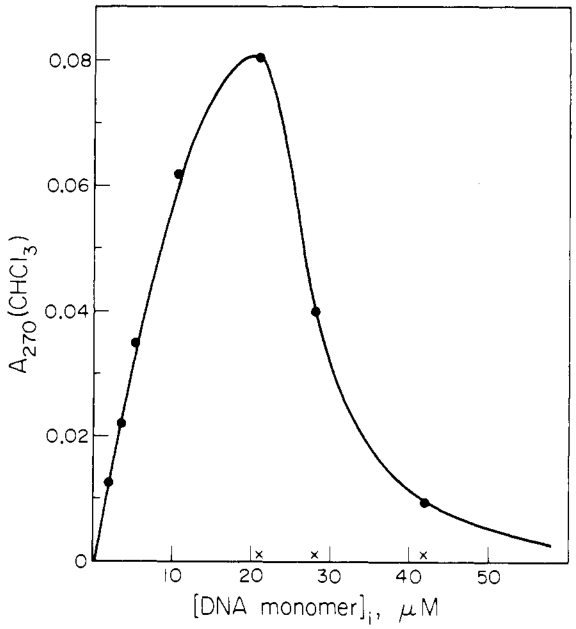

FIGURE 2: Direct phase transfer of ultrasonically irradiated calf thymus DNA by GrS ($2 \times 10^{-5}$ M), pH 7. The presence of a precipitate in the aqueous layer in individual runs is indicated (×).

GrS interaction at this pH is 1:2 (Krauss & Chan, 1983). The dependence of the absorbance at 270 nm in the organic phase after extraction ($A_{270,\mathrm{CHCl_3}}$) on the ratio of initial concentrations, [DNA monomer]$_i$/[GrS]$_i$ ($\equiv r$), is as follows: For $0 < r < 1$, $A_{270,\mathrm{CHCl_3}}$ increases gradually with $r$, achieving a maximum when $r = 1$; when $1 < r < 2$, $A_{270,\mathrm{CHCl_3}}$ decreases sharply toward a limiting value of ~0.005, thereafter remaining negligible for all $r > 2$. A precipitate was evident in the aqueous phase after extraction for all $r \geq 1$. This inhibition of ATP phase transfer by calf thymus DNA when $r > 1$ is indicative of a direct interaction between GrS and the nucleic acid. That it is just complete when $r = 2$ suggests that the nucleic acid can accommodate a maximum of 0.5 equiv of GrS per DNA residue.

GrS was indeed found to mediate DNA phase transfer (Figure 2), albeit in a manner qualitatively different from the free nucleotides. With the latter, the quantity transferred by a fixed concentration of GrS increases linearly with nucleotide concentration up to the point that the ratio of initial concentrations is equivalent to the stoichiometry of the complex; the quantity transferred at higher nucleotide concentrations is simply equal to that transferred at the point of stoichiometric equivalence. In contrast, while DNA transfer increases up to $r = 1$, a *decrease* with $r$ is observed for higher values of this parameter. The nonlinear dependence of $A_{270,\mathrm{CHCl_3}}$ on $r$ and the appearance of a precipitate after extraction are first noted for $0.5 < r < 1$. When the precipitate is present, the sum of the absorbances of the two phases is less than the initial absorbance of the aqueous phase, indicating that the precipitate contained DNA as a GrS conjugate. In these experiments, the aqueous layer was visibly turbid prior to extraction.

Table I: Phase Transfer of DNA: Effect of Sonication

| sonication time (min)[a] | $r$[b] | ppt[c] | $A_{270,\mathrm{aq}}$ | $A_{270,\mathrm{CHCl_3}}$ |
|---|---|---|---|---|
| 0 | 0.5 | | 0.003 | 0.011 |
| 0 | 2 | + | 0.002 | 0.000 |
| 0.5 | 0.5 | | 0.002 | 0.040 |
| 0.5 | 2 | + | 0.005 | 0.004 |
| 5 | 0.5 | | 0.002 | 0.048 |
| 5 | 2 | + | 0.007 | 0.004 |

[a] Actual time of irradiation: 50% duty cycle (see Experimental Procedures). [b] $r \equiv$ [DNA monomer]$_i$/[GrS]$_i$; in all instances, [DNA monomer]$_i$ = $10^{-5}$ M. [c] Presence of a precipitate after extraction.

Figure 1 should thus exhibit the interplay of three phenomena: (i) the direct phase transfer of DNA, which results in an increase in $A_{270,\mathrm{CHCl_3}}$ for $0 < r < 1$; (ii) competitive inhibition of the ATP extraction by DNA, assisted by a stoichiometry change, which yields a decrease in $A_{270,\mathrm{CHCl_3}}$ for $r > 0$ that partially cancels the increase attributable to (i); and (iii) a decrease in DNA transfer when $r \geq 1$ which contributes, with (ii), to the sharp decrease in $A_{270,\mathrm{CHCl_3}}$ for $1 < r < 2$. It was possible to reverse the inhibition of ATP transfer almost completely at $r = 2$ ([ATP]$_i$ and [GrS]$_i$ as in Figure 1) by the addition of 0.005 M 1,4-butanediamine (putrescine) dihydrochloride, which should bind DNA extensively at this concentration (Mahler & Mehrotra, 1963). On the other hand, a 5-fold molar excess of ATP over DNA, again with $r = 2$, afforded no reversal of the inhibition.

The presence of DNA in $CHCl_3$ after direct extraction was verified by UV spectrophotometry. The absorption spectrum of the transferred complex, measured between 300 nm and the solvent cutoff (245 nm), is consistent with the near-UV absorption envelope of a nucleic acid. The contribution of D-Phe ($\lambda_{\max}$ 260 nm) is, in comparison, negligible, and the peptide group absorbance becomes appreciable only at shorter wavelengths.

*Interaction of GrS with Native DNA: Stoichiometry of the DNA–GrS Complex.* Unsonicated calf thymus DNA was found to be only slightly transferred by GrS into $CHCl_3$. The native strands partition quantitatively into the precipitate, as indicated by the low absorbance of both phases. Phase transfer is markedly enhanced by brief ultrasonic irradiation (Table I).

The extraction–competition experiment was repeated with unsonicated DNA in order to measure the inhibition of ATP phase transfer as a function of DNA monomer concentration without concurrent transfer of nucleic acid. As shown in Figure 3, the fraction of ATP phase transfer blocked by unsonicated DNA (as measured by $A_{270,\mathrm{CHCl_3}}$) is just $0.5r$, providing more compelling evidence that the stoichiometry of the interaction between the nucleic acid and GrS is indeed two DNA residues per peptide molecule, as expected for a predominantly electrostatic mode of binding. Thus, when $r \leq 2$, the precipitate formed between unsonicated DNA and GrS should possess an overall stoichiometry of 2:1 (DNA-P:GrS).

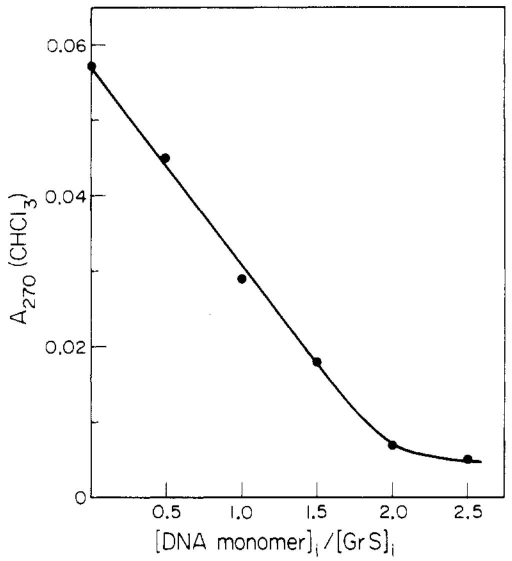

FIGURE 3: Extraction–competition employing native calf thymus DNA. The aqueous phase prior to extraction contained $10^{-5}$ M ATP, 2 × $10^{-5}$ M GrS, and DNA, pH 7.

The same overall binding stoichiometry for sonicated DNA will afford, for $r \leq 2$, an electrically neutral complex capable of undergoing phase transfer. The linearity of the observed decrease in $A_{270,\mathrm{CHCl_3}}$ (Figure 3) suggests that the overall affinity of GrS for DNA is considerably higher than it is for free nucleotides.

Reexamining Figure 2, it is apparent that direct phase transfer of sonicated DNA occurs only when GrS is present in stoichiometric or higher concentrations. If GrS is randomly distributed among available (monomer) binding sites on the nucleic acid molecules and if nucleic acid transfer requires quantitative charge neutralization, then phase transfer should be negligible when the concentration of GrS is substoichiometric ($r > 2$). The decrease in DNA extraction efficiency for $1 < r < 2$ likely derives from incomplete binding to all available polymer sites. It is also conceivable that an excess of free peptide is necessary to prevent aggregation of the DNA–GrS complexes in the aqueous phase and promote quantitative transfer, as was observed with the nucleotides (Krauss & Chan, 1983).

For low relative concentrations of nucleic acid ($r < 1$), sonicated DNA is transferred quantitatively to CHCl$_3$. From the initial slope of the phase transfer curve in Figure 2, one calculates $\epsilon_{270} \simeq 6 \times 10^3$ for the complex in the organic phase. If $\epsilon_{270}/\epsilon_{258} = 0.8$, as measured for calf thymus DNA in aqueous buffer, then this value of the extinction coefficient represents a hyperchromicity of between 10 and 15%. In water, the difference spectrum between complexed and uncomplexed DNA is complicated by light-scattering effects, but the enhancement of the near-UV absorbance appears smaller ($\sim$4%). The hyperchromicity in CHCl$_3$ represents, at least in part, a change in the solvation state of the nucleic acid.

*Interaction of GrS with RNA.* GrS was in addition found to bind soluble yeast RNA containing tRNA$^{\mathrm{Val}}$ and tRNA$^{\mathrm{Tyr}}$. The interaction qualitatively resembled that with sonicated calf thymus DNA: direct phase transfer was obtained, ATP phase transfer was blocked, and RNA transfer ceased with high $r$, the RNA–GrS complexes instead partitioning into a precipitate. The shut-off of direct transfer at high nucleic acid concentration occurs at higher values of $r$ than for calf thymus DNA, however, with significant phase transfer still observed when $r = 2$.

*Interaction of GrS Analogues with Sonicated DNA.* In view of the importance of ion coordination in DNA binding, it was of interest to examine the affinity of the monofunctional derivative AcGrS for DNA. As shown in Table II, AcGrS promotes phase transfer of sonicated calf thymus DNA, albeit significantly less efficiently that the native peptide. While the separate extinction coefficients of the complex in the two phases are not known, the increase in total absorbance of the AcGrS conjugates in the two phases is consistent with a significantly larger (20–30%) hyperchromicity than was estimated for the DNA–GrS complex in CHCl$_3$.

The results of an extraction with the quaternary analogue Me$_6$GrS are also shown in Table II. In contrast to the situation with free nucleotides, the degree of DNA binding, as indicated by the removal of nucleic acid from the aqueous layer, is quantitatively similar for GrS and the quaternary analogue. Interestingly, Me$_6$GrS fails to promote measurable phase transfer.

## Discussion

A novel interaction between GrS and nucleic acids has been discovered which, like that between GrS and nucleotides, exploits both the dicationic and amphiphilic properties of the peptide. While interactions of nucleic acids with basic polypeptides are well-known (Helene & Maurizot, 1981) and have been in many instances characterized stoichiometrically (Seipke et al., 1979) and thermodynamically (Latt & Sober, 1967; Record et al., 1976), no previous example of phase transfer by a peptide or other likely candidate, such as a suitably substituted polyamine, has been reported. Among the polypeptide antibiotics produced by other *B. brevis* strains, the tyrocidines, which are structurally related to GrS but monocationic, have been found to interact in aqueous media with DNA of diverse origins (Schazschneider et al., 1974; Ristow et al., 1975a,b; Hansen et al., 1982). The stoichiometry of the tyrocidine–DNA interaction is unknown, and neutralization of the single positive charge does not weaken the binding (Hansen et al., 1982), indicating that ion pairing contributes only secondarily to complexation here, in marked contrast to GrS.

In view of the 2:1 stoichiometry of binding and the lipophilicity of the nucleic acid–GrS complexes, the interaction should involve apposition of the Orn side chains of GrS to adjacent phosphoryl groups of the nucleic acid backbone, with exposure of the hydrophobic face of the peptide to the solvent. From what is presently known of the solution conformation of the peptide (Ovchinnikov et al., 1970; Rae & Scheraga, 1978; Kuo et al., 1979; Huang et al., 1981; Krauss & Chan, 1982), a mode of binding to double-stranded DNA is proposed in which GrS is oriented with the peptide ring plane and ring minor axis parallel to the DNA helix axis, each peptide molecule thus forming up to eight interamide hydrogen bonds with the backbone moieties of its neighbors on opposite sides of the grooves. At saturation, the nucleic acid is then virtually ensheathed by bound peptide, the resultant complex being markedly hydrophobic. Interestingly, the sense of the ring twist in GrS (internal Orn$^{i}NH_3^{+}$–O═C–D-Phe$^{i+2}$ hydrogen bonding; Hull et al., 1978; Krauss & Chan, 1982) appears specifically to favor interaction with a right-handed helix since the axis connecting the terminal amino groups of Orn in the peptide and that connecting adjacent phosphates in the nucleic acid are inclined similarly to the helix axis, when the peptide is apposed. Structural/conformational studies of the extracted complex are presently in progress.

The affinity of GrS for DNA is apparently significantly higher than that for free nucleotides, for which two rationales may be offered. (i) The interanionic distances in the nucleic

Table II: Phase Transfer of DNA by GrS Congeners[a]

| peptide | $[\text{peptide}]_i$ ($\times 10^{-5}$ M) | ppt | $A_{270,\text{aq}}$ | $A_{270,\text{CHCl}_3}$ |
|---|---|---|---|---|
| GrS | 2.0 | + | 0.001 | 0.049 |
| AcGrS[b] | 2.0 | | 0.033 | 0.029 |
| AcGrS[b] | 4.0 | | 0.019 | 0.056 |
| $Me_6GrS$ | 2.0 | + | 0.002 | 0.003 |

[a] $[\text{DNA monomer}]_i = 10^{-5}$ M in all instances. [b] Added initially to the $CHCl_3$ phase.

acid are relatively fixed and closely approximate the intercationic spacing in the peptide. The di- and triphosphate moieties of the nucleotides, in contrast, are conformationally mobile, and GrS binding selects a limited set of rotameric isomers which optimize the coordination of oppositely charged groups, an entropically unfavorable process. (ii) The monomeric nucleotide–GrS complexes in the organic phase contain exposed amide and uncharged phosphoryl groups, which are solvophobic. Association of the complexes by intermolecular hydrogen bonding (cross-$\beta$-aggregation) yields ribbonlike aggregates with highly apolar surfaces (Krauss & Chan, 1983); this enhances the efficiency of phase transfer but requires diffusional encounters of the complexes in three dimensions. On the other hand, the nature of the GrS–nucleic acid interaction is such that interamide hydrogen bonding occurs between neighboring peptide molecules on the polynucleotide strand. The resulting complex presents, as transferred, minimal solvophobic contacts in the organic phase.

The behavior of AcGrS toward DNA differs from that of both GrS and the tyrocidines. AcGrS transfers DNA to $CHCl_3$ less efficiently than does the native peptide, reflecting the lower stability of the complex involving a single ion pair. In addition, there is a large hyperchromic effect. If charge neutralization by the peptide is a prerequisite for phase transfer, the stoichiometry should approach 1:1 (DNA-P: AcGrS), and DNA unwinding is inevitable if all the bound AcGrS is to be accommodated. Unlike AcGrS, the monocationic tyrocidines induce significant *hypo*chromicity (Ristow et al., 1975b), supporting the contention that tyrocidine–DNA interactions involve hydrogen bonding and/or base stacking through participation of the Asn, Gln, Phe, and Trp residues and thus differ fundamentally from the interactions of nucleic acids with GrS or its derivative.

Binding of nucleic acids by GrS provides a plausible molecular basis for the lag between germination and the onset of RNA synthesis exhibited by wild-type *B. brevis* Nagano spores or GrS-negative mutants supplemented with GrS, but not by untreated mutants or wild-type spores from which GrS is chemically extracted (Nandi & Seddon, 1978; Seddon & Nandi, 1978; Mahariel et al., 1979). If the peptide is physically associated with the bacterial chromosome, as suggested by its strong interaction with DNA in vitro, its removal will be necessary for translation to proceed. Since the DNA-P content of a *Bacillus* spore is typically $\sim 5 \times 10^{-17}$ equiv (Doi, 1969), which is comparable to the GrS content of the wild-type spore of the producer, $4 \times 10^{-17}$ equiv (Nandi & Seddon, 1978), the association may be close to stoichiometric. The possibility is thus raised by the present study that the physiological role of GrS in the producer is to protect the dormant chromosome against chemical degradation. Preliminary data obtained elsewhere have indeed shown diminished heat resistance in GrS-negative spores (Mahariel et al., 1979). Additional studies of the susceptibility of the wild-type and mutant dormant chromosomes to environmental damage and of the thermal and chemical stability of GrS–DNA complexes in vitro will be required to establish definitively the role of the peptide in sporulation.

**Registry No.** GrS, 113-73-5; AcGrS, 60687-10-7; $Me_6GrS$, 83573-52-8.

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