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Silver Sulfadiazine: Interaction with Isolated Deoxyribonucleic Acid

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Silver sulfadiazine (AgSu) was found to interact with *isolated* deoxyribonucleic acid (DNA) to form nondissociable complexes. These complexes differ in physical and chemical properties from those that are established when silver nitrate is added to DNA. The reaction between AgSu and DNA is visualized as occurring in two stages: (i) a weak and reversible interaction (intercalation) between DNA and the sulfadiazine moiety and (ii) a tight binding involving the silver atom. In the first stage, sodium sulfadiazine competes with AgSu for the DNA.

Silver sulfadiazine (AgSu) was synthesized by Fox, who also established its effectiveness for the prevention and treatment of burn infections due to pseudomonads (8–10, 28). Even though AgSu is a sulfonamide drug, its antibacterial activity is not a function of the sulfonamide moiety because its effectiveness is not reversed by *p*-aminobenzoic acid (10). Silver nitrate ($AgNO_3$) reacts with deoxyribonucleic acid (DNA) in vitro (14, 31; see also 3 and 33); hence, it has been suggested (10) that AgSu reacts with the cellular DNA and that this forms the basis of its antibacterial action. It has been proposed further (10) that when AgSu reacts with the cellular DNA it dissociates and that only the silver ion becomes associated with DNA while the sulfadiazine portion is released. However, data on both the in vivo and in vitro effects of AgSu are scarce, and therefore an examination of its mode of action was undertaken. The present study shows that in vitro AgSu forms a complex with DNA which is unlike that formed when DNA is mixed with $AgNO_3$; but it is also shown (25; J. E. Coward, H. S. Carr, and H. S. Rosenkranz, *in preparation*) that this in vitro reaction between DNA and AgSu is *not* the basis of the latter's antibacterial action. The biological activity of AgSu appears to derive from its effect on the external bacterial structures, as documented in the accompanying report (25; also Coward, Carr, and Rosenkranz, *in preparation*).

MATERIALS AND METHODS

Deoxyribonucleates. Calf thymus DNA was obtained either from Nutritional Biochemicals Corp. or from Calbiochem. *Pseudomonas aeruginosa* DNA was prepared by the procedure of Marmur (20), and bacteriophage DNA was isolated from *Klebsiella* phage E-1, currently under investigation in this laboratory. ^{3}H-labeled DNA derived from chicken embryos was prepared as described previously (13). All DNA specimens were dissolved in 0.005 M $NaNO_3$ and extensively dialyzed against 0.005 M $NaNO_3$ to remove all traces of chloride (capable of reacting with $AgNO_3$ to form AgCl).

Sulfadiazines. AgSu (29.1% silver) and sodium sulfadiazine (NaSu) were generously provided by Marion Laboratories, Inc., Kansas City, Mo. Only freshly prepared solutions (in 0.005 M $NaNO_3$) were used.

Chemical analysis. The phosphorus content of DNA specimens was determined by the procedure of Fiske and Subbarow (7).

Spectral analysis. The spectra of DNA solutions were taken with a Carey model 11 recording spectrophotometer, and the absorbance at selected wavelengths was checked with a Beckman DU-2 spectrophotometer.

Centrifugal procedures. Sedimentation velocity studies were carried out on dilute solutions in 12-mm Kel-F centerpiece centrifuge cells. A Spinco model E analytical ultracentrifuge equipped with an ultraviolet optical system was operated at 50,740 rev/min, and pictures were taken at 2-min intervals. The photographs were traced with a Joyce-Loebl Mark III B microdensitometer, and sedimentation coefficients ($S_{50\%}$) and distributions of sedimentation coefficients were calculated by a modification (H. S. Rosenkranz, Ph.D. thesis, Cornell Univ., Ithaca, N.Y., 1959) of the procedure of Schumaker and Schachman (27). The ultracentrifugal heterogeneity of DNA specimens was expressed as the value of one standard deviation divided by the $S_{50\%}$ value (24; Rosenkranz, Ph.D. thesis). The amount of ultraviolet-absorbing nonsedimenting material was calculated from the microdensitometer tracings as previously described (13).

The banding properties of DNA in gradients of CsCl were determined as described by Schildkraut et al. (26). Portions of the DNA, together with a reference sample (*Micrococcus lysodeikticus* DNA, 1.731 g/cm³), were placed in a CsCl solution (density, 1.70 g/cm³) and centrifuged at 44,770 rev/min for 24 hr. The bands formed by the specimens at their equilibrium positions were photographed and traced as above.

For Cs_2SO_4 density gradient analyses, mixtures containing 0.3 ml of radioactive DNA solution, 1.7 ml of 0.005 M $NaNO_3$, and 1.33 ml of saturated Cs_2SO_4 (23 C) solution were spun at 30,000 rev/min in the SW-50 rotor of a Spinco model L-2 ultracentrifuge for 39 hr. Fractions were collected as previously described (5) and were assayed for acid-precipitable radioactivity. The refractive indices of selected fractions were determined. These values were used to calculate buoyant densities (30).

For zonal centrifugations in gradients of sucrose, radioactive DNA (0.3 ml) was layered on top of 4.5 ml of a gradient (5 to 20%) of sucrose dissolved in 0.01 M tris(hydroxymethyl)aminomethane (Tris) buffer, *p*H 7.0. The solutions were spun at 30,000 rev/min in the SW-50 rotor of a Spinco model L-2 ultracentrifuge for 8 hr. Fractions were collected and processed as described above.

Thermal transition profiles. Thermal transition profiles were measured with a Beckman DU spectrophotometer in the manner described by Marmur and Doty (21). All DNA specimens were dissolved in 0.005 M $NaNO_3$.

RESULTS

Effect of AgSu on spectral properties. Addition of $AgNO_3$ and AgSu to DNA resulted in a shift of the absorption maxima from 257.5 to 263 nm (Fig. 1). Such an effect of $AgNO_3$ has been described previously (14). Further analysis indicated, however, that the effects of the two silver compounds were not identical, as evidenced by differences in hyperchromic shifts and ratios of absorbancies at selected wavelengths (Table 1). The lack of effect of AgSu (and $AgNO_3$) on the absorbance in the visible range (300 to 450 nm) indicates that no precipitation of DNA occurs when these substances interact with DNA. Contrariwise, when polyamines or basic polypeptides are complexed with DNA, this results in an absorbance in the visible range due to aggregation (light-scattering effect; 2, 4, 6, 11, 12).

Effect on the sedimentation coefficient of DNA. The addition of increasing amounts of $AgNO_3$ to calf thymus DNA resulted in increases in the sedimentation coefficients (Table 2, experiment V). On the other hand, no such concentration dependence was observed when AgSu was added to DNA (Table 2). Thus, a maximal effect was observed when the molar ratio of AgSu to DNA-phosphorus (AgSu/DNA-P) was 0.5. Moreover, it was consistently found that the maximal effect

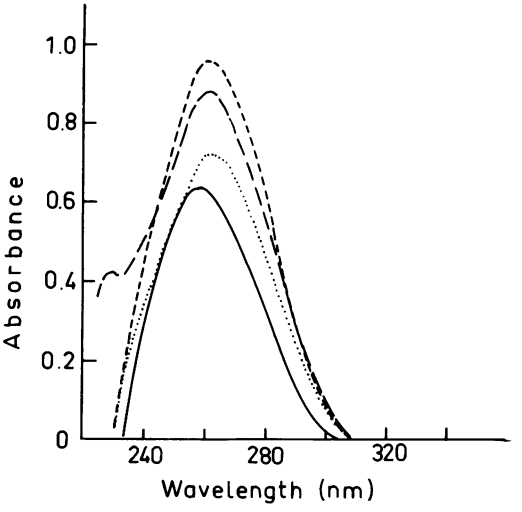

FIG. 1. *Spectra of DNA and silver-DNA complexes. Solutions of calf thymus DNA (35 μg per ml of 0.005 M $NaNO_3$) were mixed with $AgNO_3$ or AgSu (silver/DNA-P = 1.0), and 24 hr later spectra were determined. Solid line, DNA read against 0.005 M $NaNO_3$; dotted line, $AgNO_3$-DNA read against $NaNO_3$; long-dashed line, AgSu-DNA read against AgSu; short-dashed line, AgSu-DNA read against $NaNO_3$. (The curves show no minima at 232.5 nm because the presence of $NaNO_3$ makes the reading of absorbancies below 235 nm impossible.)*

TABLE 1. *Effect of silver on the absorbance of DNA*[a]

Addition	Maximal absorbance (nm)	Increase in A_{260} (%)	A_{240}/A_{260}	A_{280}/A_{260}
$NaNO_3$.....	257.5	—	0.45	0.52
$AgNO_3$ in $NaNO_3$.....	263	11	0.53	0.63
AgSu in $NaNO_3$.....	263	38	0.49	0.66

[a] The preparation of the solutions is described in the legend of Fig. 1. The absorbance at selected wavelengths was checked with a Beckman DU-2 spectrophotometer.

of AgSu was always smaller than that produced by the addition of equimolar amounts of $AgNO_3$ (Table 2). (In no instance was there a decrease in the sedimentation coefficient of DNA upon exposure to either AgSu or $AgNO_3$; i.e., DNA was not degraded.)

Analysis of the amount of ultraviolet-absorbing material sedimenting in mixtures of AgSu and DNA revealed that such mixtures contained ultraviolet-absorbing nonsedimenting species, presumably unbound AgSu (AgSu absorbs in the ultraviolet). However, as the time of exposure of

TABLE 2. *Effect of silver on the sedimentation coefficient of DNA*[a]

Expt	Addition	Ag/DNA-P	Sedimentation coefficient
I	None	0	14.1*S*
	$AgNO_3$	0.5	19.6*S*
	$AgNO_3$	1.0	20.1*S*
	AgSu	0.5	15.1*S*
	AgSu	1.0	15.1*S*
II	None	0	11.3*S*
	$AgNO_3$	1.0	19.8*S*
	AgSu	1.0	14.0*S*
III	None	0	13.3*S*
	$AgNO_3$	1.0	19.7*S*
	AgSu	1.0	14.5*S*
IV	None	0	10.5*S*
	$AgNO_3$	1.0	15.8*S*
	AgSu	1.0	11.4*S*
V	None	0	12.9*S*
	$AgNO_3$	0.022	14.5*S*
	$AgNO_3$	0.216	15.1*S*
	$AgNO_3$	2.16	16.2*S*
	$AgNO_3$	21.6	19.2*S*
	$AgNO_3$	216	22.8*S*

[a] Small amounts of $AgNO_3$ or AgSu (in 0.005 M $NaNO_3$) were added to solutions of calf thymus DNA (40 μg per ml of 0.005 M $NaNO_3$). Solutions were kept in the dark for 24 hr and analyzed in a Spinco model E analytical ultracentrifuge.

TABLE 3. *Amount of nonsedimenting AgSu in mixtures of DNA and AgSu*[a]

Addition	Time (hr)	Per cent nonsedimenting
DNA (control)	1	0
DNA (control)	24	0
DNA + $AgNO_3$	2	0
DNA + $AgNO_3$	25	0
DNA + AgSu	1	91
DNA + AgSu	3	83
DNA + AgSu	6	72
DNA + AgSu	12	60
DNA + AgSu	24	54
DNA + AgSu	30	49

[a] The sedimenting boundaries of mixtures of DNA + AgSu and DNA + $AgNO_3$ (in each case the ratio of DNA-phosphorus to silver was 1.0) were analyzed as a function of time. Calculation of the percentage of nonsedimenting material was performed as described previously (13).

the DNA to AgSu increased, the amount of unbound ultraviolet-absorbing material (AgSu) decreased (Table 3). This finding suggests that the reaction between DNA and AgSu is a slow one and that it will be difficult to determine the exact molar ratio of AgSu bound per DNA-P when equilibrium is reached. (Nonsedimenting material was absent in complexes of DNA plus $AgNO_3$ [Table 3].)

Distributions of sedimentation coefficients of DNA, DNA plus $AgNO_3$, and DNA plus AgSu are shown in Fig. 2. The control DNA exhibited the usual (23) spread of sedimentation coefficients associated with mammalian DNA. Even though the addition of either AgSu or $AgNO_3$ caused increases in sedimentation coefficients ($S_{50\%}$), the distribution of sedimentation coefficients was not altered. This can be expressed numerically in terms of the spread of sedimentation coefficients (Table 4). This means that neither AgSu nor $AgNO_3$ reacted preferentially with DNA molecules of certain sizes (i.e., the DNA of various sizes present in native calf thymus DNA reacted equally well with $AgNO_3$ and AgSu, even though the extent of the reaction between DNA and $AgNO_3$ and AgSu varied [Table 2]). In confirmation of this conclusion, it was shown (Fig. 3) that DNA of low molecular weight (obtained by sonic treatment) also reacted with AgSu to the same extent as native (undegraded) DNA; moreover, although this DNA showed a shift to higher sedimentation coefficients (Fig. 3) upon addition of AgSu, it still exhibited the same distribution of sedimentation coefficients (Table 4). The sedimentation coefficients of high-molecular-weight DNA species (*Klebsiella* bacteriophage and *P. aeruginosa*) also increased upon addition of AgSu (Fig. 3). In these cases, too, there was no significant change in the distribution of sedimentation coefficients (Table 4).

These data indicate that DNA species of dissimilar molecular weights, different sources (mammalian, bacterial, and viral), and various base compositions (guanine plus cytosine contents of calf thymus, *Klebsiella* bacteriophage E-1, and

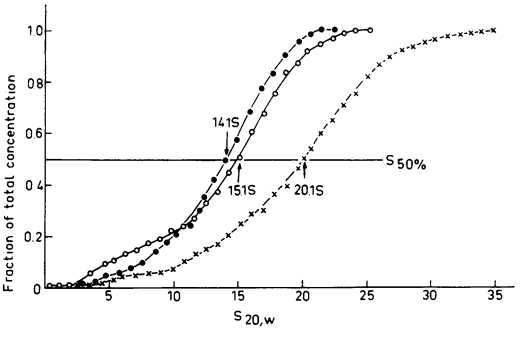

FIG. 2. *Distributions of sedimentation coefficients of DNA and silver DNA complexes.* (●) *Control DNA;* (○) *AgSu-DNA (AgSu/DNA-P = 1.0);* (×) *$AgNO_3$-DNA ($AgNO_3$/DNA-P = 1.0).*

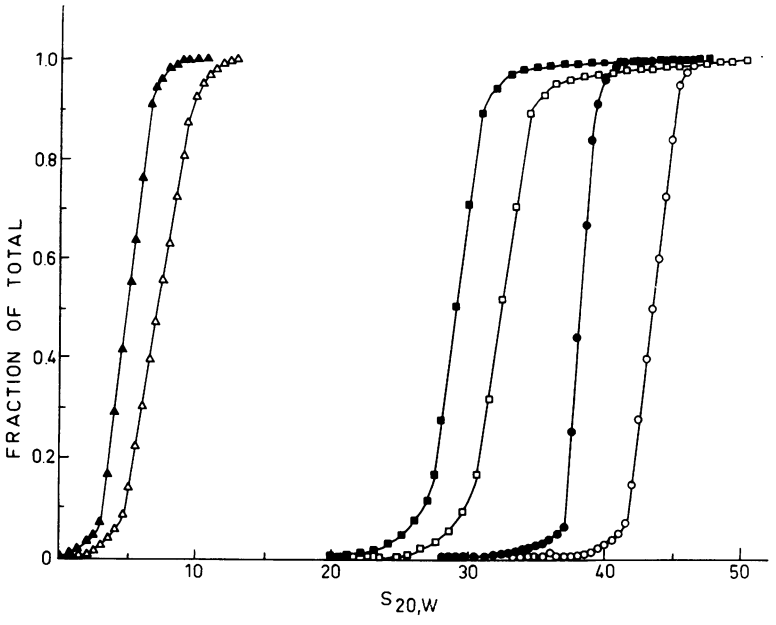

FIG. 3. *Effect of DNA size on complex formation—distribution of sedimentation coefficients. DNA specimens were mixed with AgSu (AgSu/DNA = 1.0) and analyzed in an ultracentrifuge 24 hr later. (▲ and △) Sonically treated calf thymus DNA before and after the addition of AgSu. (■ and □) DNA from Pseudomonas aeruginosa before and after addition of AgSu. (● and ○) DNA from Klebsiella phage before and after addition of AgSu.*

TABLE 4. *Heterogeneity of DNA and DNA-silver complexes*[a]

DNA	Addition	$S_{50\%}$	$S_{83\%}$	$\Delta S\sigma$	$\Delta S\sigma/S_{50\%}$
Calf thymus	None	14.1	17.6	3.5	0.25
	AgSu	15.1	18.8	3.7	0.25
	$AgNO_3$	20.1	25.2	5.1	0.25
Calf thymus, sonically treated	None	4.9	6.3	1.4	0.28
	AgSu	7.2	9.2	2.0	0.28
Pseudomonas aeruginosa	None	29	30.5	1.5	0.05
	AgSu	32.4	34.2	2.0	0.06
Klebsiella phage	None	38.2	39.0	0.8	0.02
	AgSu	43.6	44.9	1.3	0.03

[a] $S_{83\%}$ and $S_{50\%}$ are the cumulative sedimentation coefficients of 83% and 50% of the sedimenting species, respectively. The values are obtained from the 0.83 and 0.50 intercepts of Fig. 2 and 3. $\Delta S\sigma$ is the difference between $S_{83\%}$ and $S_{50\%}$. $\Delta S\sigma/S_{50\%}$ is a numerical expression of the heterogeneity.

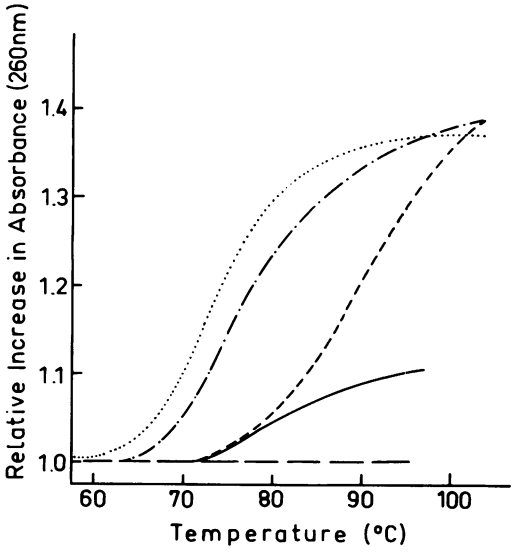

FIG. 4. *Effect of $AgNO_3$ on thermal-helix transition profile of calf thymus DNA. Calf thymus DNA (18 μg per ml of 0.005 M $NaNO_3$) was supplemented with increasing amounts of $AgNO_3$. Dotted line, control DNA; dash-dot line, $AgNO_3$/DNA-P = 0.02; short-dashed line $AgNO_3$/DNA-P = 0.21; solid line, $AgNO_3$/DNA-P = 0.5; long-dashed line, $AgNO_3$/DNA-P = 1.0.*

P. aeruginosa are 39, 47, and 67%, respectively) are all modified by the addition of AgSu.

Helix-to-coil thermal transitions. The presence of $AgNO_3$ had a profound effect on the thermal denaturation profile of calf thymus DNA (Fig. 4). Thus, low levels of $AgNO_3$, i.e., $AgNO_3$/DNA-P of 0.02 and 0.21, increased the T_m, the midpoint of the thermal transition curve, by 3.1 and 15.2 C, respectively. Under these conditions, however, complete denaturation was achieved, as evidenced by the extent of the hyperchromic shift (Fig. 4). At an $AgNO_3$/DNA-P ratio of 0.5, denaturation required higher temperatures and was incomplete even at 95 C. When the molarity of $AgNO_3$ equaled that of the nucleotides (i.e., $AgNO_3$/DNA-P = 1.0), denaturation was completely prevented (Fig. 4).

These effects of $AgNO_3$ on the thermal denaturation of DNA were reproducible, and they were independent of the period of incubation which preceded the measurement.

AgSu also had a profound effect on the thermal denaturation profile of DNA (Fig. 5). This effect depended upon AgSu concentration and was also influenced by the duration of the contact between DNA and AgSu (Fig. 5). As the time of incubation increased, the effect on the thermal denaturation profile became more pronounced. A similar effect was also observed by sedimentation velocity analysis (Table 3). However, unlike the effects of silver substances on the sedimentation behavior of DNA (Table 2), AgSu was almost as potent as $AgNO_3$ in modifying the "melting-out" behavior of DNA (compare Fig. 4 and 5).

Although AgSu-DNA complexes exhibited increased resistance to thermal denaturation, once denaturation occurred, it was irreversible, as illustrated by the data summarized in Fig. 6. This indicates that AgSu did not cross-link the DNA strands and thereby cause renaturation.

Cesium chloride density gradient centrifugation. Specimens of DNA, DNA plus AgSu, and DNA plus $AgNO_3$ were placed in CsCl and analyzed in an analytical ultracentrifuge by buoyant density gradient centrifugation. The tracings of the DNA bands at equilibrium are shown in Fig. 7. The behavior of the $AgNO_3$-DNA complex was similar to that of the control DNA (Fig. 7A and B). This presumably resulted from the presence of concentrated CsCl and dissociation of the $AgNO_3$-DNA complex owing to the formation of insoluble AgCl (14). However, the banding behavior of the DNA-AgSu complex (Fig. 7C) differed from that of the others both in position and in shape. This unexpected finding suggests that, whereas the $AgNO_3$-DNA complex is dissociated by CsCl, the AgSu-DNA complex is not. The increase in band width of the AgSu-DNA complex (Fig. 7C) indicates a heterogeneity of attachment sites and a possible preferential reaction of AgSu with certain species of DNA.

Cesium sulfate density gradient centrifugation. AgCl is insoluble, but Ag_2SO_4 is not; this has permitted the use of Cs_2SO_4 density gradient centrifugation for the analysis of the properties of $AgNO_3$-DNA complexes (14). It was thus found (Fig. 8) that the $AgNO_3$-DNA complex had a buoyant density higher than that of the control DNA; this confirms the earlier finding of Jensen and Davidson (14) and presumably reflects the increased molecular weight of the silver nucleate

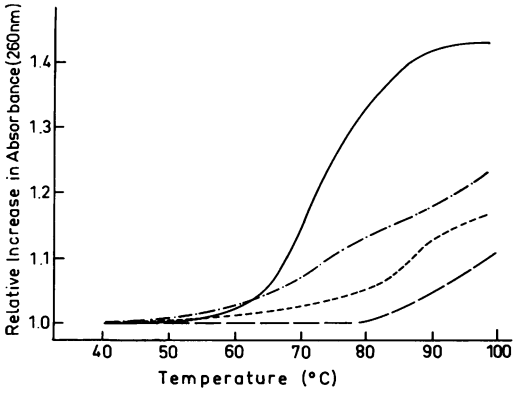

FIG. 5. *Effect of AgSu on the thermal helix-coil transition profile of DNA. Calf thymus DNA (18 μg per ml of 0.005 M $NaNO_3$) was supplemented with various amounts of AgSu. The solutions were incubated in the dark (4 C), and 2 and 24 hr later thermal denaturation profiles were determined. Solid line, control DNA at t = 2 and 24 hr; dash-dot line, AgSu/DNA-P = 0.5 at t = 2 hr; short-dashed line and long-dashed line, AgSu/DNA-P = 1.0 at t = 2 and 24 hr, respectively.*

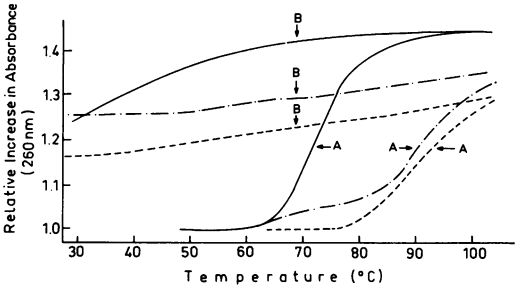

FIG. 6. *Effect of AgSu on second cycle of thermal denaturation. Upon completion of the first cycle of heating (curves A), the samples were gradually cooled to 20 C and subjected to a second heating cycle (curves B). Solid lines, control DNA; dash-dot line, AgSu/DNA-P = 0.5; dashed line, AgSu/DNA-P = 1.0.*

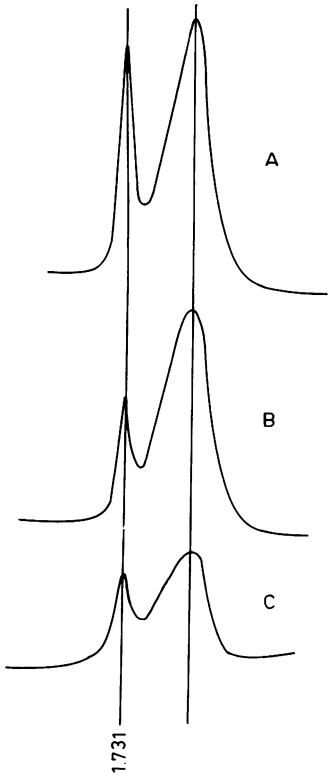

FIG. 7. *Buoyant densities in CsCl of (A) normal calf thymus DNA; (B) $AgNO_3$-DNA, $AgNO_3$/DNA-P = 1.0; and (C) AgSu-DNA, AgSu/DNA-P = 1.0. The band at the extreme left represents the position of the marker DNA (Micrococcus lysodeikticus DNA; 1.731 g/cm³).*

(3, 14, 33). The AgSu-DNA complex, on the other hand, showed a *decreased* buoyant density in Cs_2SO_4 (Fig. 8). Moreover, although the AgSu-DNA and $AgNO_3$-DNA complexes differed in their banding behavior, it should be noted that neither was dissociated by high salt concentrations (i.e., Cs_2SO_4), as evidenced by the differences between their banding behavior and that of the control DNA.

Zonal centrifugation in sucrose. The rate of migration of macromolecules in gradients of sucrose is a function of their molecular weights (22). Analyses of silver-DNA complexes in such gradients showed (Fig. 9) that the rate of sedimentation of the $AgNO_3$-DNA complex was far greater than that of unmodified DNA, as shown by the observation that, in the time it took for the control DNA to reach the middle of the gradient, the $AgNO_3$-DNA complex had reached the bot-

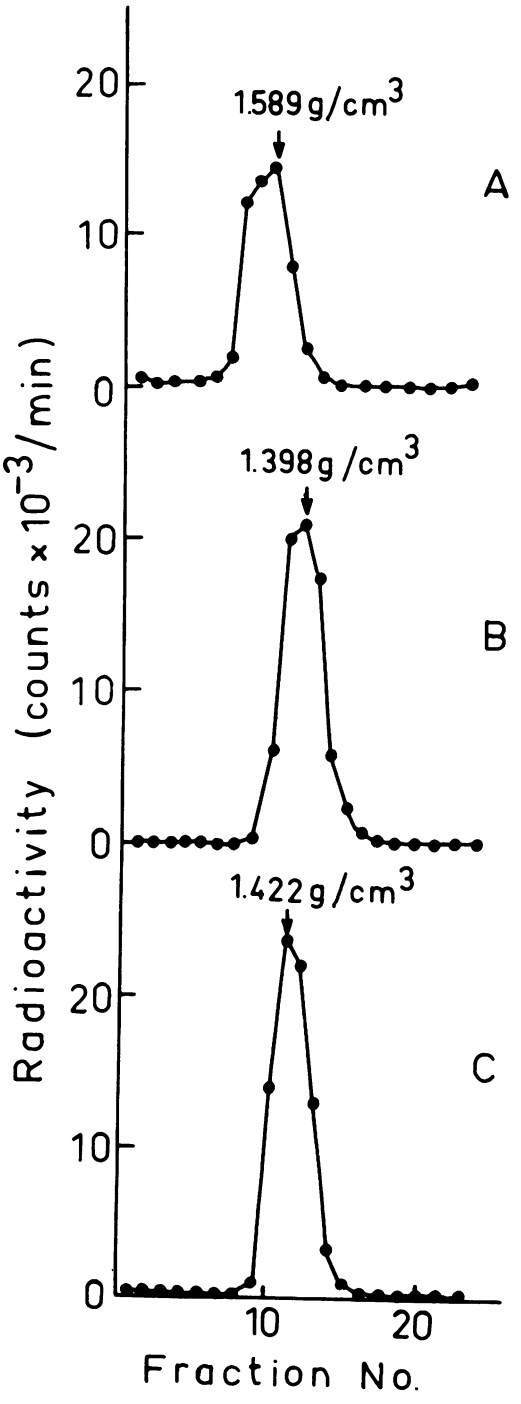

FIG. 8. *Banding properties of silver-DNA complexes in Cs_2SO_4. (A) $AgNO_3$-DNA ($AgNO_3$/DNA-P = 1.0); (B) AgSu-DNA (AgSu/DNA-P = 1.0); and (C) control ³H-labeled DNA from chicken embryos.*

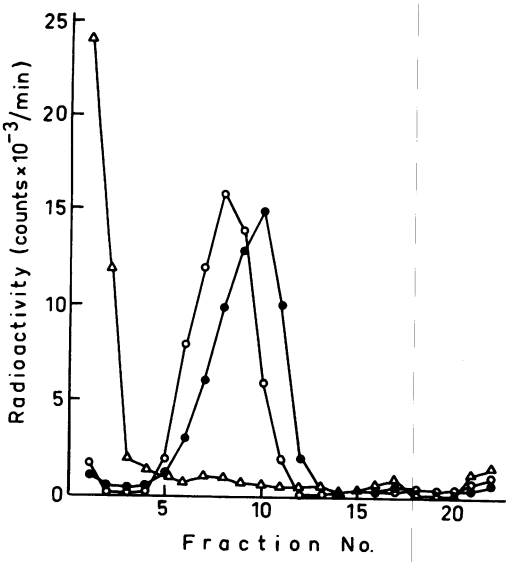

FIG. 9. *Zonal centrifugation in 5 to 20% sucrose gradients.* (●) *Control* ^{3}*H-DNA (chicken embryos);* (○) *AgSu-DNA (AgSu/DNA-P = 1.0); and* (△) *$AgNO_3$-DNA ($AgNO_3$/DNA-P = 1.0).*

tom of the tube. On the other hand, the rate of migration of the AgSu-DNA complex was only slightly, but reproducibly, faster than that of the control DNA (Fig. 9).

Effect of cyanide on silver-DNA complexes. Silver ions form soluble complexes with cyanide (32), and indeed it has been reported that $AgNO_3$-DNA complexes can be dissociated by cyanide (3, 14). This reversal of the $AgNO_3$-DNA complexes was confirmed in the present study: (i) KCN caused a decrease in the sedimentation coefficients of $AgNO_3$-DNA complexes (*unpublished data*) and (ii) addition of KCN to $AgNO_3$-DNA complexes caused the thermal helix-coil transition profiles to become indistinguishable from that of untreated DNA (*unpublished data*). However, the effect of KCN on AgSu-DNA complexes could not be studied by this technique because KCN forms strongly ultraviolet-absorbing complexes with both AgSu and NaSu (*unpublished data*). To overcome this effect of KCN, advantage was taken of the availability of ^{3}H-labeled DNA. It was thus shown that addition of KCN to $AgNO_3$-DNA restored the rate of sedimentation in a sucrose gradient to a level similar to that of the unmodified DNA (Fig. 10B); i.e., the complex dissociated. On the other hand, addition of KCN to the AgSu-DNA complex did not result in dissociation of the complex (Fig. 10A).

Effect of NaSu in DNA. The addition of NaSu to DNA did not significantly affect the sedimentation behavior of calf thymus DNA (Table 5). An analysis of the sedimentation boundaries did not reveal any NaSu co-sedimenting with the DNA.

Although NaSu had no effect on the sedimentation behavior of DNA, it greatly influenced the thermal helix-coil transition profile (Fig. 11). This effect was not due to a contribution of NaSu to the ionic strength of the solution as an equivalent amount of $NaNO_3$ or NaCl had no such effect. Dialysis of NaSu-DNA mixtures against 0.005 M $NaNO_3$ completely reversed this effect of NaSu. (*Bound* AgSu was not removable by dialysis [see below].)

Exposure of DNA to mixtures of NaSu and AgSu revealed that NaSu competed with AgSu for the polydeoxynucleotide. Thus, the maximal effect of AgSu on the sedimentation coefficient of DNA was inhibited by the simultaneous presence of NaSu (Table 5). On the other hand, addition of NaSu after the formation of the AgSu-DNA complex was without effect on the sedimentation coefficient. Thus, the 1:1 *Klebsiella* phage DNA-AgSu complex had a sedimentation coefficient of 43.6*S*. When NaSu (NaSu/DNA-P = 1.0) was added after the formation of this complex (t = 24 hr) and the mixture was incubated for another 24

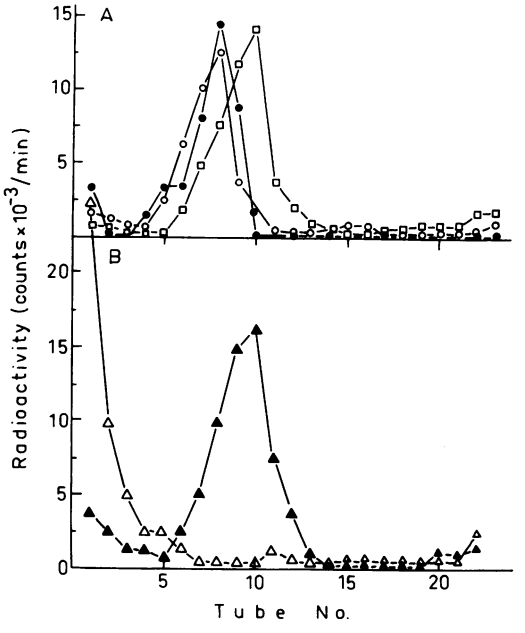

FIG. 10. *Effect of KCN on sedimentation behavior of silver-DNA complexes in gradients of sucrose. A:* (□) *Control* ^{3}*H-labeled DNA (chicken embryos);* (○) *AgSu-DNA (AgSu/DNA-P = 1.0);* (●) *AgSu-DNA + KCN. B:* (△) *$AgNO_3$-DNA ($AgNO_3$/DNA-P = 1.0);* (▲) *$AgNO_3$-DNA + KCN.*

TABLE 5. *Competition for DNA binding between AgSu and NaSu*

Expt	DNA	Addition	AgSu/DNA-P	NaSu/DNA-P	Sedimentation coefficient
I	Calf thymus	None	—	—	11.3*S*
		AgSu	0.5	—	15.1*S*
		NaSu		0.5	11.9*S*
II	Calf thymus	None	—	—	13.3*S*
		AgSu	0.5	—	14.5*S*
		NaSu	—	0.5	13.2*S*
		AgSu + NaSu	0.5	0.5	13.8*S*
		$AgNO_3$	0.5	—	19.7*S*
		$AgNO_3$ + NaSu[a]	0.5	0.5	16.0*S*
III	*Klebsiella* phage	None	—	—	38.2*S*
		AgSu	1.0	—	43.6*S*
		NaSu	—	1.0	38.0*S*
		AgSu + NaSu	1.0	0.1	43.1*S*
		AgSu + NaSu	1.0	0.2	42.7*S*
		AgSu + NaSu	1.0	0.5	40.6*S*
		AgSu + NaSu	1.0	1.0	39.4*S*

[a] AgSu was formed by reaction between $AgNO_3$ and NaSu.

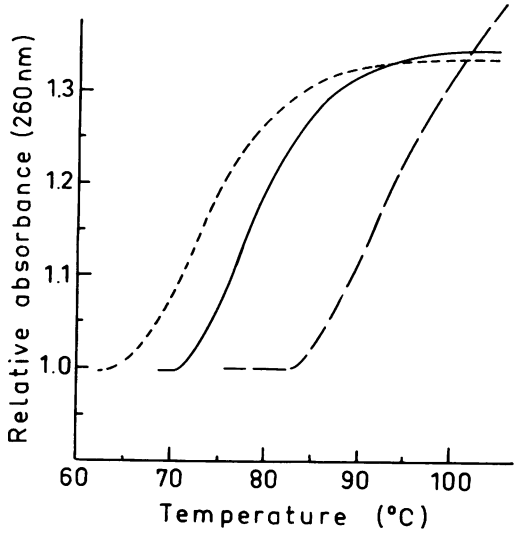

FIG. 11. *Effect of sodium sulfadiazine (NaSu) on the thermal helix-coil transition of calf thymus DNA. Short-dashed line, control DNA; solid line, NaSu + DNA (NaSu/DNA-P = 0.5); long-dashed line, NaSu + DNA (NaSu/DNA-P = 1.0).*

hr, the sedimentation coefficient was found to be 43.4*S* (i.e., unchanged within experimental error).

Effect of NaCl on silver-DNA complexes. The AgSu-DNA complex appears to be undissociated by either CsCl or Cs_2SO_4 as determined by buoyant density centrifugation (see Fig. 7 and 8). The above results with NaSu suggest, however, that, in addition to this "irreversible" binding, a weak interaction involving the sulfadiazine moiety was also playing a role in the binding of AgSu to DNA. Such a weak interaction could conceivably be reversed by salt.

The data of Table 6 summarize the effects on the sedimentation coefficients of the addition of NaCl (0.2 M). NaCl lowered the sedimentation of both silver-DNA complexes. In the case of the $AgNO_3$-DNA, it was ascertained that this was due to the formation of insoluble AgCl (i.e., dissociation of the complex had occurred). On the other hand, analysis of the sedimenting boundaries of the AgSu-DNA complex containing NaCl revealed that the amount of AgSu co-sedimenting with the DNA remained unchanged (i.e., there was no increase in the amount of nonsedimenting ultraviolet-absorbing material; see Table 3). This suggested that even in the presence of NaCl, the AgSu was still bound to the DNA. When the NaCl was removed by dialysis and *no* further AgSu was added, the sedimentation coefficient increased once more (Table 6); moreover, *unbound* AgSu had been removed (Table 6).

AgSu and intercalating agents. Deoxyribonucleates form complexes with ethidium bromide, chloroquine, and proflavine; in each of these instances, there is intercalation of the chemical between adjacent base pairs. These interactions with DNA can be detected in the spectra of these substances (1, 16–18). It was found that even excessive amounts of AgSu (AgSu/DNA-P = 1.0) did not interfere in the formation of complexes between DNA and ethidium bromide, proflavine, and chloroquine (*unpublished data*).

TABLE 6. *Effect of NaCl addition on the sedimentation behavior of silver DNA complexes*[a]

Prepn	No additions		NaCl added		Subsequent dialysis	
	Sedimentation coefficient	Percent non-sedimenting	Sedimentation coefficient	Percent non-sedimenting	Sedimentation coefficient	Percent non sedimenting
Control DNA	13.3*S*	0	13.2*S*	0	13.2*S*	0
$AgNO_3$-DNA	19.7*S*	0	15.2*S*	0	13.8*S*	0
AgSu-DNA	14.5*S*	54	13.2*S*	56	15.0*S*	0
AgSu (alone)	0	100	0	100	0	0

[a] DNA-silver complexes (silver/DNA-P = 1.0) were prepared, and portions of each were supplemented with NaCl (final concentration, 0.2 M). Samples were then analyzed in an ultracentrifuge. Portions of the specimens containing NaCl were dialyzed extensively against 0.005 M $NaNO_3$ and analyzed once more in an ultracentrifuge. In each case, the amount of nonsedimentable material was determined.

DISCUSSION

The antibacterial effectiveness of AgSu has been attributed (10) to its ability to penetrate into the bacterial cell wherein it dissociates, thereby allowing the silver ion to interfere with the base pairs of DNA in a manner similar to the one shown to occur in vitro between DNA and $AgNO_3$ (3, 14, 33). In the present study, it is shown that AgSu interacts with isolated DNA but that the product is different in all respects from that obtained when $AgNO_3$ is added to DNA. It would appear that one of the foremost differences between $AgNO_3$ and AgSu is the inability of AgSu to ionize. (Thus, addition of NaCl to AgSu does not result in formation of insoluble AgCl.) AgSu must thus be viewed as a nondissociable molecule.

It is shown that AgSu reacts with DNA species of various sizes (4.9 to 38.2*S*), sources (mammalian, avian, bacterial, and viral), and base compositions (47 to 67% guanine plus cytosine).

The sedimentation coefficients of macromolecules are determined by size and shape, whereas rates of migration in gradients of sucrose have been equated with size (22). $AgNO_3$-DNA complexes show great increases in sedimentation coefficients and in rates of sedimentation in sucrose, which suggests that they possess vastly increased molecular weights. AgSu-DNA complexes also display increased sedimentation coefficients, but their rates of migration in sucrose gradients are nearly identical to that of unmodified DNA. These facts can be interpreted as signifying that the main effect of AgSu on DNA involves an alteration in shape with a minimal effect on the molecular weight. This is in agreement with the observation that even when the $AgNO_3$ concentration greatly exceeds the DNA-phosphorus ratio (>200:1) the sedimentation coefficient still increases (Table 2). On the other hand, AgSu exerts its maximal effect at an AgSu/DNA-P ratio of 0.5, and even at that concentration a large proportion of the AgSu remains unbound.

The observation that unlike $AgNO_3$-DNA, AgSu-DNA is not dissociated by NaCl, CsCl, and KCN may be a result of the lack of ionization of the AgSu molecule, or it may indicate that the AgSu to DNA bond is very tight. The fact that the complex is not dissociated by dialysis against NaCl suggests the second possibility. The observation that after dialysis of the AgSu-DNA complex the amount of nonsedimentable material, i.e., unbound AgSu, was eliminated (Table 6) indicates that the dialysis procedure was efficient and that the "real" amount of AgSu bound is much smaller than the amount added. Moreover, the temporary return, in the presence of NaCl, to a lower sedimentation coefficient may indicate that the shape of the molecule was restored even while AgSu was still attached. This could be due to "swinging out" of the sulfadiazine moiety while silver was still attached (see below), thereby leading to a temporary restoration of shape.

The most puzzling observation is that of a weak association between NaSu and DNA. This association appears to be involved also in the reaction between AgSu and DNA, as AgSu and NaSu appear to compete for the DNA. On the other hand, if AgSu is added first, NaSu no longer competes with it. This leads to the suggestion that the reaction between AgSu and DNA proceeds in two steps: a weak interaction between DNA and the sulfadiazine moiety, followed by a strong binding between the silver atom and the DNA. It is possible that the sulfadiazine moiety puts the AgSu molecule in place so that the second step can take place.

Concerning the nature of these reactions, conceivably the interaction between the sulfadiazine moiety and the DNA could involve an intercalation: indeed, the spectral hyperchromicity (Fig. 1) and the decreased buoyant density (Fig. 8) support such a suggestion (15, 16, 19, 29). The nature of this hypothetical intercalation remains to be elucidated. AgSu does not compete with pro-

flavine, ethidium bromide, or chloroquine, which may indicate either that a different sort of intercalation is involved or that the binding between DNA and these dyes is much more efficient than between it and AgSu. This last possibility is reinforced by the fact that the binding between DNA and AgSu is a slow process and that in reality only a few molecules of AgSu are bound per DNA molecule, even when the ratio of added AgSu to DNA-P is 1.0.

It has been shown that the shift in the absorption maximum that occurs when $AgNO_3$ is added to DNA is due to binding of the Ag^+ to base-pairs. It is interesting, therefore, that the AgSu-DNA complex also exhibits this shift (Fig. 1), and by analogy it can be suggested that the same bonds are involved in the binding of AgSu to DNA. If this is true, then it can be visualized that the binding of AgSu involves (i) intercalation between base pairs followed by (ii) a strong binding between AgSu and base pairs. Since the binding of silver is strongest for guanine-cytosine base pairs (3, 14), this in effect would make AgSu an intercalator with a preference for GC group base pairs.

In the present study, it was shown that AgSu was capable of binding to DNA species of different base compositions but, owing to the difficulty in assessing exact binding, it was not possible to determine whether the interaction was greater for some preparations than for others. However, the finding that after addition of AgSu to calf thymus DNA there was an increased banding heterogeneity (in CsCl) suggests that there are some molecules that are preferential substrates for AgSu. The recent availability of radioactive AgSu should permit a better determination of the "binding constant" as well as the role of base composition in the binding. In addition, the nature of the binding (intercalation) may also be revealed by use of radioactive AgSu. Such studies are proceeding in this laboratory.

It is interesting as well as important to note that none of the interactions that have been detected in the present study appear to be determinants of the biological activity of AgSu. Studies on the antibacterial action of AgSu are presented in the accompanying report (25; also Coward, Carr, and Rosenkranz, *in preparation*).

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