

The Interaction of Calf Thymus DNA with Mercuric Acetate and 3,6-Bis-(acetatomercurimethyl)-dioxane. Small-Angle X-Ray Scattering and Viscosity Studies

Peter Zipper, Gertraud Ribitsch, and Josef Schurz

Institut für Physikalische Chemie, Universität Graz, Heinrichstraße 28, A-8010 Graz, Austria

and

Hans Bünemann

Institut für Genetik, Universität Düsseldorf, Universitätsstraße 1, D-4000 Düsseldorf, Bundesrepublik Deutschland

Z. Naturforsch. 37 c, 824–832 (1982); received February 11/May 17, 1982

Dedicated to Prof. O. Kratky on the Occasion of His 80th Birthday

DNA, Interaction with Mercurials, Small-Angle X-Ray Scattering, Viscosity, Structural Changes

The binding of Hg^{2+} and 3,6-bis-(acetatomercurimethyl)-dioxane (BAMD) to sonicated calf thymus DNA was studied by small-angle X-ray scattering and viscosity measurements. The scattering experiments with DNA complexed by different amounts of mercurials (for Hg^{2+} $r_b = 0-0.79$, for BAMD $r_b = 0-0.86$ mol of mercurial bound per mol of base pairs) established that the rod-like character of the DNA molecules is maintained up to high binding ratios. They revealed further a steady decrease of the cross-section radius of gyration R_c for the $\text{DNA} \cdot \text{Hg}^{2+}$ complex and a similar decrease of R_c for the $\text{DNA} \cdot \text{BAMD}$ complex up to $r_b = 0.35$. This behaviour is certainly caused by the incorporation of both mercurials near the axis of the DNA helix. Binding of BAMD at $r_b > 0.35$ led to an increase of R_c which behaviour obviously reflects the location of mercury atoms at large distances from the axis, possibly on the surface of the helix. The increase of the mass per unit length M_c upon binding of the mercurials was found to be much higher than expected. This finding established that the length of the DNA helix decreases by 0.10 ± 0.01 nm per bound mercurial at low binding ratios (*i.e.* up to $r_b = 1/3$ for BAMD, up to possibly $r_b = 0.5$ for Hg^{2+}). A similar conclusion was also drawn from the observed decrease of intrinsic viscosity $[\eta]$ with increasing r_b . The analysis of M_c at high binding ratios suggests that every BAMD molecule bound beyond $r_b = 1/3$ decreases the length of the DNA by 0.21 ± 0.05 nm whereas Hg^{2+} when bound beyond $r_b = 0.5$ causes no change of the length.

Introduction

Mercury as Hg^{2+} or as CH_3Hg^+ binds to native and to denatured DNA with a remarkable specificity for AT-rich polynucleotides [1]. The binding process is cooperative and results in a disturbance of the structure of the helix.

Recently, the binding of 3,6-bis-(acetatomercurimethyl)-dioxane (BAMD) and of 4,5-dibromo-2,7-di-(acetatomercuri)-fluorescein (DDMF) to DNAs of different base composition was also studied [2, 3]. Both substances bind also cooperatively to native DNA, but binding is by more than one order of magnitude weaker as compared to Hg^{2+} . However, DDMF shows the same and BAMD shows even an enhanced specificity for AT-base pairs. The

AT-specificity of Hg^{2+} and of BAMD can be used for the separation of DNA species of different base composition [4–6].

Hg^{2+} as well as BAMD form intramolecular complexes only [7]. Hg^{2+} is assumed to be able to form both interstrand and intrastrand crosslinks between thymine residues [8, 9]. The classical CD spectrum of B-form DNA is practically reversed by binding of Hg^{2+} [10]. The CD spectrum of $\text{DNA} \cdot \text{Hg}^{2+}$ complexes resembles that of DNA in organic polymer solutions [11] or even that of DNA with left-handed Z-structures [12–14]. When at least 0.5Hg^{2+} ions are bound per base pair, $\text{DNA} \cdot \text{Hg}^{2+}$ complexes do not show any melting transition (A. Walter, unpublished results). Under the same conditions, $\text{DNA} \cdot \text{BAMD}$ only shows a small continuous increase of absorption at 260 nm upon heating to 80°C (unpublished results). Generally, the changes in the UV spectrum observed upon addition of BAMD to DNA solutions

resemble those observed after addition of Hg^{2+} ; however, the helical structure seems to be disturbed by BAMD much more than by Hg^{2+} [2].

In order to get further insight into the binding of Hg^{2+} and BAMD to native DNA and to compare the structural changes induced by the binding of these mercurials, we performed small-angle X-ray scattering (SAXS) and viscosity measurements with DNA and with DNA · Hg^{2+} and DNA · BAMD complexes. Due to the use of low molecular weight DNA for this study, the viscosity measurements were expected to yield direct information about changes of the overall shape and size of the rodlike DNA molecules. The SAXS method has already been used successfully for the investigation of complexes between DNA and actinomycin or actinomine [15]. Some preliminary results of the present study have been reported elsewhere [16, 17].

Materials and Methods

Preparation of DNA and DNA complexes

Mercuric acetate was purchased from Merck; 3,6-bis-(acetatomercurimethyl)-dioxane (BAMD) was prepared from allyl alcohol and mercury (II) oxide and recrystallized from glacial acetic acid [18]. Calf thymus DNA, type I from Sigma Chemical Company, was dissolved in 0.2 M NaCl, 0.01 M sodium phosphate buffer (pH 7.1) and purified by several phenol extractions. Low molecular weight DNA (M_r about 140 000) was obtained from purified DNA by effective sonic degradation [19] at ice bath temperature for a total of 30 min of sonification with a MSE ultrasonicator. The DNA samples were clarified by centrifugation and dialyzed in visking dialysis tubes (Serva) several times against a large volume of buffer containing 0.02 M sodium acetate, 0.1 M sodium perchlorate (pH 7.4) for preparation of mercuric acetate complexes or against 0.1 M sodium sulfate, 0.005 M sodium tetraborate (pH 9.1) for measurements with BAMD. The molecular weight of DNA was calculated from sedimentation coefficient or intrinsic viscosity [20].

Complexes of DNA and mercuric acetate or BAMD were formed by equilibrium dialysis to guarantee a uniform complex formation. For a particular experiment 5 ml samples were filled into pretreated dialysis tubes (*cf.* [2]) and dialyzed separately in closed glass tubes against 20 ml of buffer

containing the calculated amount of mercuric acetate or BAMD respectively. The buffer was stirred by a magnetic stirrer. The amount of mercuric acetate or BAMD bound to DNA was calculated from dithizone measurements at 490 nm as described earlier [2]. 8.33×10^{-8} mol of BAMD and 6.11×10^{-8} mol of Hg^{2+} were equivalent to a difference of absorbance of 1.00 (measured at 490 nm in 1 cm cells) if samples of 3 ml of experimental solution were used. The DNA concentration was calculated by using the molar absorption coefficient $\epsilon_{260}^{260} = 6540 \text{ M}^{-1} \text{ cm}^{-1}$ [21]. It ranged from 6.7 mg/ml to 9.6 mg/ml for the different samples.

Small-angle X-ray scattering

Small-angle X-ray scattering (SAXS) experiments were performed by means of a Kratky camera [22] similarly as in a previous study on DNA ([15] and references therein). Measurements were taken at 4 °C in an angular range from 2.5 to 125 mrad.

To avoid a noxious influence of radiation damages, a frequent exchange of the samples turned out to be essential especially in the case of the DNA · BAMD complexes. The evaluation of the experimental data was performed by means of several computer programs [23]. Each scattering curve was plotted as $\log I(2\theta)$ vs. $(2\theta)^2$; the approximation of the data in this cross-section Guinier plot by a straight line yielded the radius of gyration of the cross-section, R_c , and the mass per unit length, M_c [24, 25]. This parameter was obtained by applying the multicomponent formalism described previously ([15]; *cf.* also Density Measurements).

Density measurements

The apparent isopotential specific volumes Φ'_2 [26] of unliganded DNA and of the various complexes were determined by means of density measurements by using a digital density meter DMA [27]. The measurements were performed at the same temperature as the SAXS measurements. As measurements on independently prepared series of solutions yielded slightly different results, possibly because of differences in the preparation procedure, buffer composition, and/or concentration standards, slightly different Φ'_2 values were used for the evaluation of the various SAXS measurements.

To estimate the apparent specific volumes Φ'_L of the ligands, the experimental Φ'_2 values were ana-

lyzed according to the linear relation

$$(\Phi'_2)_r (1 + x) = (\Phi'_2)_o + \Phi'_L x.$$

The subscripts o and r refer to unliganded DNA and to a complex of binding ratio r_b , and x is related to the molar mass M_L of one ligand and to the molar mass M_{NP} of one nucleotide pair by $x = r_b M_L / M_{NP}$. Some typical results of such an analysis are the following: $(\Phi'_2)_o = 0.537 \text{ cm}^3 \text{ g}^{-1}$, $\Phi'_L = 0.062 \text{ cm}^3 \text{ g}^{-1}$ for Hg^{2+} , and $\Phi'_L = 0.307 \text{ cm}^3 \text{ g}^{-1}$ for BAMD. The Φ'_L were only used for the estimation of the excess electrons of the ligands in the analysis of the cross-section radii of gyration.

Viscosity measurements

Viscometric studies were performed by means of a micro Ubbelohde viscometer type Ic at 25 °C. Measurements were performed at several concentrations, the intrinsic viscosity $[\eta]$ was obtained by extrapolation of the reduced viscosities to zero concentration. An extrapolation to zero shear rate was not performed because a pilot experiment with a sample of unliganded DNA showed almost no variation of $[\eta]$ with the shear rate.

Results and Discussion

1. Small-angle X-ray scattering

Some typical cross-section Guinier plots for unliganded DNA and for $\text{DNA} \cdot \text{Hg}^{2+}$ and $\text{DNA} \cdot \text{BAMD}$ complexes at several binding ratios r_b (r_b = mol of mercurial bound per mol of base pairs) are shown in Fig. 1. The curves 1–6 for the unliganded DNA, for the $\text{DNA} \cdot \text{Hg}^{2+}$ complexes and for the $\text{DNA} \cdot \text{BAMD}$ complexes up to $r_b = 0.567$ are of similar shape except for the different slopes. The linear course over a wide angular range is characteristic of rodlike particles with uniform cross-section. The curve for the $\text{DNA} \cdot \text{BAMD}$ complex with $r_b = 0.861$ (curve 7) is at smaller angles considerably steeper than the other curves, but its slope decreases towards larger angles. The linear course at small angles suggests that this curve too reflects the presence of rodlike particles. The cross-section of these particles is possibly non-uniform or inhomogeneous as may be concluded from the shape of the curve.

The analysis of the scattering curves for the unliganded DNA established a value of $R_c = 0.845 \text{ nm}$ for the radius of gyration of the cross-section of the

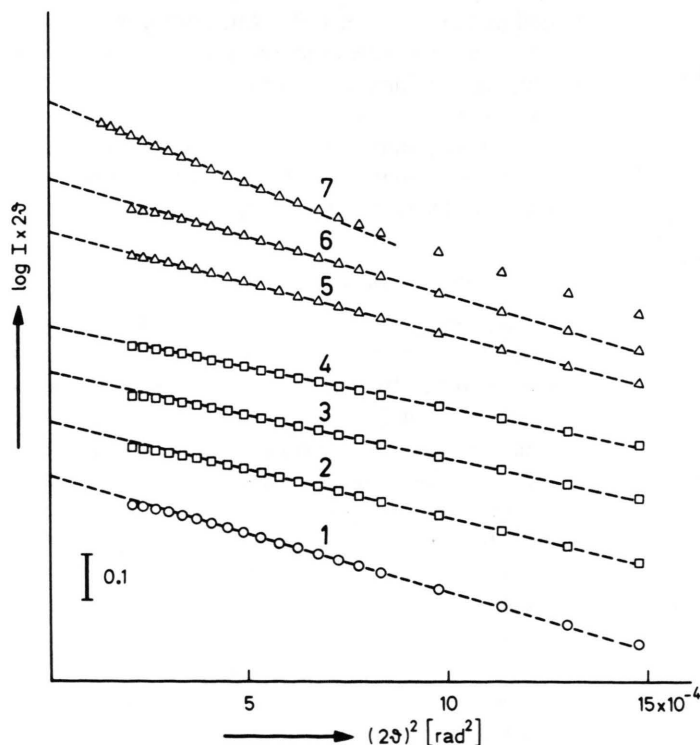


Fig. 1. Cross-section Guinier plots of selected scattering curves for unliganded DNA ($\circ$, curve 1) and $\text{DNA} \cdot \text{Hg}^{2+}$ ($\square$, curves 2–4) and $\text{DNA} \cdot \text{BAMD}$ ($\triangle$, curves 5–7) complexes. The binding ratios r_b (= mol of mercurial bound per mol of base pairs) are: 2 $r_b = 0.290$; 3 $r_b = 0.610$; 4 $r_b = 0.791$; 5 $r_b = 0.262$; 6 $r_b = 0.567$; 7 $r_b = 0.861$.

Table I. Molecular parameters R_c , M_c , l , $[\eta]$ and L for unliganded DNA and for DNA · Hg²⁺ and DNA · BAMD complexes

	r_b	R_c [nm]	M_c [g mol ⁻¹ nm ⁻¹]	l [nm]	$[\eta]$ [ml g ⁻¹]	L [nm]
unliganded DNA	0	0.845	1947	0.340		
DNA · Hg ²⁺	0.043	0.826				
	0.087	0.847	2008	0.338		
	0.182	0.773	2171	0.318		
	0.248	0.793	2308	0.308		
	0.275	0.796	2313	0.310		
	0.290	0.768	2281	0.316		
	0.610	0.719	2652	0.295		
	0.791	0.714	2848	0.288		
	0.036	0.847	(2235) ^a		70 ^b	62
	0.073				64.7	61
	0.127	0.819	(2289)		58.1	60
	0.192	0.811	(2489)		55	58
	0.221	0.797	(2649)		51.4	57
	0.278	0.789	(2764)		52	56
DNA · BAMD	0.050	0.804	2029	0.339	84	69
	0.114	0.789	2177	0.331	84	67
	0.153	0.774	2319	0.319	72	66
	0.262	0.800	2582	0.309		
	0.264	0.774	2462	0.324	66	62
	0.304	0.773	2602	0.314	54	61
	0.354	0.758	2824	0.299	46	60
	0.567	0.850	3822	0.250		
	0.861	0.990	5114	0.216		

^a The M_c values for this series are probably affected by a systematic error; for the corresponding unliganded DNA, $M_c = 2228 \text{ g} \cdot \text{mol}^{-1} \cdot \text{nm}^{-1}$.

^b For the corresponding unliganded DNA, $[\eta] = 73.5 \text{ ml} \cdot \text{g}^{-1}$.

DNA double helix and a value of $M_c = 1947 \text{ g mol}^{-1} \text{ nm}^{-1}$ for its mass per unit length. The values for both parameters are in good agreement with the results from previous investigations on DNA [15]. According to the mean mass of one nucleotide pair, $M_{NP} = 661.9 \text{ g mol}^{-1}$, the experimental M_c corresponds to a length increment of the DNA helix of $l = 0.340 \text{ nm}$ per nucleotide pair. This result is consistent with the B form of DNA. The value for R_c is a measure for the overall cross-section of the DNA double helix plus a shell of counterions (*cf.* [28, 15]).

As can be seen from the data listed in Table I, the formation of complexes between DNA and mercurials leads to significant changes of the parameters R_c and M_c . This behaviour is not surprising because the occurrence of changes of R_c and M_c upon ligand binding can be expected from the attachment of additional masses to DNA in any case, even when no structural changes of DNA should accompany the binding process.

The dependence of R_c on the amount of Hg²⁺ ions or BAMD residues bound to DNA is shown graphically in Fig. 2. As can be seen, the binding of Hg²⁺

causes a decrease of R_c in any case. At a binding ratio of about 0.8 Hg²⁺ ions per nucleotide pair the decrease of R_c amounts to about 0.13 nm or more than 15% as compared to the R_c value for the unliganded DNA. The formation of the DNA · BAMD complex, on the other hand, leads to a decrease of R_c only at low binding ratios. At $r_b = 0.354$, the decrease of R_c amounts to nearly 0.09 nm. A further increase of r_b , however, causes an increase of R_c , eventually even above the value for the unliganded DNA.

The observed decrease of R_c upon binding of mercurials to DNA does not necessarily reflect an actual decrease of the DNA cross-section. This can be concluded from a thorough analysis of the experimental R_c values by means of a procedure established previously [15]. If one assumes that for each ligand bound to DNA a number of n nucleotide pairs are structurally influenced by the ligand, while all other nucleotide pairs are unaffected, then the following equation

$$(R_c)_r^2 = \frac{Z_{NP} (R_c)_0^2 (1 - r_b n) + r_b (R_c)_b^2 (Z_{NP} n + Z_L)}{Z_{NP} + r_b Z_L}$$

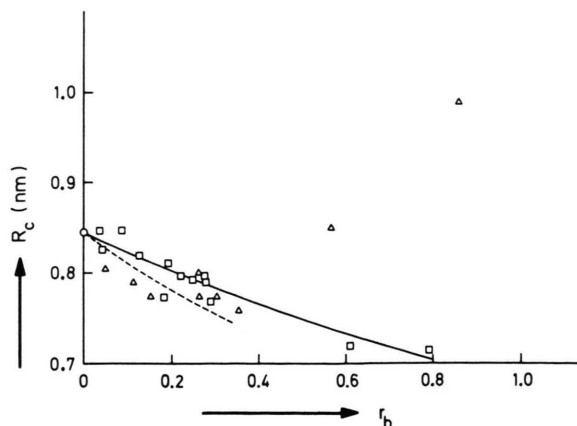


Fig. 2. Plot of the cross-section radius of gyration R_c versus the binding ratio r_b for DNA · Hg²⁺ (□) and DNA · BAMD (Δ) complexes. The solid and dashed curves have been calculated for models as described in the text.

relates the average cross-section radius of gyration (R_c)_r at a given r_b to the cross-section radius of gyration (R_c)₀ of unliganded DNA and to the cross-section radius of gyration (R_c)_b of a DNA segment consisting of n nucleotide pairs and the bound ligand. In the above formula Z_{NP} and Z_L designate the number of excess electrons of one nucleotide pair and of one ligand, respectively. By using the appropriate specific volumes (*cf.* Materials and Methods), Z_{NP} was calculated as 141, and Z_L was estimated to be about 72 for the Hg²⁺ ion and about 133 for the BAMD residue.

The absence of an obvious change in the dependence of R_c for the DNA · Hg²⁺ complex on r_b at high binding ratios suggests $n = 1$ for this complex. On this basis, a value of (R_c)_b = 0.678 ± 0.026 nm follows from the R_c values given in Table I (*cf.* also the corresponding curve drawn through the points in Fig. 2). This value corresponds to the cross-section radius of gyration for a DNA · Hg²⁺ complex with $r_b = 1$. It agrees within the limits of error with the theoretical value of $R_c = 0.688$ nm that can be calculated on the assumption that one Hg²⁺ per nucleotide pair is bound in the helix axis without any change of the DNA cross-section.

The obvious change in the dependence of R_c for the DNA · BAMD complex on r_b at binding ratios above 0.354 suggests $n = 3$ for this complex. With this assumption, the analysis of the experimental R_c values (*cf.* Table I) yields (R_c)_b = 0.746 ± 0.037 nm. This value corresponds to the cross-section radius of gyration for a DNA · BAMD complex with $r_b = 1/3$.

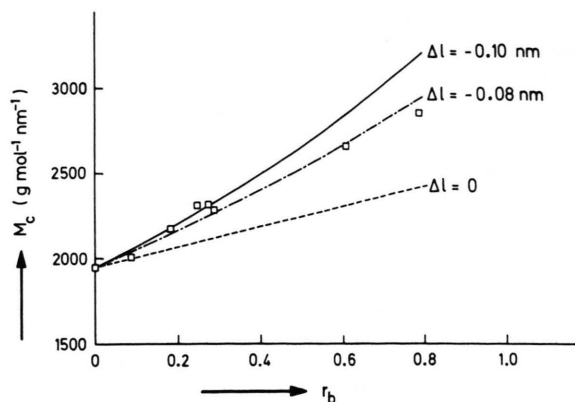


Fig. 3. Plot of the mass per unit length M_c of DNA · Hg²⁺ complexes versus r_b . The lines have been calculated on the assumption of the following changes of the length increment l upon ligand binding: (—) $\Delta l = -0.10$ nm; (---) $\Delta l = -0.08$ nm; (····) $\Delta l = 0$.

The theoretical cross-section radius of gyration of such a complex would amount to 0.737 nm when all mercury atoms of bound BAMD are placed in the helix axis of B-form DNA.

The observed increase of R_c at high binding ratios of BAMD could indicate a structural change of DNA leading to an enhancement of the DNA diameter, but it could as well reflect a change in the binding mechanism for BAMD by which mercury atoms would be placed in positions on the surface of the DNA or at still larger distances from the helix axis. Possibly both effects contribute simultaneously to the increase of R_c . A simple estimation shows that, without any change of the DNA cross-section, the experimental R_c value at $r_b = 0.861$ could only be achieved when either all mercury atoms are placed at a radial distance of about 1.15 nm from the helix axis or when the presence of mercury atoms at smaller radial distances is compensated by the location of other mercury atoms at significantly larger distances. For instance, the location of one BAMD per three base pairs in the helix axis would have to be compensated by the location of the mercury atoms of all further bound BAMD molecules at radial distances of about 1.35 to 1.45 nm.

The dependence of the mass per unit length of the DNA · Hg²⁺ and DNA · BAMD complexes on r_b is shown in Figs. 3 and 4. As the figures convincingly demonstrate for both kinds of complexes, the actual increase of M_c with r_b exceeds by far the increase which can be expected for a mere binding of the ligands. Since the observed decrease of R_c rules out a

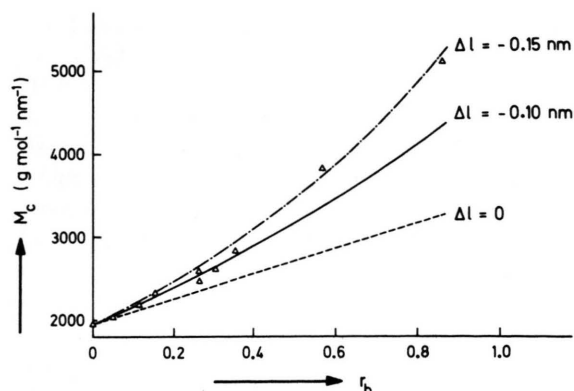


Fig. 4. Plot of the mass per unit length M_c of DNA · BAMD complexes versus r_b . The lines have been calculated on the assumption of the following changes of the length increment l upon ligand binding: (---) $\Delta l = 0$; (—) $\Delta l = -0.10$ nm; (- · - · -) $\Delta l = -0.15$ nm.

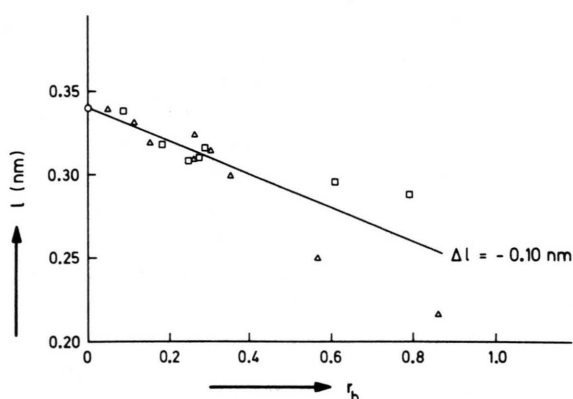


Fig. 5. Plot of the length increment l versus r_b for DNA · Hg²⁺ (□) and DNA · BAMD (Δ) complexes. The straight line is the best approximation of the data for both kinds of complexes at low binding ratios.

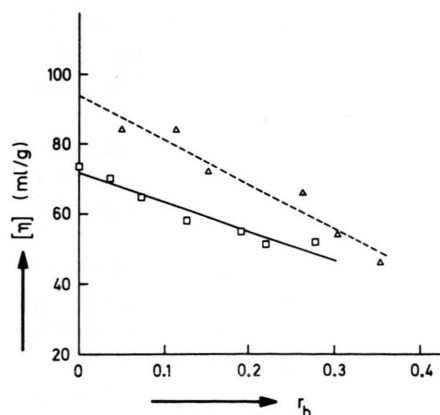


Fig. 6. Plot of the intrinsic viscosity $[\eta]$ versus r_b for DNA · Hg²⁺ (□) and DNA · BAMD (Δ) complexes.

lateral association of DNA molecules upon complex formation, the behaviour of M_c obviously indicates a decrease of the length increment l . The extent of this decrease can be derived from a plot of l versus r_b (Fig. 5). The values for l which are also listed in Table I were calculated from M_c according to (cf. [15]).

$$l = \frac{M_{NP} + r_b M_L}{M_c}$$

As can be seen from Fig. 5, the data for both kinds of complexes can be approximated at low binding ratios by the same straight line. The slope of this straight line corresponds to a change of the length increment by $\Delta l = -0.10 \pm 0.01$ nm per bound ligand. The inclusion of the l values at high binding ratios leads to mean values of $\Delta l = -0.08$ nm for the DNA · Hg²⁺ complex and of $\Delta l = -0.15$ nm for the DNA · BAMD complex (cf. also Figs. 3 and 4).

In order to verify the reliability of the experimentally determined Δl values, the possible influence of counterions and preferential interaction on our results was carefully checked. It was found that the Δl values do not depend much on these factors; an insufficient consideration of counterions and preferential interaction might possibly result in a slight underestimation but hardly in an overestimation of the Δl values.

2. Viscometry

The determination of the intrinsic viscosities of the DNA · Hg²⁺ and DNA · BAMD complexes established a decrease of $[\eta]$ with increasing r_b (cf. Table I and Fig. 6). Apart from the small contribution of the bound ligands, this behaviour of $[\eta]$ might reflect a decrease of length of the DNA molecules or a decrease of their rigidity as well. In order to distinguish between these two effects, measurements would have to be performed on several samples of sufficiently different molecular weights [29]. In our case, however, a decrease of length has already been established by SAXS. Therefore we chose the following procedure: we analyzed $[\eta]$ for the changes of the length increment, thereby neglecting any changes of rigidity, and alternatively we estimated from $[\eta]$ the changes of rigidity by using the Δl from the SAXS investigation.

On the assumption that the DNA molecule is a circular cylinder of length L and radius r , thus its axial ratio is given by $p = L/2r$, the intrinsic viscosity

may be expressed by (cf. [30])

$$[\eta] = \frac{2 N_A V_{h2} A(p)}{3 M_2}.$$

M_2 is the molar mass of the particles, V_{h2} is their hydrated volume, N_A is Avogadro's number and $A(p)$ is given by [31]

$$A(p) = \frac{p^2}{5 (\ln 2p - A)} + \frac{p^2}{15 (\ln 2p - B)} + \frac{14}{15}$$

with $A = 1.5$ and $B = 2.5$ [32, 33]. The ratio V_{h2}/M_2 in the above formula can be replaced with a ratio of the SAXS parameters R_c and M_c as follows

$$\frac{V_{h2}}{M_2} = \frac{r^2 \pi L}{M_2} = \frac{2 R_c^2 \pi}{M_c}$$

because $L = M_2/M_c$ and $r = R_c \sqrt{2}$.

Thus from $[\eta]$, R_c and M_c first $A(p)$ and then p can be obtained. L can be calculated from p and R_c . Assuming a linear dependence of L on r_b , one obtains the molar mass M_2 of the unliganded DNA from its length L_0 and its mass per unit length, and Δl from the slope (dL/dr_b) after multiplication by the factor M_{NP}/M_2 . It has turned out to be useful to refine the parameters thus obtained in the following way. By using L and the molar mass of the complexes (as calculated from M_2 for the unliganded DNA and from M_L and r_b) new M_c values can be obtained which can serve as a modified basis for a recalculation of L from $[\eta]$, R_c and M_c . This refinement procedure can be repeated several times until constant results are obtained. In our analysis, the entire evaluation of $[\eta]$ was performed by means of a computer program. The R_c of unliganded DNA was used as a measure for the radius of the complexes as well. Due to the refinement procedure, the final results depend only on the values of $[\eta]$ for the complexes and on the SAXS data for R_c and M_c of the unliganded DNA. The Δl values are practically independent of the unknown molecular weight distribution of the DNA.

The analysis of the data for the DNA · BAMD complexes yields a value of $M_2 = 138\,000 \pm 6\,000$ g mol⁻¹ for the unliganded DNA (this corresponds to $L_0 = 71 \pm 3$ nm) and a value of $\Delta l = -0.15 \pm 0.06$ nm per bound BAMD residue. For the DNA · Hg²⁺ complexes the analogous analysis yields $L_0 = 63 \pm 2$ nm and $\Delta l = -0.13 \pm 0.04$ nm. The length L for the various samples is given in Table I.

The analysis of $[\eta]$ on the basis of the formalism developed by Reinert [29] yielded similar Δl values.

On the assumption, that the persistence length [34] of DNA does not change upon ligand binding, we derived values of $\Delta l = -0.13$ nm for DNA · BAMD and $\Delta l = -0.12$ nm for DNA · Hg²⁺. Almost the same values were also obtained by the method used in [35]. On the other hand, when Δl was assumed to be -0.1 nm for both kinds of complexes as derived from the SAXS measurements at low binding ratios, Reinert's method yielded a decrease of the persistence length by about 0.1 to 0.2 nm per bound mercurial.

Conclusions

Our SAXS measurements on DNA · Hg²⁺ and DNA · BAMD complexes have established that at least up to binding ratios of about 0.8 for Hg²⁺ and 0.6 for BAMD the rodlike character of sonicated DNA is not destroyed by the binding of the mercurials. The maintenance of the rodlike character at even higher binding ratios of BAMD is probable. On the other hand the occurrence of significant structural changes during complex formation is clearly indicated by the decrease of the length of the liganded DNA molecule. This decrease has been established unambiguously by the SAXS results and has been confirmed by the viscosity measurements. The viscometric results have possibly indicated the occurrence of slight changes of rigidity too. These would be too small to affect the scattering behaviour in the angular range of our SAXS experiments.

In spite of the structural changes, which may include a successive loss of base pair stacking and hydrogen bonding as suggested by the previous investigations [1, 2] the mercurials must certainly hold the opposite strands in register to each other: upon addition of complexing agents for the mercurials native DNA is readily regenerated from both kinds of complexes [2, 4]. This finding fully agrees with the observed maintenance of the rodlike structure.

The results of our experiments are in best agreement with those models which place both mercurials in the axis (or very near to it) of the rodlike DNA molecule.

Our data for the cross-section radius of gyration of the complexes suggest that the incorporation of Hg²⁺ into the helix takes place in a steady and somehow regular manner up to high binding ratios, whereas the binding of BAMD obviously changes drastically above $r_b = 0.35$. At low binding ratios BAMD is certainly bound in the interior of the DNA helix similar

to Hg^{2+} . BAMD bound at $r_b > 0.35$ seems to be attached to the surface of DNA. Possibly there occurs also a structural change by which already bound BAMD is shifted from the inside to the outside of the DNA helix. Anyway, the limiting r_b value of 0.35 gives a hint for the molecular structure of the DNA · BAMD complex at low binding ratios.

From model building with CPK space-filling models one can deduce that the mercury atoms in the BAMD molecule have a distance of 0.58 nm to each other. This is about 0.10 nm less than the distance between 3 base pairs in B-form DNA. The CPK model also shows that both mercuries are able to undergo simultaneous binding by their main valencies at right angles to the long axis of the molecule. By their tetrahedron angles the methylene groups between the mercury atoms and the dioxane ring allow structures with syn- and anti-conformation for the heavy metal atoms. Both structures favour simultaneous binding of both mercuries in planes 0.58 nm distant to each other, when the molecule is oriented parallel to the direction of the DNA helix axis. This orientation might direct the complex formation in the following way. One BAMD molecule binds by one of its mercuries to a thymine in N3-position of the base, which must rotate out of its normal position to enable the binding. Binding of the second mercury to a further thymine, two base pairs apart, is possible after preceding rotation of the base into appropriate position. Stacking of the involved bases is lost and the distance between them can be lowered from 0.68 nm in the helix to 0.58 nm or less in the complex. If the dioxane ring is not strictly oriented in the direction of the helix axis distances shorter than 0.58 nm are also possible.

This binding model would explain the observed decrease of length of about 0.1–0.15 nm per bound BAMD molecule and the limiting r_b -value of 0.35. The structure seems to be saturated with BAMD when 3 base pairs are complexed by 1 BAMD molecule. Since other base pairs are also able to bind mercury [36], although with lower affinity, this sort of complex seems to be possible for GC-rich sequences too.

Similarly, when Hg^{2+} is to crosslink bases of two adjacent base pairs in the opposite strands this cannot be done without a reduction of the distance between the base pairs involved. In the B-form of DNA, the shortest distance between the N3-positions of nearest neighbour thymines on the opposite strands

is 0.52 nm, which is considerably larger than the N-Hg-N length of about 0.41 nm. Even a decrease of the length increment of the helix by 0.1 nm would not reduce the distance between the thymine nitrogens in the required extent. Thus apparently a larger decrease of the length increment and/or other structural changes too are necessary for this kind of crosslinking.

At least at the limiting r_b -values of 0.35 for BAMD and of 1.0 for Hg^{2+} , both complexes might adopt a structure which resembles that of Z-DNA so far as partially stacked bases are wound helically around a core which contains the more or less regularly spaced mercurials.

Though our results certainly do not allow definite statements on the location of the sites to which the mercurials are attached, the proposed model is in good agreement with our data. The limiting values for the cross-section radii of gyration of the complexes, $(R_c)_b = 0.678$ nm for DNA · Hg^{2+} and $(R_c)_b = 0.746$ nm for DNA · BAMD, imply that the mercury atoms of bound mercurials (in the case of BAMD only at $r_b < 0.35$) cannot be situated at too far distances from the helix axis. Model calculations show that any placement of mercurials distant to the helix axis would lead to an enhancement of $(R_c)_b$. This effect could only be compensated by a simultaneous decrease of the cross-section radius of gyration of the DNA itself. The required extent of this decrease can be estimated from the relations $\Delta R_c = -0.3 r_L^2$ for Hg^{2+} and $\Delta R_c = -0.2 r_L^2$ for BAMD, where r_L designates the mean radial distance of the mercury atoms. However, since both the assumed rotation of bases out of the helix axis and the reduction of the distance between adjacent base pairs impede a decrease of the cross-section radius of gyration of DNA and would even favour an increase, the binding of the mercurials at radial distances exceeding a few tenths of nanometer is clearly ruled out.

The drastic change in the formation of the DNA · BAMD complex at $r_b > 0.35$ is not only reflected by the increase of the cross-section radius of gyration but possibly also by the behaviour of the mass per unit length and of the length increment derived therefrom (*cf.* Fig. 4 and Table I). Fig. 5 suggests that the length increment decreases at high binding ratios more than at low binding ratios.

The opposite behaviour can be observed for the DNA · Hg^{2+} complexes (*cf.* Figs. 3 and 5): here the

length increment decreases at high binding ratios less than at low binding ratios. A plausible explanation for this behaviour would be that at a binding ratio of $r_b = 0.5$, when on the average one base per base pair is involved in cross-linking, the structure of the DNA has already transformed in such a way as to enable the binding of further Hg^{2+} without an additional decrease of length. This explanation is supported by the fact that the length increments found for the DNA · Hg^{2+} complexes at r_b values above 0.5

(*cf.* Table I) are close to the value of $l = 0.29$ nm which can be calculated on the assumption of $\Delta l = -0.1$ nm for every Hg^{2+} bound up to $r_b = 0.5$ and $\Delta l = 0$ for any further Hg^{2+} bound beyond this limiting ratio. An analogous analysis for BAMD, performed on the assumption of $\Delta l = -0.1$ nm per BAMD bound up to $r_b = 1/3$, results in a value $\Delta l = -0.21 + 0.05$ nm for every BAMD bound beyond the limiting r_b .

- [1] V. A. Bloomfield, D. M. Crothers, and I. Tinoco, *Physical Chemistry of Nucleic Acids*, pp. 420–429, Harper and Row Publishers, New York 1974.
- [2] H. Bünemann and N. Dattagupta, *Biochim. Biophys. Acta* **331**, 341–348 (1973).
- [3] N. Dattagupta, H. Bünemann, and W. Müller, *Biochim. Biophys. Acta* **378**, 44–53 (1975).
- [4] U. S. Nandi, J. C. Wang, and N. Davidson, *Biochemistry* **4**, 1687–1696 (1965).
- [5] J. Cortadas, G. Macaya, and G. Bernardi, *Eur. J. Biochem.* **76**, 13–19 (1977).
- [6] G. Macaya, J. Cortadas, and G. Bernardi, *Eur. J. Biochem.* **84**, 179–188 (1978).
- [7] D. Riesner and H. Bünemann, *Proc. Natl. Acad. Sci. USA* **70**, 890–893 (1973).
- [8] S. Katz, *Biochim. Biophys. Acta* **68**, 240–253 (1963).
- [9] M. Liebermann, D. J. Harvan, D. E. Amacher, and J. B. Patterson, *Biochim. Biophys. Acta* **425**, 265–277 (1976).
- [10] A. Walter and G. Luck, *Nucl. Acids Res.* **4**, 539–550 (1977).
- [11] C. F. Jordan, L. S. Lerman, and J. H. Venable Jr., *Nature New Biol.* **236**, 67–70 (1972).
- [12] F. M. Pohl and T. M. Jovin, *J. Mol. Biol.* **67**, 375–396 (1972).
- [13] A. H. J. Wang, G. J. Quigley, F. J. Kolpak, G. van der Marel, J. H. van Boom, and A. Rich, *Science* **211**, 171–176 (1981).
- [14] J. Klysik, S. M. Stirdivant, J. E. Larson, P. A. Hart, and R. D. Wells, *Nature* **290**, 672–677 (1981).
- [15] P. Zipper and H. Bünemann, *Eur. J. Biochem.* **51**, 3–17 (1975).
- [16] P. Zipper, G. Ribitsch, J. Schurz, and H. Bünemann, *Chem. Zeitg.* **102**, 225 (1978).
- [17] P. Zipper, G. Ribitsch, J. Schurz, and H. Bünemann, *Hoppe-Seyler's Z. Physiol. Chem.* **359**, 1170 (1978).
- [18] J. T. Edsall, R. H. Maybury, R. B. Simpson, and R. Straessle, *J. Am. Chem. Soc.* **76**, 3131–3138 (1954).
- [19] O. C. Richards and P. D. Boyer, *J. Mol. Biol.* **11**, 327–340 (1965).
- [20] P. Doty, B. B. McGill, and S. A. Rice, *Proc. Natl. Acad. Sci. USA* **44**, 432–438 (1958).
- [21] R. B. Inman and D. O. Jordan, *Biochim. Biophys. Acta* **42**, 530–532 (1960).
- [22] O. Kratky and Z. Skala, *Z. Elektrochem.* **62**, 73–77 (1958).
- [23] P. Zipper, *Acta Phys. Austriaca* **36**, 27–38 (1972).
- [24] G. Porod, *Acta Phys. Austriaca* **2**, 255–292 (1948).
- [25] O. Kratky and G. Porod, *Die Physik der Hochpolymeren* (H. A. Stuart, ed.) **Vol. 2**, pp. 515–534, Springer Verlag, Berlin, Göttingen, Heidelberg 1953.
- [26] D. W. Kupke, *Physical Principles and Techniques of Protein Chemistry* (S. J. Leach, ed.) part C, pp. 1–75, Academic Press, New York 1972.
- [27] O. Kratky, H. Leopold, and H. Stabinger, *Z. Angew. Phys.* **27**, 273–277 (1969).
- [28] S. Bram and W. W. Beeman, *J. Mol. Biol.* **55**, 311–324 (1971).
- [29] K. E. Reinert, *J. Mol. Biol.* **72**, 593–607 (1972).
- [30] C. Sadron, *Progr. Biophys. Biophys. Chem.* **3**, 237–304 (1953).
- [31] R. Simha, *J. Phys. Chem.* **44**, 25–43 (1940).
- [32] S. Broersma, *J. Chem. Phys.* **32**, 1626–1631 (1960).
- [33] G. Cohen and H. Eisenberg, *Biopolymers* **8**, 45–55 (1969).
- [34] O. Kratky and G. Porod, *Rev. Trav. Chim. Pays-Bas* **68**, 1106–1122 (1949).
- [35] K. R. Fox, N. L. Harrison, and M. J. Waring, *FEBS Lett.* **133**, 305–310 (1981).
- [36] R. B. Simpson, *J. Am. Chem. Soc.* **86**, 2059–2065 (1964).