A SMALL-ANGLE X-RAY SCATTERING STUDY ON THE INTERACTION OF ACTINOMYCIN C_3 WITH DEOXYRIBONUCLEIC ACID FROM CALF THYMUS

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Received 7 June 1972

1. Introduction

Actinomycin C_3 is an antibiotic containing two cyclic oligopeptides which by binding to double helical DNA acts as an inhibitor of the DNA-primed RNA synthesis [1–4]. Based on the specificity for guanine, on the behaviour of N-alkylated actinomycin derivatives and on fiber diffraction patterns Hamilton et al. [5] proposed a model for the complex, in which the actinomycin is hydrogen-bonded to the outside of the DNA helix, with a hydrogen bond between the actinomycin quinone oxygen and the quanine group and two further hydrogen bonds between the amino group of actinomycin and the N_3 of guanine and the deoxyribose ring oxygen, respectively. Müller and Crothers [6] suggested from their results, from equilibrium, kinetic and hydrodynamic studies, a model in which the chromophore of actinomycin intercalates between adjacent base-pairs with the peptide rings in the narrow groove of the DNA helix. The specificity for guanine is explained by electronic interactions between the GC pair and the chromophore in the intercalation complex. Recently Sobell et al. [7] investigated a crystalline actinomycin–deoxyguanosine complex and showed that in it the actinomycin chromophore is intercalated between two guanines and hydrogen bonds are formed between the cyclic oligopeptides and the guanine 2-amino groups.

This report describes small-angle X-ray scattering experiments performed with actinomycin–DNA complexes in solution and demonstrates that the results from these experiments are inconsistent with an outside-binding model, but strongly favour an intercalation model

2. Materials and methods

All small-angle X-ray scattering measurements were performed in BPES-buffer: 0.008 M Na_2PHO_4, 0.002 M NaH_2PO_4, 0.18 M NaCl, 0.001 M diNaEDTA (pH 7.1).

Calf thymus DNA samples were purchased from Sigma Chemical Company and further purified by phenol extraction. Low molecular weight DNA (90,000) was obtained by sonic degradation at ice bath temperature for a total of 60 min of sonication. The molecular weight was calculated from the intrin-

sic viscosity, using the equation of Doty et al. [8]. The actinomycin–DNA complex solutions were prepared from calculated amounts of solid dye and 5 ml samples of DNA solution (conc. 8.6 mg/ml). After 60 hr equilibrium dialysis with 10 ml BPES-buffer the r-values, ratio of bound actinomycin to total DNA base pairs, were calculated as described elsewhere [6].

Small-angle X-ray scattering measurements were performed using the experimental techniques and the theory and evaluation procedures described previously [9–12], except that a multicomponent formalism similar to that proposed by Eisenberg and Cohen [13] was used for the calculation of the mass per unit length. The additivity of the apparent partial specific volumes of NaDNA and of actinomycin in the complexed state was confirmed by density measurements using a digital density meter [14].

3. Results and discussion

We investigated several samples of uncomplexed NaDNA and of actinomycin–NaDNA complexes of various binding ratios r. The scattering curves obtained therefrom are all of the rod type and are very similar to each other. In a Guinier plot of the cross section factors the steepest region in the curves was approximated by straight lines. The slope of these straight lines decreases with increasing binding ratio r, and in the same way the cross section radius of gyration R_c of the DNA double helix decreases from 8.7 Å in the absence of actinomycin to 8.1 Å, the value obtained for the complex in which one actinomycin molecule is bound to about six base pairs. As shown in fig.1 the dependence of the cross section radius of gyration on the binding ratio is approximately linear.

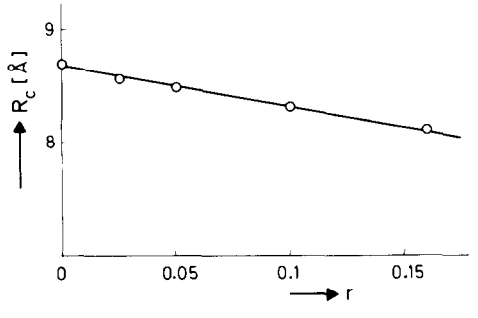

Fig. 1. Dependence of the cross section radius of gyration R_c of NaDNA–actinomycin complexes on the binding ratio r.

When the mass per unit length is determined from the limiting values for the cross section factors at zero angle, as obtained by an extrapolation of the straight lines, then the values found for the NaDNA–actinomy complexes are much lower than one would expect from the mass of the bound actinomycin. This discrepancy can be explained only by the assumption that an extension of the DNA double helix has occurred when the actinomycin was bound. The ratio of the double helix in the complexed state, L, to the length in the absence of actinomycin, L_0, can be easily calculated from the experimentally found masses per unit length. The results from this calculation, performed for two different binding ratios, are presented in fig.2. This figure shows that the length ratio L/L_0 is a linear function of the binding ratio r. From the slope of the straight line, 1.38, the extension of the double helix can be easily derived as 4.6 ± 0.2 Å per bound actinomycin molecule.

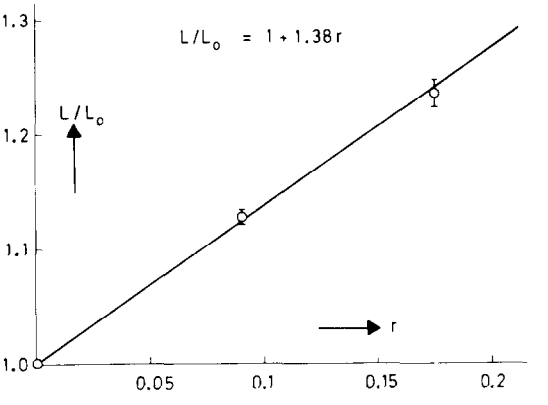

Fig. 2. Ratio of length of the NaDNA–actinomycin complex to the length of the uncomplexed NaDNA, shown as a function of r.

The volume of a rod-like particle of circular and hom geneous cross section can be calculated from the lengt of the rod and from its cross section radius of gyratio When this method is used to determine the volume of the DNA double helix, then the volume V_{exp} thus obtained is much larger than the theoretical specific volume V_{theor} that can be calculated from the partial

specific volume of NaDNA. This discrepancy is mainly due to the fact, that actually, when only a few base pairs are considered, the cross section of the double helix is neither circular nor homogeneous. It is convenient to assume the space, given as the difference between V_{exp} and V_{theor}, being occupied by solvent and to define the ratio of the volumes formally as a degree of swelling $q = V_{exp}/V_{theor}$.

The degree of swelling so defined was determined for NaDNA–actinomycin complexes of various binding ratios, whereby the extension of the double helix by the bound actinomycin was taken into account in every case. When the values for q_r obtained in this way are divided by q_0, the degree of swelling of NaDNA in the absence of actinomycin, the curve presented in fig.3 results. As can be seen from this figure the degree of swelling of the NaDNA–actinomycin complexes is much lower than that of the uncomplexed NaDNA. This result implies that a certain amount of solvent has been expelled from the DNA when the actinomycin was bound. A rough estimation of the volume of the expelled solvent yiels a value of about the same magnitude as the volume of the peptide rings of actinomycin.

The extension of the double helix by the bound actinomycin agrees excellently with the findings of Müller and Crothers [6]. This extension can be hardly explained on the basis of the outside-binding model [5], but it is consistent with any intercalation model [6, 7].

The strong decrease of the cross section radius of

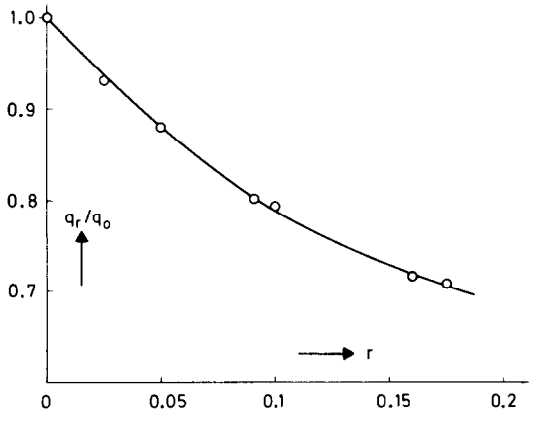

Fig. 3. Ratio of the degree of swelling of the NaDNA–actinomycin complex to the degree of swelling of the uncomplexed NaDNA, in dependence upon r.

gyration, when actinomycin is bound, could be caused by several factors. One, an extension of the double hel is believed to require a local distortion of the helix, th leading to a reduction of the lateral dimensions of the DNA backbone [15, 16]. The same effect could be favoured by the possible hydrogen bonding between the phosphate of the DNA and the amino groups of the dye chromophore. Both would cause a decrease of the cross section radius of gyration, and with NaDNA–proflavine complexes such a decrease was actually found by small-angle X-ray scattering [17]. A decrease of the cross section radius of gyration would also result if the peptide rings of actinomycin are located relatively near to the helix axis, that means at distances less than 8.7 Å. Location of the peptide rings at larger distances would, on the other hand, lead to an increase of the cross section radius of gyration. Thus the experimentally found behaviour of the cross section radius of gyration can be regarded as further evidence against outside-binding, since the peptide rings can be brought much closer to the helix axis in an intercalation complex than in an outside-binding complex.

The decrease of the degree of swelling with increasing amount of bound actinomycin can be easily understood if one assumes that the cyclic oligopeptides are in close contact with the DNA helix, being located in the narrow groove as suggested by the intercalation models [6,7]. Only in this case can they expel such a large amount of solvent that was originally, i.e. in the absence of actinomycin, contained in the overall volume of the DNA. A close contact between the peptide rings and the DNA backbone was suggested from the different behaviour of actinomycin and of the synthetic actinomine in kinetic studies [6].

The change of the degree of swelling observed with the DNA–actinomycin complexes was compared also with the corresponding quantities for DNA–actinomine and DNA–proflavine complexes, calculated from the data given by Wawra et al. [18] and Luzzati et al. [17]. The comparison shows that the decrease of the degree of swelling is much less when actinomine in which the bulky peptide rings of actinomycin are replaced by short side chains, is boun to DNA. Practically no change of the degree of swelling can be observed with the complexes of DNA with proflavine, which has no side chains at all.

Returning to our results it can be said that particularly the extension of the double helix, but also the strong decrease of the cross section radius of gyration are incompatible with the outside-binding model, whereas, on the other hand, all our results agree well with the predictions of the intercalation models.

Acknowledgements

The Austrian Fonds zur Förderung der wissenschaftlichen Forschung has supported this work by the supply of apparatus. Part of this work has been also supported by grant P.L. 480 from the United States Department of Agriculture.

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