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STRUCTURAL STUDIES OF DNA GYRASE

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DNA gyrase, a prokaryotic type II topoisomerase, can introduce negative supercoils into DNA in an ATP-dependent manner and relax negative supercoils in the absence of ATP. Gyrase is a tetramer of two A subunits (MW 105,000) and two B subunits (MW 95,000), and it interacts with about 140 base pairs of DNA (based on staphylococcal nuclease and DNase I protection experiments). We have undertaken an electron microscopic study of gyrase and its binding to DNA, in order to determine how DNA wraps in its complex with the protein.

ELECTRON MICROSCOPY OF GYRASE. Figure 1 shows images of gyrase (M. luteus) sprayed onto mica in glycerol-containing buffer and visualized by rotary shadowing with Pt. We find images that vary in appearance from heart-shaped to lozenge-shaped. This apparent shape variability can be accounted for by a gyrase molecule of constant shape that adsorbs with different orientations to the mica substrate. Inspection of large numbers of pictures shows that a common characteristic of many images is an approximate mirror line that bisects the molecule, suggesting a molecular dyad. (This symmetry is compatible with the A_2B_2 chemical structure.) The gallery in Fig. 1 includes a number of these images. Figures 1a-k display molecules with their dyad oriented approximately vertically; the height from top to bottom is 20-22 nm (this and following measurements are uncorrected for Pt grain size). The model in Fig. 2 summarizes these views. For each of the images in 1a-k, the angle of view about the dyad (with respect to the axes shown next to the model in Fig. 2) is indicated. Figure 1ℓ shows a molecule viewed along the twofold axis (lozenge-shaped image). The reconstruction in Fig. 2 accounts for this image as well as for many others viewed in oblique directions.

ELECTRON MICROSCOPY OF A SUBUNIT DIMERS. The A subunit of DNA gyrase yields images with a striking 'V' shape when sprayed in glycerol onto mica and rotary shadowed with Pt. Some selected images are shown in Figure 3. The A subunit is believed to dimerize in solution, and we suggest that each arm of the 'V' corresponds to a single A subunit. The maximum separation measured at the top of the 'V' is 25 nm, similar to the maximum separation of the ears in intact gyrase. The maximum height of the 'V' shape complex is 16 nm, roughly two-thirds the height of the complete A_2B_2 molecule. We have observed a small and limited variation in angular spread of the A subunits (Figure 3b-m). We cannot be sure at present whether this variation corresponds to some flexibility at a hinge between the subunits or to different projected views of the A subunits as they adsorb in various orientations to the substrate. The latter interpretation is consistent with the fact that we can also find some images that appear as single rods with a total length of 25 nm (see, for example, Figure 3a). This length is the same as the maximum distance between the ears of intact gyrase (see the example in Figure 3n), and we suggest that this image corresponds to a top (or bottom) view of the A dimer, along the molecular dyad.

Negatively stained images of the A subunits reveal the same 'V' shape assembly seen by rotary shadowing, an example of which is given in the electron micrograph of Figure 4. Likewise, there are presumed 'top views'

with a characteristic zig-zag appearance: two small rods of 10 nm in length, joined at their ends side by side, thus displaying an apparent dislocation.

Our current interpretation of the images of DNA gyrase and of the A subunit dimer are summarized by the superposed reconstruction in Fig. 2 and by the diagram in Fig. 7. The B subunits have been drawn with a separation between them, consistent with the absence of B_2 dimers in chemical cross-linking experiments.

COMPLEXES WITH DNA. Micrographs of shadowed complexes of gyrase with a 541 bp restriction fragment of DNA are shown in Fig. 5. The upper row shows images with both DNA tails emerging from the tip of the heart-shaped molecule. We have measured the length of the DNA tails in a large number of such images and compared the results with apparent lengths of control DNA (Fig. 5c). The results show that about 120 bp of DNA must be wrapped onto or within the gyrase 'heart'. This measurement is in good agreement with the results of nuclease protection and footprinting experiments. Other images, such as those in the bottom row of Fig. 5, show DNA entering at the tip and exiting from an ear. There is no significant length decrement in these complexes. We interpret such structures as incomplete binding modes, based on the conclusion from protection experiments that "complete" interaction involves 120-140 bp. Both "full" and "incomplete" binding can also be seen on 541 bp covalently closed circles (not shown).

The A subunit dimer binds independently to DNA, as shown in Fig. 6. The double-stranded cut made by gyrase leaves one A subunit attached to each newly generated 5' end, and the symmetrical disposition of DNA across the dyad of the 'V', seen in most of the images, is consistent with the requirements of the cleavage. These images, together with the interpretation of images of gyrase, gyrase A_2, and gyrase/DNA complexes just outlined, suggest the overall mode of DNA binding drawn in Fig. 7. We emphasize that this is a very tentative model. It appears to be the simplest picture consistent with available EM and biochemical data.

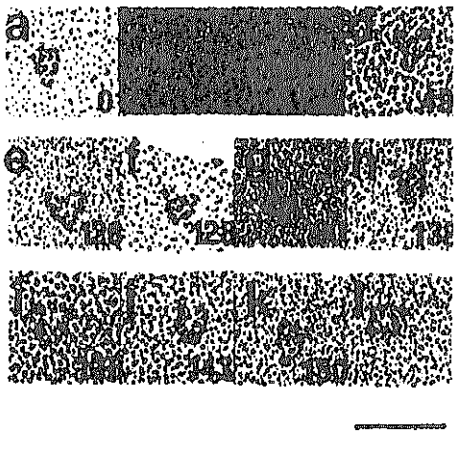


Figure 1. Selected images of Pt rotary shadowed DNA gyrase. Angle of view about the dyad is indicated. Referring to axes in Fig. 2, 0° is a view toward origin along y and 90° is a view toward origin along x. Bar: 50 nm.

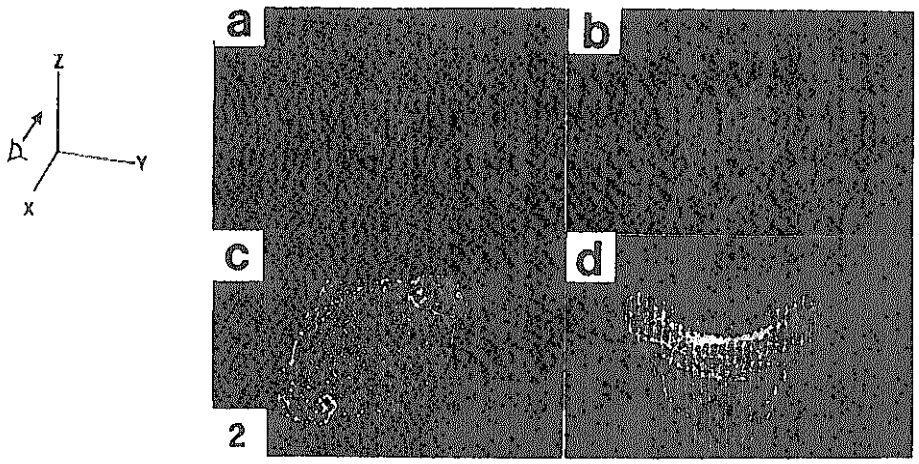


Figure 2. Model for DNA gyrase obtained by a simple direct-space reconstruction of the shadowed outline of the molecule. Figure 2d includes a reconstruction of the A subunit dimer. Axes are reference frame for angles in Fig. 1; they apply to orientation of model in (2a).

Figure 3. Selected images of Pt rotary shadowed A subunit dimers (a-m); DNA gyrase shown for comparison in (n). Bar: 50 nm.

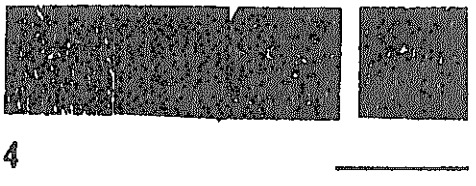
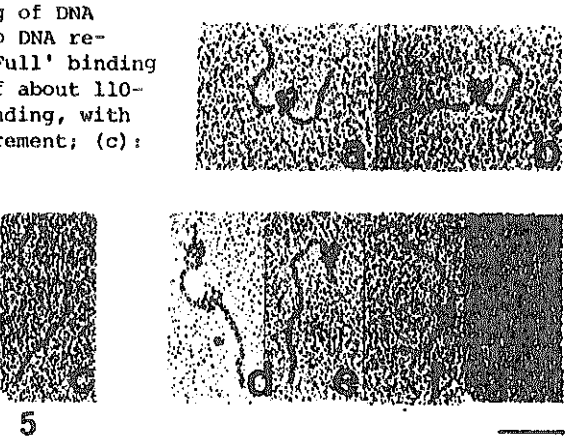


Figure 4. Negatively stained images (1.5% uranyl acetate) of A subunit dimers. (a-c) Characteristic 'V' shape; (d) 'zig-zag' (presumptive top view). Bar: 50 nm.

Figure 5. Pt rotary shadowing of DNA gyrase complexed with a 541 bp DNA restriction fragment. (a-b): 'Full' binding with decrease in DNA length of about 110-115 bp; (d-g) 'incomplete' binding, with no significant DNA length decrement; (c): control DNA. Bar: 50 nm.



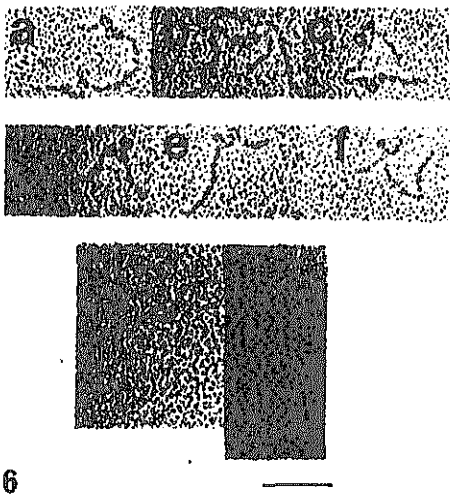


Figure 6. Pt rotary shadowing of A subunit dimer with 541 bp DNA restriction fragment. No significant length decrement of DNA is observed. Bar: 50 nm.

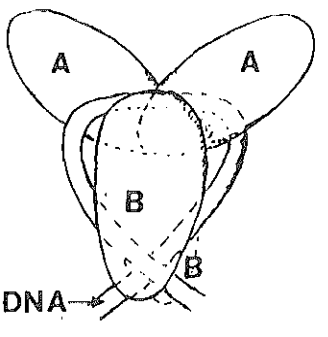


Figure 7. A tentative model for DNA binding to DNA gyrase.