Synapsis by Tn3 resolvase: speed and dependence on DNA supercoiling.

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The transposon Tn3 encodes a protein known as resolvase. During transposition, resolvase catalyzes a site-specific recombination between two directly repeated copies of the transposon DNA [1]. Resolvase acts by binding to 120 bp sites on the DNA, known as res sites, each of which contains three sub-sites called I, II and III [2]. During recombination, three resolvase dimers bind co-operatively to each res site, forming a resolvosome. Two resolvosomes then interact to form a synaptic complex, in which the strand transfer reactions take place. Resolvases from other Tn3-like transposons such as Tn21 act in the same way. However, Tn21 resolvase has no activity at Tn3 res sites and Tn3 resolvase has no activity at Tn21 sites [2-4]. Both resolvases, however, have an absolute requirement for a supercoiled substrate.

The recombination reactions by either Tn3 or Tn21 resolvases generally take several minutes to convert the DNA substrate to the recombinant product [5, 6]. However, the synapsis of two res sites is complete within 0.5 seconds [6]. This was shown by using plasmids such as pSH1 that contain two Tn3 res sites interspersed with two Tn21 res sites [7]. On a plasmid of this type, synapsis of the res sites for one resolvase segregates the other pair of res sites into different topological domains. This segregation inhibits recombination by the second enzyme [6] and synapsis can then be followed by quantifying the extent of inhibition.

The aims of the current work were two-fold: first, to establish the speed of synapsis using rapid reaction techniques; second, to determine the supercoiling requirement of the steps in the reaction that lead to the formation of the synaptic complex.

We have used a rapid-reaction quenched-flow apparatus, constructed by Dr. C. Bagshaw, (Department of Biochemistry, University of Leicester) to measure the rate of formation of the Tn3 synaptic complex. With this apparatus, solutions of pSH1 DNA and Tn3 resolvase were loaded separately into two sample chambers, mixed together and then expelled at high velocity into a quench solution. The time between mixing and quenching was varied between 5 and 1000 milliseconds. Typical experiments used 9 nM pSH1 and 270 nM Tn3 resolvase as reactants, with 900 nM Tn21 resolvase as quench solution. The amounts of the products from both Tn3 and Tn21 recombination formed after 20 minutes were then determined. When the mixture of Tn3 resolvase and DNA was added to Tn21 resolvase after 200 ms, the DNA yielded almost exclusively Tn3 product: i.e., the Tn3 synaptic complex is formed in less than this time interval. Subsequent work indicated that the speed of synapsis increased if the concentration of Tn3 resolvase was doubled. Thus, the rate-limiting step in synapsis appears to be the association of the protein with the DNA to form the resolvosome.

The effects of substrate superhelicity (\(\phi_0\)) on the rate of recombination and on the stability of the synaptic complex were also examined. Substrates that contained either more or fewer supercoils than native pSH1 were produced by relaxing the plasmid with calf thymus topoisomerase I in the presence of ethidium bromide [8]. The linking differences were measured from the altered distributions of topoisomers in the treated samples compared with native pSH1, as observed on chloroquine gels. The rates of recombination by both Tn3 and Tn21 resolvases were reduced when \(\phi_0\) was altered from -0.062 (native DNA) to -0.021. Tn21 recombination was also reduced on DNA with a \(\phi_0\) value of -0.038. Tn3 recombination, however, proceeded at comparable rates on DNA with \(\phi_0\) values of -0.038, -0.062 or -0.073. These results with Tn3 resolvase are similar to an earlier study on the effect of supercoiling on the rate of Tn21 recombination [5], except that the Tn21 enzyme is more sensitive to reductions in superhelical density.

The stabilities of synaptic complexes on pSH1 with varying \(\phi_0\) values were determined by using the assay described previously [6]. With native pSH1 of \(\phi_0\) -0.062, the addition of one resolvase before the second lead to the majority (>75%) of the DNA being committed to recombination by the first resolvase, even when the second enzyme was in large excess over the first. The commitment to the first enzyme was unaltered on DNA of \(\phi_0\) -0.073. However, on DNA with \(\phi_0\) -0.038, approximately equal amounts of each product were formed (i.e. less commitment to the first resolvase was observed). This implies that the synaptic complexes formed on DNA of reduced superhelicity are less stable than those on native DNA.

The results measuring the speed of synapsis have implications for the dynamics of supercoiled DNA molecules. For example, distant sites on supercoiled DNA may encounter each other by random diffusion [9]. However, the speed of interaction between res sites determined here is too fast to be explained by simple diffusion within a DNA molecule modelled as a dynamic worm-like coil. An alternative proposal is that the sites encounter each other by an ordered 'slithering' motion of the DNA. Further experiments are planned to explore this possibility.

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