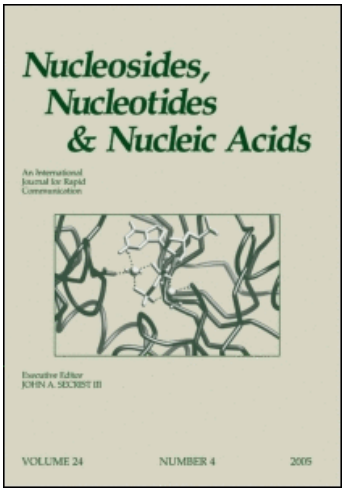

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Synthesis of Dumbbell-Shaped Circular DMA-Molecules

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SYNTHESIS OF DUMBBELL-SHAPED CIRCULAR DNA-MOLECULES.

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Abstract. The syntheses of two dumbbell-shaped circular DNA-molecules of chain lengths 140 and 150 nt are described. They are formed by enzymic ligation of chemically synthesized oligonucleotides containing self-complementary 5' protruding ends.

We have investigated the possibility of synthesizing dumbbell-shaped circular DNA-molecules according to the following steps:

1. Chemical synthesis of deoxyoligonucleotides containing sequences which allow the formation of fold-back structures with single stranded protruding 5' ends. The two examples selected are CGGGATCCCGGGTACCTCGAGCCGGGATGGTAGAACCCGTCGGGG AGGCCTTTAAATAACTCGAGGTACC (I) and GGGAGATCTCCCTATAGTGAGTCG TATTAAGATGGTAGAACCCGTCGGGGAGGGCTCTTAATACGACTCACTATA (II). Their self-complementary regions are marked by pairs of inverted arrows and the respective fold-back structures I and II are depicted schematically in the phosphorylated form in Fig. 1.

2. Enzymic phosphorylation of the 5' ends. Introduction of ^{32}P-labelled 5' phosphates from [γ^{32}P]ATP catalyzed by T_4-polynucleotide kinase is a prerequisite for the subsequent ligation step and allows characterisation of the ligation products by phosphatase sensitivity, nearest neighbour analysis and by restriction endonuclease cleavage at sites contained in the stems or in the newly formed double stranded regions such as the BamHI site in structure IL_2 and the BglII site in structure IIL_2. Gel electrophoretic charac-

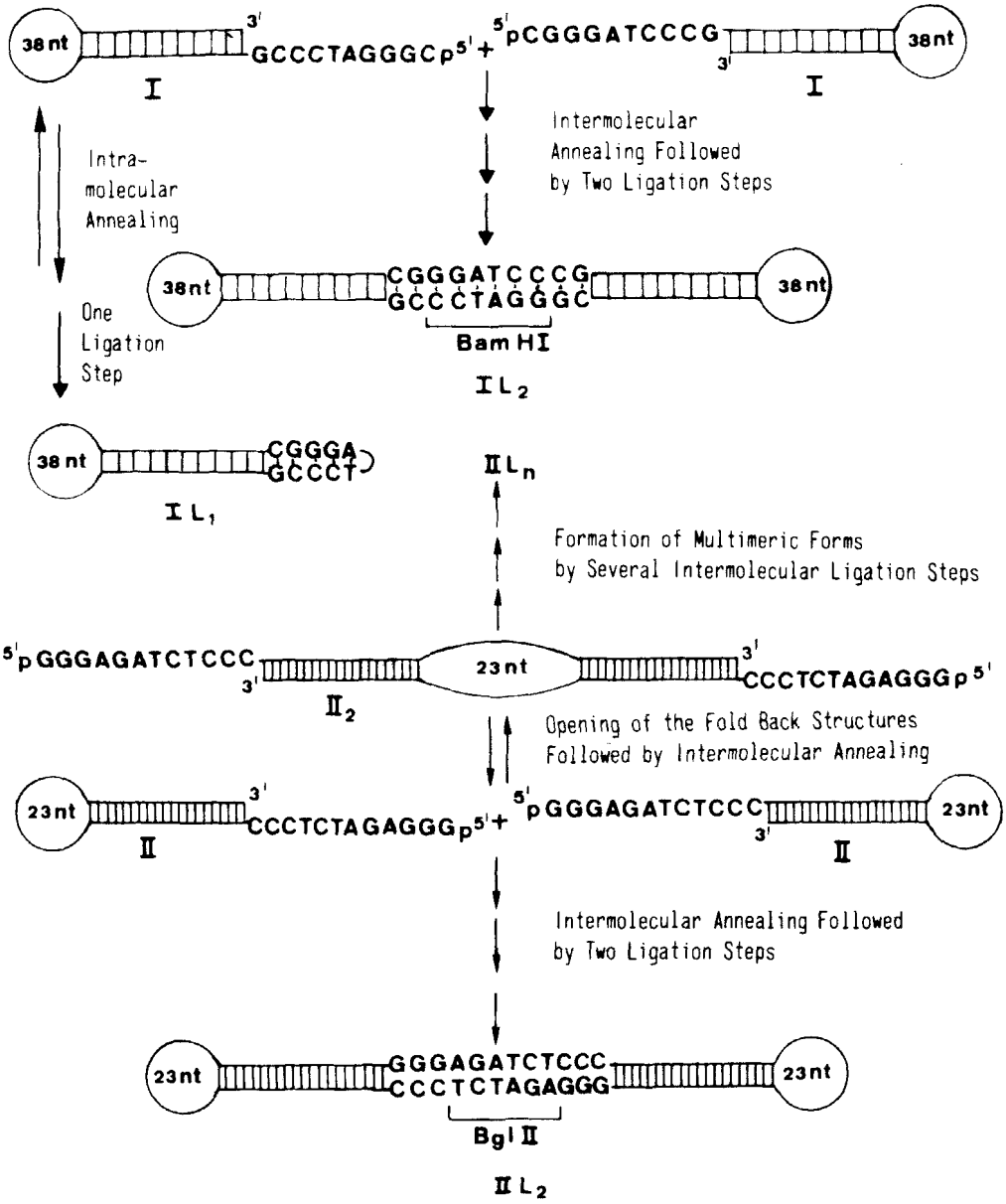

FIGURE 1. Synthesis of the dumbbell-shaped circular DNA molecules IL_2 and IIL_2 by ligation of two fold-back structures I and II. The side products IL_1 and IIL_n are also depicted.

terisation of the phosphorylated chains I and II is shown in FIG. 2, lanes Ia and IIa.

3. Intermolecular annealing of the single stranded protruding 5' ends. Due to the self-complementarity of the single stranded protruding 5' ends, two molecules of I and II, respectively, can form dimers by intermolecular base pairing. They correspond to the structures IL_2 and IIL_2 except that they still contain two single strand breaks. Alternative structures, however, can be formed by intramolecular base pairing of the protruding 5' ends such as structure IL_1 (unligated) or by opening of the fold-back structures and intermolecular head-to-tail reannealing as depicted in structure II_2.

4. Formation of dumbbell-shaped circular DNA-molecules by enzymic ligation. Formation of the products IL_2 and IIL_2 requires two ligation steps catalyzed by T_4-DNA ligase in the presence of ATP. Incomplete ligation and/or incomplete phosphorylation during step 2 leads to dumbbell structures containing one unligated position (IL_2* and IIL_2*). Other side products are self ligated monomeric circles such as structure IL_1 and multimeric forms (IIL_n) derived from ligation of the dimeric annealing products II_2. Both these types of side products are formed from both the chains I and II in variable amounts depending on, for example, the concentration of the monomers. Product patterns of the ligation reactions, analyzed by denaturing polyacrylamide gel electrophoresis are shown in FIG. 2., lanes Ib and IIb. Besides unligated starting material I and II, several ligation products are observed. Their characterization and structural assignment was achieved as follows.

Product IL_2 is completely resistant to phosphatase and, after degradation with micrococcal and spleen phosphodiesterase leads to [^{32}P]-3'-dCMP as the sole radioactive product. The latter observation is consistent with the CpC nearest neighborhood expected for an end to end ligation. Cleavage with BAMHI produces a single product of

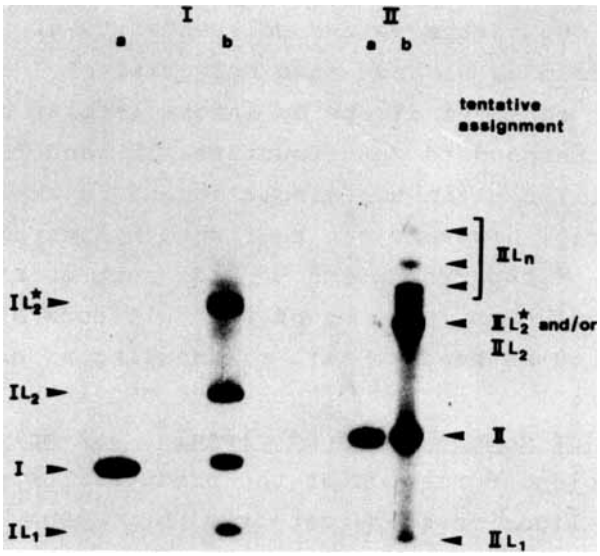

FIGURE 2. Characterization of ligation products by electrophoresis on 5% polyacrylamide gels containing 7 M urea. In lanes Ia and IIa the unligated chains I and II, respectively, in the [^{32}P]-5'-phosphorylated form are shown. In lanes Ib and IIb products of the respective ligation reactions are shown. The designation of the products, given alongside, is according to FIG. 1.

chain length identical with the original starting material I. These observations and the position of the product relative to the starting material has led us to conclude that product IL_2 is the fully ligated circular dumbbell-shaped DNA-molecule as depicted in FIG. 1.

Product IL_2*, in contrast, is not completely resistant to phosphatase but again shows CpC nearest neighborhood and partial cleavage by BamHI. From this and from its relative mobility with respect to IL_2 we conclude that IL_2* represents a mixture of nicked dumbbell-shaped DNA-molecules which are either not phosphorylated at the nick position and therefore have escaped the second ligation step or are phosphorylated but not completely ligated. The latter species would cause the observed partial degradation by phosphatase. The existence of a nick in the double stranded region would cause the molecule to unfold under denaturing conditions to the linear single stranded form, which is in agreement with its slower mobility in the denaturing electrophoretic system.

From the resistance to phosphatase and its higher mobility as compared with the unligated starting material, product IL_1 is concluded to be the closed circular monomeric form of the original fold-back structure as depicted in FIG. 1.

Similar analyses have led to the tentative assignments of the main products derived from ligation of oligonucleotide II (see lane IIb of FIG. 2). This product pattern shows a weak ladder of slower moving products (IIL_n) which are interpreted as the multimers of the alternatively annealed dimeric form (II_2) as depicted in FIG. 1.

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