RESEARCH NOTE

Non-intertwined strands of plasmid DNA contradicts the Watson and Crick model of DNA structure [version 1; peer review: awaiting peer review]

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Abstract
According to Watson and Crick (W/C) model, a DNA molecule consists of two antiparallel polynucleotide chains, intertwined with each other. Although the W/C model is accepted widely, a number of researchers have raised questions against it and proposed alternative structures for DNA. In the present study, the W/C model was examined using plasmid DNA. It was hypothesized that two strands of plasmid DNA will remain intertwined and not separate from each other under denaturing conditions, if it follows the W/C model. To test this, plasmid DNA was denatured using sodium hydroxide (NaOH) and analyzed by gel electrophoresis. It was observed that addition of NaOH to pUC19 and pBR322 plasmids resulted in new form of DNA with higher electrophoretic mobility in agarose gel. DNA corresponding to higher electrophoretic mobility band of pUC19 (hmP19) was single-stranded and circular, indicating the separation of two strands of pUC19 plasmid. Next, we examined whether hmP19 DNA can re-anneal to form native pUC19 plasmid. It was observed that neutralization of NaOH resulted in the appearance of native pUC19 plasmid in denatured DNA. Native pUC19 was also formed by hmP19 DNA extracted from agarose gel and was found to be digestible with Hind III. Ability to confer ampicillin resistance in transformed Escherichia coli demonstrated the functionality of pUC19 plasmid formed by extracted hmP19 DNA. Reversible separation of two strands of plasmid into single-stranded circular DNA shows that DNA strands are not intertwined with each other and contradicts the W/C model of DNA structure.

Keywords
DNA structure, Watson and Crick model, plasmid denaturation, single-stranded circular DNA, non-helical structure, side-by-side model.
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Abbreviations
DNA, Deoxyribonucleic acid
NaOH, Sodium hydroxide
HCl, Hydrochloric acid
hmP19, higher electrophoretic mobility band of pUC19 plasmid.

Introduction
DNA is the genetic material of all organisms, with the exception of some viruses. The currently accepted model of DNA structure was proposed by James Watson and Francis Crick in 1953. According to this model, a DNA molecule consists of two antiparallel polynucleotide chains, intertwined with each other. Although the Watson and Crick (W/C) model is accepted widely, some researchers have raised questions against it and proposed alternative models for DNA structure. Among these, Rodley’s model which envisaged that two strands of a DNA molecule are held side-by-side has generated significant interest and curiosity in the scientific community.

In the present study, the W/C model of DNA structure was examined with the help of plasmid DNA. It was hypothesized that two strands of a plasmid will remain intertwined and not separate into single-stranded circular DNA molecules under denaturing conditions, if it follows the W/C model. To test this, pUC19 and pBR322 plasmids were denatured using sodium hydroxide (NaOH) and analyzed by gel electrophoresis. Interestingly, addition of NaOH to pUC19 and pBR322 plasmids resulted in new form of DNA showing higher electrophoretic mobility in agarose gel. DNA corresponding to higher electrophoretic mobility band of pUC19 (hmP19) was found to be single-stranded and circular, suggesting the separation of two strands of plasmid DNA. Under suitable conditions, hmP19 DNA re-annealed to form native pUC19 plasmid. These results showed that two strands of a DNA molecule are not intertwined with each other and contradicted the W/C model of DNA structure.

Methods
Plasmid isolation, denaturation and agarose gel electrophoresis
pUC19 and pBR322 plasmids were isolated from E. coli strains [cultured in Luria Bertani broth (HiMedia, catalogue# M1245) in a shaking incubator at 37°C] by alkaline-lysis method as described previously. For denaturation, approximately 5 µg plasmid DNA (concentration determined using NanoDrop spectrophotometer) was added with an equal volume (5 µl) of NaOH solution of indicated concentration.

Digestion of plasmid DNA and polymerase chain reaction
NaOH in denatured pUC19 solution was neutralized using 5 µl HCl (concentration, 0.5 M) and plasmid was incubated with Hind III (SibEnzyme, catalogue# E073), S1 nuclease (Promega Corporation, catalogue# E576A) or exonuclease I and alkaline phosphatase (Thermo Scientific, catalogue# EN0581 and EF0651, respectively) at room temperature for 30 min. Samples were immediately run on 1% agarose gel. A mix of forward primer (5’-CTGCTTTCTGCTGTATGTC-3’) and reverse primer (5’-AAGCCCTTGCTTCTTATACT-3’) (experimental control) was also digested with exonuclease I and alkaline phosphatase. Digested and undigested primers were used in polymerase chain reaction [initial denaturation, 95°C/3 min followed by 30 cycles of (i) 95°C/30 sec, (ii) 55°C/30 sec, (iii) 72°C/30 in the same order] to amplify target sequence in A2780 cell line genomic DNA. PCR product was analyzed on 1% agarose gel.

DNA extraction from agarose gel
hmP19 DNA bands were cut with the help of a clean knife. DNA was purified using an extraction kit, as suggested by manufacturer (FairBiotech, catalogue# DE0100). Briefly, gel was dissolved in DE buffer and passed through a column. After washing with buffers, DNA was eluted in 50 µl nuclease free water.

Bacterial transformation
Transformation of E. coli strain DH5-alpha with gel-extracted plasmid DNA was carried out by heat-shock method (42°C for 30 sec) using water bath. Transformed and non-transformed bacteria were spread on ampicillin-nutrient agar plates supplemented with 25 µl X-Gal (Himedia, catalogue# MB0690). Plates were kept overnight in a 37°C incubator.

Results and discussion
Two strands of a DNA molecule are held together by non-covalent interactions, which can be disrupted by increasing the pH of DNA solution. In the present study, approximately 5 µg of plasmid DNA was denatured by adding NaOH solution of increasing concentration. It was observed that addition of 0.5 N NaOH to pUC19 resulted in a new form of DNA showing higher electrophoretic mobility in agarose gel (Figure 1a, Underlying data). Similar results were obtained with pBR322 plasmid added with 0.5 N NaOH (Figure 1b, Underlying data). Formation of higher electrophoretic mobility DNA in pBR322 plasmid added with NaOH has also been reported previously.

DNA corresponding to higher electrophoretic mobility band of pUC19 (hmP19) was characterized using DNA modifying enzymes in next experiments. Incubation with Hind III, which acts on double-stranded DNA, digested pUC19 plasmid but not hmP19 DNA (Figure 1c, Underlying data). S1 nuclease, which digests single-stranded DNA, degraded hmP19 DNA but not pUC19 plasmid (Figure 1d, Underlying data). Exonuclease I and alkaline phosphatase, which would digest single-stranded linear DNA, degraded neither of pUC19 or hmP19 DNA (Figure 1e, Underlying data). These results showed that hmP19 DNA is single-stranded and circular. Exonuclease I- and alkaline phosphatase-digested primers (experimental control) did not form product in PCR reaction (Supplementary Figure 1, Extended data).

We asked whether hmP19 DNA was generated by separation of two strands of pUC19 or breakage of one strand followed by release of another one. Formation of a single band of higher electrophoretic mobility by denatured pUC19 (instead of two, which would have been the case when one strand was linearized and another was circular) suggested that hmP19 DNA was generated by separation of two strands of the plasmid (Figure 1a, b). To confirm this notion, hmP19 DNA was subjected to renaturing conditions. If hmP19 DNA were formed due to separation of two strands of pUC19, it would reanneal to form
Figure 2. Single-stranded circular hmP19 DNA annealed to form double-stranded pUC19 plasmid. pH of denatured pUC19 plasmid DNA was normalized using HCl solution of indicated concentration. Agarose gel electrophoresis showed the formation of native pUC19 plasmid DNA in denatured plasmid DNA solution added with 0.25 M HCl (a). hmP19 DNA was extracted from agarose gel and approximately 3 µg of it was rerun on 1% agarose gel. Band pattern of pUC19 plasmid formed by extracted hmP19 DNA was same as that of native pUC19 plasmid (b). Gel-extracted hmP19 DNA was incubated with Hind III and run on 1% agarose gel. Hind III digested the pUC19 plasmid formed by gel-extracted hmP19 DNA (c). E. coli strain DH5-alpha was transformed with gel-extracted hmP19 DNA by heat-shock method. hmP19 DNA-transformed (d), but not non-transformed (e) bacteria formed colonies on ampicillin-X-Gal-nutrient agar plates. Representative data of two-three independent experiments are shown.
due to reversible separation of two strands of pUC19 plasmid DNA.

Next, we characterized pUC19 plasmid formed by reannealing of gel-extracted hmP19 DNA. Interestingly, similar to the native plasmid, pUC19 plasmid formed by gel-extracted hmP19 DNA was also degraded by Hind III (Figure 2c). Functionality of pUC19 formed by gel-extracted hmP19 DNA was demonstrated by its ability to transform E. coli. hmP19 DNA-transformed bacteria acquired ampicillin resistance and formed colonies on ampicillin-nutrient agar plates supplemented with X-Gal (Figure 2d, Underlying data). No colonies were formed by non-transformed bacteria (Figure 2e). These results showed that pUC19 plasmid formed by re-annealing of hmP19 DNA was structurally and functionally similar to native plasmid DNA.

Concludingly, reversible separation of two strands of plasmid DNA into single-stranded circular DNA molecules shows that DNA strands are not intertwined with each other. These findings contradict the W/C model of DNA structure and provide evidence for the side-by-side structure of DNA.

Data availability

Underlying data

Figshare: Addition of sodium hydroxide (NaOH) to pUC19 plasmid resulted in a distinct band of higher electrophoretic mobility DNA. https://doi.org/10.6084/m9.figshare.7751093.v6

This project contains the following underlying data:
- puc naoh.tif (Gel image demonstrating plasmid mobility in varying NaOH concentrations)

Figshare: Addition of sodium hydroxide (NaOH) to pBR322 plasmid resulted in a distinct band of higher electrophoretic mobility DNA. https://doi.org/10.6084/m9.figshare.7751084.v5

This project contains the following underlying data:
- pbr naoh.tif (Gel image demonstrating plasmid mobility in varying NaOH concentrations)

Figshare: Higher electrophoretic mobility band of pUC19 (hmP19) DNA is single-stranded in nature. https://doi.org/10.6084/m9.figshare.7751090.v3

This project contains the following underlying data:
- puc s1nuclease.tif (Gel image showing plasmid products following incubation with S1 nuclease)

Figshare: Higher electrophoretic mobility band of pUC19 (hmP19) DNA is not double-stranded in nature. https://doi.org/10.6084/m9.figshare.7751087.v3

This project contains the following underlying data:
- puc hind.tif (Gel image showing plasmid products following incubation with Hind III)
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