

Abstract

Structural Polymorphism of DNA in Solution of Homologous Duplexes and Its Consequences for Heteroduplex Formation Procedure

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After invention of PCR heteroduplex formation procedure (HF) has become widely used in practical medicine for detection of point mutations of unknown location. DNA solutions for HF represent mixtures of homologous duplexes differing by just a single base pair or a very small region at the position of mutation. As the result of HF, four types of DNA duplexes are formed: two homoduplexes and two heteroduplexes. Identification of heteroduplexes or mismatch cleavage are used to reveal the unknown mutation. Modern practical medicine performs detection of unknown point mutations predominantly using following HF-based methods: Chemical or Enzyme Cleavage of Mismatches, Heteroduplex Analysis, Denaturing Gradient Gel Electrophoresis, and Denaturing High-Performance Liquid Chromatography. A linear structure of homo- and heteroduplexes is critical for the analyses: formation of branched DNA structures during HF causes undesirable background. However, spontaneous DNA-DNA interaction in HF has never been analyzed and now it is not taken into consideration. For the first time the phenomenon of spontaneous formation of branched DNA by linear duplexes was demonstrated 10 years ago by Kallenbach et. al. on the model of four synthetic hemihomologous DNA duplexes. Then spontaneous DNA-DNA interaction was shown on the model of homologous DNA fragments with CA-repeats restricted from the genome of simian virus 40 and linearized plasmids, and recently this phenomenon was demonstrated for the wide range of homologous duplexes on the models of purified PCR products and linearized plasmids. In our previous investigation we found out the mechanism and peculiarities of the interaction. It was shown that spontaneous DNA-DNA interaction between homologous linear duplexes occurs via nucleation of dissociated ends of complementary fragments with formation of Holliday junctions resolving via branch migration into new or previously existing duplexes. On the whole, spontaneous DNA-DNA interaction with formation of branched DNA is considered nowadays to be a characteristic peculiarity of DNA, and there is equilibrium between linear duplexes, HJs and more complex branched DNA structures in solutions of homologous duplexes. Conditions used by different authors for HF vary considerably, and some of them facilitate DNA branching, which is expected to occur also in solutions of heteroduplexes, and thus increase undesirable background in the analyses requiring linear DNA structure. Formation of Holliday junctions, branched DNA structures, was demonstrated in solutions of homologous duplexes after heteroduplex formation procedure on the model of four different PCR products. Presence of DNA structures of approximately double the weight of duplexes was revealed by gel electrophoresis. Electron-microscopy of DNA fraction of double the weight of duplexes demonstrated symmetrical χ -structures. We have shown the influence of buffer ion composition and DNA concentration on Holliday junction appearance after heteroduplex formation. Duplexes with homologous central regions, but non-complementary sequences at the fragment ends were shown not to form hybrid heteroduplexes or hybrid Holliday junctions after completion of heteroduplex formation procedure. Concentration of Holliday junctions after heteroduplex formation depends on duplex end sequences: equilibrium concentration of Holliday junctions in solutions of fragments flanked by GC sequences was greatly less than in solutions of the same fragments but with AT sequences on the ends. Our results demonstrate the perspectives of multiplex heteroduplex formation procedure and necessity of special mixture composition, optimal DNA concentration and GC-clamp usage to prevent intensive Holliday junction formation and to increase the fidelity of linear duplex formation by heteroduplex formation procedure.