

between spermine and protonated C^{+} , as a major cause of low pK destabilization of sp-ODNs triplexes.

Effect of mismatches. Thermal denaturation studies of triplexes containing a single Hoogsteen mismatched triad of 5-Me-dC- N^4 -spermine with Watson-Crick doublets CG, TA and AT showed that the inverted doublet CG also formed triplex, although less effective than the original GC (Fig. 2, Table 1). Of the other pairs, AT and TA, only the former exhibited any detectable triplex formation. With both CG and AT doublets, a carbonyl group (O6 of G and O4 of T) is available in the first base of the triad as a hydrogen bond acceptor in major groove [20]. It is possible that the N^4 -spermine side chain from the third strand can span across the major groove to form additional hydrogen bonds from its imino/amino group with the O6/O4 carbonyls of G and T. Thus C and A are well tolerated in the central purine position of the triad, with pronounced requirement of C in the third strand assuming less significance and hence favouring triplex even at neutral pH. This may have utility for employing 5-Me-dC- N^4 -spermine as a Hoogsteen mismatch tolerant, without loss of stability. Spermine chain may also participate in hydrogen bonding with WC base pair of adjacent triads and this possibility should lead to considerable sequence dependence of triplex stabilization and may be understood better by a study of nearest neighbour effect in sp-ODN triplex.

Cationic ion effects. From a recent study of electrostatic effects in triplexes in intermolecular triplexes [46], it was observed that electrostatic interactions between protonated C (C^{+}) of third strand and phosphate backbone offer considerable stabilization in a global, sequence independent manner. However, repulsion between adjacent C^{+} can cause successful negation of the above electrostatic advantage, thereby imparting local sequence dependent effects. Application of polyelectrolyte theory to DNA suggests that DNA melting is accompanied by release of a part of counter-ions from DNA bound state to bulk as the condensed charge density is reduced in the melting process. In zwitterionic DNA, such as that from sp-ODNs 8-8 described here, or 2-thiacytosine-2'-dC incorporated DNA reported earlier [18], the high charge density in triplex/duplex form is partially balanced by the covalently bound, non-diffusible cations, offering a unique stabilization effect.

Electrostatic neutralization. The intermolecular phosphate neutralization by polycations spermine leading to a net diminished charge for sp-ODNs 8-8 is clearly apparent from melting retardation seen for them in gel electrophoresis and better association of sp-ODN in triplex formation. Also spermine (from ODN 8 migrates equal to an unsubstituted 24mer B and this migration is systematically retarded as a function of degree of spermine substitution. Such a correspondence illustrates the zwitterionic character of sp-ODNs 8-8 similar to those reported by Switzer et al. [16]. An analogous effect of interstrand phosphate charge neutralization is possible in triplex. Such charge neutralization effects by externally added cations are known to lead to electrophoretic retardation of high molecular weight (1-3 kb) linear/plasmid DNA due to counter-ion condensation effects [11]. The third strand of DNA asymmetrically partitions the major groove [37] and the N^4 -linked spermine chain may position itself across the major groove accessing the phosphate backbone of both of WC duplex strands.

Effect of spermine on dissociation/association equilibrium. The nature of the observed hysteresis (Fig. 6) and its salt dependence (Fig. 7) indicated that the appended spermine may assist third strand association with complementary duplex. Normally, hysteresis in triplex melting as seen with d N^4 B N^9 arises from a slower association of third strand with duplex during cooling as compared to dissociation rate during heating [28]. The insignificant hysteresis phenomena seen in sp-ODN triplex (100 mM NaCl) suggests that the association rate is comparable to that of dissociation rate, i.e., the association is enhanced relative to that seen in unsubstituted triplex. In presence of highest salt concentration (500 or 100 mM NaCl with 20 mM MgCl₂), the hysteresis is marginally increased (LJ₅₀, -3°C) due to a slower association. This perhaps arises from accumulated salt cations on duplex surface which electrostatically disfavors the approach of cationic spermine chain. Thus, stable triplex formation by sp-ODNs at physiological pH may be a combined consequence of intermolecular hydrogen bonding and electrostatic stabilization leading to a favored association event.

The overall experimental results presented here suggest that when spermine molecule is covalently attached to the third strand, it may still be accumulated in the deep major groove of the duplex DNA along with the third strand, yielding a stable triplex helix. However, it is difficult at this juncture to delineate the extent of contributions by each of the above factors for enhanced stability of sp-ODN triplexes.

Conclusions

This paper reports studies on stability of triplex helices employing spermine conjugated ODNs as third strand. Triplexes are observed in the absence of MgCl₂ at physiological pH, conditions under which ODNs containing dC N^4 or 5-Me-dC N^4 fail to show triplex hybridization. The simple and site-specific spermine conjugation permits introduction of polycations at desired, multiple internal sites in oligonucleotide sequences, thereby imparting significant zwitterionic character to DNA. No pronounced loss is observed for triplex formation by sp-ODNs since the corresponding loss in stability is more than compensated for by spermine's favorable interactions with duplex DNA. The increased stability of sp-ODN triplexes is also a consequence of favoured association of third strand with the duplex. The observed nature of mismatch tolerance may enlarge the scope of sp-ODN applications in triplexes. A lower net negative charge arising from multiple substitution, may assist the cellular uptake of ODNs. This conjecture is strengthened by the recent findings that neutralization of ODNs with cationic lipids significantly enhances the membrane permeability [33,34]. The synthetic DNA-lipid complexes have also found utility in receptor mediated gene transfer [37] and the presence of spermine binding sites on the cell surface [35] may aid uptake of sp-ODNs. Since polycations can also form metal complexes, the compounds such as those synthesized here, may in addition have potential utility in site-directed cleavage of DNA.

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Laura E. Berk



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Technology to Assess Sleep, An Issue of Sleep Medicine Clinics Thomas Penzel, 2016-10-31 Guest editor Thomas Penzel has assembled an expert team of authors on the topic of Technology to Assess Sleep Article topics include Sleep Apps using new sensor technologies Sleep Apps to assess sleep quality Pulse wave analysis Peripheral arterial tone to assess sleep disordered breathing Pulse transit time to assess cardiovascular function A computer model of sleep and breathing regulation Upper airway imaging Quantifying leg movement disorders Multi center sleep studies Cardiorespiratory coupling during sleep Quantifying airflow limitation and snoring and more **Sleep Research** ,1998 **Sleep and Dreaming** Edward F. Pace-Schott, 2003-02-27 How and why does the sleeping brain generate dreams Though the question is old a paradigm shift is now occurring in the science of sleep and dreaming that is making room for new answers From brainstem based models of sleep cycle control research is moving toward combined brainstem forebrain models of sleep cognition itself The book presents five papers by leading scientists at the center of the current firmament and more than seventy five commentaries on those papers by nearly all of the other leading authorities in the field Topics include mechanisms of dreaming and REM sleep memory consolidation in REM sleep and an evolutionary hypothesis of the function of dreaming The papers and commentaries together with the authors rejoinders represent a huge leap forward in our understanding of the sleeping and dreaming brain The book s multidisciplinary perspective will appeal to students and researchers in neuroscience cognitive science and psychology Reviews in Food and Nutrition Toxicity, Volume 3 Victor R. Preedy, 2005-03-22 Including the latest reviews of the most current issues related to food and nutrition toxicity Reviews in Food and Nutrition Toxicity Volume 3 distills a wide range of research on food safety and food technology Put together by a strong team with a wealth of broad experience the continuation of this important new series includes contributions f The International Conference on Health Informatics Yuan-Ting Zhang, 2013-11-20 This volume presents the proceedings of the International Conference on Health Informatics ICHI The conference was a new special topic conference initiative by the International Federation of Medical and Biological Engineering IFMBE held in Vilamoura Portugal on 7 9 November 2013 The main theme of the ICHI2013 was Integrating Information and Communication Technologies with Biomedicine for Global Health The proceedings offer a unique forum to examine enabling technologies of sensors devices and systems that optimize the acquisition transmission processing storage retrieval of biomedical and health information as well as to report novel clinical applications of health information systems and the deployment of m Health e Health u Health p Health and Telemedicine **Index of Conference Proceedings** British Library. Document Supply Centre, 1998 **Ambient Intelligence** Ioannis Chatzigiannakis, Boris De Ruyter, Irene Mavrommati, 2019-11-06 This book constitutes the refereed proceedings of the 15th European Conference on Ambient Intelligence AmI 2019 held in Rome Italy in November 2019 The 20 full papers presented together with 10 short papers were carefully reviewed and selected from 50 submissions The papers

cover topics such as embedded devices that can merge unobtrusively and in natural ways using information and intelligence hidden in the network connecting these devices e.g. the Internet of Things The main topic of Aml 2019 was Data driven Ambient Intelligence which follows the vision of Calm Technology where technology is useful but does not demand our full attention or interfere with our usual behavior and activities

Plasticity in the Adult Brain: From Genes to Neurotherapy M.A. Hofman, G.J. Boer, Eus JW Van Someren, J. Verhaagen, D.F. Swaab, A.J.G.D. Holtmaat, 2002-10-23 In the past decade neuronal plasticity has become a major theme of modern neurobiology from cellular and molecular mechanisms of synapse formation in worms and insects to behavioural recovery from strokes in elderly humans For this reason the focus of interest in the present volume of Progress in Brain Research is on the topic of neuroplasticity in mature organisms including humans Contributions range from neurogenesis and synaptic plasticity in the adult primate brain to neural mechanisms of learning and memory and the influence of environmental factors and aging on the functional potential of the central nervous system Several contributions focus on recent developments in neural regeneration and brain repair providing challenging evidence that the use of stem cell neurotherapy may be beneficial to humans suffering from various neurological and psychiatric diseases This volume integrates new information on the cellular and molecular mechanisms of neuroplasticity and highlights challenging future questions in this exciting and topical area of neuroscience

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