nature communications

Article

https://doi.org/10.1038/s41467-024-49534-0

A non-B DNA binding peptidomimetic channel alters cellular functions

Received: 8 August 2022

Accepted: 7 June 2024

Published online: 20 June 2024

Check for updates

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DNA binding transcription factors possess the ability to interact with lipid membranes to construct ion-permeable pathways. Herein, we present a thiazole-based DNA binding peptide mimic **TBP2**, which forms transmembrane ion channels, impacting cellular ion concentration and consequently stabilizing G-quadruplex DNA structures. **TBP2** self-assembles into nanostructures, e.g., vesicles and nanofibers and facilitates the transportation of Na^+ and K^+ across lipid membranes with high conductance (~0.6 nS). Moreover, **TBP2** exhibits increased fluorescence when incorporated into the membrane or in cellular nuclei. Monomeric **TBP2** can enter the lipid membrane and localize to the nuclei of cancer cells. The coordinated process of time-dependent membrane or nuclear localization of **TBP2**, combined with elevated intracellular cation levels and direct G-quadruplex (G4) interaction, synergistically promotes formation and stability of G4 structures, triggering cancer cell death. This study introduces a platform to mimic and control intricate biological functions, leading to the discovery of innovative therapeutic approaches.

Bio-inspired peptides exhibit a remarkable ability for self-assembly into nanostructures in specific microenvironments, enabling the formation of membrane channels upon insertion into biological or model lipid bilayers[1,2]. Some of these peptides induce cytotoxicity or potent antitumor activity[2]. Transmembrane channel proteins play a vital role in cellular homeostasis by transporting ions across lipid bilayer membranes[1–3]. However, their practical utility is limited by structural complexity and critical molecular mechanisms[4–6]. Efforts have been directed towards the development of synthetic ion channels with nucleic acids, natural transcription factors and peptides, and synthetic molecules by mimicking characteristics of biological ion channels[7–16]. Only a few small molecule ion transporters have been reported to exhibit therapeutic properties[17–23]. For instance, Zhang et al. reported a small molecule based cation transporter with the ability to kill cancer cells[19]. Crafting synthetic molecular ion transporters with therapeutic potential and understanding their biophysical and biological properties could provide critical insights into their functional mechanisms[24–36]. Peptide mimetics[37–41], due to their unique structural diversity and potential role in biological systems, hold promise as essential chemotherapeutic agents by targeting DNA or its secondary structures.

Non-B-DNA four stranded secondary structures e.g., G-quadruplexes (G4s) play a crucial role in cellular growth by regulating the replication and transcription machinery[42–44]. G4s are commonly found in the promoter G-rich region of various proto-oncogenes (e.g., *c-MYC*, *c-KIT*), as well as in telomeres (*h-TELO*) of cancer cells. The *c-MYC* proto-oncogene predominantly controls cell proliferation, apoptosis, and drug resistance in various cancer types such as cervical, breast, and lung cancers[44–48]. The NHE (nuclease hypersensitive element) III_1 region of *c-MYC* promoter is responsible for approximately 90% of its transcriptional activity, contains G4-forming sequences that act as transcriptional repressors[45–52]. Stabilizing these G4 structures with synthetic molecules has emerged as a potential strategy for cancer therapeutics, offering promising avenues for targeted cancer treatment[53–59].

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How to cite: *Angew. Chem. Int. Ed.* **2023,** *62,* e202215245
International Edition: doi.org/10.1002/anie.202215245
German Edition: doi.org/10.1002/ange.202215245

G-quadruplexes

Expanding the Toolbox of Target Directed Bio-Orthogonal Synthesis: *In Situ* Direct Macrocyclization by DNA Templates

*Ritapa Chaudhuri, Thumpati Prasanth, and Jyotirmayee Dash**

Dedicated to Professor Ayyappanpillai Ajayagosh on his 60th birthday

Abstract: Herein, we demonstrate for the first time that noncanonical DNA can direct macrocyclization-like challenging reactions to synthesize gene modulators. The planar G-quartets present in DNA G-quadruplexes (G4s) provide a size complementary reaction platform for the bio-orthogonal macrocyclization of bifunctional azide and alkyne fragments over oligo- and polymerization. G4s immobilized on gold-coated magnetic nanoparticles have been used as target templates to enable easy identification of a selective peptidomimetic macrocycle. Structurally similar macrocycles have been synthesized to understand their functional role in the modulation of gene function. The innate fluorescence of the *in situ* formed macrocycle has been utilized to monitor its cellular localization using a G4 antibody and its *in cell* formation from the corresponding azide and alkyne fragments. The successful execution of *in situ* macrocyclization *in vitro* and in cells would open up a new dimension for target-directed therapeutic applications.

Introduction

Target-guided synthesis (TGS) has emerged as a powerful tool for identifying target-selective leads without prior synthesis and high-throughput screening.[1–3] It has been efficiently used for the facile synthesis of inhibitors for various therapeutic proteins as well as ribonucleoprotein targets.[4–7] However, DNA/RNA targets as templates remain less explored for the design and development of small molecules for modulating gene function.[8–11] The Dervan group first employed duplex DNA as a template to conjugate two minor groove-binding anti-parallel hairpin polyamides by azide-alkyne cycloaddition, demonstrating its feasibility for specific genome targeting.[12] Balasubramanian and co-workers explored noncanonical DNA/RNA G-quadruplexes (G4s) as templates to discover their high-affinity binders.[13,14] Herein, we delineate the *target-directed macrocyclization* of bifunctional building blocks to generate a new class of peptidomimetic macrocycles using G4s as targets. Owing to the structural constraints of peptide and peptidomimetic macrocycles, they exhibit metabolic stability and enhanced binding affinity for biomolecular targets.[15] The large surface area of peptidomimetic macrocycles prevents intercalative binding to double-stranded DNA and shows selectivity towards DNA quadruplexes.[10,16–19] However, the synthesis of macrocycles, especially ring-closing reactions, is always cumbersome and challenging. The azide-alkyne cycloaddition has been utilized as a tool for the construction of macrocyclic scaffolds that find applications in peptide and carbohydrate chemistry, supramolecular systems and medicinal chemistry.[6,20–22]

Double cycloaddition between bifunctional fragments leading to macrocyclization requires high dilution conditions, often undergoes polymerization and is unachievable at high temperatures.[15,23] The target-guided synthesis of peptides such as macrocyclic ligands by *in situ* double click reaction would not only enable the efficient construction of macrocyclic structures but also assist in identifying the binders of a biological target without synthesizing all of them. However, the identification of *in situ*-generated ligands from the reaction mixture has always been challenging. In this work, we present that G4s could selectively guide the synthesis of a macrocyclic peptidomimetic from a pool of bis-alkyne and bis-azide fragments (Scheme 1). This macrocyclic ligand has also been synthesized in live cells from its corresponding fragments and shows a promising biological profile.

Tetrahelical DNA G4s are abundantly present in the telomeric region of chromosomes and the promoter region of oncogenes.[24,25] Small molecule-mediated G4 stabilization has been considered an evolving approach for modulating gene expression as well as maintaining telomere length.[26–29] Since G4s contain a G-quartet having a cyclic structure as the basic structural motif, we thus presumed that the G4 DNA targets could template the formation of macrocyclic binders of similar size among the indefinite possibilities of generating linear open chain products as well.

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