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DESIGN, SYNTHESIS AND DNA-CLEAVAGE OF GLY-GLY-HIS-NAPHTHALENE DIIMIDE CONJUGATES

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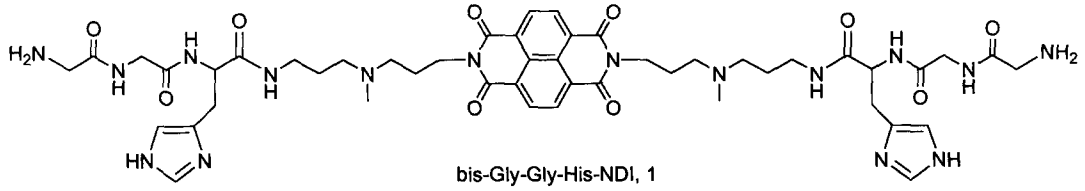
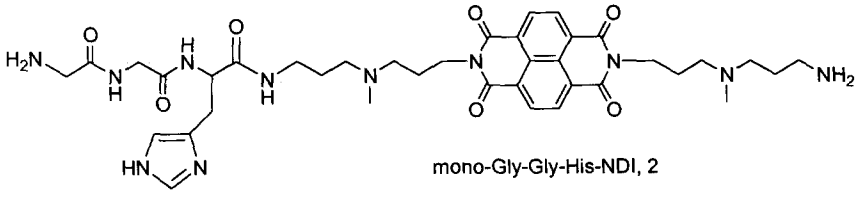
Abstract: Naphthalene diimides with one and two metal-chelating Gly-Gly-His (GGH) motifs have been synthesized. Both conjugates induce single-stranded cleavage of plasmid pBR322 DNA in the presence of nickel and Oxone and are approximately 100-fold more efficient than Ni(II)•GGH-$CONH_2$ itself.

Introduction

There has been extensive recent interest in the oxidative cleavage of DNA using transition metal complexes.[1-6] Transition metal complexes can be targeted to DNA. Examples of metal-based cleavers conjugated to intercalators include systems based on the intercalators methidium,[7,8] dipyridoimidazole,[9] anthraquinone,[10-12] ellipticine,[13,14] acridine,[15-20] and porphyrins.[21]



bis-Gly-Gly-His-NDI, 1



mono-Gly-Gly-His-NDI, 2

Figure 1. Structures of Gly-Gly-His-naphthalene diimide conjugates.

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The goal of the current study was to probe the structural factors in metal-based intercalator-cleavers that lead to effective cleavage. We attached the known metal chelating peptide Gly-Gly-His (GGH)[3] to a bis-substituted naphthalene diimide (NDI) (Figure 1). Naphthalene diimides with this substituent pattern are threading intercalators.[22] Herein we report the plasmid cleavage of the naphthalene diimide bearing two Ni(II)•GGH substituents (1) in comparison with that bearing only one Ni(II)•GGH substituent (2) and Ni(II)•GGH-$CONH_2$ itself.

Results and Discussion

The two GGH-naphthalene diimide molecules in this study[23] were designed to probe the effect of an appended NDI intercalator on Ni(II)•GGH cleavage of plasmid DNA. The side chains were long enough [eight rotatable bonds between the diimide nucleus and the Ni(II)•GGH moiety] that the Ni center can reach the H-4' in the minor groove (the expected site of attack).[24-26] In addition, the peptide side chains were designed to have a net charge of +1 at physiological pH (the protonated $CH_2N^+HMeCH_2$ moiety). Comparisons of various Ni(II)•Xaa-Xaa-His species as DNA cleavers shows that a Lys or Arg in the first Xaa position enhances cleavage by approximately 20-fold,[26,27] presumably because the positive charge of the terminal amino acid enhances the DNA binding of the neutral Ni(II)•GGH complex (the GGH is bound to the nickel through the histidine imidazole nitrogen, two deprotonated amide nitrogens and the terminal α-NH_2).[3,28]

The DNA cleavage ability of Ni(II)•GGH-$CONH_2$, mono-Ni(II)•GGH-NDI and bis-Ni(II)•GGH-NDI were compared using Oxone ($KHSO_5$) as the oxidant.[29] Ni(II)•GGH-NDI complexes readily converted the supercoiled form (Form I) of plasmid pBR322 into nicked (Form II) and linear (Form III) forms (Figure 2).[30] Significant conversion (around 60% after 10 min) of the supercoiled plasmid to the nicked form was achieved

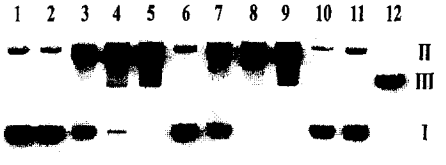

Figure 2. Cleavage of pBR322 with Ni(II)•GGH complexes. The reactions were run in 20 uL sodium phosphate buffer (10 mM, pH 7.5). Mixtures contained pBR322 (0.5 ug) and following additions: lane 1, none; lanes 2, 3, 4 and 5, bis-GGH-NDI at 0.2, 0.5, 1.0 and 1.5 uM, Oxone and $Ni(OAc)_2$ at 0.4, 1.0, 2.0 and 3.0 uM, each, respectively; lanes 6, 7, 8 and 9, mono-GGH-NDI, Oxone and $Ni(OAc)_2$ at 0.2, 0.5, 1.0 and 1.5 uM, respectively; lane 10, Oxone and $Ni(OAc)_2$ at 50 uM; lane 11, GGH, Oxone and $Ni(OAc)_2$ at 5 uM; lane 12, 1 uL of EcoR I.

at 0.5 uM concentrations of **1** and **2**. The same degree of cleavage by Ni(II)•GGH requires 100 times higher (50 uM) peptide concentration.[31] Three control reactions did not give observable cleavage of pBR322: (1) Oxone and Ni(II) alone (50 uM each); (2) **1** or **2** alone (100 uM); and (3) starting *N,N'*-bis(*N*-3,3'-diamino-*N*-methyldipropyl)-1,4,5,8-naphthalenetetracarboxylic diimide, Oxone and $Ni(OAc)_2$ (50 uM each). The

Ni(II)•GGH-NDI conjugates are two orders of magnitude more efficient than the tripeptide itself under the same conditions. This presumably results largely from a higher local concentration of the GGH-nickel conjugate in the grooves due to intercalation of the naphthalene diimide. Dicationic naphthalene diimides have binding constants of $\sim 10^5$ M^{-1}.[22]

Concentration dependence cleavage experiments were also carried out; cleavage increased with increasing concentration of the intercalator. At 0.2, 0.5, 1.0, and 1.5 uM intercalator concentrations, bis-Ni(II)•GGH-NDI showed 6, 61, 92, and 92% nicked DNA as well as 0, 2, 3, and 7% linear DNA, respectively [one equivalent of Oxone and $Ni(OAc)_2$ per equivalent of peptide; 6% background nicked plasmid]. At the same concentrations of intercalator, mono-Ni(II)•GGH-NDI produced 8, 51, 97, and 95% nicked DNA as well as 0, 2, 2, and 5% linear DNA. The observation of quantifiable amounts of linear DNA only after significant nicked DNA has been formed indicates that the linearized DNA arises from statistical approximation of two nicks on opposite strands of the DNA rather than from specific double stranded cleavage.[32]

For the bis-substituted derivative, the small extent of double-stranded cleavage may have a number of origins. To the extent that cleavage occurs with low efficiency relative to the concentration of nickel centers, double strand cleavage may be rare simply because the statistical chance of cleavage arising from both centers is rare. Our experiments used stoichiometric amounts of GGH-NDI, Ni(II), and Oxone to reduce the extent of background cleavage. However, this also means that the Ni(II) center is unlikely to be reactivated in the experiment. The lack of significant double-stranded cleavage may also be because the naphthalene diimide dissociates from the DNA after cleaving one strand but before effecting cleavage of the second strand. The dissociation rate constants for naphthalene diimide threading intercalators are in the range of 1 - 10 s^{-1}.[22] Finally, lack of double-stranded cleavage may be because the orientation of the oxidizing nickel complex in the major groove does not favor ready hydrogen abstraction. Previous work on Ni(II)•GGH has indicated that most of the cleavage occurs via abstraction of the H-4', which is found in the minor groove.[24,25] When the links tethering the Ni(II)•GGH motif to the DNA binding motif are long enough, conjugates in which the DNA binding moieties are in the major groove can cleave the DNA, presumably because the linkers allow the Ni(II)•GGH motif to reach the minor groove. Examples include GGH attached to the DNA-binding domain on Hin recombinase [Ni(II)•GGH(Hin139-190)],[24] the zinc finger-based cutter Sp1GGH,[3,33] M(II)•Gly-Lys-His-Fos(138-211),[34] and a four-stranded Ni(II)•PNA-DNA bundle.[35] Molecular modeling (Sybyl, Tripos Associates) of bis-Ni(II)•GGH-NDI intercalated into duplex DNA shows that the GGH arm is long enough to reach around the phosphate backbone and approach the H-4'. Even though the GGH arm can reach the H-4' position, the orientation may not favor ready abstraction. This explanation was proposed by Footer et al. to explain their

observation of only a maximum of 20% cleavage in the Ni(II)•PNA-DNA bundle.[35]

Conclusions

This study shows that the Ni(II)•GGH-NDIs are effective in cleaving plasmid DNA. The addition of a second Ni(II)•GGH does not increase the cleavage efficiency significantly. The Ni(II)•GGH motif appended to a naphthalene diimide intercalator is approximately 100-fold more effective in cleaving DNA than the Ni(II)•GGH moiety itself. The enhancement compares favorably with other systems bearing a metal-chelating moiety appended to an intercalator. Enhancement of cleavage is not always observed when a metal binding moiety is attached to an intercalator: a series of cationic metalloporphyrin-ellipticine complexes were less effective cleavers than the cationic porphyrins alone.[13] However, most intercalator-metal binding conjugates do show enhanced cleavage. For example, ellipticine-salen-Cu conjugates cleave supercoiled DNA approximately three-fold more efficiently than salen-Cu itself.[14] The acridine hybrid of Cu(II)•Clip-Phen was approximately four-fold more active than Cu(II)•Clip-Phen itself.[20] A Cu(II)•polyamine-anthraquinone derivative was approximately eight-fold more effective than the copper•polyamine complex alone.[11] A series of Cu(II)•salen-anthraquinone conjugates showed cleavage efficiencies within ten-fold of the Cu(II)•salen moiety itself.[12] A porphyrin bearing a chelated manganese cleaved plasmid DNA >ten-fold more effectively than the manganese chelate alone.[21] A series of dipyridoimidazole-hemin molecules averaged >50-fold more effective cleavage than hemin itself.[9] Methidiumpropyl-EDTA•iron(II), widely used as a footprinting agent, cleaves >100-fold more efficiently than Fe(II)EDTA itself.[8] The Ni(II)•GGH-NDI conjugates in the current study, approximately 100-fold more efficient than the Ni(II)•GGH-$CONH_2$ alone, show one of the highest cleavage enhancements found to date.

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containing 0.5 ug/mL ethidium bromide in the gel and in the buffer. The electrophoresis was carried out in 1 x TAE buffer, pH 8.0, for 1.5 h at 5 V/cm. The gel was scanned with a Molecular Dynamics FluorImager SI. Bands corresponding to nicked supercoiled (Form I), nicked (Form II) and linear (Form III) plasmids were quantitated with ImageQuaNT gel scanning software. The data were corrected for the decreased stainability of Form I DNA, assuming that the supercoiled plasmid was taken to be 1.22 times more fluorescent than the relaxed DNA at the concentrations of ethidium used.[7] Cleavage percentages were calculated as: [(Form I)/(Forms (I + II +III))] × 100. Control linearized DNA was made with EcoR I (New England Biolabs Inc., 20,000 units/mL).

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