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DNA-fiber EPR investigation of the influence of amino-terminal residue stereochemistry on the DNA binding orientation of Cu(II)•Gly-Gly-His-derived metallopeptides

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Abstract

DNA fiber EPR was used to investigate the DNA binding stabilities and orientations of Cu(II)•Gly-Gly-His-derived metallopeptides containing D- vs. L-amino acid substitutions in the first peptide position. This examination included studies of Cu(II)•D-Arg-Gly-His and Cu(II)•D-Lys-Gly-His for comparison to metallopeptides containing L-Arg/Lys substitutions, and also the diastereoisomeric pairs Cu(II)•D/L-Pro-Gly-His and Cu(II)•D/L-Pro-Lys-His. Results indicated that L-Arg/Lys to D-Arg/Lys substitutions considerably randomized the orientation of the metallopeptides on DNA whereas the replacement of L-Pro by D-Pro in Cu(II)•L-Pro-Gly-His caused a decrease in randomness. The difference in the extent of randomness of D- vs. L-Pro-Gly-His complexes was diminished through the substitution of Gly for Lys in the middle peptide position, supporting the notion that the ε-amino group of Lys triggered further randomization, likely through hydrogen bonding or electrostatic interactions that disrupt binding of the metallopeptide equatorial plane and the DNA. The relationship between the stereochemistry of amino acid residues and the binding and reaction of M(II)•Xaa-Xaa'-His metallopeptides with DNA are also discussed.

Keywords

Copper; DNA-fiber; EPR; Gly-Gly-His

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1. Introduction

Investigations of DNA and RNA recognition by peptide-based metal complexes [1], including metallopeptides of the general form $M(II)\bullet Gly-Gly-His$ (Fig. 1) [2-3], have contributed to our fundamental understanding of low molecular weight agent-nucleic acid recognition principles. In these studies a well-structured peptide is generated by ligation to a metal center and subsequently exploited to present to a DNA or RNA target the same fundamental amino acid side chain recognition elements (e.g., amides, amines, and guanidinium functional groups) used by proteins or peptide-based drugs to achieve an efficient, selective interaction. Additionally, redox-active metallopeptides can be activated to induce DNA damage, further permitting their use as models to understand factors that influence small molecule DNA cleavage efficiency [4] and site-selectivity [2,3]. Indeed, the much-studied DNA cleaving antitumor agent bleomycin is, at its functional core, a metallopeptide [5]. Unlike most naturally-occurring systems, however, synthetic metallopeptides can be generated in a straightforward fashion to include alternative amino acid compositions and alpha carbon configurations, allowing detailed structure-function relationships to be examined conveniently.

Among the systems studied to date, those involving metallopeptides derived from the tripeptide Gly-Gly-His have served to illustrate the impact of amino acid composition and stereochemistry on small molecule-DNA cleavage site-selectivity and strand scission efficiency [1-3,4,6]. The Gly-Gly-His tripeptide, and derivatives with alternative amino acids at the first two peptide positions, bind Cu^{2+} or Ni^{2+} to form 1:1 complexes with high affinity through chelation of the peptide terminal amine, two intervening deprotonated peptide amides, and the His imidazole (Fig. 1); the approximately square-planar structures formed are well-characterized, stable at physiological pH values [7,8], and permit the side chains of amino acids located at the first two positions to project from the periphery of the final metal complex to participate in DNA recognition; the interactions of these systems with DNA appear to be dominated by amino acid side chain-DNA interactions. As Cu^{2+} complexes, these tripeptides are known to interact stereospecifically [9] with the DNA minor groove and can be activated to induce DNA strand scission [4]; as Ni^{2+} complexes, these metallopeptides interact similarly with DNA [9-11] and when activated with peroxides, induce well-resolved acts of DNA strand scission selectively at A/T-rich regions through C4'-H abstraction [10]. More recently, experimental [11,12] and theoretical [11-13] studies have established that $M(II)\bullet Gly-Gly-His$, and derivatives that include Arg, Lys, or Pro in the first peptide position, insert their amino-terminal/His imidazole "edge" into the minor groove to form hydrogen bonds between the amino-terminal N-H and His imidazole pyrrole N-H donors with H-bond acceptors located on the minor groove floor, e.g., the N3 or adenine or O2 of thymine residues. These interactions lead to a minor groove-bound metallopeptide that directs the side chain present in the first peptide position into the minor groove while amino acid side chains present in the second peptide position are exposed at the surface of the DNA-bound complex. Consequently, while the amino acid present in the amino-terminal position can be a major determinant of overall activity, the amino acid present at the second peptide position influences activity to a far lesser extent; however some substitutions can indeed lead to interesting DNA cleavage site-selectivities [14].

Given the position of the first amino acid residue in a minor groove-bound $M(II)\bullet Gly-Gly-His$ metallopeptide, the nature of the amino acid and also its stereochemistry can influence DNA reactivity. In the case of $Ni(II)\bullet L-Arg/Lys-Gly-His$ vs. $Ni(II)\bullet D-Arg/Lys-Gly-His$, L-side chains produce an isohelical metallopeptide with an extended, stabilizing "outrigger" that complements well the overall structure and curvature of the DNA minor groove while D-side chains appear to result in less structural compatibility [12]. These differences lead to increased DNA cleavage site-selectivity in the case of the L-substituted isomers and decreased site-selectivity, but increased overall cleavage efficiency, in the case of the D-isomers. However,

with side chains such as Pro in the first peptide position (*e.g.*, as in $M(II)\bullet_{L/D}\text{-Pro-Gly-His}$), the influence on DNA cleavage site-selectivity is less, likely due to the compact nature of this side chain which serves to diminish the structural differences between these two diastereoisomeric metallopeptides.

To further examine the impact of amino acid stereochemistry on the DNA binding stability and orientation of $M(II)\bullet\text{Gly-Gly-His}$ derived metallopeptides, we have employed DNA fiber EPR to investigate a series of metallopeptides containing *D*- vs. *L*-amino acid substitutions in the first peptide position. DNA fiber EPR spectroscopy provides unique information on the DNA binding stereospecificities and dynamics of many paramagnetic metal complexes, in particular those of $Cu(II)$ [6,9,15-18]. Indeed, we have employed DNA fiber EPR successfully in the past [9] to verify the parallels between $Cu(II)\bullet\text{Xaa-Gly-His}$ derived metallopeptides and their $Ni(II)$ -containing analogues. If a paramagnetic complex binds with a specific orientation to double helical DNA and the double helices are highly oriented in the DNA fibers, the EPR spectra will change as a function of the angle between the fiber axis and the static magnetic field; simulations of the resulting spectra provide information on the orientation and the randomness of the complexes bound to double helical DNA. If the complexes are randomly oriented on the DNA double helices, the spectra become independent of the angles and the line shapes show the same patterns as those of frozen solutions or powders; if the complexes are mobile on the DNA relative to the EPR time scale, EPR spectra are observed with the same line shapes as those of solutions [17,18].

The series of metallopeptides examined herein included $Cu(II)\bullet_D\text{-Arg-Gly-His}$ and $Cu(II)\bullet_D\text{-Lys-Gly-His}$, for comparison to metallopeptides containing *L*-Arg/Lys substitutions studied in a previous DNA fiber EPR investigation [9], and also the diastereoisomeric pairs $Cu(II)\bullet_{D/L}\text{-Pro-Gly-His}$ and $Cu(II)\bullet_{D/L}\text{-Pro-Lys-His}$ to determine, through direct comparison, the influence of stereochemistry on the orientation and stability of these particular amino acid substitutions.

2. Experimental

2.1. Materials

The tripeptide-ligands used in this study, $H_2N\text{-D-Lys-Gly-L-His-CONH}_2$, $H_2N\text{-D-Arg-Gly-L-His-CONH}_2$, $H_2N\text{-D/L-Pro-Gly-L-His-CONH}_2$, $H_2N\text{-D/L-Pro-Lys-L-His-CONH}_2$, were synthesized using standard Fmoc protocols on Rink-amide resin to yield tripeptides having free aminotermini and amidated carboxy-termini. All synthetic tripeptides were purified using reverse phase HPLC and verified by ESI-MS analyses. Salmon testes DNA was purchased from Sigma Ltd. Co. All other chemicals were of reagent grade and used without further purification.

All $Cu(II)\bullet$ metallopeptides formed from the above apo-peptides were verified, via EPR analyses of frozen solutions (pH 7.4, -150°C), to contain similar, planar quadridentate structures as shown in Fig. 1; $Cu(II)\bullet_{D/L}\text{-Arg-Gly-His}$ and $Cu(II)\bullet_{D/L}\text{-Arg-Gly-His}$: $g_{\parallel} = 2.187$ and $A_{\parallel} = 0.0203\text{ cm}^{-1}$; $Cu(II)\bullet_{D/L}\text{-Pro-Gly-His}$: $g_{\parallel} = 2.185$ and $A_{\parallel} = 0.0205\text{ cm}^{-1}$; and $Cu(II)\bullet_{D/L}\text{-Pro-Lys-His}$: $g_{\parallel} = 2.182$ and $A_{\parallel} = 0.0205\text{ cm}^{-1}$. These control studies further verified that the replacement of an amino-terminal *D/L*-Arg or *D/L*-Lys in the tripeptides with *D/L*-Pro and replacement of Gly for Lys in the second peptide position did not change the N_4 inner coordination spheres of the complexes.

2.2. Preparation of DNA pellets and fibers

The details of the preparation of DNA-pellets and fibers were reported previously [15,16]. Salmon testes DNA was dissolved in 20 mM NaCl to produce a solution of approximately 1 mM DNA base pair concentration. Subsequently, metallopeptide solutions were added dropwise to the DNA, and stirred for approximately 12 hrs at 4°C . The resulting solutions were

adjusted to a pH of 7.4 with sodium hydroxide and hydrochloric acid throughout the experiments. The amount of total DNA-bound Cu(II) was assayed by atomic absorption spectroscopy of the remaining Cu in the supernatant following ultracentrifugation of the mixed solution of DNA and the complex. Atomic absorption spectra were obtained using a Shimadzu AA-640-01 atomic absorption spectrophotometer.

2.3. EPR spectra

X-band (9.2 GHz) EPR spectra at ambient temperature and -150°C were measured on a JEOL FR-2X spectrometer with 100 kHz field modulation of 0.5 G. The magnetic field was calibrated with an NMR field meter EFM-2000 (ECHO Electronics LTD) and the microwave frequency was measured on an Anritsu MF2412A frequency counter. Simulations of the EPR spectra of Cu complexes on DNA fibers were performed according to the algorithm described previously [16]. The definitions of the angles and the relative orientations of the fiber axis, magnetic field, and the g-tensor axes are shown in Fig. 2. EPR spectra were measured at different angles of Φ between the fiber axis and the static magnetic field. The orientation of a complex on the double helical DNA was specified by an angle θ_0 and the randomness of θ by $\Delta\theta$ on assumption that the distribution of θ can be represented by a Gaussian function of the form

$$G(\theta) = \frac{1}{\Delta\theta \sqrt{2\pi}} \exp \left\{ \frac{-(\theta - \theta_0)^2}{2(\Delta\theta)^2} \right\}$$

2.4. Modeling and Simulations

All calculations were performed using SPARTAN 5.1 [19], MacroModel 7.0 [20] and AMBER 7 [21] employing procedures described in detail elsewhere [12].

3. Results and discussion

3.1. EPR spectra of Cu(II)•_D-Arg-Gly-His and Cu(II)•_D-Lys-Gly-His

DNA fiber EPR spectra of Cu(II)•_D-Arg-Gly-His (Fig. 3) and Cu(II)•_D-Lys-Gly-His acquired at room temperature are shown in Fig. 4(a) and (c). These spectra do not exhibit a conspicuous Φ dependence compared to spectra reported previously for Cu(II)•_L-Arg-Gly-His and Cu(II)•_L-Lys-Gly-His [9], indicating that the _D-peptide complexes are oriented more randomly on the DNA fibers. To compare the extent of observed randomness quantitatively, we estimated the $\Delta\theta$ values for these complexes, reproducing the observed change in the relative intensities of the $g_{\parallel}$ signals for the $M_I = 3/2$ transition as a function of Φ by simulation of the EPR line shapes (Fig. S1, Supporting Information). The $g_{\parallel}$ region of the spectra is a sensitive indicator of the degree of randomness because the signal is well separated from the $g_{\perp}$ region where various transitions other than those of the $g_{\perp}$ component overlap: The more randomly the complexes orient, the smaller the change in the relative intensities of the $g_{\parallel}$ signals. In Fig. 5(a), the observed and calculated relative intensity changes are compared for the _D- and _L-Arg-Gly-His complexes. Though it was difficult to precisely fit the observed changes to the calculated ones, it is obvious that the Φ dependence of the relative intensity varies with different $\Delta\theta$ values. The simulated spectra with $\theta_0 = 45^\circ$ and $\Delta\theta = 40^\circ$ are shown in Fig. 4(b) and (d).

In comparison to Cu(II)•_L-Arg-Gly-His and Cu(II)•_L-Lys-Gly-His [9], the angles for θ_0 (45°) estimated for both Cu(II)•_D-Arg-Gly-His and Cu(II)•_D-Lys-Gly-His bound to B-form DNA are in good agreement with those ($\theta_0 = 50^\circ$) estimated for Cu(II)•_L-Arg-Gly-His and Cu(II)•Gly-Gly-His-^{-COO⁻}; however, the $\Delta\theta$ values (40°) are considerably larger than those reported previously for Cu(II)•_L-Arg-Gly-His and Cu(II)•_L-Lys-Gly-His (20°) [9]. This difference can be illustrated via a Gaussian distribution of θ shown in Fig. 5(b).

The estimated $\Delta\theta$ values include randomness in the orientation of DNA double helices in the fibers and the misorientation of some fibers in a static magnetic field. Therefore, the actual randomness in the orientation of the complexes on the double helical DNA should be smaller than those evaluated from the $\Delta\theta$ values alone. Despite the fact that $\Delta\theta$ values include potential experimental errors, the present results clearly indicate that the stereochemistry of the terminal amino acid residue within a Cu(II)•Gly-Gly-His-derived metalloprotein is crucial to the stereo-specific DNA binding of these metalloprotein complexes just as observed with their Ni(II)-containing analogues [12].

These results support the notion that the D-amino acid substituted metalloproteins studied above participate in an overall DNA-bound orientation that is similar to the orientation determined previously for the L-substituted diastereoisomeric systems [9]; however, as indicated by their $\Delta\theta$ values (40°), notable differences occur in the stabilities of their resulting DNA-bound complexes. This result is in good agreement with the outcome of studies involving DNA cleavage recognition and molecular dynamics simulations of Ni(II)•L-Arg-Gly-His and Ni(II)•L-Lys-Gly-His vs. their analogous D-amino acid-containing diastereoisomers [12]. While there can be inherent differences between the DNA interactions of some Cu vs. Ni complexes, especially when the metal ion directly interacts with the DNA, M(II)•Gly-Gly-His-DNA interactions are dominated by amino acid side chain-DNA contacts, as pointed out earlier. These studies determined that Ni(II)•L-Arg-Gly-His and Ni(II)•L-Lys-Gly-His target their DNA cleavage activity to a sub-set of available A/T-rich sites and that their dynamic behavior is closely coupled to that of the DNA indicating a favorable interaction. In contrast, their diastereoisomeric counterparts, Ni(II)•D-Arg/Lys-Gly-His, lead to a much less selective interaction with the DNA and exhibit a less stable, more dynamic behavior that is independent of the DNA [12] as observed with the Cu analogues examined herein. These differences have been attributed to the stereochemistry of the alpha carbon of the amino-terminal amino acid and its location in the minor groove relative to the bound equatorial plane of the metalloprotein: An extended side chain provided by an L-Arg or L-Lys residue leads to an overall metalloprotein structure that complements sterically the curvature of the minor groove while a D-Arg/Lys leads to a less stable interaction due to a steric clash between the wall of the minor groove and the D-side chain of a DNA-bound metalloprotein.

3.2. EPR spectra of Cu(II)•D-Pro-Gly-His and Cu(II)•L-Pro-Gly-His

The observed DNA fiber EPR spectra of Cu(II)•L-Pro-Gly-His and Cu(II)•D-Pro-Gly-His (Fig. 6) bound to B-form DNA fibers acquired at room temperature are shown in Fig. 7 (a) and (c). These same spectra expanded at the $g_{\parallel}$ regions are shown in Fig. S2 (Supporting Information). In distinct contrast to the behavior of Cu(II)•D-Arg/Lys-Gly-His vs. the Cu(II)•L-Arg/Lys-Gly-His complexes, the intensity of the $g_{\parallel}$ region changed more markedly as a function of Φ for Cu(II)•D-Pro-Gly-His than for Cu(II)•L-Pro-Gly-His, indicating that Cu(II)•D-Pro-Gly-His in this particular case binds to DNA more stereo-specifically than Cu(II)•L-Pro-Gly-His. The calculated spectra of Cu(II)•D-Pro-Gly-His fit well to the observed spectra with $\theta_0 = 55^\circ$ and $\Delta\theta = 20^\circ$ while those of Cu(II)•L-Pro-Gly-His fit well with $\theta_0 = 55^\circ$ and $\Delta\theta = 40^\circ$ as shown in Fig. 7 (b) and (d). The EPR spectra observed at -150°C (Fig. S3; Supporting Information) show a similar Φ dependence, indicating that the complexes are fixed at room temperature and that freezing of water in the DNA fibers did not disturb the orientation of the complexes.

The EPR results reported here are wholly consistent with observations made during the simulation of these two diastereoisomers bound to DNA as Ni(II) complexes [12]. In simulations of Ni(II)•L-Pro-Gly-His and Ni(II)•D-Pro-Gly-His bound to an A/T-rich site, it was observed that the D-Pro isomer was able to fit better in the minor groove when compared to the L-Pro isomer. This difference in minor groove fit appears to be due to the overall shape of the D-Pro isomer which is structurally more compact in comparison to the L-Pro isomer: The L-Pro

isomer displays its C-terminal amide and Pro side chain on opposite “faces” of the metallopeptide equatorial plane while the *D*-Pro isomer displays both the C-terminal amide and the Pro side chain on the same face of the metallopeptide equatorial plane. Consequently, the *L*-Pro isomer is slightly “thicker” than the corresponding *D*-isomer and sterically less compatible with the DNA minor groove width (Fig 8). Thus, while it appears that these subtle structural differences can lead to differences in the relative stabilities of their individual DNA-bound Cu (II) complexes, DNA cleavage results via the analogous Ni(II) complexes indicate nearly identical selective cleavage patterns [12]; thus, in this case, the stability of the DNA interaction appears not to influence the overall site-selectivity of DNA cleavage.

In further support of the DNA fiber EPR results presented above, MD simulations also revealed that: (1) the *D*-Pro diastereoisomer exhibits a lower relative DNA binding energy (-82 Kcal/mol) compared to the *L*-Pro diastereoisomer (-77 Kcal/mol); (2) the RMS deviations of *D*-Pro are more stable (*L*-Pro slides out of the minor groove while the *D*-isomer remains in the groove); and (3) there are more, and qualitatively different, H-bonds formed between the DNA and the *D*-Pro isomer in comparison to the *L*-Pro isomer.

It is worthy to note in light of the above observations that any diastereoisomeric differences observed for Ni(II) complexes vs. Cu(II) complexes may be accentuated further by their differences in ligand affinity at the apical position and their overall geometries: The Cu(II) complexes assume a tetragonal pyramidal form while the Ni(II) complexes prefer a tetragonal planar structure. These geometric differences perhaps lead to a further increased molecular thickness in the case of the Cu complexes allowing their diastereomeric differences to be magnified.

3.3. EPR spectra of Cu(II)•*L*-Pro-Lys-His and Cu(II)•*D*-Pro-Lys-His

In addition to the Cu(II)•*L/D*-Pro-Gly-His system, similar diastereoisomeric metalloptides containing Lys in the second peptide position, Cu(II)•*L/D*-Pro-Lys-His (Fig. 9), were also examined to investigate the effects of this positively-charged substitution on DNA binding stability and orientation. Interestingly, this combination of amino acids, Pro in the amino-terminal position and Lys in the second peptide position, was selected as exhibiting “optimal” DNA cleavage activity from among a library of metallotriptides when activated in the presence of Ni [22]. The observed DNA fiber EPR spectra of Cu(II)•*L*-Pro-Lys-His and Cu(II)•*D*-Pro-Lys-His acquired at room temperature are shown in Fig. 10 (a) and (c). These same spectra expanded at the $g_{\parallel}$ regions are shown in Fig. S4 (Supporting Information). These spectra exhibit a similar dependence on Φ , indicating that both θ_0 and $\Delta\theta$ of the complexes in the DNA fibers are similar to each other.

The EPR spectra of Cu(II)•*L*-Pro-Lys-His and Cu(II)•*D*-Pro-Lys-His could be reproduced by the same θ_0 and $\Delta\theta$ values (Fig. 10, (b) and (c)). Thus, despite their diastereoisomeric differences, and the diastereomeric differences observed for the Cu(II)•*L/D*-Pro-Gly-His system, both Cu(II)•Pro-Lys-His metalloptides behaved similarly; each metalloptide generated $\Delta\theta$ values of 30° indicative of a dynamic behavior when bound to DNA. It appears that the Lys residue of both Cu(II)•Pro-Lys-His metalloptides prevented a distinct stereospecific orientation of their DNA bound structures in comparison to the Cu(II)•*L*-Arg-Gly-His, Cu(II)•*L*-Lys-Gly-His and Cu(II)•*D*-Pro-Gly-His complexes.

The above differences can be explained based on previous studies. While the positively-charged Lys residue of both Cu(II)•Pro-Lys-His complexes increased their overall DNA binding in comparison to the charge-neutral Cu(II)•Pro-Gly-His complexes, earlier studies of the analogous Ni(II) complexes indicated that a Lys residue at the second peptide position does not promote a diastereoisomer-specific DNA cleavage pattern [12]; Ni(II)•Gly-*L/D*-Lys-His metalloptides generate almost identical DNA cleavage patterns just as Cu(II)•*D*-Pro-Lys-His

and Cu(II)•*L*-Pro-Lys-His generate nearly identical DNA fiber EPR spectra. In this instance, MD simulations of the Ni(II) complexes [12] suggested that this particular positioning of the charged Lys side chain disrupts the usual groove-bound metallopeptide structure by bending toward the minor groove and interacting electrostatically and via H-bonding to acceptors along its edge leading to a destabilized interaction with the DNA that is structurally reminiscent to that of M(II)•Gly-Gly-His. In support of this, site-selective DNA cleavage studies [12] have revealed that Ni(II)•Gly-Gly-His produced a pattern of DNA modification that was nearly identical to that produced by Ni(II)•Gly-*L/D*-Lys-Gly-His. The DNA fiber EPR results presented herein provide clear support for this model and also indicate that Lys present in the middle peptide position can override the influence of the amino acid contained in the amino-terminal peptide position.

4. Conclusions

The DNA fiber EPR results presented here provide a structural basis for understanding the effects of amino acid stereochemistry on DNA recognition by Cu(II)•*L/D*-Xaa-Gly-His and Cu(II)•*L/D*-Xaa-Lys-His metallopeptides; the results reported herein are consistent with experimental and theoretical studies of analogous metallopeptide systems. With Cu(II)•*L/D*-Arg/Lys-Gly-His, the DNA fiber EPR results presented here and in earlier studies [9] indicate that an extended, positively-charged side chain provided by an *L*-Lys or *L*-Arg in the amino-terminal peptide position leads to a stereospecifically-oriented, stable metallopeptide interaction with B-form DNA while the *D*-configuration leads to destabilization of the system.

In contrast, a non-extended, compact side chain provided by a Pro residue in the amino-terminal position leads to decreased metallopeptide stereospecificity when in the *L*-configuration relative to the *D*-configuration. While this finding appears counter to the behavior of other M(II)•*L/D*-Xaa-Gly-His based systems, the difference in this instance appears to derive from alterations that occur as part of the overall structure of the metallopeptide coordination plane; the position of an *L*-Pro side chain relative to the equatorial plane and the *L*-His carboxy-terminal amide creates a metallopeptide that is sterically less conducive to a stable DNA binding interaction. Finally, in comparison, the influence of the above structural consequence can be overridden by the presence of a Lys residue in the second peptide position, a modification that appears to disrupt a stable metallopeptide-minor groove interaction. The results presented here support and assist in explaining earlier findings in which a Lys residue in the second peptide position leads to a less selective metallopeptide-DNA cleavage interaction with decreased stability. Thus, it appears that a controlled destabilization of metallopeptide-DNA association can lead to less site-selectivity but a concomitant overall increase in DNA cleavage activity [22].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Fig. 1.
Structure of $M(II) \cdot \text{Gly-Gly-His}$ metallopeptides.

Fig. 2.
 Coordinate systems: $\mathbf{B}$, Static magnetic field; (X_f, Y_f, Z_f) , DNA fiber axes; $(g_{//}, g_{\perp})$, g-tensor axes; Φ , The angle between $\mathbf{B}$ and Z_f ; θ , The angle between $g_{//}$ and Z_f .

Fig. 3.

Comparison of the structures of $\text{Cu(II)} \cdot \text{D-Arg-Gly-His}$ and $\text{Cu(II)} \cdot \text{L-Arg-Gly-His}$. These structures are analogous to metallopeptides containing Lys substitutions.

Fig. 4. EPR spectra of Cu(II)•D-Arg-Gly-His and Cu(II)•D-Lys-Gly-His on B-form DNA-fibers at room temperature. (a) Observed and (b) calculated spectra of Cu(II)•D-Arg-Gly-His. Magnetic parameters used for the calculations: $g_{//} = 2.17$, $g_{\perp} = 2.05$, $A_{//} = 193$ G, $A_{\perp} = 25$ G, $A_{N//} = 10$ G, $A_{N\perp} = 15$ G, $\Delta B_{//} = 20$ G, $\Delta B_{\perp} = 19$ G, $\theta_0 = 45^\circ$, $\Delta\theta = 40^\circ$. (c) Observed and (d) calculated spectra of Cu(II)•D-Lys-Gly-His. Magnetic parameters used for the calculations: $g_{//} = 2.17$, $g_{\perp} = 2.05$, $A_{//} = 193$ G, $A_{\perp} = 29$ G, $A_{N//} = 13$ G, $A_{N\perp} = 17$ G, $\Delta B_{//} = 13$ G, $\Delta B_{\perp} = 18$ G, $\theta_0 = 45^\circ$, $\Delta\theta = 40^\circ$.

Fig. 5.

(a) The relative intensities of the $g_{//}$ signals for the $M_I = 3/2$ transition. (i) Observed and (ii) calculated values with $\theta_0 = 45^\circ$ and $\Delta\theta = 40^\circ$ for $\text{Cu(II)}\bullet\text{-Arg-Gly-His}$. (iii) Observed and (iv) calculated values with $\theta_0 = 45^\circ$ and $\Delta\theta = 20^\circ$ for $\text{Cu(II)}\bullet\text{-Arg-Gly-His}$. (b) Gaussian distribution of θ for $\theta_0 = 45^\circ$ and $\Delta\theta = 20^\circ, 30^\circ$ and 40° . In a previous report [9] we also estimated $\theta_0 = 50^\circ$ for $\text{Cu(II)}\bullet\text{-Arg-Gly-His}$.

$\text{Cu(II)} \cdot \text{L-Pro-Gly-His}$

$\text{Cu(II)} \cdot \text{D-Pro-Gly-His}$

Fig. 6.
Comparison of the structures of $\text{Cu(II)} \cdot \text{L/D-Pro-Gly-His}$.

Cu(II)•**L**-Pro-Lys-His Cu(II)•**D**-Pro-Lys-His**Fig. 7.**

EPR spectra of Cu(II)•*D*-Pro-Gly-His and Cu(II)•*L*-Pro-Gly-His on B-form DNA-fibers at room temperature. (a) Observed and (b) calculated spectra of Cu(II)•*D*-Pro-Gly-His. Magnetic parameters used for the simulation; $g_{\parallel} = 2.17$, $g_{\perp} = 2.05$, $A_{\parallel} = 199$ G, $A_{\perp} = 25$ G, $A_{N\parallel} = 18$ G, $A_{N\perp} = 17$ G, $\Delta B_{\parallel} = 21$ G, $\Delta B_{\perp} = 23$ G, $\theta_0 = 55^\circ$, $\Delta\theta = 20^\circ$. (c) Observed and (d) calculated spectra of Cu(II)•*L*-Pro-Gly-His; $g_{\parallel} = 2.17$, $g_{\perp} = 2.05$, $A_{\parallel} = 194$ G, $A_{\perp} = 27$ G, $A_{N\parallel} = 17$ G, $A_{N\perp} = 15$ G, $\Delta B_{\parallel} = 18$ G, $\Delta B_{\perp} = 16$ G, $\theta_0 = 55^\circ$, $\Delta\theta = 40^\circ$.

Ni(II)•D-Pro-Gly-His

Ni(II)•L-Pro-Gly-His

Fig. 8. Space-filling molecular models of the average structures resulting from the simulation of Ni(II)•L-Pro-Gly-His and Ni(II)•D-Pro-Gly-His bound to the minor groove of d(CGCGAATTCGCG)₂; the DNA-bound orientations of these metalloproteins are consistent with the fiber EPR results reported herein. These models emphasize the relative differences in the thickness of each diastereoisomeric metalloprotein.

$\text{Cu(II)} \cdot \text{L-Pro-Lys-His}$

$\text{Cu(II)} \cdot \text{D-Pro-Lys-His}$

Fig. 9.
Comparison of the structures of $\text{Cu(II)} \cdot \text{L/D-Pro-Lys-His}$.

Fig. 10.

EPR spectra of Cu(II)•L-Pro-Lys-His and Cu(II)•D-Pro-Lys-His on B-form DNA-fibers at room temperature. (a) Observed and (b) calculated spectra of Cu(II)•L-Pro-Lys-His. Magnetic parameters used for the calculations: $g_{\parallel} = 2.17$, $g_{\perp} = 2.05$, $A_{\parallel} = 198$ G, $A_{\perp} = 23$ G, $A_{N\parallel} = 13$ G, $A_{N\perp} = 16$ G, $\Delta B_{\parallel} = 14$ G, $\Delta B_{\perp} = 18$ G, $\theta_0 = 45^\circ$, $\Delta\theta = 30^\circ$. (c) Observed and (d) calculated spectra of Cu(II)•D-Pro-Lys-His. Magnetic parameters used for the calculations: $g_{\parallel} = 2.17$, $g_{\perp} = 2.05$, $A_{\parallel} = 202$ G, $A_{\perp} = 27$ G, $A_{N\parallel} = 10$ G, $A_{N\perp} = 20$ G, $\Delta B_{\parallel} = 20$ G, $\Delta B_{\perp} = 22$ G, $\theta_0 = 45^\circ$, $\Delta\theta = 30^\circ$.