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Nucleobase Recognition by Alloxazine and Pteridine Derivatives at an Abasic Site in a DNA Duplex and Their Application to Gene Analysis

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論 文 内 容 要 旨

Introduction:

Studies on the chemistry of DNA-binding drugs and/or low molecular weight ligands are of on-going interest due to their promising functions and biological activities, including anti-cancer properties and regulation of gene expression. Of particular interest to us is the development of a class of ligands available for gene detection, especially for SNPs (single-nucleotide polymorphisms) typing. Our group has recently discovered a series of aromatic ligands that can bind to a nucleobase opposite an abasic site (AP site) in DNA duplexes, and has proposed a new strategy of ligand-based fluorescence assay for SNPs typing. An AP site-containing probe DNA is hybridized with a target DNA so as to place the AP site toward a target nucleotide, by which a hydrophobic binding pocket is provided for aromatic ligands to bind to target nucleotides with a fluorescence signaling. In this work, I have successfully discovered a fluorescence ligand with a useful affinity and selectivity for an adenine base. In addition, I discuss a simple, but effective chemical modification of pteridine derivatives for an improvement in the binding affinity and selectivity.

Development of an adenine-selective ligand:

In present system, the selectivity and high affinity of the ligand-nucleotide binding are achieved by a combination of stacking and hydrogen-bonding interactions. In case of guanine-selective 2-amino-6,7-dimethyl-4-hydroxypteridine (diMe-pterin),[1b] for example, it is highly likely that, by stacking with two nucleobases flanking the AP site, the ligand is located at the AP site and facing the Watson-Crick edge of a target guanine base, where three point hydrogen bonds are formed. However, in case of adenine-binding, the challenge is to achieve the binding selectivity by the formation of two point hydrogen bonds along the edge

of adenine bases, keeping a deep insertion of the aromatic ligand within the AP site so as to effectively stabilize the ligand binding. In this work, I focused on alloxazine or pteridine derivatives as a candidate for adenine-selective ligands. From the examination of their binding to AP site-containing DNA duplexes (5'-TCC AGX GCA AC-3'/3'-AGG TCN CGT TG-5', X=AP site, N = target nucleotide) in solutions buffered to pH 7.0 (I = 0.11 M, at 5 ℃), alloxazine is found to selectively bind to adenine with a dissociation constant K_d of 0.82 μM. Comparison with the binding abilities of structurally-related riboflavin or lumiflavin, it is highly likely that the polar group along the edge of the alloxazine ring is involved in the adenine recognition. Alloxazine was effectively applicable to the analysis of G>A present in 107-mer DNAs (*K-ras* gene, codon 12, sense strand) obtained by asymmetric PCR. Fluorescence quenching of alloxazine is observed for the A-containing mutation sequence, while the ligand shows almost no response to the G-containing wild type sequence. The analysis requires no time-consuming steps such as purification of PCR products and careful control of temperature and the result is readily obtained after PCR. These promising functions of alloxazine are discussed based on the examination by fluorescence and ITC (isothermal titration calorimetry) measurements.

Effect of a methyl group for the improvement of base selectivity and binding affinity:
It has been proposed that, by introducing a methyl group specific binding and selectivity for particular target base is achieved. Isoxanthopterin (**IX**) has two sets of hydrogen-bond forming sites suitable for target nucleotides, one of which binds to thymine (T) and the other site which binds to cytosine (C) with strong affinity compared to adenine (A) and guanine (G), but the base selectivity for T against C (*vice versa*) is moderate. In order to improve both binding affinity and base selectivity for T against C (*vice versa*), IX is modified by introducing a methyl group at N-3 or N-8 position for the binding site of T and/or C, which are known as 3-methyl isoxanthopterin (**3-MIX**), and 8-methyl isoxanthopterin (**8-MIX**), by which binding affinity and base selectivity for C or T is significantly enhanced. Indeed, **3-MIX** selectively binds to T more strongly than **IX** with a binding constant of 1.5×10^6 M^{-1} and loses its binding affinity for C. Similarly **8-MIX** selectively binds to C more strongly than **IX** with a binding constant of 1.0×10^6 M^{-1} and loses its binding affinity for T and also for A.

論文審査の結果の要旨

本論文の目的は、DNA 内部に形成した微小疎水場空間における有機小分子リガンドと核酸塩基との水素結合を介した分子認識機構の解明と蛍光分光分析による遺伝子診断法への応用である。第一章では、DNA 結合性リガンドに関する研究の現状についてまとめている。第二章では、アロキサジンとイソアロキサジン誘導体をリガンドとして、各種分光および熱力学的手法によりアデニン塩基に対する選択性を評価している。互変異性体であるアロキサジンとイソアロキサジン骨格の違いにより塩基選択性が変化し、さらに基本骨格の拡張およびメチル基の有無によって結合定数を制御可能であることを明らかとした。第三章では、イソキサントプテリジン誘導体におけるメチル基導入による標的塩基選択性と結合能の向上への寄与について検討している。複数の水素結合性官能基を有するイソキサントプテリジン骨格の各官能基をメチル基置換することで、水素結合能パターンおよび選択性への置換基効果について系統的に明らかにした。第四章では、前二章で開発したリガンド類を用いた DNA 一塩基変異の蛍光分光分析手法への応用を行っている。水素結合性リガンドとしてアデニン塩基選択性を有するアロキサジン誘導体、グアニン塩基選択性を有するプテリジン誘導体を用いている。標的 DNA と脱塩基部位含有 DNA 鎖を混合することで標的塩基向かい側に微小疎水場空間を有する DNA 二本鎖として用いている。その結果、標的塩基向かい側の疎水場空間を分子認識反応場として水素結合性リガンドが標的塩基を認識していること、標的塩基の種類に応じた蛍光消光応答を示すことを明らかにした。また二つのリガンドを併用することにより一塩基変異の決定（タイピング）への適用についても検討し、二波長蛍光分光手法で解析を行っている。

以上の研究成果は論文提出者が自立して研究活動を行うために必要な高度の研究能力と学識を有することを示している。したがって、Burki Rajendar 君提出の論文は博士（理学）の学位論文として合格と認める。