Functional reactivity of oxanine: its biological meanings and biotechnological applications

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ABSTRACT

Oxanine (Oxa), generated as one of the major products from guanine by nitrosative oxidation, has been focused as a mutagenic lesion. Here, Oxa was explored in terms of its unique property to react with –NH₂ or –SH group since Oxa possesses O-acylisourea conformation in the base-ring structure. In particular, Oxa has been characterized in terms of its formation and mechanism of DNA-protein cross-link (DPC). In addition, Oxa was testified as a new carboxy-linker for activation-free covalent bonding with NH₂-molecules, which can be usefully employed for the design of biotechnological or nano/biotechnological systems.

INTRODUCTION

Oxanine (Oxa) has been considered as a unique lesion since we firstly reported in 1996 that Oxa is generated as one of the main deamination products from guanine (Gua) by nitric oxide (NO) or nitrous acid (HNO₂)-induced nitrosative oxidation [1]. It was demonstrated that Oxa is formed together with xanthine (Xan) with the molar ratio of 1:3 when 2-deoxyguanosine (dGua) or DNA is treated with NO or in weakly acidic HNO₂.

Since then, Oxa has been intensively explored in terms of its biochemical or biophysical properties. Once Oxa is produced in DNA sequence, Oxa exists for a long time due to the stable N-glycosidic bond between the base and sugar moieties of its nucleoside, deoxyxoxanosine (dOxa) [2]. It was found that Oxa is incorporated opposite cytosine (Cyt, C) and thymine (Thy, T) in the DNA polymerase chain elongation [3] and also that Oxa in template DNA can induce mis-incorporation of Thy opposite Oxa during the DNA replication [4]. Although DNA duplexes containing O:C base pairing showed low melting temperature than normal DNA duplexes containing G:C [4,5], the biophysical analyses including CD or NMR analysis revealed that Oxa makes base-pairing and stacking in the DNA duplex and therefore does not cause any severe distortion in the whole DNA structure [6,7]. It was also determined that several restriction endonucleases recognize and cleave the specific base-sequence even when Gua was substituted by Oxa in the sequence [6,7]. In addition, in the responses to other several DNA-relevant enzymes such as T4 polynucleotide kinase, T4 ligase or Tth ligase, Klonef fragment (exo’), Oxa was not distinguished as abnormal DNA base [6,7]. These results imply a high possibility that Oxa in DNA strands could be considered similarly as Gua, especially, in terms of molecular and biological recognition.

Since Oxa produced in DNA sequence could induce severe genotoxic and cytotoxic damages, for instance GC to AT transversion mutagenesis, the biological repairing mechanism for Oxa has been expected. However, it was reported that the general base-excision repairing (BER) systems are not effective for repairing Oxa in DNA strands [8]. Moreover, Oxa mediates DNA-protein cross-link (DPC) with some BER enzymes or DNA-binding proteins [9]. Therefore, it is more plausible that in the case of Oxa-repairing, the nucleotide excision and recombination repair (NER) system would play a more effective role by excising the Oxa-mediated DPC product [8].

Oxa has an O-acylisourea formation in its nucleobase as shown in Figure 1. That is, Oxa has two different sides, a stable conformation and an activated-carboxylate function. Although the reactivity of Oxa is not so high, Oxa is ready to change to another formation if Oxa encountered with –NH₂ or –SH containing biomolecules. Although we reported previously the phenomena of Oxa-mediated DPC [9], the reaction mechanism has not been elucidated. In this report, we explored such functional reactive property of Oxa in terms of its biological meanings and biotechnological applications.

RESULTS AND DISCUSSION

To understand the reactivity patterns of Oxa, dOxo (2.5 mM) was incubated with several amine-containing...
molecules (equivalent) for 2 hr in several universal buffer systems (pH 5-12) composed of borate, ascorbate and phosphate. It was found that the productivity of amide-bond formation is quite related to pKₐ values of amino group. For instance, in the case of hexylamine (pKₐ = 10.63), the high product conversion was observed at above pH 9, but in the biophysiological conditions (pH 7.4 and 37°C) the conversion is below 5%. Under biophysiological conditions, the Oxa reactivity was further investigated by incubating dOxo with several N⁰-acetyl amino acids, which has amino or thiol group in residue chain. The productivities of dOxo with His (pKₐ of residue's amino group = 6.10), Lys (pKₐ = 10.54) and Arg (pKₐ = 12.48) were ~20%, ~5% and 0%, respectively. It should be noted that the productivity of Cys is ~50%.

However, when Oxa-containing DNA oligomer was employed in stead of dOxo monomer, the productivities for Cys, His and Lys were decreased up to below 5% although the productivities were increased at alkali condition (pH 9.5). In the cases of spermine (bio-polyamine), which has biding interaction with DNA oligomer, cross-link product was observed. When most of the DNA-relevant enzymes such as several DNA polymerases, DNA ligases and restriction endonucleases were incubated with Oxa-containing DNA oligomer, no cross-link product was detected in biophysiological conditions except for histone or some BER enzymes (hOGG1, hNEIL1&2, Fpg, EndoIII, EndoVIII etc.). These results indicated that the interactive access and the following physiological change of amino or thiol group of the binding biomolecules are required for inducing the cross-link reaction of Oxa in DNA strands.

On the other hand, the reactivity of Oxa can be employed usefully in the bioconjugation applications based on amine-directed reactions. For instance, as shown in Figure 2, Oxa can be used as chemical linker for the immobilization of DNA oligomer on amine-functionalized surface. Generally, condensation reagents such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) should be employed for carboxyl group to make amide-bond formation with amino group on the surface. In the case of Oxa, without any activation step, DNA oligomer can be immobilized directly on the amine-functionalized surface.

![Figure 2](image_url) Immobilization of probe DNA oligomer on the NH₂-functionalized surface through the reactivity of Oxa

**CONCLUSION**

In this study, the reactive property of Oxa was explored more in detail by incubating the dOxo and Oxa-containing DNA oligomer with several –NH₂ or –SH containing compounds and biomolecules. The obtained results are useful for understanding the reactivity of Oxa in biological system, in particular, Oxa-induced DPC phenomena. In addition, amine-directed reactivity of Oxa can be employed for the immobilization of probe DNA oligomer on the amine-functionalized surface.

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**REFERENCES**


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