Nucleic Acids Chemistry beyond the Watson-Crick Double Helix (66) : Effect of molecular crowding on replication along non-natural DNAs

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Unnatural nucleic acids are promising materials to expand genetic information beyond the natural bases.[1] During replication, substrate nucleotide incorporation should be strictly controlled for optimal base pairing with template strand bases. Base-pairing interactions occur via hydrogen bonding and base stacking, which could be perturbed by the chemical environment.[2] However, the chemical environmental effect on the replication of unnatural nucleic acids is less understood. In this study, we investigated the effect of molecular crowding on the efficiency and preference of single primer extension with native dNTPs along a template containing different unnatural bases (inosine, 5-methyl-isocytosine, and isoguanine) and different sugars (DNA, hexitol nucleic acids, and arabinose nucleic acids).[3] Although dNTPs were non-cognate substrate against the unnatural nucleobases on the template, Klenow Fragment (KF) DNA polymerase preferred to polymerize a certain dNTP. Interestingly, the trend of polymerization basically indicated the high efficiency of the incorporation of preferred pyrimidine dNTPs with low fidelity but the low efficiency of the incorporation of preferred purine dNTPs with high fidelity. However, in the presence of 20 wt% PEG 200 (average molecular weight 200), the efficiency of the incorporation of preferred pyrimidine dNTPs decreased, whereas that of preferred purine dNTPs increased, resulting in all the efficiencies showing almost similar levels irrespective of the chemical structure of the templates (Figure). These findings indicate that preferred pyrimidine dNTPs depend on hydrogen bond formation, which was destabilized by molecular crowding due to a decrease in the water activity. However, the incorporation of preferred purine dNTPs through base-stacking interaction was facilitated by molecular crowding. Our finding suggests that the crowding conditions in a solution and in an enzyme could be key factors determining the efficiency and fidelity of DNA polymerization along unnatural nucleosides.

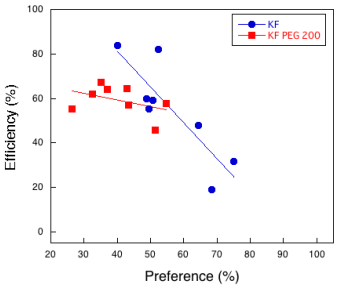

Figure. Plots of the efficiency versus preference of the primer extension by KF in the absence and presence of PEG 200.

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