Journal of Sciences, Islamic Republic of Iran 16(1): 11-14 (2005) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Short Communication

Enzymatic-Like Properties of dsDNA and Its Comparison with ssDNA

M. Ghollasi,[1] B. Farzami,[2,*] S.Z. Bathaie,[3] and B.L. Rad[1]

[1] *Department of Biology, Faculty of Sciences, Alzahra University, Tehran, Islamic Republic of Iran*
[2] *Department of Medical Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran*
[3] *Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Islamic Republic of Iran*

Abstract

A highly sensitive spectroscopic method using dichlorofluorescin (LDCF) was employed to study the rate of electron-transfer reaction in presence of dsDNA, ssDNA and some metal ions and imidazole derivative (N-trans cinnamoyl imidazole).Our results show that both kinds of DNA possess an enzyme-like catalytic activity in oxidative conversion of non fluorescent LDCF to fluorescent DCF. A biphasic saturation curve was observed when the reaction velocities were measured at fixed concentration of dsDNA, ssDNA and variable amounts of cinnamoyl imidazole. Each of biphasic phase gave the values of $Km_1=1\times10^{-6}$ M, and $Vm_1=20.8$ ΔF/min with $Km_2=1\times10^{-4}$ M, $Vm_2=8.7$ ΔF/min for dsDNA and $Km_1=2\times10^{-6}$ M, $Vm_1=16.62$ ΔF/min and $Km_2=4\times10^{-4}$ M, $Vm_2=29.2$ ΔF/min for ssDNA. Among investigated metal ions Mn and Cd caused inhibition of ET reaction in presence of DNA and imidazole while Cu and Ca activated the reaction. A linear correlation was found between conductivity of each metal ion and activation or inhibition of reaction in presence of dsDNA and ssDNA. A model was devised based on the catalytic properties of DNA in reaction with cinnamoyl imidazole and metal ions in which the release of electron produced by reaction could be facilitated by the presence of the cinnamoyl imidazole which could transfer the electron through its conjugated structure to the DNA chord via metal ion. The more conductive metal, the higher was the catalysis.

Keywords: DNA; Catalysis; Single strand; Fluorescence; Electron transfer

1. Introduction

The linked π-system of DNA has been reported to serve as a wire to convey electrons through DNA [1-6]. The electric potential differences has also been measured using Ru(II) metal bound to two ends of DNA double strand [7]. Furthermore a catalytic activity has been detected when a double stranded DNA was used in presence of an electron transfer (ET) reaction [6]. All the investigation regarding the electronic properties of

* *E-mail: bfarzami@neda.net*

DNA points to the fact that the charge transfer does occur at distances where it could allow for shuttling of a charge from remote locations on a DNA strand to a certain site. Damage at any site could produce a "mutational hot spot". Also G among other bases has the lowest ionization potential that can lead to oxidative damage and mutation [1].

Contradictory reports exist regarding the higher rate of ET in dsDNA than in ssDNA [8]. The ET rates in ssDNA decrease to a much slower rates than those for dsDNA when the number of nucleotides between the radical cation site and the nearest G increases [3]. This may point to the fact that a more linear structure, with a specified distance could be envisaged for a dsDNA that may not allow exchange of charges by random proximity. Another factor that may effect ET in DNA are metal ions. Some metal ions have carcinogenic potential [9,10]. These metals could bind DNA and result in ET blockage. The toxcicocity of heavy metal ions may depend partly on their binding to specific DNA sites and their role in conductivity [11].

Considering what is mentioned, we decided to investigate the effect of dsDNA, ssDNA and some metal ions on ET reaction in presence of N-trans cinnamoyl imidazole using a highly sensitive spectrofluorometric method. The reaction involves oxidation of diacetyl dichlorofluorescin (LDADCF) to a nonfluorescent dichlorofluorescin (LDCF) and to fluorescent dichlorofluorescein (DCF) in presence of NaOH (0.01 N) and H2O2, peroxidase or hematin, respectively [12-14]. Hematin is used in this reaction to accelerate the effect of H2O2. It is thought that a complex is formed between hematin and H2O2 that could dissociate into a ferroxyl-oxo compound and a hydroxyl radical which assist oxidation of LDCF to DCF.

2. Materials and Methods

2.1. Materials

2′,7′-dichlorofluorescindiacetate (LDADCF), N-trans cinnamoyl imidazole, Hematin were bought from Sigma Chemical Co. H2O2, chloride salts of Ca, Cu, Mn, Cd were bought from Merck Chemical Co. High molecular weight DNA was extracted and purified from calf-thymus as explained elsewhere [15].

2.2. Methods

2.2.1. Dichlorofluorescin Solution

Stock solution of LDADCF (1 mM) was made in ethanol and stored in the dark. LDADCF is stable for months under this condition. Activation of LDADCF to DCF for assay required dilution of 1:4 V/V of alcoholic solution of LDADCF and 0.01 N of NaOH. The mixture was allowed to stand at room temperature for 30 min. LDCF has a high rate of auto oxidation, so it required being prepared freshly each day.

2.2.2. Hematin Solution

The hematin solution (0.01 mg/ml) was prepared by dissolving 1 mg of hematin in 0.5 ml of 0.2 N NaOH and then diluted to 100 ml with 0.05 M *tris*-base buffer. This solution was made freshly each day.

2.2.3. ssDNA Solution

Single stranded DNA was prepared by denaturing double stranded DNA by heating at 100°C for 15 min with subsequent cooling in ice-bath. The concentration of stock DNA solution was calculated from their absorbance at 256 nm [16].

2.2.4. Sample Preparation

Seven milliliters of hematin was made up to 50 ml 0.05 M *tris*-base buffer at pH 8 and boiled for 15 min, while purging with nitrogen. This solution was cooled on ice. A 2.47 ml volume of this solution was mixed with 9.75 µl of activated DCF (1.3×10^{-7} M) and 22 µl of H2O2 (1.3×10^{-7} M). Ultimately DNA, the imidazole containing compounds and metal ions were added. Then the sample mixture was incubated at 25°C for 5 min. The relative fluorescence was determined using a spectrofluorometer (Shimatzu. model RF-5000) with a 4 ml fluorescence cell. The excitation and emission wavelengths were 499.2 and 521.6 nm, respectively. Both the excitation and emission slits were in 5 nm bandwidth. For the determination of optimum pH, all the required amounts of reagents were made in tris-base buffer adjusted to the desired pH between 7 to 10 with 0.4 pH units intervals. The metal ion concentrations in samples ranged from 10^{-8} to 10^{-2} M.

3. Results

3.1. The Effect of dsDNA, ssDNA and N-trans Cinnamoyl Imidazole

Initially optimum conditions for oxidative conversion of LDCF to DCF were determined using all compounds of the reaction in tris buffer (0.05 M). Optimum pH was found to be 8.0 for such reaction and was used for all reactions (data not shown).

The rate of conversion of LDCF to DCF in the presence of fixed concentration of N-trans cinnamoyl imidazole (2×10^{-5} M) and variable amounts of DNA was

determined. The rate of the reaction was found to increase with increasing concentrations of DNA (Fig. 1).

The higher catalytic activity of dsDNA compared to ssDNA was observed from the higher value of Vm/Km (Table 1)

Figure 2 shows the rate of reaction in presence of fixed concentration of ssDNA and dsDNA (2×10^{-6} Mbp or 4×10^{-6} Mb) and without DNA and variable amounts of *N*-trans cinnamoyl imidazole from 10^{-8} to 2×10^{-3} M. The Vm and Km values are obtained for each segment (Table 1).

3.2. The Effect of Metal Ions

The metal ions produced effects that portrayed the role of metal ions as activators or inhibitors. Metal ions such as Mn2+ caused 60%-70% inhibition in ssDNA and dsDNA while Cd2+ showed slight inhibitory and activation effect in dsDNA and ssDNA, respectively. Ca2+ and copper enhanced the rate of the reaction in both DNA forms. It is worthwhile to mention that ssDNA showed a slight higher sensitivity towards the activating effect of metal ions although this effect was not found to be significant (Fig. 3).

4. Discussion

The oxidative conversion of LDCF to DCF (the fluorescent product) is performed in the presence of H2O2 as an oxidative agent. The optimum conditions for DCF reaction in presence of DNA was determined and used throughout the experiments. Considering that both concentrations of DNA and trans cinnamoyl imidazole could enhance the rate of the reaction at pH 8, it could be assumed that the electron produced in the course of reaction could be directed through the conjugated π electronic system of cinnamoyl imidazole towards the DNA chord in which the electron could be transported along the DNA chord. This process may be enhanced by more conductive metals that can bind to DNA structure on one side and N-trans cinnamoyl imidazole on the other side mediating the transfer of electrons more effectively. In fact our results show that more conductive metals could enhance the effect more efficiently. Our former report indicated biphasic stages in DNA reaction using carnosine (Ala-His) as a cofactor [5]. Initially it was assumed that the major and minor grooves may be related to the biphasic trend of the reaction but the existence of the same trend for ssDNA could negate this assumption.

The behaviour of metal ions may also be revealed in the catalytic reaction. Some metal ions have inhibitory effects while others have none. We assumed that the enhancing effect of metals may be correlated to their electronic structures. The more conductive metals were more effective in rate enhancement. It could be rationalized that metal ions bind to the backbone of DNA and mediate transfer of electrons. This effect supports the model that metals act as a bridge in transferring the received electrons from the center of reaction via the imidazole to DNA structure. Considering our results it seems that this model could also be used for ssDNA. However, further studies are needed to establish more insights in to the mechanism of DNA catalysis.

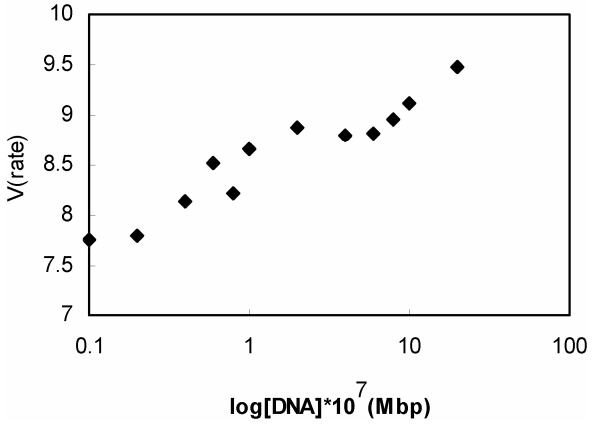

Figure 1. The rate of DNA reaction as a function of increasing concentration of DNA (logarithmic scale).

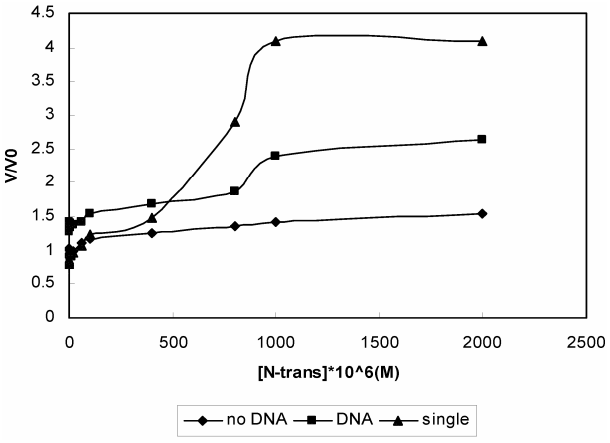

Figure 2. Relative rate of reaction in the absence and presence of dsDNA and ssDNA. V0 is related to the sample that does not have *N*-transcinnamoyl imidazole.

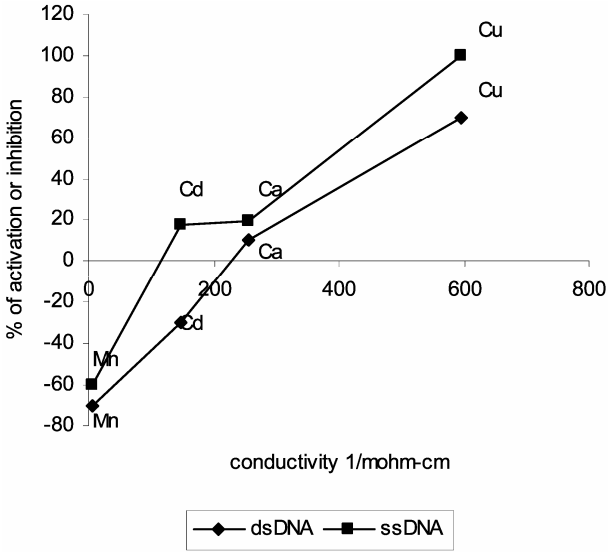

Figure 3. DNA activities of single and double stranded forms as a function of conductivities of metal ions.

Table 1. The kinetic parameters for double stranded DNA and single stranded DNA obtained from Figure 1

Kinetic parameter	dsDNA	ssDNA
Vm1	20.8	16.62
Vm2	8.7	29.2
Km1	1×10^{-6}	2×10^{-6}
Km2	10^{-4}	4×10^{-4}
Vm1/Km1	20.8×10^{6}	8.32×10^{6}
Vm2/Km2	8.7×10^{4}	7.3×10^{4}

5. References

1. Gullick B.M. Charge transfer through DNA. Literature seminar.
2. Tran P. and Alavi B.G. Charge transport along the lambda –DNA double helix. *Physical Review Letters*, **85**(7): 1564-1567 (2000).
3. Meggers E., Dussy A., Schafer T., and Giese B. Electron transfer in DNA from guanine and 8-oxoguanine to a radical cation of the carbohydrate backbone. *Chem. Eur. J.*, **6**(3): 485-492 (2000).
4. Treadway C., Hill M., and Barton J. Charge transport through a molecular Π-stack: double helical DNA. *Chemical Physics*, **281**: 409-426 (2002).
5. Symon M. Electron movement through proteins and DNA. *Free Radical Biology and Medicine*, **22**(7): 1271-6 (1997).
6. Farzami B., Bathaie S.Z., Sadeghi R., and Shamsaie A. DNA as an enzyme:the effect of imidazole derivatives as cofactors and metal ions as activator or inhibitors. *Clinical Biochemistry*, **36**: 353-358 (2003).
7. Krider E.S. and Meade T.J. Electron transfer in DNA: covalent attachment of spectroscopically unique donor and acceptor complexes. *JBCI*, **3**: 222-225 (1998).
8. Giese B. and Wessely S. The influence of mismatch on long-distance charge transport through DNA. Angew. Chem. Int. **39**(19): 3490-3491 (2000).
9. Hartwing A. and Schwerdtle T. Interaction by carcinogenic metal compounds with DNA repair process: toxicological implications. Toxcicology Letters, **127**: 47-54 (2002).
10. Hartwing A. Carcinogenicity of metal compounds: possible role of DNA repair inhibition. *Ibid.*, **102-103**: 235-239 (1998).
11. Anastassopoulou J. Metal-DNA interactios. *Journal of Molecular Structure*, **19**(26): 651-653 (2003).
12. Cathcart R., Shwiers E., and Ames B.N. Detection of picomole levels of hydroperoxides using a fluorescent dichlorofluorescin assay. *Anal. Biochem.*, **134**: 111-116 (1983).
13. Howell R.R. and Wyngnarden J.B. On the mechanism of uric acid by hemoproteins. *J. Biol. Chem.*, **235**: 350-354 (1960).
14. Zhang K., Mao L., and Cai R. Stopped-flow spectrophotometric determination of hydrogen peroxide with hemoglobin as catalyst. *Talanta*, **51**: 179-186 (2000).
15. Bathaie S.Z., Moosavi-Movahedi A.A., and Saboury A.A. Energetic and binding properties of DNA upon interaction with dodecyl thrimethyl ammonium bromide. *Nucl. Acids. Res.*, **27**: 1001-5 (1999).
16. Marin D., Perez P., Teijeiro C., and Palecek E. Interactions of surface-confined DNA with acid-activated mitomycin C. *Biophysical Chemistry*, **75**: 87-95 (1998).