CHEMISTRY LETTERS, pp. 1889-1892, 1989.

A DNA Probe of Ruthenium Bipyridine Complex Using Photocatalytic Activity

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A method is presented for labelling DNA and for detection of the complimentary sequence using photocatalytic activity of tris-(2,2'-bipyridine)ruthenium(Ⅱ). A hybridization experiment with a model system has shown that plasmid DNA of ca. 0.6 μg can well be detected by a dye-forming reaction in photocatalytical redox cycles.

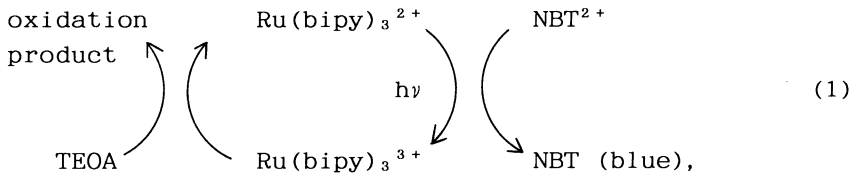
Recently there has been considerable attention focussed on interactions of polypyridineruthenium(Ⅱ) complexes with DNA, including enantiomeric selection in binding to DNA by intercalation,[1] or enhancement of the efficiency of photo-induced electron transfer by DNA mediation.[2] We report here on a novel DNA probe for a given sequence using photocatalytic activity of tris(2,2'-bipyridine)-ruthenium(Ⅱ) complex, $Ru(bipy)_3^{2+}$.

It is well known that a molecular photocatalyst such as $Ru(bipy)_3^{2+}$ undergoes a redox cycle under light when it is coupled with suitable counter reactions.[3] If we set a dye-forming reaction (i.e., indicator reaction) in the latters, we can visualize a trace of photocatalyst. That is, the photocatalyst can play a tracer. The problems for making a DNA probe are, then, how to label a DNA molecule with a photocatalytic source and how to get photocatalytic activity after hybridization. We found that cis-dichlorobis(2,2'-bipyridine)ruthenium(Ⅱ), cis-$Ru(bipy)_2Cl_2$,[4] coordinates to a single-stranded DNA under reflux with DNA in water-ethanol solution and that ruthenium was recovered in the form of $Ru(bipy)_3^{2+}$ when the Ru-DNA complex solution was refluxed with an excess of 2,2'-bipyridine. Thus, it became possible to use the photocatalyst as a DNA probe.

Figure 1 shows the complex formation of cis-$Ru(bipy)_2Cl_2$ with DNA and the recovery of $Ru(bipy)_3^{2+}$ from the DNA complex. Spectra a) and b) show absorption of the solution of cis-$Ru(bipy)_2Cl_2$ and oligo-DNA(69-mer) before and after reflux in ethanol-water(1:1 in volume), respectively. Spectrum c) is after reflux with an excess of 2,2'-bipyridine in ethanol-water(7:3). The latter coincides with that of $Ru(bipy)_3^{2+}$. Spectrum d) is of resulting solution of cis-$Ru(bipy)_2Cl_2$ with 2'-deoxyguanosine-5'-monophosphate(dGMP) after reflux in ethanol-water(7:3). It resembles Spectrum b) (the Ru-DNA complex). The other three 2'-deoxyribonucleotide-5'-monophosphates give an absorption peak at 490-495 nm. Therefore, a likely site of coordination may be the guanine residue. This is similar to the case of cis-dichlorobis(1,10-phenanthroline)ruthenium(Ⅱ), which was reported by Barton et al.[5] The binding to DNA was stable. We observed the migration of the complex, by its reddish color, toward the positive direction in electrophoresis. The separation of the DNA-Ru complex from the unbound Ru complex was carried out by the addition of an excess ethanol to the solution. The DNA complex was precipitated

by centrifuge, with the unbound Ru remaining in solution.

For an indicator reaction, we employed the following redox cycle using a tetrazolium salt:



(1)

where NBT^{2+} is 2,2'-di(p-nitrophenyl)-5,5'-diphenyl-3,3'-(3,3-dimethoxy-4,4'-diphenylene)ditetrazolium(Ⅱ) and TEOA is triethanolamine. The former is irreversibly reduced to give a blue color.[6] The indicator solution was basically composed of NBT^{2+} and TEOA in water-ethanol(1:1), but additional reagents were needed to improve the sensitivity and stability of the coloring. We adjusted the pH of the solution to 10.5 and added DNA as a mediator of electron transfer from Ru(bipy)$_3^{2+}$ to NBT^{2+}. In Table 1 on the lines 1 and 2, the effect of DNA(calf thymus DNA, Sigma Type Ⅱ) is shown on the efficiency of the reduction of NBT^{2+} in the Ru(bipy)$_3^{2+}$/NBT^{2+}/TEOA system. The sample solutions were irradiated for 2 h with a xenon lamp of 500 W with a short wave-cut filter at 450 nm(Toshiba Y-45). The turnover of the redox cycle was determined by comparing the optical absorbance of NBT at 530 nm when NBT^{2+} was reduced with β-NADH disodium salt of known quantity instead of Ru(bipy)$_3^{2+}$.[7] We also tested Ru(phen)$_3^{2+}$ as the photocatalyst on the lines 3 and 4 in the table. In the both photocatalysts, the addition of DNA greatly enhances the efficiency of the photoreduction of NBT^{2+}.

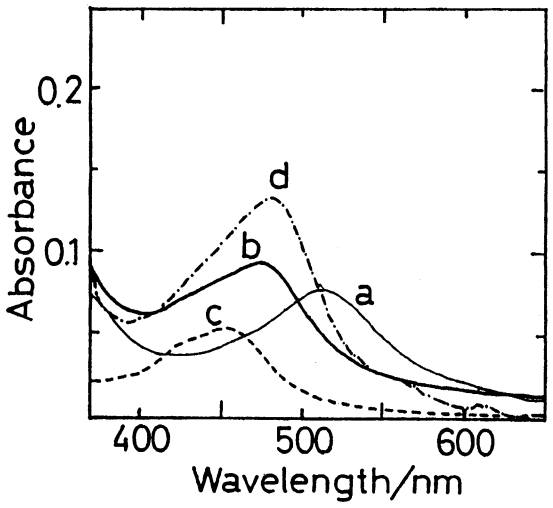


Fig.1. Absorption spectra of Ru(bipy)$_2$-DNA and related systems.
(a) cis-Ru(bipy)$_2$Cl$_2$ 9.5×10^{-6} M/l and origo-DNA (69-mer) 1.9×10^{-5} M/l in ethanol-water(1:1) before reflux. (b) ibid. after reflux for 30 min. (c) The Ru-DNA complex 2.6×10^{-6} M/l and 2,2'-bipyridine 10^{-2} M/l in ethanol-water (7:3) after reflux for 30 min. (d) cis-Ru(bipy)$_2$Cl$_2$ 1.9×10^{-5} M/l and dGMP 3.8×10^{-4} M/l in ethanol-water (7:3) after reflux for 1 h.

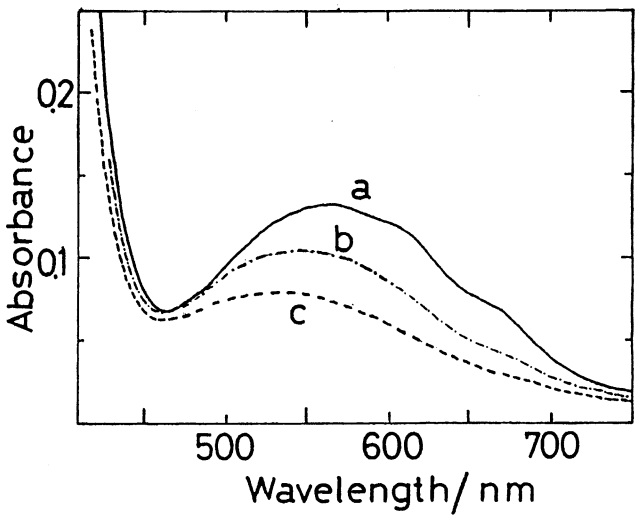


Fig.2. Absorption spectra of NBT after the indicator reaction in the system of Ru(bipy)$_3^{2+}$/NBT^{2+}/TEOA for the test of hybridization. The sample solutions were prepared from filter pieces containing: (a) 0.6 μg, (b) 0.3 μg of the positive plasmid, and (c) 0.3 μg of the negative plasmid after the hybridization with the Ru-DNA probe. They were irradiated with a xenon lamp for 2 h. The composition of the indicator system is as same as given in Table 1.

Hybridization and selection for DNA with a complimentary sequence were carried out using a model system. A DNA probe of Ru-DNA complex was prepared from a single-stranded DNA of 69-mer[8] which was chemically synthesized (Nippon Zeon Genet A-Ⅱ) for another purpose.[9] Two kinds of plasmid for selection were prepared; one was constructed by inserting into plasmid pUC19 a 42-mer DNA whose sequence was identical to that in the probe; the other was plasmid pBR322 which has no common sequence. A mixture of the DNA of 69-mer, 7.0×10^{-10} M and cis-Ru $(bipy)_2Cl_2$, 1.4×10^{-9} M was heated to reflux in water-ethanol(1:1) of 1 ml for 30 min and the solvent was evaporated off by a concentrator(Savant SVC-100H). Following the standard procedure by Maniatis et al.,[10] the two kinds of plasmid were immobilized on filter pieces of an area of 1x1 cm^2 (Schleicher Schnell B85) and were subject to the hybridization with the DNA probe overnight. After the filters were washed carefully, they were refluxed with an excess of 2,2'-bipyridine in ethanol for 30 min. Then, the supernatant was concentrated and subject to the indicator reaction. After the irradiation for 2 h, the positive sample turned blue from a yellowish color of the original solution, whereas the negative one remained pale purple. Figure 2 shows the absorption spectra of the resulting solutions, which were prepared from filter pieces containing the hybridzed plasmid of 0.6 μg (a) and 0.3 μg(b), and the non-hybridized one of 0.3 μg(c), respectively. The difference could well be recognized by visual observation if the quantity of plasmid was over 0.6 μg. Consequently, we may conclude that the DNA probe of photocatalyst works effectively.

There have been considerable interests in exploring non-radioactive DNA probes.[11] The present process has notable merits in simplicity in preparation of probes and in economy of reagents, compared with enzymatic methods, for example, the biotinylation method by Ward et al.[12] Because an enzyme such as alkaline phosphatase is expensive and deactivated easily, it is not suitable for applications of a large scale. Also, involvements of indigenous biotin seriously increases a background level. However, a drawback of the present method is in sensitivity at this stage. Since the conditions of hybridization are not optimized

Table 1. The effect of the addition of DNA on the efficiency of the photocatalytic redox cycle of $Ru(bipy)_3^{2+}$ and $Ru(phen)_3^{2+}$

System[a]				Absorbance (background)	Turnover[b,c]
$Ru(bipy)_3^{2+}$	1.0×10^{-6} M/ℓ	DNA	1.2mM/l[d]	0.27(0.05)	3.2×10^2
$Ru(bipy)_3^{2+}$	1.0×10^{-6} M/ℓ	DNA	—	0.08(0.03)	—
$Ru(phen)_3^{2+}$	1.0×10^{-6} M/ℓ	DNA	1.2mM/l	0.28(0.09)	2.9×10^2
$Ru(phen)_3^{2+}$	1.0×10^{-6} M/ℓ	DNA	—	0.09(0.04)	—

a) The solution is composed of NBT^{2+} 0.25 mM/l, TEOA 50 mM/l in ethanol-buffer(1:1); the buffer is composed of Tris 5 mM/l, NaOH 5 mM/l and NaCl 20 mM/l(pH 10.5). b) Irradiation is for 2 h with a xenon lamp with a short wave-cut filter at 450 nm. c) Turnover is determined by comparing the absorbance at 530 nm using β-NADH of a known quantity as the reducing substrate instead of the Ru complexes. d) The concentration is converted to that of deoxyribonucleotide.

and it is possible to reduce the solution volume for the indicator reaction, a detectable quantity of a target DNA might further be decreased. The concentration limit of $Ru(bipy)_3^{2+}$ for the present indicator reaction is about 10^{-8} M/ℓ, which is equivalent to the turnover of 10^4 for the redox cycles. To improve the sensitivity furthermore, one has to construct a more efficient redox cycle. We have seen in the table that the addition of DNA to the indicator solution greatly enhances the efficiency of the photoreduction of NBT^{2+}. According to the result of quenching experiments by Barton et al.,[1] intercalation of $Ru(phen)_3^{2+}$ into DNA enhances the efficiency of the photoelectron transfer at least by two orders, compared with the efficiency of $Ru(bipy)_3^{2+}$. As shown in the table, however, the $Ru(phen)_3^{2+}$ system is never superior to the $Ru(bipy)_3^{2+}$ in contrast to the quenching data. Therefore, the rate determining step for the indicator reaction is not in the photoelectron transfer, but in the reduction of NBT^{2+}. Presumably DNA may interact with NBT^{2+} to change the environment around NBT^{2+} ions. There will be, however, some other ways to improve the efficiency. For example, a combination of the photocatalytic redox cycle with another catalytic reaction such as cis-trans isomerization will be useful.[13]

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(Received April 27, 1989)