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ORIGINAL PAPER

Selective Fluorescence Sensing of Deoxycytidine 5′-Monophosphate (dCMP) Employing a Bis (diphenylphosphate)diimine Ligand

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Abstract A new bis(diphenylphosphate)diimine ligand (BP1) was prepared and evaluated for its ability for selective detection of deoxycytidine 5′-monophosphate (dCMP). BP1 exhibited off-type fluorescence in the presence of dCMP. The fluorescence of BP1 was significantly quenched upon the addition of 2.5×10^{-4} M dCMP and the detection limit was 1.25×10^{-5} M in MeCN-H_2O (1:1, *v/v*). The binding ratio between BP1 and dCMP was determined to be 1:1 with the binding constant of $3.98\pm0.60\times10^{-3}$ M^{-1}.

Keywords Fluorescence (FL) sensor · Deoxycytidine 5′-monophosphate (dCMP) sensor · Diimine ligand (DLs)

Introduction

Analytes are usually present in minute amounts in biological systems. Therefore, sensitive and selective detection of analytes with real-time are highly desired [1–7]. Fluorescence chemosensors have found their applications in various fields, e.g. environmental and biomedical sciences since they provide excellent degree of selectivity and sensitivity for the detection of biological and chemical analytes [8–15]. In general, the design of chemosensors bases on the incorporation of two functional moieties which are: i) ionophore (the receptor part) and ii) chromophore (the signal generating unit). The binding of the analyte to the ionophore initiates the communication between the receptor and the signaling unit to induce the change of chromophore's physical properties [16–18]. Physical changes of the chromophore can be of various types, e.g. chemiluminescence (CL), redox potentials, absorption and fluorescence (FL) [19–34]. Of these, fluorescence chemosensor receives much research attention since it provides the detection of analytes with low cost sampling and high degree of selectivity and sensitivity [35–37].

Bisphosphonate and its derivatives are well recognized for their applications as diagnostic tools in the cancer treatment [38–42]. Particularly, aminobisphosphonate (NBP) is known as an anti-cancer drug. The mode of action for an anti-cancer activity of NBP is specific for prostate cancers. Apart from its medical roles, NBP is known for its ability as a metal chelator [43–45]. Gummiena and his group demonstrated the ability of β-aminobisphosphonate derivatives as chelating agents for copper (II) ion employing electron paramagnetic spectroscopy (EPR) [46].

It is known that the bisphosphate moiety exhibits the fluorescence quenching via the photon-induced electron transfer (PET) mechanism. Diimine ligands (DLs) are known as metal chelators and also as fluorophores due to their photophysical properties [47]. Based on these facts, the incorporation of these components would emerge a new fluorescence chemosensor. Consequently, we have developed new bisphosphate derivatives based on diimine ligands and evaluated for their FL sensing towards

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nucleotides. The introduction of bisphosphate group into DL is aimed to facilitate the optimal hydrogen bonding between the receptor and nucleotide substrates. The detection deoxycytidine 5detection deoxycytidine-monophosphate (dCMP) is normally achieved employing high-performance liquid chromatography (HPLC) [48]. Up to date, the fluorescent (FL) chemosensor for dCMP is not yet reported. To the best of our knowledge, the chemosensor based on diimine bisphosphate has not been reported as a selective receptor for dCMP.

Experiments

Apparatus

Fluorescence measurements were carried out using a FP-6300 spectrofluorometer (JASCO) equipped with a xenon lamp source and a 1.0-cm quartz cell, and the scan speed was 600 nmmin^{-1}. ^{1}H spectrum was recorded on Bruker DPX 400 MHz spectrometer in $CDCl_3$ using TMS as the internal standard. Mass spectra were recorded on Bruker Esquire and Finnigan MAT INCOS 50 mass spectrometers.

Reagents

All reagents for the synthesis of diimine ligand phosphates obtained commercially were used without further purification. The stock solution of diimine ligand phosphates was prepared in acetonitrile with the concentration of 10 mM. The corresponding nucleotide monophosphates [deoxycytidine 5′-monophosphate (dCMP); deoxyadenosine 5′-monophosphate (dAMP); deoxycyclicadenosine 5′-monophosphate (dcAMP); deoxythymidine 5′-monophosphate (dTMP); deoxyguanosine 5′-monophosphate (dGMP); uridine 5′-monophosphate (UMP); adenosine 5′-monophosphate (AMP); guanosine 5′-monophosphate (GMP); cytidine 5′-monophosphate (CMP); thymidine 5′-monophosphate (TMP)] used in this study were purchased from Sigma Aldrich (USA) and used without further purification. Methanol was used as a HPLC grade. All other chemicals used were supplied from Sigma Aldrich (USA) as analytical grade and used without further purification. MilliQ water was used throughout this study. The concentration of stock solution of metal ions was 1 mM.

General Synthesis of Diimine Ligands

DL was synthesized from the condensation between a corresponding benzaldehyde (1 mmol) and *o*-phenylenediamine (1 mmol) in the presence of distilled water (10 mL). After the reaction mixture was left stirring for 6 h, the crude solid was collected by filtration and crystallized from methanol to obtain the final product in good yields.

3,3′-[1,2-Phenylenebis(nitrilomethylidene)]bis-phenol (DL1)

DL1 was obtained as white crystals (2.16 g, 74 %). ^{1}H NMR (DMSO-*d*6) δ_H 7.65 (d, 1H), 7.60 (m, 2H), 7.41 (q, 1H), 7.19 (m, 1H), 6.89 (d, 2H), 6.64 (d, 2H), 5.41 (s, 2H). MS (ESI) $[M+H]^+$ 317.13.

4,4′-[1,2-Phenylenebis(nitrilomethylidene)]bis-phenol (DL2)

DL2 was obtained as white crystals (2.40 g, 82 %). ^{1}H NMR (DMSO-*d*6) δ_H 8.94 (s, 2H), 7.67 (d, 2H), 7.46 (m, 4H), 7.41 (m, 4H), 6.97 (m, 4H). ^{13}C NMR δ 117.6, 119.1, 119.4, 119.8, 127.9, 132.5, 133.5, 142.6, 161.5, 163.8. MS (ESI) $[M+H]^+$ 317.35.

Synthesis of *N,N*′-Phenylenebis[2-hydroxybenzylidene-2-benzylidenealdimine] (MP)

In a flame dried round bottom flask, DL1 (0.5 g, 1.58 mmol) was added followed by addition of dry THF (5 mL). The reaction mixture was stirred until the disappearance of DL1 and the solution was brought to −80 °C followed by addition of potassium *tert*-butoxide (0.19 g, 1.74 mmol). The reaction mixture was left stirring at room temperature for 3 h before the reaction solution was cooled down to −80 °C followed by the addition of diphenyl chlorophosphate (0.33 mL, 1.58 mmol). The reaction mixture was left stirring at room temperature overnight before the addition of saturated aqueous NH_4Cl solution followed by extraction with EtOAc (3×10 mL). The organic phase was washed with brine and dried over $MgSO_4$. The solvent was removed under a vacuum and the crude product was chromatographed on a silica gel column. Elution of the column with a mixture of EtOAc and hexane (2:8) afforded the desired product as clear oil (0.54 g, 62 %). ^{1}H NMR (DMSO-*d*6) δ_H 8.40 (s, 1H), 7.70 (d, 2H, *J*=8.51), 7.39 (d, 1H, *J*=8.50), 7.30 (t, 2H, *J*= 15.77), 7.22 (m, 2H), 7.08 (t, 2H, *J*=7.81 and 15.53), 6.92 (d, 1H, 7.49), 6.61 (d, 1H, *J*=7.74), 6.48 (d, 1H, *J*=7.49), 6.38 (s, 1H), 5.45 (s, 2H).^{13}C NMR δ 111.13 (Ar*C*H), 117.47 (Ar*C*H), 119.57 (Ar*C*H), 119.74 (Ar*C*H), 119.79 (Ar*C*H), 119.88 (Ar*C*H), 119.92 (Ar*C*H), 120.54 (Ar*C*H), 121.38 (Ar*C*H), 121.70 (Ar*C*H), 122.61 (Ar*C*H), 123.22 (Ar*C*H), 123.66 (Ar*C*H), 125.54 (Ar*C*H), 125.94 (Ar*C*H), 126.04 (Ar*C*H), 126.36 (Ar*C*H), 128.77 (Ar*C*H), 129.35 (Ar*C*H), 130.04 (Ar*C*H), 130.19 (Ar*C*H), 130.28 (Ar*C*H), 130.76 (Ar*C*H), 130.89 (Ar*C*H), 131.90 (Ar*C*), 135.91 (Ar*C*), 139.37 (Ar*C*), 142.53 (Ar*C*), 149.64 (Ar*C*), 149.71 (Ar*C*), 150.17 (*C*=NH), 151.69 (*C*=NH). HRMS (ESI+) $[M+Na]^+$ found 571.1392 $[M+H]^+$, calcd for $C_{32}H_{25}N_2O_5P$

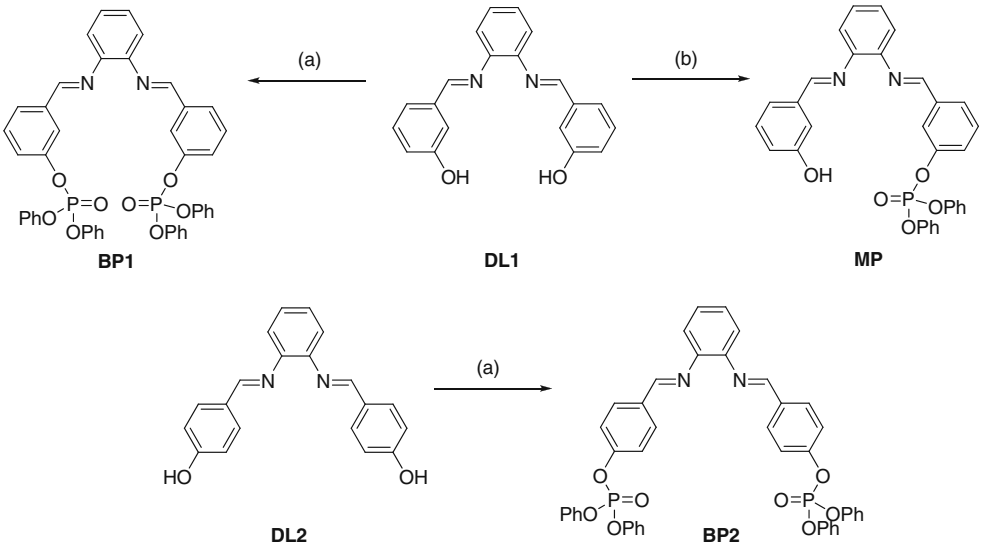

Fig. 1 Chemical structures of BP1, BP2 and MP. Conditions and Reagents; (a) (i) 2.2 equiv *tert*-BuOK, THF, room temperature, 2 hrs (ii) 2 equiv ClP(O)$(OPh)_2$, −80 °C, overnight. (b) (i) 1.1 equiv *tert*-BuOK, THF, room temperature, 2 hrs (ii) 1 equiv ClP(O)$(OPh)_2$, −80 °C, overnight

548.1501. IR (KBr, cm^{-1}) 3417, 3071, 2615, 1941, 1788, 1606, 1416, 1389, 1025, 964, 849, 777.

Synthesis of *N,N′*-Phenylenebis[2-benzylidenealdimine] phosphate (BP1)

BP1 was synthesized according to the procedure described for the synthesis of MP except 2 molar equiv of diphenyl chlorophosphate was used. ^{1}H NMR (DMSO-d6) δ_H 8.40 (s, 1H), 7.80 (d, 2H, *J*=8.67), 7.72 (d, 1H, *J*=8.96), 7.42 (m, 13H), 7.26 (m, 19H), 7.06 (d, 2H, *J*=8.61), 5.59 (s, 2H).^{13}C NMR δ 111.14 (Ar*C*H), 115.26 (Ar*C*H), 119.36 (Ar*C*H), 119.91 (Ar*C*H), 119.97 (Ar*C*H), 120.30 (Ar*C*H), 120.35 (Ar*C*H), 120.41 (Ar*C*H), 121.76 (Ar*C*H), 122.42 (Ar*C*H), 122.94 (Ar*C*H), 125.97 (Ar*C*H), 126.08 (Ar*C*H), 128.10 (Ar*C*H), 128.79 (Ar*C*H), 130.24 (Ar*C*H), 130.32 (Ar*C*H), 130.49 (Ar*C*H), 131.16 (Ar*C*), 134.53 (Ar*C*), 135.81 (Ar*C*), 142.61 (Ar*C*), 149.77 (Ar*C*), 152.13 (*C*=NH). HRMS (ESI+) $[M+Na]^+$ found 803.1687 $[M+H]^+$, calcd for $C_{44}H_{34}N_2O_8P_2$ 780.1790. IR (KBr, cm^{-1}) 3064, 2929, 2870, 2611, 1942, 1867, 1783, 1730, 1588, 1487, 1449, 1389, 1298, 1186, 1161, 1090, 1072.

Synthesis of *N,N′*-Phenylenebis[3-benzylidenealdimine] phosphate (BP2)

BP2 was similarly prepared according to the procedure for BP1 except DL2 was used as a starting material. ^{1}H NMR (DMSO-*d*6) δ_H 7.82 (d, 2H, *J*=8.65), 7.44–7.39 (m, 15H), 7.31–7.20 (m, 14H), 7.15 (d, 2H, *J*=7.15), 5.60 (s, 2H).^{13}C NMR δ 111.14 (Ar*C*H), 115.26 (Ar*C*H), 119.37 (Ar*C*H), 119.87 (Ar*C*H), 120.36 (Ar*C*H), 121.80 (*ArCH*), 122.41 (Ar*C*H), 125.61 (Ar*C*H), 126.02 (Ar*C*H), 128.10 (Ar*C*H), 130.14 (Ar*C*H), 131.16 (Ar*C*H), 135.83 (Ar*C*H), 142.64

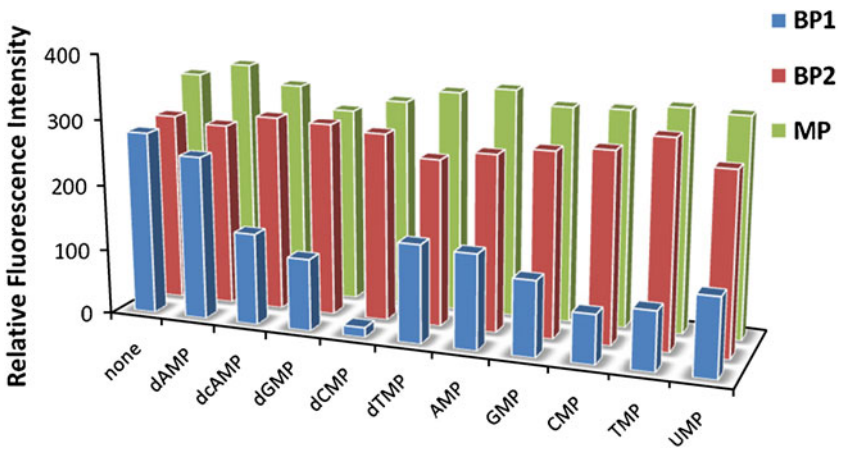

Fig. 2 The effect of various nucleotides on the FL intensities of MP, BP1 and BP2 in MeCN: H_2O (1:1; *v/v*). [receptor] = 2.5×10^{-6} M. [nucleotides] = 2.5×10^{-4} M. λ_{ex}=280 nm

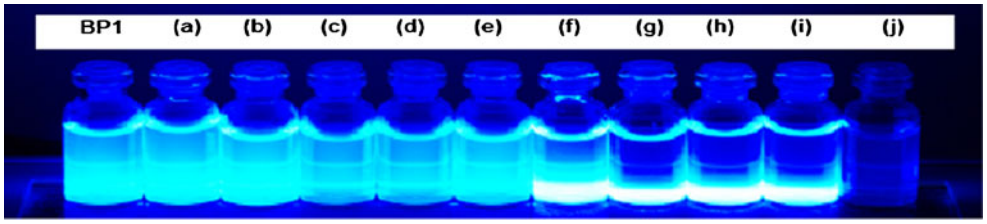

Fig. 3 FL changes of BP1 (2.5×10^{-6} M) in the presence and absence of nucleotides (a) BP1 + dAMP (b) BP1 + dcAMP (c) BP1 + dTMP (d) BP1 + dGMP (e) BP1 + AMP (f) BP1 + GMP (g) BP1 + CMP (h) BP1 + TMP (i) BP1 + UMP and (j) BP1 + dCMP. [nucleotides] = 5×10^{-4} M

(Ar*C*H), 142.77 (Ar*C*H), 149.08 (Ar*C*H), 149.83 (Ar*C*H), 150.88 (Ar*C*H), 151.51 (Ar*C*H), 152.14 (Ar*C*), 153.67 (Ar*C*), 153.95 (Ar*C*), 157.07 (Ar*C*), 159.07 (Ar*C*), 161 (*C*=NH). HRMS (ESI+) $[M+Na]^+$ found 803.1681 $[M+H]^+$, calcd for $C_{44}H_{34}N_2O_8P_2$ 780.1790. IR (KBr, cm^{-1}) 3451, 3268, 3070, 3007, 2881, 2512, 2249, 2123, 1996, 1828, 1768, 1621, 1374, 1223, 1056, 882, 759.

Results and Discussion

Syntheses of MP, BP1 and BP2

BP1 was synthesized in two steps from the reaction between DL1 with 2 equiv of diphenylchlorophosphate in the presence of potassium *tert*-butoxide as base. BP2 was also synthesized in order to investigate the positional effect of bisphosphate group. The synthesis of BP2 was similar to the procedure described for the synthesis of BP1 except DL2 was used as the starting material. After purification, BP1 and BP2 were obtained in good isolated yields (BP1= 72% and BP2=77%) (Fig. 1). Monodiphenylchlorophosphate diimine ligand (MP) was also synthesized as the control ligand for the investigation of the effect of bisphosphate group due to the fact that the presence of bisphosphate group is important for facilitating the hydrogen bonding between the receptor and analytes. The synthesis of MP was achieved by reacting DL1 with 1 equiv of diphenylchlorophosphate. BP1 was prepared in moderate yield (62%).

Fluorescence Screening of MP, BP1 and BP2 with Various Nucleotide Substrates

Obtaining BP1, BP2 and MP, FL quenching experiments of these receptors in the presence of various nucleotides were investigated. With reference to the screened nucleotides, the FL property of BP1 exhibited a significant quenching in the presence of deoxycytidine 5′-monophosphate (dCMP) (Fig. 2). It was shown that after the addition of 2.5×10^{-4} M dCMP, the FL intensity of BP1 was significantly inhibited. The presence of other nucleotides did not significantly quench the FL of BP1. BP2 and MP showed no degree of FL quenching upon treating with any nucleotides. It was shown that the presence of bisphosphate group at the *meta* position on the aromatic ring of BP1 is necessary for proper chelation between BP1 and dCMP.

In the case of MP, it lacks a phosphate group which resulted in an improper interaction between MP and dCMP. This was evident that the presence of bisphosphate group was important since no FL quenching of MP was observed in the presence of any nucleotides. BP2 possesses the

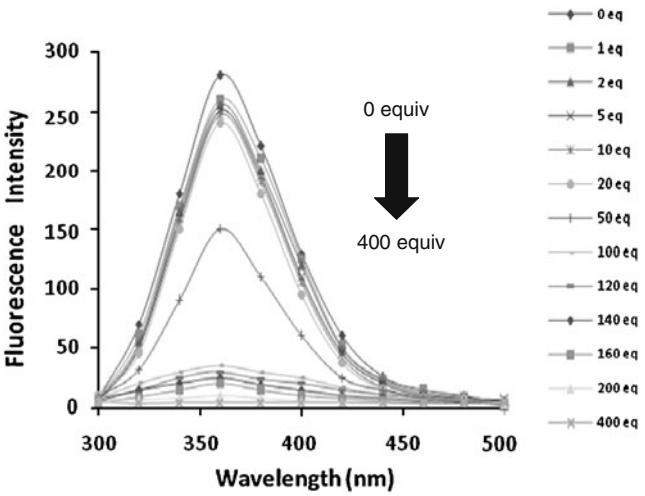

Fig. 4 FL emission spectra of BP1 (2.5×10^{-6} M) in the presence of various concentrations of dCMP

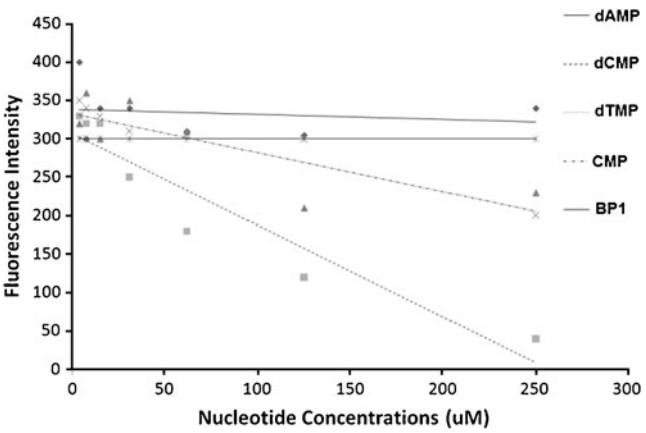

Fig. 5 FL quenching of BP1 by various concentrations of dAMP, dCMP, dTMP, and CMP

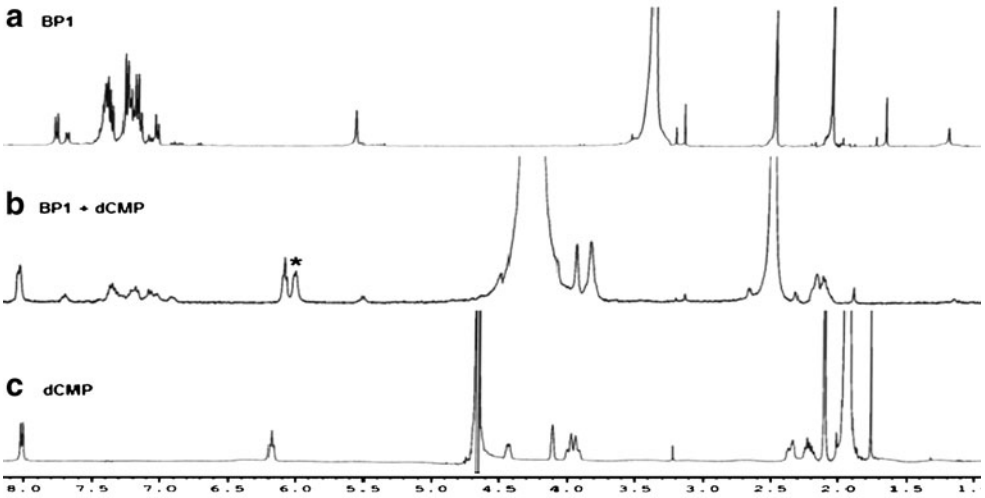

Fig. 6 ^{1}H NMR spectra (400 MHz) of (a) free BP1 (10 mM) in DMSO (top); (b) BP1 + 1 equiv of dCMP in DMSO:D_2O (1:1; *v/v*); (c) dCMP in D_2O

bisphosphate group which meets the requirement described above for the optimal interaction between the receptor and dCMP. The position of the bisphosphate group on the BP2 aromatic ring is too remote for the good binding between the receptor and dCMP. It was obvious that BP1 exhibited high degree of selectivity towards dCMP. The presence of the bisphosphate group at the *meta* position on the aromatic ring of BP1 is a prerequisite for the good recognition between the receptor and dCMP. Therefore, BP1 was used as the optimal receptor for subsequent experiments.

BP1 was shown to exhibit strong FL emission in acetonitrile. The excitation wavelength (*E*x) of BP1 was 280 nm while the emission wavelength (*E*m) was 360 nm.

Figure 3 shows the FL changes of BP1 upon the addition of various nucleotides. The presence of various nucleotides slightly to moderately affected the FL intensity of BP1. It was clearly observed that 2.5×10^{-4} M of dCMP dramatically quenched the FL of BP1.

FL emission of BP1 was measured at different concentrations of dCMP while fixed the concentration of BP1 at 2.5×10^{-6} M (Fig. 4). Increasing concentration of dCMP resulted in the decrease of the FL emission of BP1. When the concentration of dCMP reached 2.5×10^{-4} M, the FL intensity of BP1 was significantly quenched. To investigate more precisely, the quenching effect of BP1 with dCMP and other representing nucleotides at different concentrations was studied. The FL intensity of BP1 was measured in the presence of various concentrations of four nucleotides, dAMP, dCMP, dTMP and CMP. At all concentrations of dAMP, there was a little FL quenching of BP1 (Fig. 5).

Addition of dTMP and CMP resulted in slight decrease of BP1's FL intensity. The FL intensity of BP1 was significantly inhibited in the presence of dCMP even at low concentration. Further increasing concentrations of dCMP resulted in a gradual FL quenching of BP1. When the concentration of dCMP reached 400

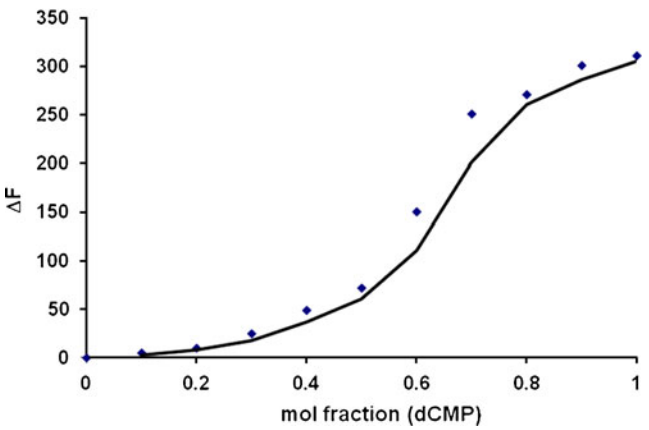

Fig. 7 Job's plot for BP1·dCMP. *Y* axis is fluorescence changes of BP1

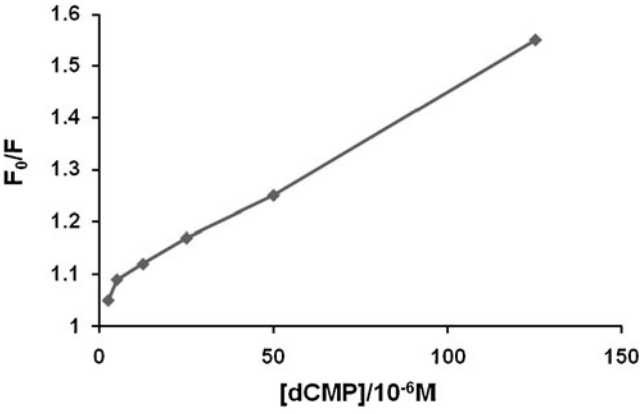

Fig. 8 Stern-Volmer plot of BP1 with increasing concentrations of dCMP. [BP1] = 2.5×10^{-6} M. [dCMP]/10^{-6} M=2.5, 5, 12.5, 25, 50, 300 (*T*=298 K)

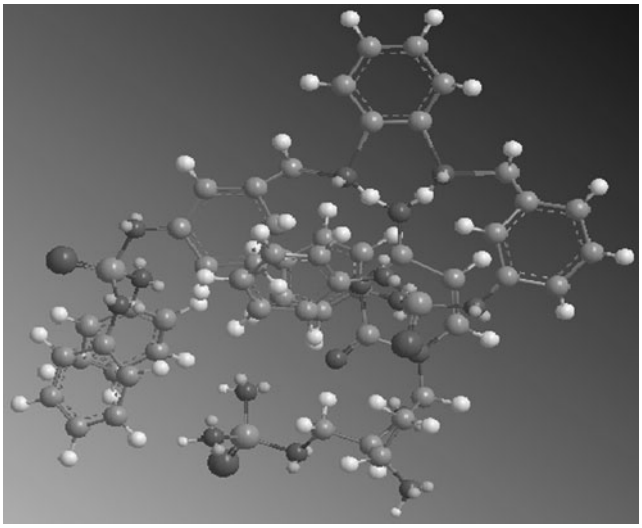

Fig. 9 Energy minimized structure of the complex between BP1 and dCMP

molar equiv of BP1, the FL intensity of BP1 was significantly quenched.

^{1}H NMR Titration of BP1 with dCMP

Of the mechanistic detail concerning interaction between BP1 and dCMP, ^{1}H NMR experiments were used for the investigation (Fig. 6). It was clearly shown that the addition of 1 equiv dCMP into BP1 solution caused a slight downfield shift of aromatic protons in BP1 indicating the disturbance of the electron density within this area. Apart from the downfield shift of protons in the aromatic region, the solution contained both BP1 and dCMP exhibited the change of the multiplicity for the designated cytosine proton. This effect was evident with the appearance of the new peak at δ 6.15 ppm (indicating as an asterisk in Fig. 6b). This new peak was the result from the perturbation for the electronic environment of the cytosine proton in dCMP due to the interaction with BP1.

Determination of the Binding Ratio and Constant for BP1 and dCMP

Attempting to obtain the binding ratio between BP1 and dCMP by mass spectrometry was unsuccessful. Therefore, Job's plot analysis was used to determine the binding ratio [49]. Job's plot analysis indicated a breaking point at 0.6 mol fraction of dCMP indicating the formation of a 1:1 complex of BP1·dCMP (Fig. 7). The binding constant between BP1 and dCMP was determined using the Stern-Volmer plot (K_{sv}). The K_{sv} was obtained from the Stern-Volmer Eq. (1) [50]

$$F_0/F = 1 + K_{SV}[Q] \quad (1)$$

Where [Q] is the concentration of the quencher (dCMP), K_{sv} is the Stern-Volmer constant. The Stern-Volmer constant indicates the constant equilibrium of BP1·dCMP complex in the static quenching process. It was shown in Fig. 8 that the Stern-Volmer plot of BP1·dCMP complex exhibited a linear relationship. The (F_0/F) was directly proportional to the increased concentration of dCMP with the correlation coefficient of 0.9953. From the plot, BP1 had a weak binding affinity to dCMP ($3.98\pm0.60\times 10^{-3}$ M^{-1}) and upon the binding between BP1 and dCMP it formed the non-fluorescent complex.

Proposed Mechanism for BP1 Binding with dCMP

The mechanistic detail for the binding between BP1 and dCMP is believed to involve the hydrogen bonding between the bisphosphate group of BP1 and the cytosine NH_2 of dCMP. The good alignment for the binding between BP1 and dCMP optimizes the interaction of these two species. Upon binding between BP1 and dCMP, the PET mechanism plays a significant role which resulted in the FL quenching of the system. The steric hindrance is also thought to play a role on the binding between BP1 and dCMP. The steric hindrance could be used to explain why the FL intensity of BP1 was not quenched upon the addition with CMP. In the case of CMP, the presence of the 2′-hydroxyl group causes the structure more bulky to fit into the receptor's pocket. Therefore, the presence of the 2′-hydroxyl group in CMP hindered proper binding between BP1 and CMP. This explained the fact that CMP is not a good substrate for BP1 due to the steric effect. The absence of the 2′-hydroxyl group in dCMP facilitates the good binding between BP1 and dCMP (Fig. 9).

Conclusions

In conclusion, we have discovered the selective FL quenching of BP1 by dCMP. The FL quenching of BP1 exhibited excellent degree of selectivity towards dCMP among various nucleotide substrates. FL and ^{1}H NMR experiments indicated the interaction between BP1 and dCMP. Job's plot analysis indicated the binding ratio between BP1 and dCMP at 1:1 with the Stern-Volmer constant of $3.98\pm0.60\times10^{-3}$ M^{-1}. It was suggested that the FL quenching of BP1 by dCMP took place via the PET mechanism. The recognition of dCMP by BP1 may be due to the extended hydrogen bonding with the proper alignment. The steric effect of the substrate also plays a determining role for good binding between BP1 and dCMP. Since BP1 shows high degree of selectivity and sensitivity to dCMP over other nucleotides it could be regarded as a new FL chemosensor for dCMP.

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References

1. Lu C, Xu Z, Cui J, Zhang R, Qian X (2007) Ratiometric and highly selective fluorescent sensor for cadmium under physiological pH range: a new strategy to discriminate cadmium from zinc. J Org Chem 72:3554
2. Tang B, Yin L, Wang X, Chen Z, Tong L, Xu K (2009) A fast-response, highly sensitive and specific organoselenium fluorescent probe for thiols and its application in bioimaging. Chem Commun 5293
3. Xue X, Wang F, Liu X (2008) One-step, room temperature, colorimetric detection of mercury (Hg^{2+}) using DNA/nanoparticle conjugates. J Am Chem Soc 130:3244
4. Kim HJ, Kim SH, Kim JH, Anh LN, Lee JH, Lee CH, Kim JS (2009) ICT-based Cu(II)-sensing 9, 10-anthraquinonecalix[4] crown. Tetrahedron Lett 50:2782
5. Maiti J, Pokhrel B, Boruah R, Dolui SK (2009) Polythiophene based fluorescence sensors for acids and metal ions. Sensors Actuat B Chem 141:447
6. Zeng X, Dong L, Wu C, Mu L, Sai-Feng X, Tao Z (2009) Highly sensitive chemosensor for Cu(II) and Hg(II) based on the tripodal rhodamine receptor. Sensors Actuat B Chem 141:506
7. Lee SJ, Lee SS, Jeong IY, Lee JY, Jung JH (2007) Azobenzene coupled chromogenic receptors for the selective detection of copper(II) and its application as a chemosensor kit. Tetrahedron Lett 48:393
8. Wu JS, Kim HJ, Lee MH, Yoon JH, Lee JH, Kim JS (2007) Anion-induced ring-opening of fluorescein spirolactam: fluorescent *OFF–ON*. Tetrahedron Lett 48:3159
9. Rieth T, Sasamoto K (1998) Detection of nitric oxide and nitrite by using a Rhodamine-type fluorescent indicator. Anal Commun 35:195
10. Soh JH, Swamy KMK, Kim SK, Sim S, Lee SH, Yoon J (2007) Rhodamine urea derivatives as fluorescent chemosensors for Hg^{2+}. Tetrahedron Lett 48:5966
11. Kikuchi K, Hashimoto S, Mizukami S, Nagano T (2009) Anion sensor-based ratiometric peptide probe for protein kinase activity. Org Lett 11:2732
12. Xue L, Liu Q, Jiang H (2009) Ratiometric Zn^{2+} fluorescent sensor and new approach for sensing Cd^{2+} by ratiometric displacement. Org Lett 11:3454
13. Wang HH, Gan Q, Wang XJ, Xue L, Liu SH, Jiang H (2007) A water soluble, small molecular fluorescent sensor with femtomolar sensitivity for zinc ion. Org Lett 9:4995
14. Xu Z, Xiao Y, Qian X, Cui J, Cui D (2005) Ratiometric and selective fluorescent sensor for Cu^{II} based on internal charge transfer (ICT). Org Lett 7:889
15. Rhee HW, Lee CR, Cho SH, Song MR, Cashel M, Choy HE, Seok YJ, Hong JI (2008) Selective fluorescent chemosensor for the bacterial alarmone (p)ppGpp. J Am Chem Soc 130:784
16. Kim NY, Chang SK (1998) Calix[4]arenes bearing two distal azophenol moieties: highly selective chromogenic ionophores for the recognition of Ca^{2+} ion. J Org Chem 63:2362
17. He X, Hu S, Liu K, Guo Y, Xu J, Shao S (2006) Oxidized bis (indolyl)methane: a simple and efficient chromogenic-sensing molecule based on the proton transfer signaling mode. Org Lett 8:333
18. Jiménez D, Martínez-Máñez R, Sancenón F, Ros-Lis JV, Benito A, Soto J (2003) A new chromo-chemodosimeter selective for sulfide anion. J Am Chem Soc 125:9000
19. Matsumoto M, Kasai D, Yamada K, Fukuda N, Watanabe N, Ijuin HK (2004) Color modulation for chemiluminescence of a dioxetane bearing a 3-(anthracen-9-yl)-5-hydroxyphenyl moiety induced by a complex of crown ether with potassium *tert*-butoxide. Tetrahedron Lett 45:8079
20. Zhu Y, Shi J, Zhang Z, Zhang C, Zhang X (2002) Development of a gas sensor utilizing chemiluminescence on nanosized titanium dioxide. Anal Chem 74:120
21. Hu X, Takenaka N, Takasuna S, Kitano M, Bandow H, Maeda Y, Hattori M (1993) Determination of ammonium ion in rainwater and fogwater by flow injection analysis with chemiluminescence detection. Anal Chem 65:3489
22. Corrales T, Abrusci C, Peinado C, Catalina F, Fluorescent sensor as physical amplifier of chemiluminescence: application to the study of poly(ethylene terephthalate). Macromolecules 37:6596
23. Kang D, Zuo X, Yang R, Xia F, Plaxco KW, White RJ (2009) Comparing the properties of electrochemical-based DNA sensors employing different redox tags. Anal Chem 81:9109
24. Cai Q, Zeng K, Ruan C, Desai TA, Grimes CA (2004) A wireless, remote query glucose biosensor based on a pH-sensitive polymer. Anal Chem 76:4038
25. Xiao Y, Qu X, Plaxco KW, Heeger AJ (2007) Label-free electrochemical detection of DNA in blood serum via target-induced resolution of an electrode-bound DNA pseudoknot. J Am Chem Soc 129:11896
26. Xiao Y, Lou X, Uzawa T, Plakos KJI, Plaxco KW, Soh HT (2009) An electrochemical sensor for single nucleotide polymorphism detection in serum based on a triple-stem DNA probe. J Am Chem Soc 131:15311
27. Watanabe S, Seguchi H, Yoshida K, Kifune K, Tadaki T, Shiozaki H (2005) Colorimetric detection of fluoride ion in an aqueous solution using a thioglucose-capped gold nanoparticle. Tetrahedron Lett 46:8827
28. Zhang C, Suslick KS (2005) A colorimetric sensor array for organics in water. J Am Chem Soc 127:11548
29. Liu J, Lu Y (2003) A colorimetric lead biosensor using DNAzyme-directed assembly of gold nanoparticles. J Am Chem Soc 125:6642
30. Zhang D, Zhang M, Liu Z, Yu M, Li F, Yi T, Huang C (2006) Highly selective colorimetric sensor for cysteine and homocysteine based on azo derivatives. Tetrahedron Lett 47:7093
31. Scrafton DK, Taylor JE, Mahon MF, Fossey JS, James TD (2008) Click-fluors: modular fluorescent saccharide sensors based on a 1, 2, 3-triazole ring. J Org Chem 73:2871
32. Yang RH, Chan WH, Lee AWM, Xia PF, Zhang HK, Li K (2003) A ratiometric fluorescent sensor for Ag^{I} with high selectivity and sensitivity. J Am Chem Soc 125:2884
33. Yao Z, Feng X, Li C, Shi G (2009) Conjugated polyelectrolyte as a colorimetric and fluorescent probe for the detection of glutathione. Chem Commun 5886
34. Mu H, Gong R, Ma Q, Sun Y, Fu E (2007) A novel colorimetric and fluorescent chemosensor: synthesis and selective detection for Cu^{2+} and Hg^{2+}. Tetrahedron Lett 48:5525
35. Hong SY, Czarnik AW (1993) A metal ion-insensitive polyanion chemosensor. J Am Chem Soc 115:3330
36. Namkung W, Padmawar P, Mills AD, Verkman AS (2008) Cell-based fluorescence screen for K^+ channels and transporters using an extracellular triazacryptand-based K^+ sensor. J Am Chem Soc 130:7794
37. Zhao J, Davidson MG, Mahon MF, Kociok-Köhn G, James TD (2004) An enantioselective fluorescent sensor for sugar acids. J Am Chem Soc 126:16179
38. Merrell MA, Wakchoure S, Ilvesaro JM, Zinn K, Gehrs B, Lehenkari PP, Harris KW, Selander KS (2007) Differential effects

of Ca^{2+} on bisphosphonate-induced growth inhibition in breast cancer and mesothelioma cells. Eur J Pharm 559:21
39. Segawa H, Kimura S, Kuroda J, Sato K, Nogawa M, Yuasa T, Yokota A, Hodohara K, Fujiyama Y, Maekawa T (2005) The anti-leukemic efficacy of the third generation bisphosphonate ONO5920/YM529. Leukemia Res 29:451
40. Cramer JA, Lynch NO, Gaudin AF, Walker M, Cowell W (2006) The effect of dosing frequency on compliance and persistence with bisphosphonate therapy in postmenopausal women: a comparison of studies in the united states, the united kingdom, and france. Clin Ther 28:1686
41. Sillero MAG, de Diego A, Silles E, Pérez-Zúñiga F, Sillero A (2006) Synthesis of bisphosphonate derivatives of ATP by T4 RNA ligase. FEBS Lett 580:5723
42. Takahashi R, Shimazaki C, Inaba T, Okano A, Hatsuse M, Okamoto A, Hirai H, Ashihara E, Nakagawa M (2001) A newly developed bisphosphonate, YM529, is a potent apoptosis inducer of human myeloma cells. Leukemia Res 25:77
43. Kamila S, Callan JF, Mulrooney RC, Middleton M (2007) A novel fluorescent chemosensor for Cu(II) in aqueous solution based on a β-aminobisphosphonate receptor. Tetrahedron Lett 48:7756
44. Matczak-Jon E, Kurzak B, Sawka-Doborwolska W, Lejczak B, Kafarski P (1998) Zinc(II) complexes of phosphonic acid analogues of aspartic acid and asparagine. J Chem Soc Dalton Trans 161
45. Gumienna-Kontecka E, Jezierska J, Lecouvey M, Leroux Y, Kozlowski HJ (2002) Bisphosphonate chelating agents: Coordination ability of 1-phenyl-1-hydroxymethylene bisphosphonate towards Cu^{2+} ions. J Inorg Biochem 89:13
46. Gumienna-Kontecka E, Galezowska J, Drag M, Latajka R, Kafarski P, Kozlowski H (2004) Coordination abilities of substituted β-aminophosphonates towards Cu^{2+} and Zn^{2+} ions. Inorg Chim Acta 357:1632
47. Jung HJ, Singh N, Jang DO (2008) Highly Fe^{3+} selective ratiometric fluorescent probe based on imine-linked benzimidazole. Tetrahedron Lett 49:2960
48. Havlis J, Madden JE, Revilla AL, Havel J (2001) High-performance liquid chromatographic determination of deoxycytidine monophosphate and methyldeoxycytidine monophosphate for DNA demethylation monitoring: experimental design and artificial neural networks optimization. J Chromatogr B 755:185
49. Schneider HJ, Yatsimirsky A (2000) Principles and methods in supramolecular chemistry. Wiley, Chichester, p 151
50. Mei X, Wolf C (2004) Enantioselective sensing of chiral carboxylic acids. J Am Chem Soc 126:14736