Interaction of a novel red-region fluorescent probe, Nile Blue, with DNA and its application to nucleic acids assay

Qiu-ying Chen,[a] Dong-hui Li,[a] Yang Zhao[b], Huang-hao Yang,[a] Qing-zhi Zhu[a] and Jin-gou Xu*[a]

[a] *Research Laboratory of SEDC of Analytical Science for Material and Life Chemistry, Department of Chemistry, Xiamen University, Xiamen 361005, China*
[b] *Department of Biology, Xiamen University, Xiamen 361005, China*

Received 11th February 1999, Accepted 6th May 1999

A novel fluorimetric method was developed for the rapid determination of DNA and RNA based on their quenching effect on the cationic red-region fluorescent dye Nile Blue (NB). In the investigation of the interaction of NB with DNA by steady-state polarization measurements, thermal denaturing study, determination of absorption and fluorescence characteristics, salt effect study and electrophoresis experiments, the results supported the suggestion that NB served as an intercalator to the stack base pairs of nucleic acids. Further evidence showed that the quenching could be ascribed to the static quenching mode. A binding constant of about 10^6 M^{-1} and a binding site size of about three base pairs were obtained by spectral methods. Under optimum conditions, the calibration curves for the determination of calf thymus DNA (CT DNA) and yeast RNA were linear over the ranges 3.0 ng mL^{-1}–2.0 μg mL^{-1} and 27 ng mL^{-1}–10 μg mL^{-1}, respectively. The detection limits were 3.0 ng mL^{-1} for CT DNA and 27 ng mL^{-1} for RNA. The relative standard deviation (n = 6) was within 2.1% in the middle of the linear range. Interferences from some interesting co-existing substances in the determination of DNA were also examined.

Investigations of the interaction of small molecules with nucleic acids has been the focus of extensive research in recent years. The small molecules studied include dyes, drugs and toxic chemicals. The aim for the research is first, to develop new dyes as probes for the determination of nucleic acids in various applications, such as in molecular biology, biotechnology, medical diagnostics and forensic analysis, and second, to provide the chemical basis for the carcinogenicity of environmental pollutants and toxic chemicals and to serve as analogues in the study of protein–nucleic acid recognition, which may be useful in designing new and promising anticancer drugs for clinical use.

In the research on dye–DNA interactions, the technique of using fluorescent dyes as probes was proposed as a common means to evaluate the feasibility of preparing proper staining dyes for low level analysis and to establish the basis for selecting new structural types of DNA intercalators through investigations of the interaction between dyes and DNA, which contributes considerably to the design of more efficient drugs for clinical use. Specifically, non-covalent labeling of fluorescent dyes was thought to be more favorable owing to its simple experimental procedure. Generally, there are two kinds of non-covalent labeling dyes that demonstrate fluorescence changes on interaction with nucleic acids. The first type, showing fluorescence enhancement, includes the classical mono-intercalating dyes such as ethidium bromide (EB),[1] Thiazole Orange, Oxazole yellow[2] and the dimeric dyes.[3–5] Ethidium bromide shows a fluorescence enhancement of about 20-fold upon interaction with DNA, but it is harmful to health. The dimeric dyes often consist of two chromophores, which show intercalative binding as the monomer linked by a cationic chain, such as Thiazole Orange homodimer (TOTO) and Oxazole Yellow homodimer (YOYO), and they typically show larger binding constants to DNA than their monomeric counterparts. However, the high cost of the dimeric dyes restricts their application to a large scale. Recently, new dyes that display enhancement of their fluorescence after interaction with DNA have been exploited, such as hypocredin, which interacts with DNA in a non-intercalative way.[6] The second type of dyes, that show a fluorescence decrease on interaction with DNA, contain a tricyclic heteroaromatic chromophore, such as Methylene Blue,[7] safranine T[8] and 9,10-anthraquinone-2-sulfonate.[9] Except for Methylene Blue and hypocredin, the aforementioned dyes possess fluorescence excitation and emission in the visible region (400–600 nm), which is subject to the interferences resulting from fluorescence of impurities and scattered light by the matrix. Hence, developing red-region or near-infrared dyes that demonstrate a considerable improvement in binding strength with DNA, decreasing the interference from the background and increasing the variations of fluorescence emission for the detection of low levels of DNA, has been a recent concern.

In this paper, the application of a red-region dye, Nile Blue (NB), as a fluorescence probe for the study of nucleic acid–dye binding for both quantitative and qualitative purposes is proposed. Nile Blue is a cationic phenoxazine dye with heterocyclic, planar and rigid structure. It has been used as an acid–base indicator and as a stain for actinomycin in pathological tissues. The planar hydrophobic phenoxazine moiety of NB is expected to facilitate the intercalation of NB into the relatively non-planar interior of the DNA helix. Compared with other DNA intercalators such as EB, TOTO and YOYO, using NB as a red-region nucleic probe has the merits of little toxcity, low cost and comparable sensitivity for DNA quantification. In this work, significant fluorescence quenching was observed when nucleic acids including calf thymus (CT) DNA and yeast RNA were added. The value of the fluorescence quenching was linear over a wide concentration range of nucleic acids, hence, nucleic acids can be determined down to the ng ml^{-1} level. The interaction between NB and DNA was also examined. The results showed that NB intercalated the helix of DNA and bound to DNA base pairs with high affinity. The results of investiga-

tion on the interaction between NB and DNA may be useful for understanding the mechanism of bioactivity of phenoxazine derivatives on the molecular level.

Experimental

Apparatus

Fluorescence intensities were measured with a Hitachi (Tokyo, Japan) Model 650 10s spectrofluorimeter with a quartz cuvette (1 × 1 cm cross-section). Absorption spectra were obtained on a Beckman (Fullerton, CA, USA) DU-7 UV/VIS spectrophotometer. A DYY-III 8B horizontal slab gel electrophoresis apparatus (Beijing Liuyi Instrument Factory, Beijing, China) was used. The working voltage was set at 60 V and the lengths of the slab and the gel were 14 and 6 cm, respectively. The running gel for electrophoresis was 0.8% Sepharose.

Reagents and solutions

Commercially available CT DNA and yeast RNA (Sigma, St. Louis, MO, USA) were used without further purification and were directly dissolved in water to give a final concentration of 100 μg mL^{-1} and stored refrigerated. A golden staphylococcus DNA sample (SA DNA, 2.2 absorbance) was kindly offered by the Cancer Research Center of Xiamen University (Xiamen, China). Its concentration was calculated to be 110 μg mL^{-1} by measuring its absorbance at 260 nm. A stock standard solution of NB was prepared by dissolving it in 95% ethanol to a final concentration of 1.0×10^{-3} mol L^{-1}. A hexamethylenetetramine (HT)–HCl buffer solution containing 0.1 mol L^{-1} HT was made by dissolving 3.50 g HT in water, adjusting the pH to 6.3 with dilute hydrochloric acid and then diluting to 250 ml. The buffer for electrophoresis was TRIS–boric acid–EDTA (TBE) which contained 0.045 mol L^{-1} TRIS–boric acid and 0.001 mol L^{-1} EDTA.

All chemicals were of analytical reagent grade or better and doubly distilled water was used throughout.

Procedures

A volume of 1.0 mL of HT–HCl buffer solution, various amounts of DNA and 1.0 mL of NB (1.0×10^{-5} mol L^{-1}) were placed in a 10 mL calibrated tube, then diluted with water to the mark and mixed. Fluorescence measurements for both the reagent blank (F_0) and the mixed solutions (F) were made at 673 nm with excitation at 627 nm after the mixture had stood at room temperature for 5 min. The fluorescence quenching [taken as the value of ($F_0 - F$)] was obtained.

For sample analysis, a 0.02 mL of SA DNA sample was used directly for analysis.

Results and discussion

Spectral characteristics

The solution of free NB displayed an excitation maximum at 627 nm and an emission maximum at 672 nm. As shown in Fig. 1, the excitation and emission maxima of NB decreased in the presence of CT DNA. Furthermore, the excitation wavelength red-shifted slightly (630 nm), whereas the emission maximum remained unchanged.

Yeast RNA was also found to quench the fluorescence of NB. The spectral changes obtained were similar to those for CT DNA.

Optimization of general procedure

The effects of apparent pH and different buffers on the quenching of NB by DNA were studied. The pH was studied in the range 5.0–9.0 and the results indicated that the optimum pH was around 6.3. Of the four kinds of buffer solutions tested, that is, TRIS–HCl, HT–HCl, Na_2HPO_4–NaH_2PO_4 and KH_2PO_4–NaOH, HT–HCl buffer solution provided the maximum quenching. As the ionic strength may affect the interaction of NB with nucleic acids, the optimum amount of HT–HCl buffer solution was also studied. The results showed that the optimum amount of HT–HCl lay in the range 0.80–1.0 mL. Therefore, 1.0 mL of 0.1 mol L^{-1} HT–HCl buffer solution was used throughout.

The influence of NB concentration was investigated with a constant concentration of DNA at pH 6.3. The results are shown in Fig. 2. It was found that the extent of fluorescence quenching increased with increase in NB concentration and the maximum fluorescence quenching was reached when NB was in the concentration range 8.0×10^{-7}–3.0×10^{-6} mol L^{-1}. However, it was also found higher NB concentrations led to poorer detection limits and a higher upper limit of the calibration graph for the determination of nucleic acids. Taking the sensitivity and the linear range together into account, therefore, a final concentration of 1.0×10^{-6} mol L^{-1} NB was chosen.

The effect of the sequence of addition of reagents on the fluorescence quenching was studied and the order of buffer, DNA and NB was found to be suitable.

Further experiments showed that the interaction of NB with DNA resulted in a constant and maximum fluorescence

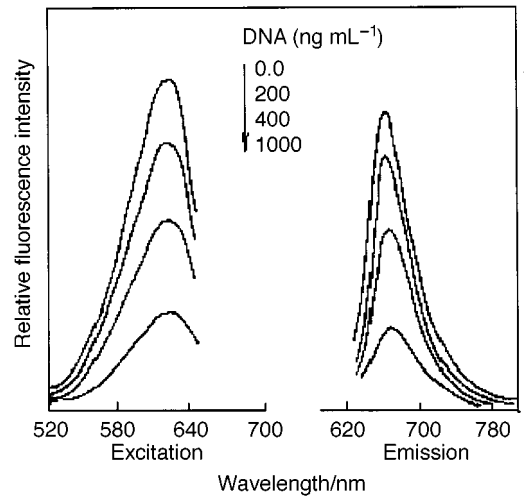

Fig. 1 Fluorescence spectra of NB in the presence of different amounts of DNA. NB, 1.0×10^{-6} mol L^{-1}.

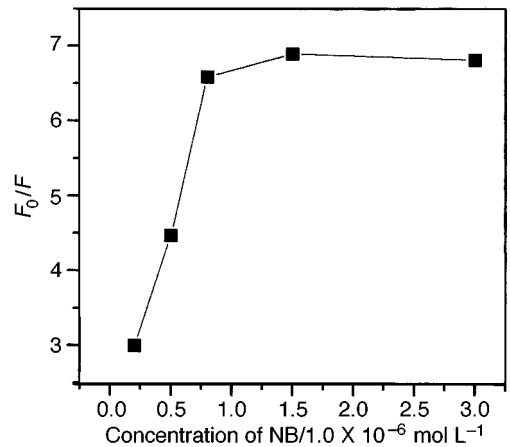

Fig. 2 Effect of Nile Blue concentration. DNA, 100 ng mL^{-1}.

quenching when the mixture was allowed to incubate at room temperature for 3–20 min.

Study of interaction of NB with DNA

Small molecules are bound to DNA by three binding modes: electrostatic binding, groove binding and intercalative binding.[10] Small molecules have selectivity to some extent when binding with DNA except by way of electrostatic binding. As is well known, the steric structures of small molecules and DNA determine the binding properties (such as affinity and binding site) and the electronic state of small molecules. In general, planarity was suggested to be one of the important features needed for efficient intercalation.[11] The composition of unfused aromatic rings with terminal basic functions was favorable for groove binding.[12] For example, the cationic nature of cyanine dyes with a long, flexible polymethine chain linking the aromatic group would favor a groove binding process as the dominant interaction mode. Electrostatic interactions, on the other hand, were usually manifested by those cationic dyes with large bulky groups which would sterically disfavor binding *via* intercalation or with major or minor grooves.[13]

In order to understand the mechanism of the interaction between NB and DNA, the following experiments were performed.

Absorption spectra study. The electronic absorption spectra of NB in the presence of DNA showed that the absorption peak decreased with increasing amount of DNA (hypochromic effect) and there was a small red shift with isosbestic points at 590 and 675 nm (Fig. 3). It was thought that the spectral red shift, the hypochromic effect and the occurrence of isosbestic points were due to the strong interaction between the electronic states of the intercalating chromophore and that of the DNA base pairs.[14]

The absorption of the base pairs of DNA at 260 nm also showed obvious hypochromicity as the concentration of NB increased (Fig. 3). It was considered that the stability of the DNA helix was strengthened in the presence of the intercalcators, and consequently the absorption of DNA bases at 260 nm would decrease.

Considering the characteristics of the absorption spectra and the fluorescence spectra mentioned above, it was suggested that NB intercalates into the helix of DNA.

Fluorescence polarization study. Fluorescence polarization measurements were performed on steady state dye–DNA solutions. The value of fluorescence polarization (p) was calculated from the equation

$$p = (I_{vv} - GI_{vH})(I_{vv} + GI_{vH})^{-1} \quad (1)$$

where I_{vv} and I_{vH} are the vertical and horizontal fluorescence emission, respectively, when the excitation polarizer was set vertically and G is the ratio of detection sensitivity between I_{vv} and I_{VH}, that is, I_{vv}/I_{vH}. When the fluorescence probe was bound to DNA and formed a stable complex with the large DNA biopolymer, the rotational diffusion of the fluorophore would be reduced, *i.e.*, its molecular rotation slowed and led to an increase in fluorescence polarization. Polarization titration of NB by increasing amounts of natural and denatured DNA was carried out. As shown in Fig. 4, an obvious enhancement of polarization was observed in the natural DNA–dye system, whereas only a slight increase was obtained with the denatured DNA–dye system. Since mere binding to the phosphate backbone or to the DNA grooves does not cause enhanced fluorescence polarization,[15] the large increase in fluorescence polarization on binding NB to CT DNA supported the intercalation of NB into the helix.

Effect of salt. Owing to the positive characteristics of NB and the negative polyphosphate skeleton of DNA, the electrostatic interaction between NB and DNA cannot be excluded. It was considered that the electrostatic interaction would be weakened by the addition of a counter ion because of the conversion of the electrostatic atmosphere of the DNA periphery. In our experiment, the salt effect was studied by adding a strong electrolyte, sodium chloride, to the free NB and NB–DNA solutions. The results indicated that adding NaCl had little effect on the fluorescence of the NB and NB–DNA solutions when the NaCl concentration was below 5.0×10^{-3} mol L^{-1}. When the concentration of NaCl exceeded 5.0×10^{-3} mol L^{-1}, the fluorescence of NB and the NB–DNA complex increased as the concentration of NaCl increased, but the extent of quenching

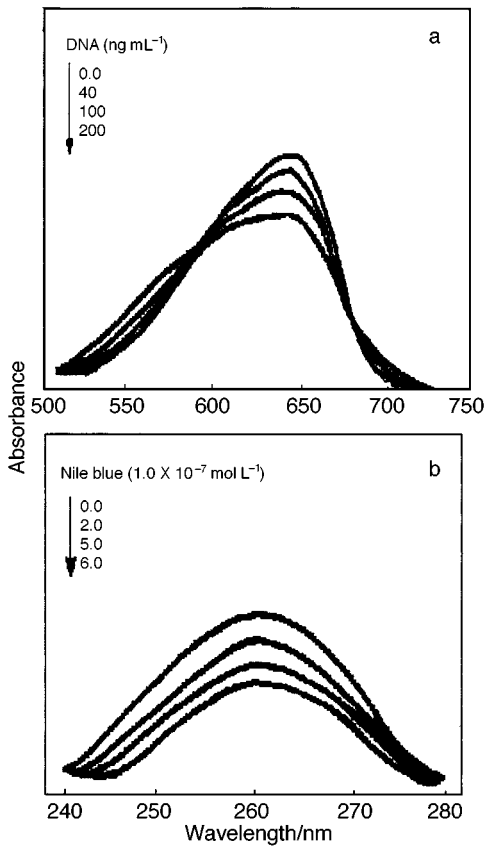

Fig. 3 Absorption spectra of (a) NB in the presence of different amounts of DNA and (b) DNA in the presence of different amounts of NB. (a) NB, 1.0×10^{-6} mol L^{-1}; (b) DNA, 200 ng mL^{-1}.

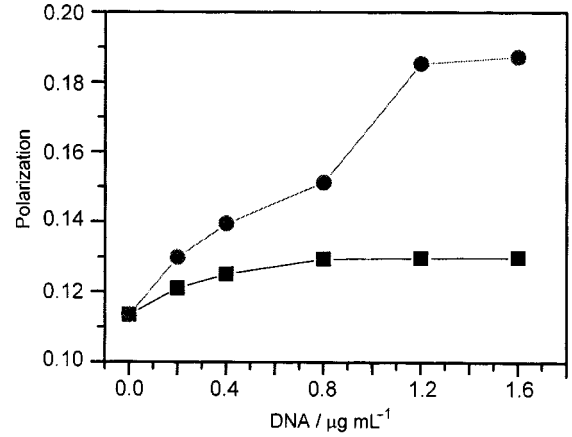

Fig. 4 Polarization titration of NB by natural and denatured DNA. NB, 1.0×10^{-6} mol L^{-1}.

($F_0 - F$) decreased and finally approached a constant value (Fig. 5). The possible reasons were that the increasing amount of Na^+ weakened the electrostatic repulsion of DNA and resulted in a tighter DNA, which was unfavorable for the intercalation of NB into DNA. Hence the fluorescence quenching decreased since there were more free dye molecules outside the helix of DNA. On the other hand, it was obvious that intercalation would not occur if NB could not be attracted to the periphery of DNA. Since the presence of external cationic ions such as Na^+ would compete with NB for the electrostatic interaction with DNA, the intercalation of NB into DNA would therefore surely be weakened. However, we found that the salt effect could not inhibit the binding of NB with DNA completely since the fluorescence intensity of the NB–DNA system at higher concentrations of NaCl was still smaller than that of NB.

The observed fluorescence enhancement of the NB–DNA system in the presence of high NaCl concentrations is consistent with intercalation[16–18] rather than groove binding, and these observations also supported the suggestion that binding of NB to DNA is predominantly controlled by intercalative interactions.

Effect of natural and denatured DNA on quenching. Comparison between the effects of the natural and denatured DNA on the fluorescence of NB was also investigated. The results are given in Table 1. Both natural and denatured DNA were capable of quenching the fluorescence of NB, but in the case of denatured DNA the fluorescence quenching was weakened owing to the partial raveling of the helix. This result was consistent with the polarization curve of denatured DNA shown in Fig. 4, so this phenomenon also supported the suggestion that NB intercalated into the interior of the double helix DNA.

Effect of temperature. The influence of temperature on the fluorescence quenching was considered to be further evidence for the static quenching mode of NB by DNA. Fig. 6 shows the fluorescence titration of NB by DNA at different incubation temperatures. The results indicate that the extent of quenching diminished as the incubation temperature was raised. This may imply that the quenching follows the static quenching mode, that is, the formation of a static complex occurs.

For static quenching, the modified Stern–Volmer equation is[19]

$$F_0/F = 1 + K_q[Q] \quad (2)$$

where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively, and K_q is the formation constant of the quenching complex. The quenching constants (the binding constants of NB with DNA) were calculated to be 4.53×10^6, 3.24×10^6 and 2.13×10^6 L mol^{-1} for the incubation temperatures of 5, 20 and 40 °C, respectively. The large binding constant for NB indicated that the phenoxazine chromophore had a high affinity for DNA base pairs.

Scatchard plot study. In order to evaluate the possibility of using NB as a potential alternative DNA intercalator, a comparison of the binding constants between some common DNA intercalators and NB was made. The binding constant and binding site size were evaluated by the Scatchard equation:

$$r/c_f = k(n - r) \quad (3)$$

where k is the binding constant, n is the binding site size, $r = c_b/[\text{DNA}]$ and c_f and c_b are the concentrations of bound and free dye, respectively. A plot of r/c_f *vs.* r at room temperature is shown in Fig. 7. A binding constant of 1.96×10^6 L mol^{-1} and a binding site size of 2.8 base pairs were obtained. It can be seen that the binding constants obtained by two methods (Stern–Volmer equation and Scatchard plot) were of the same magnitude, and comparable to that of ethidium bromide (2.6×10^6 L mol^{-1}). A comparison of the binding characteristics for some typical DNA intercalators is given in Table 2.

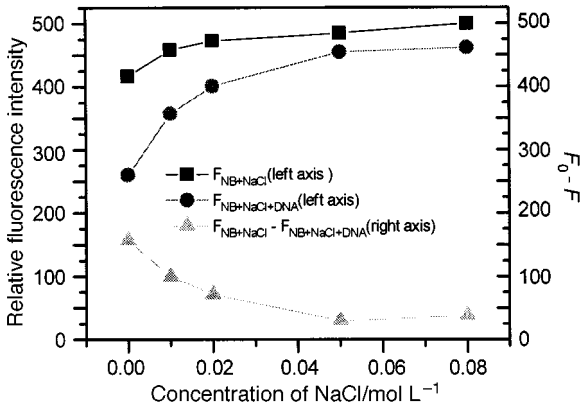

Fig. 5 Effect of NaCl on fluorescence quenching of NB. NB, 1.0×10^{-6} mol L^{-1}; DNA, 100 ng mL^{-1}.

Table 1 Comparison of the effect of natural and denatured DNA on the relative fluorescence intensity of NB (1.0×10^{-6}mol L^{-1})

Concentration of DNA/μg mL^{-1}	Fluorescence in natural DNA system	Fluorescence in denatured DNA system
0	343	343
0.2	156	179
0.4	86	164
0.6	83	108

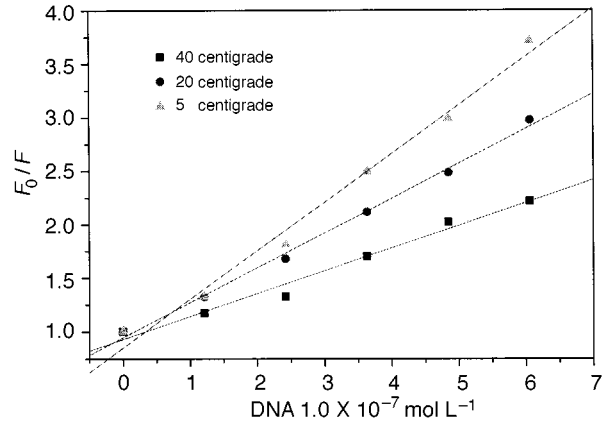

Fig. 6 Stern–Volmer plot of fluorescence quenching of NB by DNA at different incubation temperatures. NB, 1.0×10^{-6} mol L^{-1}.

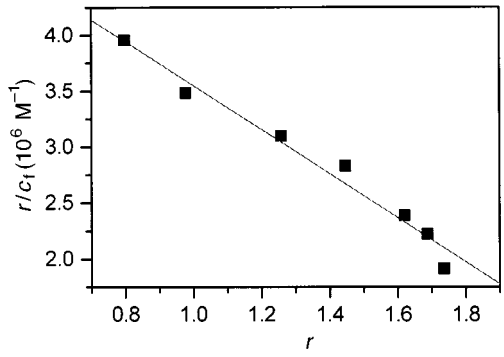

Fig. 7 Scatchard plot of the fluorescence quenching of NB by DNA. NB, 1.0×10^{-6} mol L^{-1}.

Electrophoresis experiment. The intercalation of NB into DNA was further confirmed by an electrophoresis study. For comparison, three sets of DNAs, *i.e.*, DNA, NB + DNA and EB + DNA, were electrophoresed in a slab gel containing 0.8% Sepharose and TBE. The resulting electropherograms are shown in Fig. 8. It can be seen that there were perceivable differences in the apparent mobility of NB–DNA, EB–DNA and DNA, with the mobility decreasing in the order DNA > EB–DNA > NB–DNA. Since the electrophoresis was run using the same slab gel with the same field strength, the decrease in the mobility of NB–DNA may result from two factors: the less negative charge that the DNA–NB complex possessed and the higher molecular weight of NB–DNA. As the binding force of the dye with DNA in a non-intercalative way was too small to counteract the electrical force, the apparent mobility of dye–DNA in a non-intercalative way may be the same as that of free DNA. In our experiment, it was observed that the mobility of the complex of DNA and EB, a well known DNA intercalator, was smaller than that of free DNA, and the mobility of the NB–DNA complex was even smaller than that of EB–DNA. Therefore, it could be inferred that NB intercalated into the helix of DNA. This result further confirmed the proposition that NB intercalated into DNA and had a high binding affinity with DNA.

Study of fluorescence quenching of NB by DNA

Further investigations were made of the mechanism of fluorescence quenching of NB in the presence of DNA. As mentioned above, a spectral red shift, a hypochromic effect and the occurrence of isosbestic points were observed for the absorption spectra of NB in the presence of DNA. However, a broader absorption peak of NB was observed at higher concentrations of DNA (data not shown). Further addition of DNA caused a split of the absorption peak of NB, with a main absorption peak at 675 nm and a shoulder peak at 590 nm. When even more DNA was added, the absorption at the two peaks first increased, then the shoulder peak at 590 nm gradually disappeared and a single peak at 680 nm arose. Also, as can be seen in Fig. 9, the curve for fluorescence titration of NB with DNA had a U shape, which indicated restoration of the fluorescence of NB at higher DNA concentrations. The trend of the fluorescence change of NB was consistent with the change of absorption spectra at different DNA concentrations. Based on these two phenomena, it can be inferred that the fluorescence quenching of NB may be due to the association of NB within the helix of DNA since NB molecules were in closer proximity after intercalation. However, with a further increase in DNA, more DNAs would compete with each other for binding with NB, which meant that NB would be redistributed between DNA molecules. In this case, the chance of NB–NB association was weakened and consequently contributed to the restoration of the fluorescence of NB. Further studies of the mechanism of fluorescence quenching of NB by DNA are in progress.

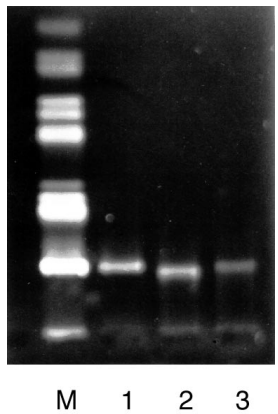

Fig. 8 Electropherogram for (1) DNA, (2) NB–DNA and (3) EB–DNA obtained by horizontal slab gel electrophoresis. DNA, 1600 ng; NB, 3.67 μg; EB, 2.0 μg; and M, λDNA/*Eco*RI + *Hin*dIII marker.

Calibration graphs and limits of detection

The calibration graphs for the determination of nucleic acids were constructed under the optimum conditions. There was good linearity between the fluorescence quenching and the concentration of nucleic acids over a wide range. Analytical characteristics for the determination of CT DNA and RNA are given in Table 3. The limit of detection (LOD) was given by the equation LOD = Ks_0/S, where K is a numerical factor chosen according to the confidence level desired, s_0 is the standard

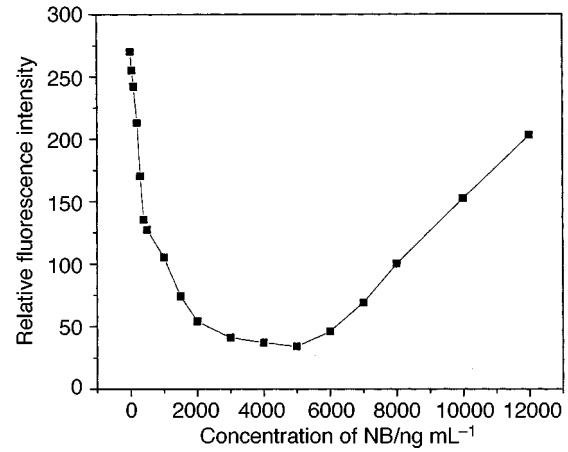

Fig. 9 Fluorescence titration of NB with DNA. NB, 1.0×10^{-6} mol L^{-1}.

Table 3 Analytical features for nucleic acid determinations

Nucleic acid	Linear range/ ng mL^{-1}	Detection limit/ ng mL^{-1}	Correlation coefficient
CT DNA	3.0–400	3.0	0.998
	400–2000		0.999
Yeast RNA	27.0–400	27	0.994
	400–10000		0.999

Table 2 Binding characteristics for some typical DNA intercalators

Intercalator	Conditions	Binding constant, K/L mol^{-1}	Binding site size base pairs	Ref.
Ethidium bromide	pH 7.5 phosphate buffer and 5 mM Na_2SO_4	2.6×10^6	2	20
Acridine Orange	pH 7.5 phosphate buffer and 5 mM Na_2SO_4	4×10^5	2	20
6-Chloro-2-methoxy-9-acridine	pH 6.50 50 mM NaCl in water–DMSO	6.6×10^4	2.2	21
(9-Anthrylmethyl)ammonium chloride	5 mM TRIS–HCl buffer	7.8×10^4	4.8	15
Safranine T	50 mM TRIS–HCl buffer, 0.01 M, pH 6.3	6.1×10^4	6.7	22
Nile Blue	Hexamethylenetetramine–HCl buffer	2.0×10^6	2.8	—

Table 4 Comparison of methods for the determination of DNA

Method	λ_{ex}/nm	λ_{em}/nm	Incubation time/min	LOD/ ng mL^{-1}	Linear range/ μg mL^{-1}	Mode of fluorescence measurement
Hypocredin[6]	470	600	5	5	0.0–0.2	Enhancement
Methlylene Blue[7]	297	672	5	82	0.0–40	Quenching
TOTO[3]	488	538	20–30	0.5	0.0005–0.1	Enhancement
YOYO[3]	470	510	20–30	0.5	0.0005–0.1	Enhancement
This method	630	673	5	3	0.003–2.0	Quenching

Table 5 Interference of foreign substances with the determination of DNA (50 ng mL^{-1})

Foreign substance	Maximum concentration	Relative error caused (%)
BSA	10 μg mL^{-1}	−3.4
Thymine	80 μg mL^{-1}	−1.8
Cytosine	80 μg mL^{-1}	−3.2
Guanine	0.3 μg mL^{-1}	0.45
Adenine	80 μg mL^{-1}	4.6
IgG	0.2 μg mL^{-1}	−8.1
PO_4^{3-}	1.0×10^{-4} mol L^{-1}	−6.3
Al^{3+}	5.0×10^{-8} mol L^{-1}	−2
Ca^{2+}	1.0×10^{-6} mol L^{-1}	−3
Mg^{2+}	5.0×10^{-7} mol L^{-1}	−3.9
Cu^{2+}	5.0×10^{-7} mol L^{-1}	0.02

Table 6 Determination of DNA in golden staphylococcus DNA sample

DNA level[a]/ ng mL^{-1}	DNA added/ ng mL^{-1}	DNA found[b]/ ng mL^{-1}	Recovery (%)
220	0	225.1	—
	20	244.2	96
	50	277.5	105
	100	323.7	99

[a] Concentration of DNA after the original sample (110 μg mL^{-1}) had been diluted 500-fold. [b] Mean of three determinations.

deviation of the blank measurements (n = 11, k = 3) and S is the slope of the calibration curve. The relative standard deviation for 0.1 μg mL^{-1} CT DNA was 2.0%. A comparison of methods for the determination of DNA is given in Table 4.

Interferences

Some substances of interest which may interfere with the determination of 50 ng mL^{-1} DNA were examined. The results are given in Table 5.

Determination of DNA real sample

DNA was determined in golden staphylococcus and the results are given in Table 6. A mean recovery of 100 ± 5% was obtained.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (No. 29775021).

References

1 J. B. Le Pecq and C. Paoletti, *Anal. Chem.*, 1966, **17**, 100.
2 H. S. Rye, M. A. Quesada, K. Peck, R. A. Mathies and A. N. Glazer, *Nucleic Acids Res.*, 1990, **19**, 327.
3 H. S. Rye, J. N. Dabora, M. A. Quesada, R. A. Matheis and A. N. Glazer, *Anal. Biochem.*, 1993, **208**, 144.
4 B. Gangain, J. Barbet, N. Capelle, B. P. Roques and J. B. Lepecq, *Biochemistry*, 1978, **17**, 5078.
5 H. S. Rye, S. Yue, D. E. Wemmer, M. A. Quesada, R. P. Hangland, R. A. Mathies and A. N. Glazer, *Nucleic Acids Res.*, 1993, **20**, 2801.
6 Q. Z. Zhu, F. Li, X. Q. Guo, J. G. Xu and W. Y. Li, *Analyst*, 1997, **122**, 937.
7 W. Y. Li, J. G. Xu, X. Q. Guo, Q. Z. Zhu and Y. B. Zhao, *Anal. Lett.*, 1997, **30**, 245.
8 X. Q. Guo, F. Li and Y. B. Zhao, *Anal. Lett.*, 1998, **31**, 991.
9 W. Y. Li, X. Q. Guo, J. G. Xu, Q. Z. Zhu and Y. B. Zhao, *Anal. Chim. Acta.*, 1997, **340**, 291.
10 Y. Chen, Y. Z. Li, W. B. Chang and Y. X. Ci, *Fenxi Kexue*, 1994, **10**, 67.
11 L. S. Lermem, *J. Mol. Biol.*, 1961, **3**, 18.
12 W. D. Wilson, F. A. Tanious, H. J. Barton, L. Strekomaski and D. W. Boykin, *J. Am. Chem. Soc.*, 1989, **111**, 5008.
13 Y. Y. Davidson, B. M. Gunn and S. A. Soper, *Appl. Spectrosc.*, 1996, **50**, 211.
14 L. J. Jiang, *Chin. Sci. Bull.*, 1990, **35**, 1681.
15 C. K. Kumar and E. H. Asuncion, *J. Am. Chem., Soc.*, 1993, **115**, 8457.
16 M. A. Sari, J. P. Battioni, D. Dupre, D. Mansuy and J. B. Le Pecq, *Biochemistry*, 1990, **29**, 4205.
17 J. B. Le Pecq and C. Paoletti, *J. Mol. Biol.*, 1967, **27**, 87.
18 R. F. Pasternack, R. A. Brigandi, M. J. Abrams, A. P. Williams and E. J. Gibbs, *Inorg. Chem.*, 1990, **29**, 4483.
19 G. Z. Chen, X. Z. Huang, J. G. Xu, Z. Z. Zheng, Z. B. Wang, *The Analytical Method of Fluorescence*, Science Publisher, Beijing, 1990, p. 118 (in Chinese).
20 A. M. Brun and A. Harriman, *J. Am. Chem. Soc.*, 1992, **114**, 3656.
21 M. Asakawa, K. Endo, H. Tol and Y. Aoyama, *Bull. Chem. Soc. Jpn.*, 1992, **65**, 2050.
22 Y. Cao and X. W. He, *Spectrochim. Acta, Part A*, 1998, **54**, 883.

Paper 9/01174I