FEBS Letters 368 (1995) 64–68 FEBS 15672

Fluorescence-detected interactions of oligonucleotides in RecA complexes

Pernilla Wittung[a,*], Mario Funk[b], Bengt Jernström[b], Bengt Nordén[a], Masayuki Takahashi[c]

[a]*Department of Physical Chemistry, Chalmers University of Technology, S-41296 Gothenburg, Sweden*
[b]*Department of Toxicology, Karolinska Insitutet, Box 60400, S-10401 Stockholm, Sweden*
[c]*Groupe d'Etude Mutagénèse et Cancérogenèsé, URA 1342 CNRS, Institut Curie, Bat. 110, Universite Paris Sud, F-91405 Orsay, France*

Received 21 April 1994; revised version received 18 May 1995

Abstract A technique has been developed to probe directly RecA-DNA interactions by the use of the fluorescent chromophore, (+)anti-benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), covalently attached to DNA. The 24-mer oligonucleotide 5'-d(CTACTAAACATGTACAAATCATCC) was specifically modified on the exocyclic nitrogen of the central guanine, to yield a trans-adduct. Upon interaction of the modified oligonucleotide with RecA we find an increase in BPDE fluorescence and a rather high fluorescence anisotropy, suggesting a restricted motion of the BPDE-oligonucleotide in the protein filament. In the presence of the cofactor ATPγS, binding of two oligonuclotides, identical or complementary in sequence, in the RecA filament is possible. The RecA-DNA complex is, however, more stable when the sequences are complementary; in addition, a shift in the BPDE emission peaks is observed. In the presence of ATP (and an ATP regeneration system), the RecA-DNA interaction between two complementary oligonucleotides is changed, and we now find protein-mediated renaturation to occur.

Key words: RecA; Recombination; Benzo(a)pyrenediolepoxide; Renaturation; Fluorescence

1. Introduction

RecA is a key component of general genetic recombination in *Escherichia coli* [1–3], and related proteins with similar functions are found in a variety of organisms [3,4]. Genetic recombination consists of strand exchange between two homologous DNA molecules, and the reaction facilitates postreplicative DNA repair and affects the organisation of the genome. Purified RecA in vitro mimics the strand exchange reaction in the presence of cofactor ATP [5,6] and has been extensively studied in order to understand the mechanism of the homologous recombination reaction (for reviews, see [3,7–9]). The physical nature of the DNA binding sites in the RecA fiber and the mechansims for these protein-mediated DNA reactions, are still, however, mostly unknown.

We have earlier investigated RecA-DNA interactions by using a fluorescent marker, BPDE (Scheme 1), covalently attached to the N^6 position [10] of adenine in poly(dA) homopolymers [11]. The BPDE chromophore is sensitive to the surrounding medium and can be used to probe the environment of the DNA binding positions in the RecA filament and interaction between DNA strands [11]. Previous experiments, in the presence of the non-hydrolysable ATP analog ATPγS, showed that interactions could occur between all of the DNA strands bound in the three DNA binding sites in the RecA filament [11]. By contrast, in presence of hydrolysing ATP, RecA promoted renaturation between complementary DNA strands within the filament [12]. To verify these conclusions also for RecA interactions with normal DNA of any sequence, we have here studied a 24-mer oligonucleotide of a mixed sequence, modified by BPDE at the N^2 position of the central guanine (Scheme 2). It has been reported [13] that RecA can promote pairing of oligonucleotides as short as 18 bases in length. By measuring the fluorescence characteristics of the BPDE chromophore, we investigate the RecA-DNA interactions in the presence of complementary or identical oligonucleotides, and also the effects of different cofactors.

2. Materials and methods

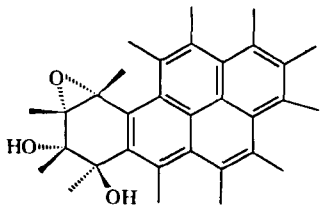
RecA was purified by HPLC (DEAE 5PW, Tosoh) as a final step, as described elsewhere [14]. The RecA concentration was determined spectrophotometrically using $\varepsilon_{280} = 2.17 \times 10^4\ M^{-1}cm^{-1}$ [15]. ATP (special quality), ATPγS, creatine phosphate and creatine kinase (from rabbit muscle) were obtained from Boehringer Mannheim. BPDE was purchased from Chemsyn Science Laboratories (Kansas, USA). Oligonucleotides were purchased from Innovàgen, Lund, Sweden. An oligonucleotide with the sequence 5'-d(CTACTAAACATGTACAAATCATCC) was chemically modified with BPDE at the N^2 position of the central guanosine and subsequently purified and characterized as described in [16]. Unmodified oligonucleotides with identical, 5'-d(CTACTAAACATGTACAAATCATCC), and complementary, 5'- d(GGATGATTTGTACATGTTTAGTAG), sequences were also used. The concentrations were determined spectrophotometrically using $\varepsilon_{260} = 274100\ M^{-1}$ for the unmodified and BPDE-modified oligonucleotide and $\varepsilon_{260} = 271400\ M^{-1}$ for the complementary oligonucleotide. Also three 18-mer oligonucleotides with the sequences 5'-d(CCATAACTACATACAACC), 5'-d(GGTTGTATGTAGTTATGG) and 5'-d(CATGGAGCAGGTCGCGGA) were studied. The sequence 5'-d(CCATAACTACATACAACC) was chemically modified with BPDE as decribed in [11]. The RecA complexes were formed in a buffer containing 5 mM cacodylate and 20 mM NaCl, pH 6.5, 1 mM $MgCl_2$, and 80 μM ATPγS. In some experiments 900 μM ATP and an ATP regeneration system containing 6 mM creatine phosphate and 60 U/ml of creatine kinase were used instead of ATPγS.

The RecA complexes with oligonucleotides, in presence of ATPγS, were formed assuming a general stoichiometry of 3 bases per protein monomer for each strand accommodated in the RecA fiber [17–21]. For this reason, 3 μM RecA was mixed with 9 μM BPDE-modified oligonucleotide and incubated for 0.5 h at 22°C in the dark. The complex with two oligonucleotides was formed by addition of 9 μM of an identical unmodified oligonucleotide or the complementary sequence to the preformed RecA:BPDE-oligonucleotide complex followed by further incubation. In another set of experiments, RecA was incubated with unmodified oligonucleotides, either of identical or complementary sequences, for 0.5 h at room temperature, followed by addition of the BPDE-oligonucleotide and further incubation. Formation of a duplex (without RecA) between the BPDE-oligonucleotide and its complementry sequence was performed by mixing stoichiometric amounts, and

*Corresponding author. Fax: (46) (31) 772 3858.

Abbreviations: ATPγS, adenosine5'-O-3-thiotriphosphate; BPDE, (+)anti-benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide; FA, fluorescence anisotropy.

SSDI 0014-5793(95)00600-1



Scheme 1

subsequent incubation for 1 h at 22°C. Studies in the presence of ATP (and an ATP regeneration system) were performed by adding 9 μM BPDE-modified oligonucleotide to 3 μM RecA and incubating for 0.5 h. Then 9 μM of the complementary sequence, or the unmodified identical oligonucleotide, was added to the mixture, and fluorescence monitored in short time intervals for 2 h.

Fluorescence emission measurements were performed on an Aminco SPF-500 Quantum Corrected spectrofluorimeter. The emission was measured from 360 nm to 460 nm with 4 nm spectral resolution upon excitation at 353 nm (4 nm band-pass), at which wavelength BPDE is efficiently excited when complexed to the oligonucleotide. Two vibrational peaks at 383 nm and 404 nm are observed. All measurements were performed at 22°C, in a 1 cm × 1 cm quartz cell. For fluorescence anisotropy measurements the spectrofluorimeter was equipped with two polaroid films, one in the excitation and the other in the emission channel. The fluorescence intensities were measured with polarizers set either vertically (v) or horizontally (h) in the excitation and emission beams. The emission was measured at 383 nm, and the excitation wavelength was 353 nm. The degree of anisotropy was calculated from the measured intensities as:

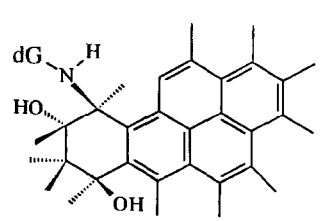
$$r = \frac{I_{vv} - I_{vh}G}{I_{vv} + 2I_{vh}G}$$

where $G = I_{hv}/I_{hh}$ is used for instrumental correction [22]. The first index refers to the setting of the excitation polarizer and the second to that of the emission polarizer. Control experiments were performed to exclude artifacts from light scattering. Compared to the relatively strongly fluorescing pyrenyl chromophore, background fluorescence was negligible when exciting above 350 nm. Bleaching of BPDE by the excitation light was also checked as a possible source of error (repeated scans reproduced the emission spectrum).

3. Results

3.1. RecA binding of 24-mer BPDE-oligonucleotide in presence of ATPγS

As shown in Fig. 1, the fluorescence emission of BPDE increases markedly upon RecA binding of the oligonucleotide. In addition, the fluorescence anisotropy, which is nearly zero for BPDE when in the free oligonucleotide, rises to nearly 0.2 upon RecA binding, as shown in Table 1. Directly after binding of RecA some decrease (15–20%) of the initially very high BPDE emission intensity was observed, reaching equilibrium after about 30 min (not shown). Such an effect was not found upon RecA binding of BPDE-modified polynucleotide, poly(dA), previously studied [11].



Scheme 2

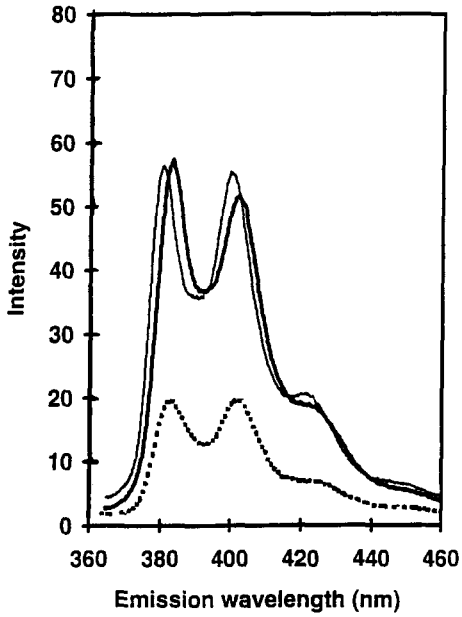


Fig. 1. Fluorescence emission spectra of the BPDE chromophore upon excitation at 353 nm, in the following complexes: BPDE-oligonucleotide (dotted line), RecA:BPDE-oligonucleotide (thin line), RecA:BPDE-oligonucleotide:complementary oligonucleotide (bold line), and RecA:BPDE-oligonucleotide:identical oligonucleotide (coinciding with thin line spectrum). All in presence of ATPγS as cofactor.

3.2. RecA binding of second oligonucleotide in presence of ATPγS

The binding of a second oligonucleotide (either complementary or identical to the first one) to a preformed RecA-oligonucleotide complex, in presence of ATPγS, was probed by FA measurements, with the BPDE-oligonucleotide added as the second strand. Also in this case the FA increased substantially upon the RecA interaction (Table 1). Furthermore, the fluorescence intensity of the BPDE-oligonucleotide increased upon addition to the preformed RecA:(unmodified oligonucleotide) complex (data not shown).

The FA of the BPDE chromophore was found to decrease faster with time when the BPDE-oligonucleotide was added to a RecA:(identical oligonucleotide) complex, compared to addition to a RecA:(complementary oligonucleotide) complex (Table 1). The decrease in FA also correlates with a decrease in the BPDE fluorescence intensity.

Experiments were also performed in which a complementary or an identical oligonucleotide was added to a RecA:BPDE-oligonucleotide complex. Upon binding of the complementary oligonucleotide, a shift of about 3 nm to longer wavelengths in the BPDE emission peaks was observed (Fig. 1). No shift was observed upon the addition of an identical oligonucleotide. In both cases, no alteration of FA was found upon binding of a second oligonucleotide (Table 1).

Experiments were also done with 18-mer oligonucleotides of complementary, identical or unrelated sequences. Upon addition of a BPDE-modified oligonucleotide to preformed RecA:oligonucleotide complexes containing any one of the three oligonucleotides, the FA increased, indicating binding. The value of FA was larger, however, when the strand in RecA was complementary in sequence to the BPDE-modified

Table 1
FA of various RecA complexes, in presence of different cofactors, as indicated

Complex	Cofactor	FA
oligo* (free)	–	0.00 ± 0.005
oligo*-duplex (free)	–	0.02 ± 0.005
RecA/oligo*	ATPγS	0.178 ± 0.005
RecA/oligo*/comp	ATPγS	0.180 ± 0.005
RecA/oligo*/ident	ATPγS	0.175 ± 0.005
RecA/oligo*-duplex	ATPγS	0.140 ± 0.005
RecA/comp/oligo* (20 min)	ATPγS	0.139 ± 0.005
RecA/comp/oligo* (120 min)	ATPγS	0.125 ± 0.005
RecA/ident/oligo* (20 min)	ATPγS	0.125 ± 0.005
RecA/ident/oligo* (120 min)	ATPγS	0.040 ± 0.005
RecA/oligo*	ATP	0.168 ± 0.005
RecA/oligo*/comp (15 min)	ATP	0.122 ± 0.005
RecA/oligo*/comp (180 min)	ATP	0.044 ± 0.005

BPDE-oligonucleotide (oligo*) and BPDE-oligonucleotide duplex (oligo*-duplex) free in solution are also included. 'Comp' stands for complementary oligonucleotide and 'ident' stands for identical oligonucleotide. Incubation times at room temperature are indicated in parenthesis after some complexes.

oligonucleotide (0.12). When the strands were either identical or unrelated in sequence, the FA:s were approx. the same and lower (0.06–0.07). Binding of identical or unrelated oligonucleotides to preformed RecA-oligonucleotide complexes gave no effect on the fluorescence emission, in contrast to the results with the complementary strand (not shown).

3.3. *RecA binding of oligonucleotide duplex in presence of ATPγS*

In Fig. 2 it is shown that, upon formation of a duplex between the BPDE-oligonucleotide and its complementary sequence, there is a decrease in the BPDE fluorescence intensity, accompanied by a shift in the emission-peak ratio, I_{383nm}/I_{404nm}, (from ca 1.05 for the free BPDE oligonucleotide to ca. 0.83 for the duplex). There is, however, no increase in FA, showing that the probe is still mobile also when in a duplex structure. The melting temperature of the duplex between the BPDE oligonucleotide and its complementary sequence was found to be 55°C, compared to 59°C for the corresponding unmodified duplex, indicating that the presence of a BPDE-adduct has a minor effect on duplex stability. The addition of RecA to the duplex increases the fluorescence intensity (Fig. 2) and the FA (Table 1). In addition, as seen in Fig. 2 an alteration of the BPDE emission-peak ratio, towards the ratio found for single stranded BPDE-oligonucleotide, was observed (I_{383nm}/I_{404nm} changes from 0.83 to 1.12).

3.4. *Renaturation of DNA in RecA filament in presence of ATP*

We also probed the RecA-DNA interactions in presence of hydrolysing ATP, instead of ATPγS, as cofactor. Similar changes in fluorescence intensitiy and FA were observed for RecA binding of the BPDE-oligonucleotide (Fig. 3 and Table 1). Upon addition of the complementary oligonucleotide to the complex, the fluorescence intensity is drastically decreased and there is a shift in the emission-peak ratio (from 1.1 to 0.9), resembling the spectrum found for a BPDE-oligonucleotide duplex (Fig. 3). The FA is found to remain large (Table 1), showing that the BPDE oligonucleotide is still in complex with RecA. As observed from a slowly fading FA with time (Table 1), the duplex dissociates from RecA possibly due to consumption of the ATP regeneration system. Upon addition of an identical oligonucleotide to the RecA:(BPDE-oligonucleotide) complex, however, no changes in BPDE fluorescence are seen (cf. Fig. 3).

4. Discussion

We find that on addition of RecA to a 24-mer BPDE-modified oligonucleotide there is an increase in the fluorescence intensity (Fig. 1) and a substantial fluorescence anisotropy (Table 1), as a result of, respectively, changed environment and restricted mobility of the BPDE chromophore upon the complex formation. We find that also a second (BPDE-modified) oligonucleotide can bind to a RecA:oligonucleotide complex, whether or not the two strands are complementary (Table 1) when in presence of the cofactor ATPγS. This possibility of accommodation of several strands without Watson–Crick binding was proposed already in 1984 [23]. It has also been verified previously with homo-polynucleotides, or single-stranded calf-thymus DNA, for which RecA can bind three strands independent of sequence [11,17,18,24]. There is, however, an effect of complementarity between bound strands in the RecA filament. If the strands in RecA are complementary to each other, the RecA complex is found to be more stable. This is even more pronounced when studying 18-mer oligonucleotides.

Upon binding of a complementary 24-mer oligonucleotide as a second strand in a RecA:BPDE-oligonucleotide complex, a red-shift of about 3 nm in the BPDE fluorescence peaks is here observed, while no effect at all is not found if the second added oligonucleotide is identical in sequence to the already bound BPDE-oligonucleotide. Studies on BPDE-modified 18 mers show a similar lack of effect upon binding to RecA-BPDE-modified oligonucleotide complex for both identical and unrelated sequences. Also in this case, the major difference is found if the strands are complementary to each other, with an effect

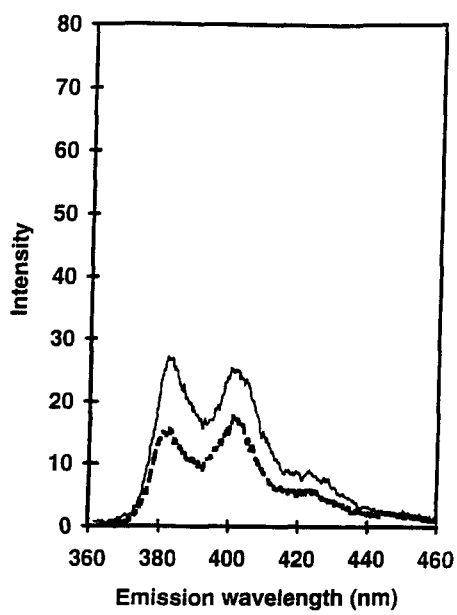


Fig 2. Fluorescence emission spectra of the BPDE chromophore when in a free BPDE-oligonucleotide duplex (dotted line) and upon RecA binding of this duplex (thin line).

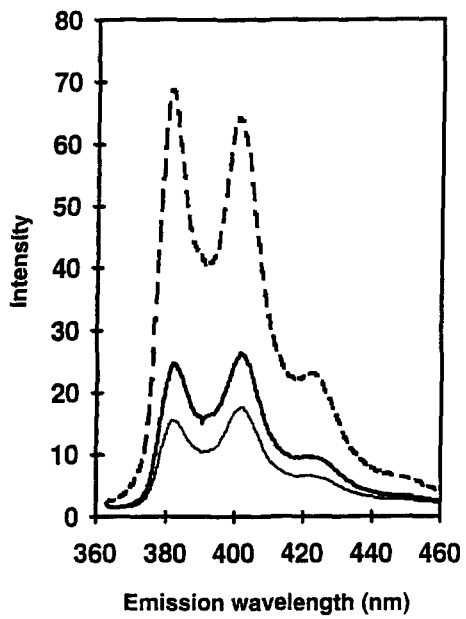


Fig 3. In presence of ATP hydrolysis. Fluorescence emission spectra of the BPDE chromophore of RecA : BPDE-oligonucleotide (dashed line) and RecA : BPDE-oligonucleotide : complementary oligonucleotide (bold line). Also shown is the fluorescence of a BPDE-oligonucleotide duplex formed in absence of RecA (thin line).

both on the fluoresence spectrum and the overall stability of the complex. The protein-mediated interaction between the DNA strands within the RecA filament may thus vary in nature depending on the degree of complementarity. This result is in contrast to recent findings by Radding et al. [25] for a RecA-DNA complex found to recognise an identical 33 mer in the same way as the complementary sequence. This difference may suggest that the nature of the RecA interaction could be dependent on the length of the oligonucleotide. It may be noted that a different, more direct method has been used here to demonstrate complex formation.

The present results support those of an earlier study of RecA complexes with BPDE-modified homo-polynucleotides [11], but there are certain variations which may relate to different environments of the probe. Upon binding of poly(dT) (or oligo(dT)$_{10}$) as the second strand in RecA : BPDE-poly(dA) (or RecA : BPDE-oligo(dA)$_{10}$) complexes, a decrease in fluorescence intensity is observed [11, and unpublished results]. Similar results have also been obtained with a mixed sequence (lacking guanines) 18-mer oligonucleotide containing BPDE-modified adenines (not shown). In the experiments presented in this report, instead a shift of the BPDE emission peaks is observed for the 24-mer oligonucleotide upon binding a complementary sequence in RecA (Fig. 1). This difference in BPDE characteristics may thus be an effect of the fact that here guanine has been selectively modified whereas it was adenine in the previous studies. BPDE chromophores linked to the exocyclic nitrogen in guanines are known to interact with the minor groove of duplex DNA and directed to the 5'-end of the binding strand [26], in contrast to pyrenyl-like chromophores linked to the exocyclic nitrogen in adenines, which have been found to mainly intercalate between bases [27]. We are currently performing studies to investigate the origin of these differences in the BPDE fluorescence characteristics upon interaction with complementary sequences in RecA.

The previous discussion concerns use of ATPγS as cofactor. We have also studied hydrolysing ATP as cofactor and found a clear difference in the RecA interactions. In the presence of ATP hydrolysis, we found renaturation between two complementary 24-mer oligonucleotides to occur within the RecA filament (Fig. 3). This is concluded from the change in ratio between the largest BPDE emission peaks, indicating a base-paired duplex, but still a high FA (Table 1), showing binding to RecA. Another possibility, that the duplex should be formed outside RecA, after a transient dissociation of the BPDE-oligonucleotide, followed by rebinding to RecA as duplex can be ruled out, since in such a case a spectrum resembling that in Fig. 2 of RecA binding a preformed duplex (peak ratio, $I_{383nm}/I_{404nm} > 1$) would have been observed. Instead the spectrum (Fig. 3) resembles that of free BPDE oligonucleotide duplex (see Fig. 3, peak ratio < 1) showing that we now have complete base-pairing. Thus, since the characteristics of the complex suggests a fully base-paired duplex, we conclude the renaturation to be mediated within the protein filament.

Our results based on direct observation of renaturation shows that ATP hydrolysis is necessary for the RecA promoted renaturation. ATP hydrolysis has earlier been found to be essential also for RecA-mediated renaturation between complementary homopolynucleotides [12]. The role of ATP hydrolysis for the RecA-DNA interactions has been widely studied, but is still an issue of controversy [28,29]. We find here the renaturated duplex to remain in complex with RecA, and not be dissociated, which can be ascribed to the to ATP regeneration system preventing accumulation of ADP. Other studies [30] have indicated ADP accumulation to promote dissociation of RecA from the DNA.

5. Conclusions

In this paper we have shown that a fluorescent probe covalently attached to a central deoxyguanosine can be used to directly monitor the interactions of RecA with mutually complementary or identical 24-mer mixed sequence oligonucleotides. In the presence of ATPγS, a RecA-oligonucleotide complex does not recognize a 24-mer oligonucleotide of identical sequence in the same way as a complementary sequence. Only in presence of ATP hydrolysis can RecA perform true base-pairing (renaturation) of two complementary oligonucleotides within the protein filament.

Acknowledgements: This work was supported by funding from the Swedish Cancer Society and the Swedish Work Environmental Fund. P.W. wishes to thank Prof. B.G. Malmström for valuable discussions regarding manuscript preparation.

References

[1] Clark, A.J. and Margulies, A.D. (1969) Proc. Natl. Acad. Sci. USA 53, 451–459.
[2] Smith G.R. (1989) Cell 58, 807–809.
[3] Roca, A.I. and Cox, M.M. (1990) Crit. Rev. Biochem. Mol. Biol. 25, 415–456.
[4] Egelman, E.H. (1993) Curr. Opin. Struct. Biol. 3, 189–197.
[5] Shibata, T., Dasgupta, C., Cunningham, R.P. and Radding, C.M. (1979) Proc. Natl. Acad. Sci. USA 76, 1638–1642.

[6] McEntee, K. Weinstock, G.M. and Lehman, I.R. (1979) Proc. Natl. Acad. Sci. USA 76, 2615–2619.
[7] Takahashi, M. and Nordén, B. (1994) Adv. Biophys. 30, 1–35.
[8] Kowalczykowski, S.C. and Eggelston, A.K. (1994) Annu. Rev. Biochem. 63, 991–1043.
[9] West, S.C. (1992) Annu. Rev. Biochem. 61, 603–640.
[10] Geacintov, N.E., Cosman, M., Ibanez, V., Birke, S.S. and Swenberg, C.E. (1990) in: Molecular Basis of Specificity in Nucleic Acid-Drug Interactions, (Pullman, P. and Jortner, J. Eds.), pp. 433–450.
[11] Wittung, P., Nordén, B., Kim, S.K. and Takahashi, M. (1994) J. Biol. Chem. 269, 5799–5803.
[12] Wittung, P., Nordén, B. and Takahashi, M. (1994) Eur. J. Biochem. 224, 39–45.
[13] Camerini-Otero, C.S. and Camerini-Otero, R.D. (1992) Proc. Natl. Acad. Sci. USA 89, 6492–6496.
[14] Takahashi, M. and Hagmar, P. (1991) FEBS Lett. 279, 270–272.
[15] Kuramitsu, S., Hamaguchi, K., Ogawa, T. and Ogawa, H. (1981) J. Biochem. (Tokyo) 90, 1033–1045.
[16] Pontén, I., Kim, S.K., Gräslund, A., Nordén, B. and Jernström, B. (1990) Cancerogenesis 15, 2207–2213.
[17] Takahashi, M., Kubista, M. and Nordén, B. (1989) J. Mol. Biol. 205, 137–147.
[18] Kubista, M., Takahashi, M. and Norden, B. (1990) J. Biol. Chem. 265, 18891–18897.
[19] Takahashi, M., Kubista, M. and Norden, B. (1991) Biochemie (Paris) 73, 219–226.
[20] DiCapua, E., Engel, A., Stasiak, A. and Koller, T. (1982) J. Mol. Biol. 157, 87–103.
[21] Stasiak, A. and Egelman, E.H. (1987) in: DNA Replication and Recombination, Vol. 47, (McMacken, R. and Kelly, T. Eds.) Alan R. Liss, New York, pp. 619.
[22] Cantor, C.R. and Schimmel, R.R. (1980) Biophysical Chemistry, 4th ed., W.H. Freeman and Co, New York.
[23] Howard-Flanders, P., West, S.C. and Stasiak, A. (1984) Nature 309, 215–220.
[24] Müller, B., Koller, T. and Stasiak, A. (1990) J. Mol. Biol. 212, 97–112.
[25] Rao, B.J. and Radding, C.M. (1993) Proc. Natl. Acad. Sci. USA 90, 6646–6650.
[26] Cosman, M., de los Santos, C., Fiala, R., Hingerty, B.E., Singh, S., Ibanez, V., Margulis, L., Live, D., Geacintov, N.E., Broyde, S. and Patel, D.J. (1992) Proc. Natl. Acad. Sci. USA 89, 1914–1918.
[27] Cosman, M., Fiala, R., Hingerty, B.E., Laryea, A., Lee, H., Harvey, R., Amin, S., Geacintov, N.E., Broyde, S. and Patel, D.J. (1993) Biochemistry 32, 12488–12497.
[28] Bryant, F.R. and Lehman, I.R. (1985) Proc. Natl. Acad. Sci. USA 82, 297–301.
[29] Jain, S.K., Cox, M.M. and Inman, R.B. J. Biol. Chem. (1994) 269, 20653–20661.
[30] Menetski, J.P., Varghese, A. and Kowalczykowski, S.C. (1988) Biochemistry 27, 1205–1217.