ISSN 0006-2979, Biochemistry (Moscow), 2009, Vol. 74, No. 7, pp. 813-818. © Pleiades Publishing, Ltd., 2009.
Published in Russian in Biokhimiya, 2009, Vol. 74, No. 7, pp. 1000-1006.

Study of the Inhibitory Effect of Fatty Acids on the Interaction between DNA and Polymerase β

Jun Yang[1]*†, Jing Yang[1,2†], Zheng-Qin Yin[3]*, Jing Xu[1], Ning Hu[1], I. Svir[4], Min Wang[1], Yuan-Yi Li[1], Lei Zhan[1], Song Wu[1], and Xiao-Lin Zheng[1]

[1]Key Laboratory of Biorheological Science and Technology (Chongqing University), Ministry of Education; Bioengineering College, Chongqing University, Chongqing 400030, China; fax: 86-23-6511-1931; E-mail: yjun1999@hotmail.com; bioyangjun@cqu.edu.cn
[2]Department of Information Engineering, Chongqing Communication College, Chongqing 400035, China
[3]Southwest Eye Hospital, Southwest Hospital, Third Military Medical University, Chongqing 400038, China; fax: 86-23-6546-0711; E-mail: qinzyin@yahoo.com.cn
[4]Kharkov National University of Radioelectronics, UA-61166 Kharkov, Ukraine

Received October 6, 2008
Revision received November 24, 2008

Abstract—The binding of human DNA polymerase β (pol β) to DNA template–primer duplex and single-stranded DNA in the absence or presence of pol β inhibitors has been studied using a surface plasmon resonance biosensor. Two fatty acids, linoleic acid and nervonic acid, were used as potent pol β inhibitors. In the interaction between pol β and DNA, pol β could bind to ssDNA in a single binding mode, but bound to DNA template–primer duplexes in a parallel mode. Both pol β inhibitors prevented the binding of pol β to the single strand overhang and changed the binding from parallel to single mode. The affinities of pol β to the template–primer duplex region in the presence of nervonic acid or linoleic acid were decreased by 20 and 5 times, respectively. The significant inhibitory effect of nervonic acid on the pol β–duplex interaction was due to both a 2-fold decrease in the association rate and a 9-fold increase in the dissociation rate. In the presence of linoleic acid, no significant change of association rate was observed, and the decrease in binding affinity of pol β to DNA was mainly due to 7-fold increase in the dissociation rate.

DOI: 10.1134/S0006297909070165

Key words: DNA polymerase β, surface plasmon resonance, fatty acid, inhibition, SPR biosensor

There are at least five types of DNA polymerases (α, β, γ, δ, and ε) in mammalian and yeast cells. Polymerase β (pol β) is the smallest, and it plays an important role in gap-filling synthesis during base excision repair [1-6]. It is known to catalyze DNA synthesis, and in a distributive model this requires that the enzyme dissociates from and rebinds to the template during each dNMP incorporation cycle [6, 7]. Commonly, pol β is expressed at low levels, similar to a number of other so-called constitutive "housekeeping" enzymes. Human pol β is a single-chain polypeptide of 335 amino acids [8-10], consisting of a 31-kDa C-terminal (C) domain that includes a polymerase active site [11, 12] and an 8-kDa N-terminal (N) domain that participates in binding to DNA and exhibits 5′-3′ deoxyribose phosphodiesterase (lyase) activity [13].

Previous biophysical studies [12, 14, 15] have provided comprehensive knowledge on the interaction between DNA and pol β. For example, both single-stranded (ss) and double-stranded (ds) nucleic acids can bind with intact pol β. The N domain has a high affinity for ssDNA, whereas the C domain binds dsDNA weakly and shows the catalytic activity. In addition, fluorescence studies indicate that human pol β forms a complex with a ssDNA in which 16 nucleotides are occluded by the enzyme, and this complex is designated as $(\text{pol }\beta)_{16}$ [16]. In the $(\text{pol }\beta)_{16}$ binding mode, the C catalytic domain of the enzyme is also involved in the interactions with ssDNA (Fig. 1a). For pol β binding to DNA template–primer duplex, it involved the protein binding to the single-stranded template and the C domain binding to the duplex in the vicinity of the 3′-end of the primer,

Abbreviations: LA, linoleic acid; NA, nervonic acid; pol β, DNA polymerase β; SPR, surface plasmon resonance.

* To whom correspondence should be addressed.
† These authors contributed equally.

and the intact pol β binding to the DNA template overhang region in which 16 nucleotides are occluded by the protein (Fig. 1b).

Linoleic acid (LA) and nervonic acid (NA) are two potent pol β inhibitors (Fig. 2a). These two fatty acids have several common characteristics: an unsaturated hydrocarbon chain containing 18 or more carbons, a free carboxyl group, and *cis*-configuration [17, 18]. NMR studies have examined the structural interaction of these fatty acids with the N domain and have shown that it is bound as a 1 : 1 complex to LA or NA with dissociation constant (K_D) of 1.02 or 2.64 mM, respectively [17]. These fatty acids were also found to suppress the binding of the N domain to ssDNA and the template–primer DNA duplex. LA and NA, when acting as pol β inhibitors, are unsaturated *cis*-configuration long-chain fatty acids, with carbon atom numbers of 18 and 24, respectively. It is well known that long-chain fatty acids can inhibit mammalian DNA polymerases. However, it is unclear whether the inhibitory effect of the fatty acids is due to their ability to affect pol β binding with DNA. Furthermore, the effects of these inhibitors on DNA binding kinetics, affinities, and the binding sites of pol β have not been determined.

The surface plasmon resonance (SPR) biosensor technique is a useful method to obtain quantitative kinetic and affinity information on biomolecular interactions [19, 20]. An SPR biosensor can translate a biospecific interaction between a ligand in solution and a binding partner immobilized on the surface into a detectable signal directly proportional to the extent of the interaction. The study of the binding process using the SPR biosensor technique has contributed significantly to the understanding of the molecular basis of DNA–protein interactions [21, 22]. The interaction of a series of DNA substrates with human pol β has been studied in real time using the SPR biosensor technique, as well as a detailed kinetic study on the various proposed binding modes between pol β with different DNA targets [23]. In this paper, the SPR biosensor technique was used to compare the binding of pol β to ssDNA and template–primer duplex DNA substrates in the presence or absence the fatty acid inhibitors, LA and NA.

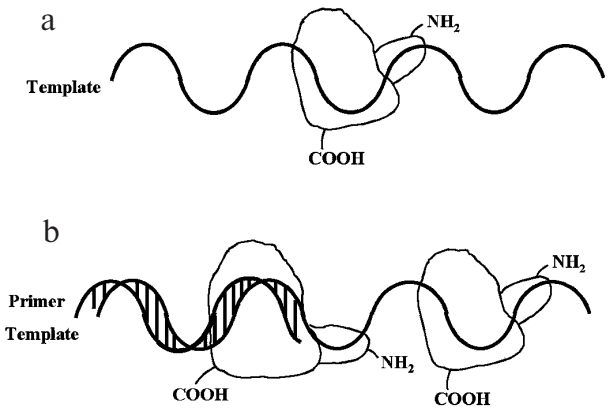

Fig. 1. Simplified models showing proteolytic domains of DNA polymerase β interacting with (a) a ssDNA in which 16 nucleotides are occluded by the enzyme to form a (pol β)$_{16}$ mode and (b) a template–primer duplex in which the 31- and 8-kDa domains are shown bound in the vicinity of the 3′ end of the primer and a single-stranded segment of the template.

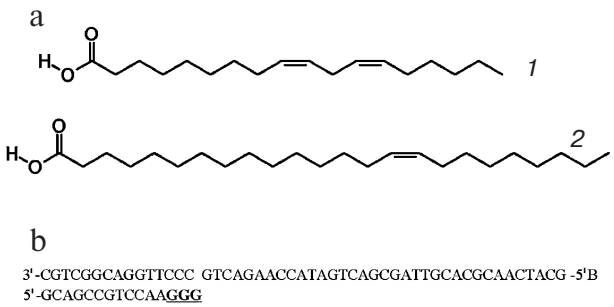

Fig. 2. a) Structures of linoleic (*1*) and nervonic (*2*) fatty acids are shown, which act as pol β inhibitors. b) DNA substrates used for polymerase binding. Single-stranded 5′-biotin-labeled DNA acts as the template. The primer (15-mer) is fully complementary to the template strand and forms the fully matched DNA template–primer duplex.

MATERIALS AND METHODS

Materials and equipment. The SPR biosensor instrument (BIAcore X) and sensor chips (SA5 research grade) were acquired from Pharmacia Biosensor (Sweden). All the experiments with pol β were carried out in TSB buffer containing 50 mM Tris-HCl, pH 7.4, and 100 mM NaCl. Purified pol β (20-100 nM) in TSB was used for the DNA–polymerase binding experiments. LA and NA fatty acids were purchased from Sigma (USA) and dissolved in 100% methanol or water, respectively. Tris, Hepes, NaCl, and $MgCl_2$ were from Sigma. HSM buffer (10 mM Hepes, pH 7.4, 150 mM NaCl, and 10 mM $MgCl_2$) was used for DNA immobilization, and TSB buffer at pH 7.4 was used as the running buffer in all the experiments.

Immobilization of DNA substrate. 5′-Biotin-labeled 50-mer single-stranded oligonucleotide (template) and unlabeled 15-mer oligonucleotide (primer) were synthesized by Biosune Company (China) and purified by HPLC. The primer was complementary to the unlabeled end of the template strand, forming a fully matched DNA duplex. The sequences of these oligonucleotides are shown in Fig. 2b. To facilitate annealing of these oligonucleotides to form duplexes, equimolar amount of oligonucleotides were mixed together in HSM buffer. The mixture was incubated at 100°C for 5 min and allowed to slowly cool to room temperature. The primer formed a

fully matched DNA duplex with the template. The streptavidin-modified (SA5) sensor surface was first equilibrated with the HSM buffer before DNA immobilization. When a stable baseline was reached, a 35 μl solution of a DNA substrate (2 μM in HSM buffer) was injected onto the sensor chip for 7 min. After the injection, the DNA-modified surface was washed with HSM buffer to remove the unbound biotin-labeled DNA molecules until a stable baseline was obtained. The immobilization procedure was carried out at 25°C and at a constant flow rate of 5 μl/min. Biotinylated DNA template–primer duplexes bound rapidly to the immobilized streptavidin molecules on the SA chip and could not be washed away by HSM buffer. A significant increase in the response was observed (ΔRU = 2738) under these experimental conditions. On the basis of the molecular weight of the DNA template–primer duplex (~20 kDa) and the calibration that ΔRU = 1000 corresponds to 1 ng of macromolecules per mm^2 change in surface density [24], the amount of DNA duplex bound to the streptavidin-modified surface was estimated to be $7.1 \cdot 10^{10}$ molecules per mm^2.

Biosensor measurement of pol β–DNA interactions. All binding experiments were carried out at 25°C with a constant flow rate of 5 μl/min in TSB buffer. The optimal experimental concentration of LA and NA was determined by examining the inhibitory effects of various concentrations of fatty acids on the binding responses obtained from the DNA–pol β interactions. The optimal experimental concentration was found to be 5 μM and was used in the subsequent experiments. A 15-μl solution comprised of different concentrations of pol β (20-100 nM) and 5 μM fatty acid (LA or NA) in TSB buffer was injected onto the DNA-modified sensor surface for 3 min, followed by washing with TSB buffer for 5 min. The DNA-modified surfaces were regenerated by injecting 10 μl 1 M NaCl solution for 2 min. The sensor surface without DNA coating was used as the control and run simultaneously for each binding experiment. The sensorgrams for all DNA–polymerase binding interactions were recorded in real time and were analyzed after subtracting the sensorgram data from the controlled channel data.

Data analysis. The binding and dissociation data were modeled and analyzed using BIAevaluation Software. A simple 1 : 1 (Langmuir) model and a heterogeneous ligand 1 : 2 (parallel binding) model were used to fit the data according to the numerical integration method. A mass transfer rate constant (k_{mt}) was included in the models to account for the diffusion of the analyte molecules (pol β) from the solution to the biosensor interface according to Fick's law [25, 26]. The data were analyzed by fitting both the association and dissociation phases for several concentrations. The degree of randomness of the residual plot and the reduced chi square (χ^2) value were used to assess the appropriateness of the various models for analyzing the sensor data.

RESULTS AND DISCUSSION

Effect of fatty acid concentration on DNA–pol β interaction. The SPR sensor surface was modified with the fully-matched DNA template–primer duplexes containing a 50-mer template hybridized with a 15-mer primer. Different concentrations of fatty acids, 0-100 μM for LA and 0-10 μM for NA, mixed with the same concentration of pol β (80 nM) were injected over the DNA-modified sensor surface. As described previously [17, 18], the longer fatty acid chains have a stronger inhibitory effect compared to short chains. NA has a longer carbon atom chain (C24) than LA (C18) and is expected to be more effective in inhibiting polymerase activity than LA. Therefore, different ranges of concentration were used for the two fatty acids. Note that 100 mM Na^+ was present in the TSB buffer in order to facilitate pol β binding [27], and no dNTP or Mg^{2+} was present in the solution to prevent the elongation of the terminal bases of the primer by the polymerase. The relationship between the relative surface binding density and various concentrations of fatty acids plotted in Fig. 3 clearly shows the inhibitory effect of fatty acids on the binding of pol β to the DNA-modified surface. The relative surface binding density decreased while the concentration LA or NA was increased, indicating that a decreasing amount of pol β was bound to the immobilized DNA. A 100% inhibition of pol β binding to the DNA duplex was observed with 100 μM LA or 10 μM NA. To compare the inhibitory effect of the two fatty acids on the binding affinity, the same concentration (5 μM) was used for both inhibitors in the subsequent experiments.

Effect of fatty acids on the binding of pol β to ssDNA. Biotinylated ssDNA substrate (50-mer) was immobilized on the sensor chip surface using the biotin–streptavidin technique. Different concentrations of pol β in the presence of 5 μM of LA or NA were injected onto the ssDNA-modified surface, and then the binding of these various concentrations of pol β to ssDNA was monitored by the SPR biosensor. The results showed that the binding response of pol β to ssDNA was extremely small com-

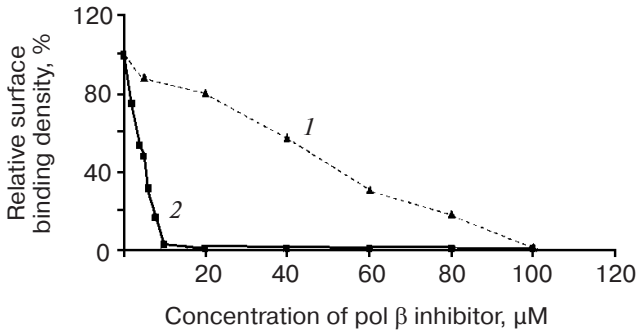

Fig. 3. Dependence of the relative surface binding density on the concentration of linoleic (*1*) or nervonic acid (*2*).

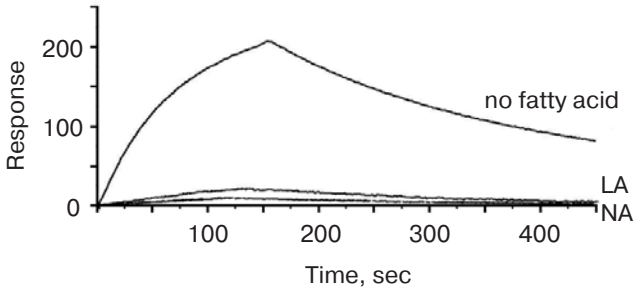

Fig. 4. Binding of pol β to ssDNA with and without fatty acids.

pared with the binding in the absence of fatty acids (Fig. 4). This indicated that LA and NA significantly suppressed the binding of intact pol β to ssDNA.

Circular dichroism and fluorescence studies of human pol β indicated that the N domain has a high affinity for ssDNA [14]. Furthermore, others [17, 18] have shown that long-chain fatty acids bind to the N-terminal 8-kDa domain of pol β. Based on the previous findings, it is reasonable to expect that the pol β inhibitors, LA and NA, would prevent the binding of the polymerase N domain to ssDNA. Although it has been shown that the 31-kDa catalytic domain of pol β is also involved in the pol β–ssDNA interaction in which 16 nucleotides were occluded by the enzyme at low protein concentration [16], the C domain seemed unaffected by the inhibitors at low concentration; an extremely small response was observed in our experiment when the fatty acid was added to the protein mixture (Fig. 4). Therefore, it can be concluded that the 31-kDa domain alone cannot bind to ssDNA. The binding mechanism of intact pol β towards ssDNA may involve a two-step process whereby the N domain initially binds to ssDNA, and the C domain then encloses the DNA target in the absence of a fatty acid.

Effect of fatty acids on pol β binding to DNA template–primer duplex. Previous studies have revealed the mechanism of pol β binding to DNA template–primer duplex [12, 15, 23]. The model involves the C and N domains binding to the single-stranded segment of the template and in the vicinity of the 3′-end of the primer. The template overhang (35-mer) was present in this DNA template–primer duplex substrate; this overhang region provides sufficient space for pol β to bind. Based on the above mechanism, a parallel model offered the best fits to the sensorgrams of pol β binding to the fully-matched DNA duplex. The sensorgrams of pol β binding to DNA duplex were obtained in the presence of the potent pol β inhibitors—LA or NA (Fig. 5). The sensorgrams were fitted to both the parallel binding model and the simple 1 : 1 Langmuir model. As shown in Fig. 5, the simple Langmuir model showed a more reasonable fit to both sensorgrams compared with the parallel model. Thus, it can be concluded that the presence of the fatty acids changed the binding characteristics of pol β to the DNA duplex. Because the binding between pol β and ssDNA was completely inhibited by the fatty acids (Fig. 4), pol β can only bind to the 3′-end of the primer of the duplex region. As a result, an equimolecular binding interaction seems to be involved.

The effects of the LA and NA were studied and compared with the results obtained previously. In the absence of an inhibitor, the affinity constants (K_A) of the two binding modes showed similar magnitudes, that is, $2.20 \cdot 10^8$ M^{-1} in the vicinity of the 3′-end of the primer and $1.70 \cdot 10^8$ M^{-1} for the single-stranded template overhang. However, in the presence of the inhibitors, the polymerase showed a single binding mode on the DNA substrate. The K_A for LA and NA were $4.12 \cdot 10^7$ and

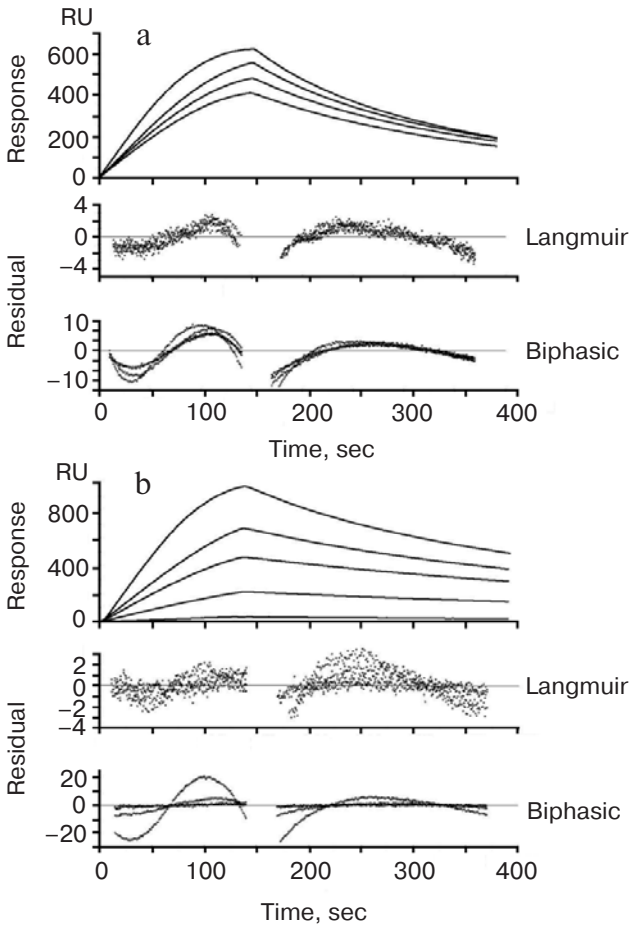

Fig. 5. Binding of pol β to DNA template–primer duplexes in the presence of fatty acids. Different concentrations (20-100 nM) of pol β were injected over the sensor surface modified with fully-matched DNA template–primer duplex in the presence of 5 μM linoleic acid (a) or nervonic acid (b) at 25°C. The residual plots fitting the interaction between pol β and fully-matched DNA duplex to the Langmuir binding and parallel models are also shown. The simple 1 : 1 model was proved to be a good model for curve fitting based on the low values (5.40 for linoleic acid, 4.50 for nervonic acid) and the random distributions of the residual plots.

Binding kinetics and affinities of human pol β to fully matched DNA template–primer duplexes in the absence or presence of two fatty acids

Fatty acid	$k_{on} \times 10^5$, $M^{-1}\cdot sec^{-1}$	$k_{off} \times 10^{-2}$, sec^{-1}	$K_A \times 10^7$, M^{-1}	χ^2
—	9.47 ± 0.06	0.43 ± 0.01	22.0 ± 0.53	0.886
Linoleic acid	12.5 ± 1.01	3.03 ± 0.25	4.12 ± 0.48	5.400
Nervonic acid	4.35 ± 0.22	4.06 ± 0.07	1.07 ± 0.06	4.500

Note: The data were locally fitted simultaneously with a simple 1 : 1 binding model with a mass transfer effect.

$1.07\cdot10^7$ M^{-1}, respectively (table). The binding affinities were decreased by 5 to 20 times compared with the situation when no fatty acid was added. The significant decrease can be explained by the difference in the binding mode of pol β to the DNA primer 3′-end. Based on the observation that fatty acids suppressed the binding of N domain to ssDNA, we also assumed that the N domain was not involved in ssDNA template binding when the intact polymerase bound to the primer 3′-end (Fig. 1). Only the C domain was involved in the ssDNA binding, and its binding affinity was lower than that of intact protein to the template–primer duplex region. Both LA and NA have an inhibitory effect on DNA–pol β interactions. The reduction of pol β binding affinity to the DNA template–primer duplex region by LA was mainly due to an increase in the dissociation rate (k_{off}) of pol β from DNA. On the other hand, the significant inhibitory effect of NA on the DNA–pol β interaction was due to both a 2-fold decrease in the association rate (k_{on}) and a 9-fold increase in the dissociation rate (k_{off}).

Because the presence of the fatty acids suppressed the binding of intact pol β to the single-stranded template overhang, the net response observed in Fig. 5 was entirely due to the binding of the 31-kDa domain of pol β to the vicinity of 3′-end primer. However, the overall sensor responses decreased as the concentration of fatty acid increased, indicating a decreasing amount of 31-kDa domain of pol β was bound to the immobilized DNA duplex region (Fig. 3). At high concentrations of pol β inhibitors (100 μM for LA and 10 μM for NA), even the binding of the 31-kDa domain to the template–primer region was totally inhibited.

In summary, we have used the surface plasmon resonance biosensor technique to characterize and obtain quantitative information on the kinetics of binding interactions between human pol β and DNA substrates including DNA template–primer duplex and ssDNA in the presence or absence of the pol β inhibitors LA and NA. The fatty acids suppressed the binding of pol β to the ssDNA because the inhibitor binds to the N domain of the protein and blocks this ssDNA binding domain. In addition, the inhibitors changed the binding of the polymerase to the DNA duplex in a way that suggested a change from a parallel reaction model to a single binding mode due to the suppression of the protein binding to the ssDNA template. The overall binding affinities of pol β to the template–primer duplex region decreased in the presence of the fatty acids and can be explained by the different binding orientations of the protein on the fully matched DNA target when the pol β inhibitor is present, in which only the C domain is involved in the interaction with DNA. When the concentration of the pol β inhibitor was increased, the binding of the C domain to the vicinity of primer 3′-end was also suppressed. This indicates that LA and NA have an inhibitory effect on the DNA–pol β interaction through inhibiting both the N and C domains, although with a much reduced extent for the latter.

The authors would like to thank Prof. Mengsu Yang at the City University of Hong Kong for his support in a previous study and Dr. T. FitzGibbon for comments on earlier drafts of the manuscript.

This work was supported by the National Natural Science Foundation of China (grant Nos. 30500120, 30870661, and 30770569), the National Basic Research Program of China (No. 2007CB512203), and the Key Science and Technology Project of Chongqing (CSTC2008AA5010).

REFERENCES

1. Beard, W. A., Prasad, R., and Wilson, S. H. (2006) *Meth. Enzymol.*, **408**, 91-107.
2. Beard, W. A., and Wilson, S. H. (2006) *Chem. Rev.*, **106**, 361-382.
3. Bergethon, P. R. (1998) *The Physical Basis of Biochemistry – the Foundations of Molecular Biophysics*, Springer-Verlag, New York.
4. Bondeson, K., Frostellkarlsson, A., Fagerstam, L., and Magnusson, G. (1993) *Anal. Biochem.*, **214**, 245-251.
5. Boozer, C., Kim, G., Cong, S. X., Guan, H. W., and Londergan, T. (2006) *Curr. Opin. Biotech.*, **17**, 400-405.
6. Boozer, C., Ladd, J., Chen, S. F., and Jiang, S. T. (2006) *Anal. Chem.*, **78**, 1515-1519.
7. Casasfinet, J. R., Kumar, A., Morris, G., Wilson, S. H., and Karpel, R. L. (1991) *J. Biol. Chem.*, **266**, 19618-19625.

8. Chang, L. M. S., and Bollum, F. J. (1971) *J. Biol. Chem.*, **246**, 5835-5837.
9. Detera, S. D., Becerra, S. P., Swack, J. A., and Wilson, S. H. (1981) *J. Biol. Chem.*, **256**, 6933-6943.
10. Hoppe, W., Lohmann, W., Markl, H., and Ziegler, H. (1983) *Biophysics*, 2nd Edn., Springer-Verlag, Berlin.
11. Jezewska, M. J., Galletto, R., and Bujalowskii, W. (2002) *J. Biol. Chem.*, **277**, 20316-20327.
12. Jezewska, M. J., Rajendran, S., and Bujalowski, W. (2001) *Biochemistry*, **40**, 3295-3307.
13. Kumar, A., Abbotts, J., Karawya, E. M., and Wilson, S. H. (1990) *Biochemistry*, **29**, 7156-7159.
14. Liu, Y. A., Prasad, R., Beard, W. A., Kedar, P. S., Hou, E. W., Shock, D. D., and Wilson, S. H. (2007) *J. Biol. Chem.*, **282**, 13532-13541.
15. Matsumoto, Y., and Kim, K. (1995) *Science*, **269**, 699-702.
16. Mizushina, Y., Ohkubo, T., Date, T., Yamaguchi, T., Saneyoshi, M., Sugawara, F., and Sakaguchi, K. (1999) *J. Biol. Chem.*, **274**, 25599-25607.
17. Murakami, S., Kamisuki, S., Takata, K. I., Kasai, N., Kimura, S., Mizushina, Y., Ohta, K., Sugawara, F., and Sakaguchi, K. (2006) *Biochem. Biophys. Res. Commun.*, **350**, 7-16.
18. Matsuda, T., Berg, B. J. V., Bebenek, K., Osheroff, W. P., Wilson, S. H., and Kunkel, T. A. (2003) *J. Biol. Chem.*, **278**, 25947-25951.
19. Pelletier, H., and Sawaya, M. R. (1996) *Biochemistry*, **35**, 12778-12787.
20. Pelletier, H., Sawaya, M. R., Kumar, A., Wilson, S. H., and Kraut, J. (1994) *Science*, **264**, 1891-1903.
21. Rajendran, S., Jezewska, M. J., and Bujalowski, W. (1998) *J. Biol. Chem.*, **273**, 31021-31031.
22. Sengupta, D. N., Zmudzka, B. Z., Kumar, P., Cobianchi, F., Skowronski, J., and Wilson, S. H. (1986) *Biochem. Biophys. Res. Commun.*, **136**, 341-347.
23. Sobol, R. W., Horton, J. K., Kuhn, R., Gu, H., Singhal, R. K., Prasad, R., Rajewsky, K., and Wilson, S. H. (1996) *Nature*, **379**, 183-186.
24. Szabo, A., Stolz, L., and Granzow, R. (1995) *Curr. Opin. Str. Bio.*, **5**, 699-705.
25. The, H. F., Peh, W. Y. X., Su, X. D., and Thomsen, J. S. (2007) *Biochemistry*, **46**, 2127-2135.
26. Tsoi, P. Y., and Yang, M. S. (2002) *Biochem. J.*, **361**, 317-325.
27. Zmudzka, B. Z., Sengupta, D., Matsukage, A., Cobianchi, F., Kumar, P., and Wilson, S. H. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 5106-5110.