Dipole-Dipole Interaction Stabilizes the Transition State of Apurinic/Apyrimidinic Endonuclease—Abasic Site Interaction*

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Human apurinic/apyrimidinic (AP) endonuclease (hAPE) initiates the repair of an abasic site (AP site). To gain insight into the mechanisms of damage recognition of hAPE, we conducted surface plasmon resonance spectroscopy to study the thermodynamics and kinetics of its interaction with substrate DNA containing an abasic site (AP DNA). The affinity of hAPE binding toward DNA increased as much as 6-fold after replacing a single adenine (equilibrium dissociation constant, \(K_D\), 5.3 nM) with an AP site (\(K_D\), 0.87 nM). The enzyme-substrate complex formation appears to be thermodynamically stabilized and favored by a large change in Gibbs free energy, \(\Delta G^\circ(-50\ \text{kJ/mol})\). The latter is supported by a high negative change in enthalpy, \(\Delta H^\circ(-43\ \text{kJ/mol})\) and also positive change in entropy, \(\Delta S^\circ(24\ \text{J/(K} \cdot \text{mol)})\), and thus the binding process is spontaneous at all temperatures. Analysis of kinetic parameters reveals small enthalpy of activation for association, \(\Delta H^\circ_{\text{ass}}(-17\ \text{kJ/mol})\), and activation energy for association \((E_a, -14\ \text{kJ/mol})\) when compared with the enthalpy of activation for dissociation, \(\Delta H^\circ_{\text{diss}}(26\ \text{kJ/mol})\), and activation energy in the reverse direction \((E_a, 28\ \text{kJ/mol})\). Furthermore, varying concentration of KCl showed an increase in binding affinity at low concentration but complete abrogation of the binding at higher concentration, implying the importance of hydrophobic, but predominately ionic, forces in the Michaelis-Menten complex formation. Thus, low activation energy and the enthalpy of activation, which are perhaps a result of dipole-dipole interactions, play critical roles in AP site binding of APE.

In all organisms, repair of DNA-containing small adducts, as well as altered and abnormal bases, occurs primarily via the base excision repair (BER) pathway, beginning with cleavage of the base by DNA glycosylase (1, 2). The next enzyme in the BER pathway is APE. APE initiates the repair of abasic sites generated either spontaneously, from attack of bases by free radicals, or during the course of the repair of damaged bases (4–6). AP sites are among the most common DNA lesions and can have deleterious consequences for the cell (7, 8). AP sites are potentially genotoxic as well as mutagenic (7, 8). As a BER enzyme, APE catalyzes the hydrolytic cleavage of the phosphodiester bond immediately 5’ to the AP site. APE can also act as a phosphodiesterase, a 3’–phosphatase, and an RNase H (4, 9–11, 13–17).

Despite the central role of APE in human BER (18–21), there have been limited data about the molecular mechanisms of recognition of AP sites by APE. However, structural studies by x-ray crystallography showed that APE electrostatically orients a rigid, preformed DNA-binding face and penetrates the DNA helix from both the major and the minor grooves, stabilizing an extrahelical conformation for target abasic nucleotides and excluding normal DNA nucleotide and racemized abasic site binding (22). To resolve the molecular basis for binding by APE in solution, we determined the thermodynamic and kinetic parameters of APE binding to DNA containing an AP site (AP DNA). Understanding damage discrimination by APE serves as a paradigm in the study of other BER proteins. Also, APE inhibitors are currently in preclinical study for cancer treatment (23). Thus, delineating the molecular basis of interaction may help find a better inhibitor for future use.

Binding of APE to AP DNA has been studied using pseudo-equilibrium methods, such as gel mobility shift assay. However, this technical approach is not amenable for use under different experimental conditions with varying parameters such as temperature and salt concentrations. Fluorescence spectroscopy, a non-invasive approach, has also been widely and successfully used to study the thermodynamics of protein-nucleic acid interactions. One important limitation to this approach is that appropriate fluorophores must be attached to the protein or nucleic acid in such a way as to give an appropriate signal change upon binding (24). Overall, most biochemical and biophysical methods available to date have limited capacity to determine the kinetic energy parameters of the interaction. Surface plasmon resonance measures binding parameters that

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3 The abbreviations used are: BER, base excision repair; AP, apurinic/apyrimidinic; APE, AP endonuclease; hAPE, human AP endonuclease.
can be used to analyze different kinetic parameters including the activation energy of Michaelis–Menten complex formation, and thus, broadens our understanding about the mechanism of macromolecular interactions. Moreover, the thermodynamic parameters alone, in many cases, are not sufficient to explain the exact mechanism for binding (25–27).

In this study, by systematic alteration of temperature and ionic strength of the binding buffer, we have been able to determine the thermodynamic, kinetic features and the nature of the interactions of APE with AP DNA. To our knowledge, this is the first report about the energetics of binding of a BER protein to its substrate. Here we showed that the stability of APE–AP DNA complex arises due to a large release of enthalpy, low activation energy, and favorable reorganization (positive entropy change) of the interacting molecules.

MATERIALS AND METHODS

Purification of Recombinant hAPE—The hAPE was purified as described previously (28).

Preparation of Oligonucleotide Substrates—A 50-mer oligonucleotide containing an AP site (tetrahydrofuran, an artificial but stable AP site analogue commonly used in the laboratory as a substrate for APE) or an adenine, biotinylated at 5’ and with sequence 5’-TCGAGGATCTCTGAGCTCGAGTCGACG-3’ (where X represents the AP site or adenine) was purchased from Gene Link (Hawthorne, NY). The complementary oligonucleotide containing T opposite the AP site or adenine was synthesized by the Recombinant DNA Laboratory Core Facility at the University of Texas Medical Branch (Galveston, TX). The oligonucleotides were purified on a sequencing gel. The AP site oligonucleotide was annealed to a complementary oligonucleotide to prepare bio-

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detailed duplex oligonucleotide as described previously (29).

DNA Binding Studies Using Surface Plasmon Resonance—In search of a mechanism of molecular interaction of APE with the AP site, we examined the effect of temperature and salt on APE/AP DNA binding, using a Biacore T100 (Biacore, Uppsala, Sweden). The Biacore T100, with its advanced features, is able to measure various binding parameters in a wide range of temperatures (5–45 °C). A 50-mer duplex oligonucleotide substrate containing an AP site at the 26th position from the 5’ end of one strand was prepared as described above and used for measuring enzyme oligonucleotide interaction. The biotinyl-

ated oligonucleotides were immobilized on streptavidin-coated Biacore chips. Control oligonucleotide-containing adene was used to check the specificity of the interaction, and NaCl (150 mM) and KCl (90 mM) were used to test the cation effect. Then we measured the binding parameters of APE (0–30 nM) for the AP site using a binding buffer (10 mM HEPES-KOH, pH 7.6, 90 mM KCl and 0.05% surfactant) at different tempera-
tures (5–37 °C). To test the salt dependence of the APE and AP DNA interactions, we used different concentrations of KCl (90–250 mM), keeping the other components in the buffer identical.

Various concentrations of APE were injected, and the surface plasmon resonance was measured for 250 s with a 60-s injection. Following each injection, the chip was regenerated with 1 M NaCl. The binding kinetics for oligonucleotides containing AP sites were established with a series of APE concentrations.

RESULTS

APE/AP DNA Binding Studies Using Surface Plasmon Resonance—In search of a mechanism of APE binding to AP DNA, we used a Biacore T100 (Biacore, Uppsala, Sweden). Previously, we used surface plasmon resonance to understand the mechanism of modulation of substrate binding of N-methylpu-
rine-DNA glycosylase by Mg2+, N-terminal tail or specific anti-
body (29–31). Here, we first standardized the binding (Fig. 1A) and examined the specificity of APE to AP DNA at 15 °C. The affinity of hAPE binding toward DNA increased as much as 6-fold after replacing a single adenine (Kd, 5.3 ± 0.4 nM) with an

The Langmuir isotherms (1:1 binding) at various protein concentra-
tions allowed us to calculate the kinetic binding param-
eters based on on/off rates and protein concentrations.

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The following classical van’t Hoff and its derivative thermody-
namic equations were used to analyze these parameters

\[
\Delta G^\circ = -RT \ln K_D = \Delta H^\circ - T\Delta S^\circ
\]  

or

\[
\ln K_D = \Delta S^\circ/R - \Delta H^\circ/RT
\]

We also used an analogous set of equations (Eyring relation-
ship) that deals with the free energy change of activation, \(\Delta G^{\circ^\ast}\), which relates to the difference between interactants or bound complexes and transition states and can also provide information on the mechanism of the interaction. The Eyring equation is as follows

\[
K^\dagger = k/T \times h/k_B
\]

where \(K^\dagger\), \(k\), \(k_B\), and \(h\) are equilibrium constants for the forma-
tion of the transition state, kinetic rate constant \((k_\text{on}\) or \(k_\text{off}\), the Boltzmann constant, and Planck’s constant, respectively. Then, by analogy with the van’t Hoff equation and using \(K^\dagger\) instead of \(K_D\) gives the relationship for transition state formation

\[
\ln K^\dagger = \ln(k/T \times h/k_B) = \Delta S^\circ/R - \Delta H^\circ/RT
\]

Thus, plotting \(\ln(k/T)\) against \(1/T\) gives \(\Delta H^\circ\) and \(\Delta S^\circ\). Now, a comparison of the Arhenius equation (\(ln k = -E_a/RT + \ln A\)) and the Eyring equation shows that \(E_a\) (energy of activation) and \(\Delta H^\circ\) or \(A\) and \(\Delta S^\circ\) are analogous quantities. However, the \(E_a\) and \(\Delta H^\circ\) are related by the following equation

\[
E_a = \Delta H^\circ + RT
\]
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AP site (K_D 0.87 ± 0.02 nM), whereas the hAPE binding to AP DNA had minor difference in the presence of Na^+ (K_D 0.2 ± 0.02 nM) when compared with K^+ (K_D 0.87 ± 0.02 nM). Then, we measured the binding parameters at different temperatures (Fig. 1B). The van’t Hoff plot changed in a linear fashion and indicated little or no change of heat capacity (ΔC_p) within our specified temperature range. The van’t Hoff plot generally deviates from linearity in cases of a change in heat capacity. The thermodynamic parameters were then evaluated from the same van’t Hoff plot (Fig. 2A). Our results showed a large negative change in standard Gibbs free energy (−50 kJ/mole) for APE/AP DNA binding, indicating a spontaneous interaction (Fig. 2B). The large change in free energy is a result of negative change in enthalpy as well as a positive entropy change at equilibrium. An Eyring plot of the kinetic data were used to clarify the formation of the Michaelis-Menten complex. In the forward binding process, the ∆G°‡ (association) is relatively low, 32 kJ/mole (Fig. 3, A and B). The transition step is facilitated by low activation energy (E_a, −14 kJ/mol) and a favorable change in enthalpy of activation (−17 kJ/mole), although the entropy-related energy of activation is significantly high (TΔS°‡, −49 kJ/mol). The ∆G°‡ (dissociation) is also appreciably high for the reverse process. The latter process seems opposed by both large enthalpy of activation (26 kJ/mol) and activation energy in the reverse direction (E_a, 28 kJ/mol), as well as large negative change in entropy of activation (TΔS°‡, −56 kJ/mol). We have also treated our data via the Arrhenius equation and found the values of E_a and E_d to be the same as those determined by Eyring equation, mentioned above. These results, thus, further ensure that the transition state of Michaelis-Menten complex formation is facilitated by low activation energy in the forward process (Fig. 4, A and B).

Salt Concentration Dependence of Binding—The salt concentration dependence of APE/AP DNA binding was deter-
A kinetic dissociation analysis was performed to quantify the thermodynamic parameters associated with the APE/AP DNA interaction. The data were fit using the standard energetic equation, revealing the contribution of some hydrophobic and predominantly ionic forces in the APE/AP DNA interaction.

**DISCUSSION**

APE, a major DNA repair protein, plays an important role by initiating the repair of AP sites. A comprehensive understanding of the mechanisms of recognition and incision requires knowledge of the nature of the specific binding of APE to AP DNA, in particular, the origin of the binding mechanism. Thus, the fundamental thermodynamic characteristics of these interactions needed to be determined. In this study, we used surface plasmon resonance spectroscopy for quantitative equilibrium thermodynamic and kinetic analysis of the interaction between APE and AP DNA, using the standard energetic equations described under “Materials and Methods”; hence, we could determine the thermodynamic and kinetic parameters for this system. Notably, alternate methods used for this type of study, for example, are stopped-flow fluorescence measurements and isothermal titration calorimetry. However, these methods either are sensitive to solvent processes (heats of mixing and dilution and solvent protonation) or provide equilibrium analysis only.

Various repair proteins, including E. coli UvrA, a photolyase, interact with DNA adducts primarily through hydrophobic forces (32–34). However, the present study is the first to characterize the energetics of a BER protein.

APE binds very tightly with AP DNA. Different studies indicated a range of values for the equilibrium binding constant (\(K_D\)), but it is noted that this variation may have arisen through a dependence on methods and salt concentrations, which also varied (35–38). In this study, we used 150 mM NaCl or “near physiological” salt concentration, i.e. 90 mM KCl (39). Under these salt concentrations, binding was slightly favored in the presence of K\(^+\), indicating a minor effect of cation in binding of APE to AP DNA. In accordance with our results, a report by Samuel H. Wilson and colleagues (36) acknowledged that the values derived from a single study could be “considered as good indicators of relative binding affinities for APE at different temperature and salt concentration since they are performed (i.e. assayed) and determined (i.e. approach) under identical conditions.”

The process of APE binding to AP DNA appears to be spontaneous at all temperatures as both enthalpy and entropy are favorable. Dipole-dipole interactions and van der Waals interactions are often dominated by large enthalpy changes. Also, the positive \(\Delta S\) indicates a favorable reorganization, which is often the result of mobility, freedom, and structure of solvent (water molecules in this study) and the molecular groups in the interacting partners and bound complexes. Hydrophobic interactions are typically dominated by entropy changes. Varying concentration of KCl showed an increase in binding affinity at low concentration but complete abrogation of the binding at higher concentration, implying the importance of hydrophobic, but predominantly ionic, forces in the Michaelis-Menten complex formation. The temperature dependence of the interaction of APE with AP DNA (Fig. 2, A and B) demonstrates a linear van’t Hoff plot, which is attributed to the temperature independence of the \(\Delta C_p\) upon binding.

Other important factors, which may dominate in a chemical interaction, are the kinetic parameters. In many cases, even thermodynamically favorable reactions do not occur due to a high kinetic barrier. Therefore, measuring the equilibrium thermodynamic parameters alone may not be adequate and does not ensure that a process with high negative free energy change will actually take place. Moreover, the energy barrier is often the determining factor for many processes. Thus, to
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understand the mechanism of macromolecular interactions in its entirety, it is necessary to comprehend the energetics of the transition state.

In this study, the kinetics showed change in enthalpy of activation for the association process as being $\sim 17$ kJ/mol and activation energy for association, $E_a$ ($\sim 14$ kJ/mol), which is extremely low when compared with the change in enthalpy of activation of the reverse process. The largely favorable forward process can significantly facilitate the transition state. Note that the negative activation energy of a reaction is not very common, but in some cases, rates of reaction decrease with increasing temperature, resulting in a negative value of activation energy. Reactions exhibiting these negative activation energies are typically barrierless reactions. Increasing the temperature leads to a reduced probability that the colliding molecules will capture one another because more glancing collisions do not lead to reaction as the higher momentum expels the colliding particles out of the reaction point. Similar observations have been described for different systems, including RNA polymerase binding to its promoter DNA (40–44).

A recent study on APE enzyme kinetics (12) showed extremely fast (hard to measure even using the KinTek rapid quench instrument in constant quench mode) catalytic rates. The extremely high burst rate (>200 s$^{-1}$) signifies the requirement of stable Michaelis-Menten complex formation. The high formation (10$^{17}$ M$^{-1}$ sec$^{-1}$) and low dissociation rate constants (0.009 s$^{-1}$) of the Michaelis-Menten complex, found in our study, support its stability and efficient catalysis of the AP site by APE. However, the overall or net reaction rate ($\sim 2$ s$^{-1}$) depends on the rate at which APE1 can dissociate from product and catalyze subsequent rounds of incision. Thus, kinetically and thermodynamically highly stable Michaelis-Menten complex formation, as we found in our study, supports the high burst rate observed by others (12).

Consequently, the transition state is facilitated by a favorable change in enthalpy of activation due to the involvement of dipole-dipole and/or van der Waals interactions at the transition state. However, the large effect of salt concentration on the binding process indicates that the transition state is facilitated predominantly by dipole-dipole interactions. Since the binding was performed under constant pressure, $\Delta H^\ddagger$ can be considered as heat of activation ($\Delta Q^\ddagger$), and the observed minimal $\Delta Q^\ddagger$ ($\sim 17$ kJ/mol) could facilitate the transition state, presumably, by dipole-dipole interactions (Fig. 6). The next question surrounds the identification of the sources of these interactions. X-ray structural studies with several co-crystals of APE bound to AP DNA revealed that hydrogen bonding plays an important role in DNA-protein interactions. Particularly of notice are the Arg-177 side chain inserts through the kinked DNA major groove, which form a hydrogen bond to the non-target, 3’-phosphate of the AP site. The target AP site 5’-phosphate is also oriented by several hydrogen bonds with Asn-174, Asn-212, and His-309. Moreover, the Asp-210 is oriented by hydrogen bonds from the Asn-212 backbone amide and Asn-68 N82 (22). Therefore, multiple hydrogen bonds appear to play major roles in AP site recognition and formation of APE-AP DNA complex. Evidently, the hydrophobic face of the extrahelical AP site in the co-crystal packs within a complementary APE1 pocket formed by the side chains of Phe-166, Trp-280, and Leu-282. These may be the source of the hydrophobic interactions in partially entropically driven binding of APE to AP DNA.

Thus, our solution binding study, which reveals the importance of dipole-dipole interaction (a characteristic of hydrogen bonding) in the transition state, appears to be consistent with the X-ray crystallographic studies of AP site recognition by APE. Analogous future studies on the energetics and kinetics of other BER proteins to their cognate substrates could provide significant insight into the detailed mechanisms of the entire BER process.

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