Human Ribosomal Protein S3 Interacts with DNA Base Excision Repair Proteins hAPE/Ref-1 and hOGG1†

Vijay Hegde,‡ Mu Wang,§ and Walter A. Deutsch*,‡

Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana 70808, and Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46202

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ABSTRACT: The human ribosomal protein S3 (hS3) possesses associated activities that suggest alternative roles beyond its participation in protein translation. For example, it is capable of cleaving apurinic/apyrimidinic (AP) DNA via a β-elimination reaction, an activity that is missing in partially purified extracts of xeroderma pigmentosum group-D fibroblasts. In a recent study, we showed by surface plasmon resonance (SPR) that hS3 also has a very high apparent binding affinity for 7,8-dihydro-8-oxoguanine (8-oxoG) and AP sites in DNA. Using the same SPR technology, it is shown here that hS3 positively interacts with the human base excision repair (BER) enzymes *N*-glycosylase/AP lyase OGG1 and APE/Ref-1. Using a DNA substrate that allows for the detection of 8-oxoG repair, we also show that hOGG1 *N*-glycosylase activity becomes increasingly more robust in the presence of hS3. Human S3 was found to co-immunoprecipitate with both hOGG1 and APE/Ref-1, indicating that these proteins physically interact with one another. These results raise the possibility that hS3 not only functions as a ribosomal protein but, in addition, may influence repair activities at sites of DNA damage.

There are several pathways that exist in human cells for the removal of DNA damage. These pathways can generally be distinguished by their initial attack on DNA. For some, such as nucleotide excision repair (NER), this means the involvement of direct-acting nucleases that cleave DNA in the vicinity of the lesion, followed by a series of reactions that involve the removal of the DNA adduct, resynthesis of DNA to return it to its original, unmodified state, and DNA ligation. For DNA that is primarily modified by reactive oxygen species (ROS) and base deaminations, the initiating event is characterized by the action of an *N*-glycosylase that liberates the modified or nonconventional base from DNA, forming in its place an apurinic/apyrimidinic (AP)[1] site. Most *N*-glycosylases contain an intrinsic β-elimination, AP lyase activity capable of cleaving the existing AP site to form a 3′-AP site terminus (*1*). Alternatively, a major activity in human cells, APE/Ref-1, exists that cleaves DNA to form a 5′-AP terminus (*2*) that is conveniently removed by DNA polymerase β (*3*) to form a one nucleotide gap that is filled in by the same polymerase, followed by DNA ligation. The importance of this pathway, termed base excision repair (BER), is supported by the abundance of *N*-glycosylases that have thus far been found in prokaryotic and eukaryotic organisms; collectively, these *N*-glycosylase enzymes are able to deal with an impressive number of oxidized purines and pyrimidines and deamination events, which arise primarily through intracellular events that ultimately can lead to mutations and a predisposition toward the onset of certain forms of cancer.

A major product of ROS damage to DNA is the formation of 7,8-dihydro-8-oxoguanine (8-oxoG). This lesion frequently mispairs with adenine, resulting in G:C→T:A transversion mutations (*4*) that are common in somatic mutations of the tumor suppressor gene *p53* found in human lung cancers (*5*). One of several glycosylases that has the potential to counter the potential deleterious consequences of 8-oxoG in human cells is OGG1. Its in vitro characterization has revealed that it is not as proficient, however, in removing 8-oxoG when compared to bacterial formamidopyrimidine DNA glycosylase (FPG) and *Drosophila* S3 (dS3). Indeed, the specificity constant for the removal of 8-oxoG from a 25mer was found to be roughly 80-fold lower for hOGG1 as opposed to FPG (*6*). We recently reported that the apparent binding affinity (K_D) for 8-oxoG was 2 orders of magnitude greater for *Drosophila* S3 vs hOGG1 (*7*). Notably, human S3 was found to have an even higher binding affinity for 8-oxoG than its *Drosophila* homologue.

The poor catalytic properties of hOGG1, and the apparent inefficient removal of 8-oxoG residues from human DNA (*8*, *9*), have prompted some investigators to examine ways to make hOGG1 more catalytically robust. This has led to the observation that hAPE/Ref-1 is able to stimulate hOGG1 activity (*10*, *11*), presumably by preventing the reassociation of hOGG1 to an AP site formed by the liberation of 8-oxoG; this action results in APE/Ref-1 cleaving the AP site instead of the hOGG1 AP lyase activity.

In a recent study, we performed surface plasmon resonance (SPR) analyses on several proteins known to interact with

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* To whom correspondence should be addressed. Telephone: (225) 763-0937. Fax: (225) 763-3030. E-mail: deutscwa@pbrc.edu.

‡ Louisiana State University.

§ Indiana University School of Medicine.

[1] Abbreviations: AP, apurinic/apyrimidinic; APE, AP endonuclease; BER, base excision repair; GST, glutathione *S*-transferase; RU, resonance unit; Ref-1, redox factor 1; SPR, surface plasmon resonance.

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8-oxoG residues (*7*). As noted above, these studies revealed that hS3 possessed a much higher apparent binding affinity for 8-oxoG than either dS3 or hOGG1. This result was surprising since, unlike dS3 and hOGG1, hS3 lacks *N*-glycosylase activity for the removal of 8-oxoG (*12*). To our knowledge, this was the first demonstration of a DNA binding protein that recognized 8-oxoG residues in DNA. It was therefore tempting to speculate that hS3 might be acting in a manner similar to XPA/RPA (*13*, *14*) by localizing sites of DNA damage that are subsequently acted on by a coordinated pathway of DNA repair. While this indeed might be the case, when we performed SPR analysis with combinations of the BER proteins hOGG1 and APE/Ref-1, a different picture emerged. Here we show by SPR analysis that hS3 positively interacts with hOGG1 and APE/Ref-1. This positive interaction is further demonstrated by hS3 increasing the catalytic activity of hOGG1 on DNA oligonucleotides containing an embedded 8-oxoG residue. Last, we show through co-immunoprecipitation experiments that hS3 can be found physically associated with hOGG1 and APE/Ref-1.

EXPERIMENTAL PROCEDURES

Bacterial Strains for Overexpression. A strain defective for AP endonuclease IV *(nfo*) and the AP endonuclease activity associated with exonuclease III (*xth*) was used, namely RPC501 *nfo*-1::*kan*Δ(*xth-pnc*A) 90 (*15*).

Purified Proteins. GST-hS3 and GST-dS3 were purified as described below. GST-hOGG1 was purchased from Trevigen, Gaithersburg, MD. GST-hAPE/Ref-1 was a gift from Dr. Mark Kelley (Indiana University School of Medicine, Indianapolis, IN).

Overexpression of GST Fusion Constructs and Purification. GST-hS3 and GST-dS3 were purified exactly as described previously (*12*). This resulted in a homogeneous preparation of fusion construct as judged by SDS−PAGE and Coomassie staining (*7*). Protein was measured by the Bradford Coomassie Brilliant Blue dye method (*16*) using Pierce protein assay reagent (Pierce Chemical Co., Rockford, IL).

Activity on 8-Oxoguanine or Abasic Site Containing DNA. Reactions were performed as previously described (*12*). Briefly, a 37 bp ^{32}P 5′-end-labeled duplex DNA fragment 5′-CTT GGA CTG GAT GTC GGC ACX AGC GGA TAC AGG AGC A-3′, where X = 8-oxoguanine (8-oxoG-37mer) (Operon Technologies) or uracil (The Midland Certified Reagent Co.) at nucleotide position 21 (labeled strand), was used as a substrate either directly or treated (20 pmol) with *Escherichia coli* uracil DNA glycosylase (Epicenter, 2 units) to form an apyrimidinic (AP) site in place of the uracil (*17*). Following phenol/chloroform extraction, the AP-site-containing oligonucleotide (AP-37mer) was precipitated with cold ethanol.

Reaction mixtures (10 μL) contained ∼1 pmol of 5′-end-labeled 37mer; in addition, reactions for GST-hS3, GST-dS3, or GST-hOGG1 contained 30 mM HEPES, pH 7.4, 50 mM KCl, 1 μg/mL bovine serum albumin (BSA), 0.05% TritonX-100, 1 mM DTT, and 0.5 mM EDTA. Reactions for GST-hAPE/Ref-1 contained 50 mM HEPES, pH 7.5, 50 mM KCl, 1 μg/mL BSA, 10 mM $MgCl_2$, and 0.05% Triton X-100. The DNA reaction products were separated on a 16% polyacrylamide gel containing 7 M urea. Dried gels were subjected to autoradiography for visualization and densitometric analysis (ChemiImager 4000, Alpha Innotech Corporation). All DNA repair proteins used in the SPR analysis were first monitored for DNA activity on 5′-end-labeled 37mers containing either 8-oxoG or an abasic site. Roughly 1.3 nM hOGG1 converted 60% of the 8-oxoG substrate to product, whereas the same concentration of hS3 was totally inactive. On the other hand, the same concentration of hS3 completely converted the abasic-site-containing 37mer to product, as did hOGG1 and APE/Ref-1.

Protein-Damaged DNA Interaction: Surface Plasmon Resonance. Interactions of hS3, hOGG1, APE/Ref-1, and dS3 with 8-oxoG or abasic DNA substrates were monitored using a surface plasmon resonance biosensor instrument, Biacore 3000 (Biacore Inc, Uppsala, Sweden). For preparation of the biosensor surface with DNA, 5′-biotinylated 37mer duplex DNA's containing specific damages of either an 8-oxoG (8-oxoG-37mer) or an apyrimidinic site (AP-37mer) were generated as previously described (*7*, *12*). The damaged duplex DNA's were diluted in a buffer containing 10 mM sodium acetate, pH 4.8, and 1.0 M NaCl and manually injected onto a streptavidin-coated surface of a BIAcore sensor chip to the desired density in different flow cells. One flow cell was left underivatized to allow for refractive index change corrections, and another immobilized with undamaged DNA to allow for refractive index change corrections and nonspecific DNA binding, respectively. Interaction analysis was performed at 25 °C, and proteins were diluted in the running buffer containing 10 mM HEPES, pH 7.4, 150 mM NaCl, and 0.005% polysorbate-20. Following protein binding, regeneration was performed with a 30-second quick injection of 0.05% SDS in running buffer. This step resets RU values to initial approximate baseline values. Protein−protein interactions in the absence of DNA were also monitored using surface plasmon resonance. For preparation of the biosensor surface, 20 nM of hOGG1 was covalently linked to a CM5 sensor chip surface through amine-coupling reaction to about 1700 RU. One flow cell was left underivatized to allow for refractive index change corrections. Interaction analysis was performed at 25 °C, and proteins were diluted in the running buffer containing 10 mM Hepes, pH 7.4, 150 mM NaCl, and 0.005% polysorbate-20. Following protein binding, regeneration was performed with a 30-s quick injection of 1 M NaCl in running buffer. This step resets RU values to initial approximate baseline values.

Surface Plasmon Resonance Analysis. To estimate the apparent association and dissociation rate constants, we used the BIA-evaluation software supplied by the vendor (Biacore Inc, Uppsala, Sweden) as described previously (*7*). When experiments were conducted in the presence of 8-oxoG- or abasic-containing DNA, an undamaged DNA substrate was used as a control. All sensorgram curves were subtracted using the curves obtained from the underivatized flow cells (*7*).

To determine whether a positive interaction existed between the BER proteins studied here, we first performed individual binding experiments for each protein (32 nM) using a 1500-RU DNA surface with an 8-oxoG or AP modification. For hS3 binding to an 8-oxoG or an AP site, an RU response of 329 and 217, respectively, was observed.

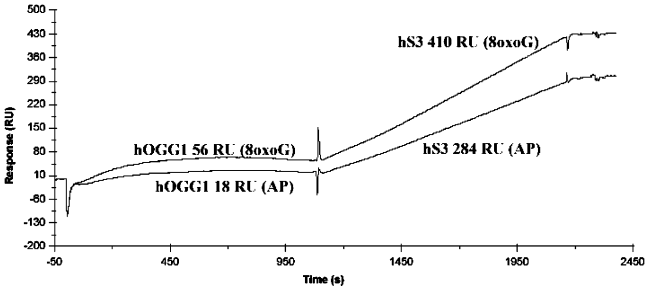

FIGURE 1: Binding response following sequential injection of 32 nM hOGG1 and hS3 on a 1500-RU DNA surface with an 8-oxoG or AP modification. The proteins were injected using the COINJECT function of Biacore 3000. The proteins (90 μL for each protein) were injected at 5 μL/min in tandem. Following association of hOGG1 over the immobilized 8-oxoG or AP 37mer oligonucleotide, hS3 was injected immediately and allowed to bind to the DNA. The binding reaction was completed by injection of running buffer (30 μL) at 5 μL/min for 6 min.

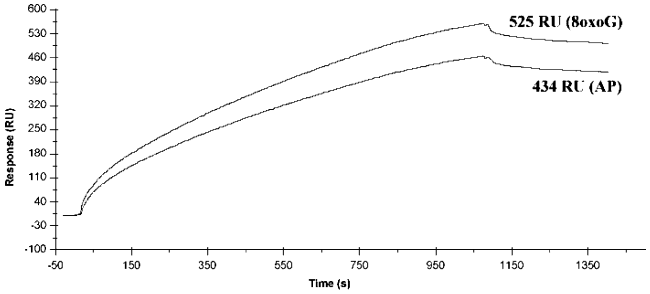

FIGURE 2: Effect of simultaneous injection of 32 nM hS3 and hOGG1 on a 1500 RU-DNA surface with an 8-oxoG or AP modification. Human S3 and hOGG1 were mixed at room temperature for 5 min and then injected on the DNA surface using the KINJECT function of Biacore 3000. The protein mix (90 μL) was injected at 5 μL/min for association followed by 30 μL of buffer injection at 5 μL/min for dissociation.

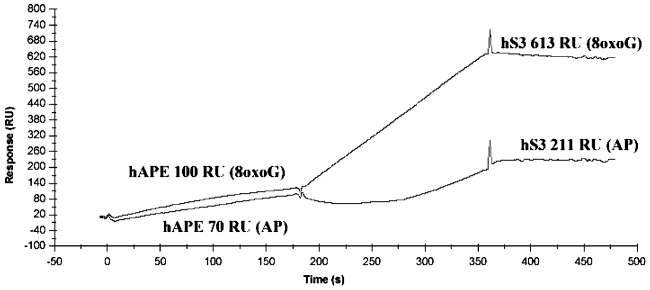

FIGURE 3: Effect of binding order of hAPE/Ref-1 and hS3 to 8-oxoG or abasic (AP)DNA. Injection of hAPE/Ref-1 (32 nM; 180 s) was followed by a subsequent injection of hS3 (32 nM; 180 s) on a 1500-RU DNA surface. The protein association for each protein was allowed for 180 s at 30 μL/min followed by a 120-s wash of buffer injection at 30 μL/min for dissociation.

For hOGG1, an RU response was 56 on an 8-oxoG substrate and 20 on an AP-containing DNA substrate. APE/Ref-1 produced an RU response of 100 on an 8-oxoG substrate and one of 70 on an AP substrate. For the experiments presented here, it was concluded that two proteins acted independently of one another if the final RU response was not substantially different than the combination of their individual responses identified above. In those experiments where one protein was sequentially added after another was bound to the chip, an expected final RU value is obtained from the sum of the RU values of two independent binding events.

Co-Immunoprecipitation of GST-hS3 with GST-hOGG1 or GST-hAPE/Ref-1. GST-hS3 (1 μg) was incubated with either 1 μg of the BER enzymes GST-hOGG1 or GST-hAPE/Ref-1 or 50 μg of nuclear extract from 293 cells in the presence of 2 μg of anti GST or anti-BER antibody at 4 °C for 4 h. Seventy microliters of 50% (v/v) protein A-Sepharose 4B (Zymed Laboratories, San Francisco, CA) was added overnight and incubated at 4 °C with rocking. The immune complexes were washed three times with 1× PBS and solubilized with 2× Laemmli sample buffer (30 μL) by heating at 95 °C for 5 min. The samples were fractionated by 12% SDS−PAGE electrophoresis and transferred to a nitrocellulose membrane. The membrane was Western blotted with either anti GST (Zymed Laboratories, San Francisco, CA) or anti-hAPE/Ref-1 antibody (Novus Biologicals, Littleton, CO), and detected by ECL plus Western blotting detection reagents.

RESULTS

Interaction of hS3 and hOGG1 by SPR Analysis. To examine the possible interaction of hS3 and hOGG1, three sets of experiments were performed using a DNA sensor chip with 1500-RU immobilized on its surface containing a single 8-oxoG or AP DNA lesion. In the first experiment, hOGG1 was first injected over the 8-oxoG- or AP-containing DNA, and after association of hOGG1, hS3 was added using the COINJECT function of the Biacore instrument. Under these reaction conditions (Figure 1), the sum of the interactions appeared to be additive for 8-oxoG (expected RUs = 385). On the other hand, when an AP substrate was tested, we found a modest increase from what would be expected (RUs = 237) if the proteins had acted independently from one another (Figure 1). When the order of addition was reversed (hS3 was injected first, followed by hOGG1), the binding events were found to be additive for both DNA substrates (not shown).

When the hS3 and hOGG1 proteins were preincubated for 5 min at room temperature before injection, the sensorgram showed an increased binding signal to the damaged DNA's, indicating that these two proteins may physically interact with one another, which was for the most part not revealed by the experiments where one protein was sequentially added after the binding of the first (Figure 1). As seen in Figure 2, for an 8-oxoG substrate, the binding signal increased from an expected RU value of 385 to an RU value of 525. DNA substrates containing an AP site revealed that the combination of hS3 and hOGG1 also resulted in an increase in the RU signal from an expected value of 237 to the observed final RU value of 434.

Interaction of hS3 and hAPE/Ref-1. When the hAPE/Ref-1 injection was followed by hS3 on an 8-oxoG template, the RU response rose from an expected RU value of 429 to 613, suggesting that hS3 interacts with hAPE/Ref-1 (Figure 3). For the AP substrate, the expected RU value was 287, which in fact is somewhat greater than what was observed. When the conditions were reversed, in which injection of hAPE/Ref-1 was followed by hS3, the binding responses were strictly additive (not shown).

As seen in Figure 4, preincubations combining both hS3 and APE/Ref-1, prior to exposure to damaged DNA, led to an increase on the 8-oxoG substrate, from an expected RU

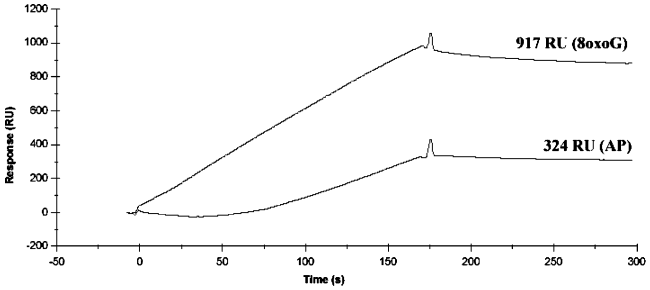

FIGURE 4: Binding affinity of a mixture of hS3 and hAPE/Ref-1 on a 1500-RU DNA surface containing 8-oxoG or an abasic (AP) substrate. The KINJECT function of Biacore 3000 was used to inject a mixture of 32 nM hS3 and hAPE/Ref-1 on the DNA surface. The protein association was allowed for 180 s followed by 120 s of buffer injection for dissociation.

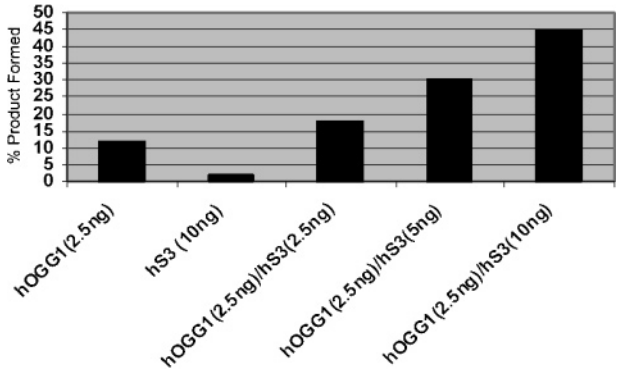

FIGURE 5: hS3 stimulates the *N*-glycosylase activity of hOGG1 on 8-oxoG-containing DNA substrate. An 8-oxoG-containing DNA substrate (~1 pmol) was incubated with 2.5 ng (0.05 pmol) of hOGG1 with increasing amounts of hS3: 2.5 ng (0.05 pmol), 5 ng (0.1 pmol), and 10 ng (0.2 pmol), respectively. This experiment was repeated three times, and the data presented are representative of those three trials.

value of 429 to a value of 917. For the AP substrate, only a modest increase was detected from an expected RU value of 287 to an observed value of 324.

Stimulation of hOGG1 N-Glycosylase Activity by hS3. On the basis of the results obtained by SPR analysis, we questioned whether the positive interactions observed between hS3 and hOGG1 in fact translated into hOGG1 becoming more efficient in the removal of 8-oxoG residues residing in a ^{32}P 5′-end labeled 37mer. Similar to the SPR analyses performed above, our analysis included three approaches that included the 8-oxoG-37mer preincubated with hS3 or preincubated with hOGG1 and conditions where hS3 and hOGG1 were preincubated together prior to the addition of DNA. In all situations, a constant amount of hOGG1 was used with increasing amounts of hS3. These experiments revealed that hS3 does in fact lead to a nearly 4-fold increase in the removal of 8-oxoG, but for the most part only when hOGG1 and hS3 are preincubated together prior to the addition of damaged DNA (Figure 5). That hS3 lacks *N*-glycosylase activity for the removal of 8-oxoG (*12*) and constant amounts of hOGG1 were used in this experiment lead to the conclusion that hS3 is somehow influencing hOGG1 to become more active, perhaps by accelerating the turnover or k_{cat} of hOGG1. Similar attempts to see if hS3 stimulated hAPE/Ref-1 activity on an AP-37mer were inconclusive. This is because APE/Ref-1 produces a hydrolytic DNA product that migrates between the β- and

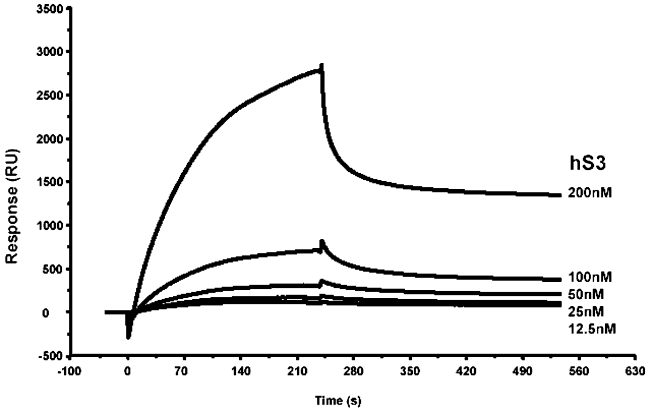

FIGURE 6: Analysis of hS3 binding to hOGG1 by SPR analysis. For preparation of the BIA sensor chip surface, 20 nM of hOGG1 was covalently linked to the CM5 sensor chip surface through amine-coupling reaction to about 1700 RU. One flow cell was left underivatized to allow for refractive index change corrections. Interaction analysis was performed at 25 °C and proteins were diluted in the running buffer containing 10 mM Hepes, pH 7.4, 150 mM NaCl, and 0.005% polysorbate-20. Following protein binding, regeneration was performed with a 30-second quick injection of 1 M NaCl in running buffer. This step resets RU values to initial approximate baseline values. Five different hS3 concentrations were used to obtain the kinetic parameters for hS3 and hOGG1 interaction.

δ-elimination products produced by hS3. The close migration of these products therefore made the results difficult to interpret.

Is the Interaction between hS3 and BER Proteins DNA-Dependent? For the most part, the ability of hS3 to stimulate hOGG1, as well as hAPE/Ref-1, appeared in the above experiments to depend on preincubations that were conducted in the absence of DNA. To test whether the interactions we observed were in fact DNA-dependent, we turned to SPR analysis, where any of the three proteins being studied here was first immobilized on a BIAcore sensor chip and its interaction with a second protein was established when a change in the response units (RU) on the chip surface was observed. When hS3 was covalently linked to the chip, no binding to either hOGG1 or hAPE/Ref-1 was observed. Likewise, when hAPE/Ref-1 was covalently linked to the chip, no binding to hS3 was observed. However, when hOGG1 was covalently linked to the chip, a substantial increase in binding was observed (Figure 6). The apparent association rate constant (k_a) was 1.26×10^5/M/s. The apparent dissociation rate constant (k_d) was 3.45×10^{-3}/s. The equilibrium dissociation constant (K_D), determined from the ratio of these two kinetic constants (k_d/k_a) was 2.75×10^{-8} M.

Co-immunoprecipitation of hS3 with hOGG1 and hAPE/Ref-1. Overall, the experiments presented above show compelling evidence for the strong interaction of hS3 and the BER enzymes hOGG1 and APE/Ref-1. We next questioned whether a physical interaction could be shown between hS3 and hOGG1/APE/Ref-1 using a more classic method, such as co-immunoprecipitation followed by Western blot. Our first attempts examined the possible interaction between GST-hS3 and GST-hOGG1 and whether they formed a complex that can be co-immunoprecipitated. Conditions were chosen in which GST-hS3 and GST-hOGG1 were mixed at 4 °C for 4 h with a monoclonal antibody prepared against hOGG1. Protein A Sepharose 4B was added

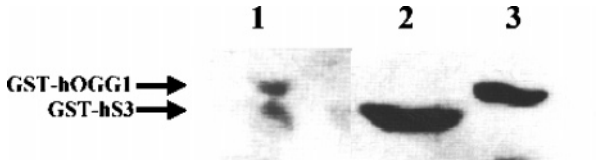

FIGURE 7: Co-immunoprecipitation of GST-hS3 and GST-hOGG1. A monoclonal antibody prepared against hOGG1 was used to co-immunoprecipitate (Co-IP) GST-hS3 and GST-hOGG1. The immunoprecipitated proteins were Western blotted with a monoclonal antibody against GST. Lane 1 shows the co-immunoprecipitated GST-hS3−GST-hOGG1, and lanes 2 and 3 show the GST-hS3 and GST-hOGG1 purified proteins, respectively.

and incubated overnight at 4 °C. The immune complexes were washed four times with PBS and then solubilized with 2× Laemmli sample buffer. The samples were fractionated on a 12% SDS−PAGE, transferred to nitrocellulose, and then probed with an anti-GST monoclonal antibody. As seen in Figure 7, this method proved successful in showing the co-immunoprecipitation of hS3 and hOGG1 (lane 1).

A similar co-immunoprecipitation was performed to test whether in fact hS3 also physically interacts with hAPE/Ref-1. In this case, GST-hS3 and GST-APE/Ref-1 were combined, and then immunoprecipitated with antibody to APE/Ref-1. Westerns were then performed using antibody to GST. As can be seen in Figure 8, two proteins were co-immunoprecipitated by the APE/Ref-1 antibody (lane 1) that correspond in molecular weight to GST-hS3 (lane 2) and GST-APE/Ref-1 (lane 3). We also performed a co-immunoprecipitation with GST-hS3 and nuclear extracts of human embryonic kidney (HEK) 293 cells, once again immunoprecipitating with antibody to APE/Ref-1. The blot was first probed with the GST antibody, in which the presence of GST-hS3 is clearly present (upper band, lane 4). The blot was then stripped and re-probed with antibody to APE/Ref-1, in which endogenous APE/Ref-1 is clearly visible (for clarity we have merged the GST and APE/Ref-1 antibody probes into a single lane, 4). The remaining lane (5) is nuclear extracts of 293 cells probed with the APE/Ref-1 antibody.

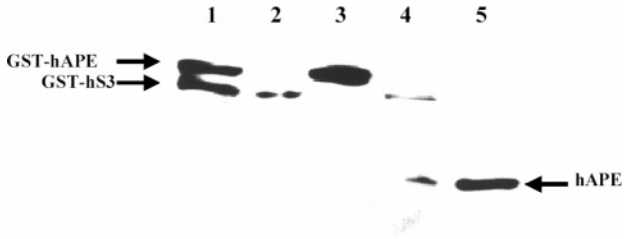

FIGURE 8: Co-immunoprecipitation of mixed GST-hS3−GST-hAPE or in HEK 293 nuclear extracts. A monoclonal antibody prepared against hAPE/Ref-1 was used to co-immunoprecipitate (Co-IP) GST-hS3 and GST-hAPE/Ref-1, also GST-hS3 and HEK 293 nuclear extract. The immunoprecipitated proteins were Western blotted with a monoclonal antibody against GST and a monoclonal antibody against hAPE/Ref-1. Lane 1 shows the co-immunoprecipitated GST-hS3−GST-hAPE, and lanes 2 and 3 show the GST-hS3 and GST-hAPE/Ref-1 purified proteins respectively Western blotted with GST antibody. Lane 4 shows the co-immunoprecipitated GST-hS3 and HEK 293 nuclear extract. The membrane was first blotted with the GST antibody, stripped, and reblotted with hAPE/Ref-1 antibody. For clarity, we have merged the GST and APE/Ref-1 antibody probes into a single lane, 4. Lane 5 shows the HEK 293 nuclear extract Western blotted with hAPE/Ref-1 antibody.

DISCUSSION

Human S3 was originally defined as a protein that was part of a ribosomal domain for the initiation of protein translation. It can be cross-linked to eIF-2 and eIF-3 and appears to be directly involved in mRNA-aminoacyl tRNA interactions during protein synthesis (*18*). Then, Linn and co-workers found that one of the AP lyases they had characterized in human cells (*19*) was in fact hS3 (*20*). In an earlier study, it was determined that this same AP lyase activity was absent in xeroderma pigmentosum group D fibroblasts (*21*), which may be linked to its inability to bind DNA-containing AP sites (Deutsch, unpublished observation). Subsequent studies have shown that hS3 contains an iron−sulfur cluster that is reminiscent of the abasic β-elimination catalyst *E. coli* endonuclease III (*22*).

We have found that the *Drosophila* homologue of hS3 has robust *N*-glycosylase activity for the removal of 8-oxoG residues residing in a ^{32}P 5′-end labeled 37mer oligonucleotide (*23*, *24*). Human S3, on the other hand, lacks this activity (*12*), even though it is 80% identical to *Drosophila* S3 (*25*). Nevertheless, hS3 has an apparent binding affinity (K_D) for 8-oxoG of 4.82×10^{-11} M, which is an order of magnitude greater than that of *Drosophila* S3 and 3 orders of magnitude greater than that of hOGG1 (*7*). Human S3 has a 3.2×10^{-13} M apparent K_D for an AP site, which is 2 orders of magnitude greater than that of *Drosophila* S3 and roughly 5 orders of magnitude greater than those of hOGG1 and APE/Ref-1 (*7*). Although there are a limited number of studies where DNA binding proteins have been subjected to SPR analysis, to our knowledge hS3 possesses one of the highest apparent K_D values thus far measured for binding to a damaged DNA site, the closest being replication protein A at $K_D = 2.02 \times 10^{-10}$ M (*26*).

In the present study, we were interested in determining whether hS3 interacted with two different proteins that have an established role in BER. Using SPR technology, a positive interaction between hS3 and either of the BER proteins would only be established if the response unit (RU) established by SPR for the presence of two proteins would be greater than the combination of the binding response values established for each protein acting alone. Many of our tests in fact failed to show that the expected response units involving two proteins under different conditions were no greater than the additive combination of RU values determined for proteins interacting with DNA on their own. The most obvious exception to this, however, was when hS3 was combined with hOGG1 or hAPE/Ref-1 prior to their injection into the Biacore sensor chip. In these cases, binding responses increased roughly 1.5−2-fold above that expected if the proteins acted independently. These results suggest that hS3 interacts with these BER proteins prior to any transactions with DNA. The same appears to be the case for XPC, which physically interacts with XPA independent of DNA (*27*). Likewise, tests performed in the present study appear to indicate that the interaction between hS3 and hOGG1 is also DNA independent. The interaction between hS3 and APE/Ref-1 is less clear as to its dependence on DNA, since we failed to show any positive interaction between these two proteins under conditions in which one was immobilized on a sensor chip. This could be due to other factors beyond a strict dependence upon the presence of DNA, including the possible masking of potential interaction sites as a result of

the covalent linkage to the sensor chip.

The ability of hS3 to positively influence hOGG1 was further demonstrated using a ^{32}P end-labeled 37mer containing a single 8-oxoG site. Reactions containing a constant amount of hOGG1, but increasing amounts of hS3, led to a roughly 4-fold increase in the amount of 8-oxoG liberated by hOGG1. We previously have shown that hS3, at levels 10 times that used here (*12*), possessed only negligible amounts of *N*-glycosylase activity for 8-oxoG lesions. Thus, we conclude that the results provided here could only be due to hS3 positively influencing the *N*-glycosylase catalytic activity of hOGG1.

We performed co-immunoprecipitation experiments to determine if the results obtained by SPR analysis were due to the existence of a physical interaction between hS3 and the BER proteins. For testing OGG1 and hS3 interactions, immunoprecipitation was performed with a monoclonal to hOGG1, followed by Western analysis using an antibody specific for GST. Interactions involving hS3 and hAPE/Ref-1 used antibody specific for hAPE/Ref-1 for immunoprecipitation, followed once again by Western analysis using antibody to GST. Both tests showed that hS3 could be captured in a complex with the BER proteins. Perhaps most compelling in regards to the association of these proteins was the finding that hS3 and hAPE/Ref-1 were co-immunoprecipitated by antibody to hAPE/Ref-1 in nuclear extracts of HEK 293 cells. Thus, concerns regarding whether the stickiness some ribosomal proteins possess contributed to the mixing experiments were ruled out.

The results presented here, and elsewhere (*7*, *12*), show that hS3 binds to sites of DNA damage and physically interact with proteins known to be involved in their repair. It remains unclear, however, whether the DNA binding and protein−protein interactions act in concert with one another. On the basis of our SPR interaction studies, it appears that hS3 is most effective in stimulating both hAPE/Ref-1 and hOGG1 binding activity before exposure to DNA. Thus, it is unlikely, on the basis of our results, that hS3 is replacing hOGG1 (or APE/Ref-1) on an AP-damaged DNA substrate, as appears to be the case for hAPE/Ref-1 and its influence on hOGG1 (*10*, *11*). We also show here that hS3 positively affects the catalytic *N*-glycosylase activity of hOGG1 in removing 8-oxoG from a synthetic DNA oligonucleotide. Overall, our results point to hS3 positively affecting the repair of DNA damage recognized by BER. Conversely, the extremely high apparent binding affinity of hS3 to 8-oxoG and AP sites (*7*) cannot be overlooked, since our data suggests that once this occurs, the benefits to BER appear to be minimal. Indeed, it would not be unexpected to find that hS3, with its high binding affinity to 8-oxoG, could in fact create an obstacle to the BER pathway accessing 8-oxoG. In summary, our data suggest that hS3 can provide a benefit to BER enzymes through protein−protein interactions, but if hS3 reaches a site of DNA damage, those positive effects could be negated by hS3 creating an obstacle to other enzymes destined to interact with the same site of DNA damage.

REFERENCES

1. Dodson, M. L., Michaels, M., and Lloyd, R. S. (1994) Unified catalytic mechanism for DNA glycosylases. *J. Biol. Chem. 269*, 32709−32712.
2. Warner, H. R., Demple, B. F., Deutsch, W. A., Kane, C. M., and Linn, S. (1980) Apurinic/apyrimidinic endonucleases in repair of pyrimidine dimers and other lesions in DNA. *Proc. Natl. Acad. Sci. USA 77*, 4602−4606.
3. Matsumoto, Y., and Kim, K. (1995) Excision of deoxyribose phosphate residues by DNA polymerase beta during DNA repair. *Science 269*, 699−702.
4. Grollman, A. P., and Moriya, M. (1993) Mutagenesis by 8-oxoguanine: An enemy within. *Trends Genet. 9*, 246−249.
5. Hollstein, M., Shomer, B., Greenblatt, M., Soussi, T., Hovig, E., Montesano, R., and Harris, C. C. (1996) Somatic point mutations in the p53 gene of human tumors and cell lines: Updated compilation. *Nucleic Acids Res. 24*, 141−146.
6. Asagoshi, K., Yamada, T., Terato, H., Ohyama, Y., Monden, Y., Arai, T., Nishimura, S., Aburatani, H., Lindahl, T., and Ide, H. (2000) Distinct repair activities of human 7,8-dihydro-8-oxoguanine DNA glycosylase and formamidopyrimidine DNA glycosylase for formamidopyrimidine and 7,8-dihydro-8-oxoguanine. *J. Biol. Chem. 275*, 4956−4964.
7. Hegde, V., Mu. W, and Deutsch, W. A. (2003) Characterization of human ribosomal protein S3 binding to 7, 8-dihydro-8-oxoguanine and abasic sites by surface plasmon resonance. *DNA Repair (Amst) 3*, 121−126.
8. Cappelli, E., Degan, P., and Frosina, G. (2000) Comparative repair of the endogenous lesions 8-oxo-7, 8-dihydroguanine (8-oxoG), uracil and abasic site by mammalian cell extracts: 8-oxoG is poorly repaired by human cell extracts. *Carcinogenesis 21*, 1135−1141.
9. Jaruga, P., and Dizdaroglu, M. (1996) Repair of products of oxidative DNA base damage in human cells. *Nucleic Acids Res 24*, 1389−1394.
10. Vidal, A. E., Hickson, I. D., Boiteux, S., and Radicella, J. P. (2001) Mechanism of stimulation of the DNA glycosylase activity of hOGG1 by the major human AP endonuclease: Bypass of the AP lyase activity step. *Nucleic Acids Res. 29*, 1285−1292.
11. Hill, J. W., Hazra, T. K., Izumi, T., and Mitra, S. (2001) Stimulation of human 8-oxoguanine-DNA glycosylase by AP-endonuclease: Potential coordination of the initial steps in base excision repair. *Nucleic Acids Res. 29*, 430−438.
12. Hegde, V., Kelley, M. R., Xu, Y., Mian, I. S., and Deutsch, W. A. (2001) Conversion of the bifunctional 8-oxoguanine/beta-delta apurinic/apyrimidinic DNA repair activities of Drosophila ribosomal protein S3 into the human S3 monofunctional beta-elimination catalyst through a single amino acid change. *J. Biol. Chem. 276*, 27591−27596.
13. Matsuda, T., Saijo, M., Kuraoka, I., Kobayashi, T., Nakatsu, Y., Nagai, A., Enjoji, T., Masutani, C., Sugasawa, K., Hanaoka, F., and et al. (1995) DNA repair protein XPA binds replication protein A (RPA). *J. Biol. Chem. 270*, 4152−4157.
14. Vasquez, K. M., Christensen, J., Li, L., Finch, R. A., and Glazer, P. M. (2002) Human XPA and RPA DNA repair proteins participate in specific recognition of triplex-induced helical distortions. *Proc. Natl. Acad. Sci. USA 99*, 5848−5853.
15. Cunningham, R. P., Saporito, S. M., Spitizer, S. G., and Weiss, B. (1986) Endonuclease IV (*nfo*) mutant of *Escherichia coli*. *J. Bacteriol. 168*, 1120−1127.
16. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem. 72*, 248−254.
17. Lindahl, T. (1980) Uracil-DNA glycosylase from *Escherichia coli*. *Methods Enzymol. 65*, 284−295.
18. Bommer, U. A., Lutsch, G., Stahl, J., and Bielka, H. (1991) Eukaryotic initiation factors eIF-2 and eIF-3: Interactions, structure, and localization in ribosomal initiation complexes. *Biochimie 73*, 1007−1019.
19. Kane, C. M., and Linn, S. (1981) Purification and characterization of an apurinic/apyrimidinic endonuclease from HeLa cells. *J. Biol. Chem. 256*, 3405−3414.
20. Kim, J., Chubatsu, L. S., Admon, A., Stahl, J., Fellows, R., and Linn, S. (1995) Implication of mammalian ribosomal protein S3 in the processing of DNA damage. *J. Biol. Chem. 270*, 13620−13629.
21. Kuhnlein, U., Lee, B., Penhoet, E. E., and Linn, S. (1978) Xeroderma pigmentosum fibroblasts of the D group lack an apurinic DNA endonuclease species with a low apparent Km. *Nucleic Acids Res. 5*, 951−960.

22. Lee, C. H., Kim, S. H., Choi, J. I., Choi, J. Y., Lee, C. E., and Kim, J. (2002) Electron paramagnetic resonance study reveals a putative iron−sulfur cluster in human rpS3 protein. *Mol Cells 13*, 154−156.
23. Yacoub, A., Augeri, L., Kelley, M. R., Doetsch, P. W., and Deutsch, W. A. (1996) A Drosophila ribosomal protein contains 8-oxoguanine and abasic site DNA repair activities. *Embo J. 15*, 2306−2312.
24. Deutsch, W. A., Yacoub, A., Jaruga, P., Zastawny, T. H., and Dizdaroglu, M. (1997) Characterization and mechanism of action of Drosophila ribosomal protein S3 DNA glycosylase activity for the removal of oxidatively damaged DNA bases. *J. Biol. Chem. 272*, 32857−32860.
25. Wilson, D. M. I., Deutsch, W. A., and Kelley, M. R. (1993) Cloning of the *Drosophila* ribosomal protein S3: Another multifunctional ribosomal protein with AP endonuclease DNA repair activity. *Nucleic Acids Res. 21*, 2516.
26. Wang, M., Mahrenholz, A., and Lee, S. H. (2000) RPA stabilizes the XPA-damaged DNA complex through protein−protein interaction. *Biochemistry 39*, 6433−6439.
27. You, J. S., Wang, M., and Lee, S. H. (2003) Biochemical analysis of the damage recognition process in nucleotide excision repair. *J. Biol. Chem. 278*, 7476−7485.

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