Nitric Oxide-Mediated Inhibition of Hdm2−p53 Binding†

Christopher M. Schonhoff,‡ Marie-Claire Daou,‡ Stephen N. Jones,§ Celia A. Schiffer,‡ and Alonzo H. Ross*,‡

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, Massachusetts 01605, and Department of Cell Biology, University of Massachusetts Medical School, 55 Lake Avenue, Worcester, Massachusetts 01655

Received June 7, 2002; Revised Manuscript Received September 17, 2002

ABSTRACT: It has become increasingly evident that nitric oxide exerts its effects, in part, by S-nitrosylation of cysteine residues. We tested in vitro whether nitric oxide may indirectly control p53 by S-nitrosylation and inactivation of the p53 negative regulator, Hdm2. Treatment of Hdm2 with a nitric oxide donor inhibits Hdm2−p53 binding, a critical step in Hdm2 regulation of p53. The presence of excess amounts of cysteine or dithiothreitol blocks this inhibition of binding. Moreover, nitric oxide inhibition of Hdm2−p53 binding was found to be reversible. Sulfhydryl sensitivity and reversibility are consistent with nitrosylation. Finally, we have identified a critical cysteine residue that nitric oxide modifies to disrupt Hdm2−p53 binding. This cysteine is proximal to the Hdm2−p53 binding interface and is conserved across species from zebrafish to humans. Mutation of this residue from a cysteine to an alanine does not interfere with binding but rather eliminates the sensitivity of Hdm2 to nitric oxide inactivation.

The tumor suppressor p53 is an important regulatory molecule that is involved in cell cycle arrest during development and acts as a mediator of apoptosis in response to stress (*1*−*3*). Mutation of p53 is one of the most common genetic lesions in human cancer. Although p53 is dispensable for normal cell growth and development, cells lacking functional p53 show a predisposition to cell proliferation, chromosomal abnormalities, and tumorigenesis, suggesting that p53 plays a critical role in the maintenance of genome integrity (*2*). p53 functions as a transcription factor that activates transcription of genes involved in cell cycle arrest (p21WAF1 and cyclin G), DNA damage (GADD45), and apoptosis (Bax) (*2*). Mdm2 is another transcriptional target of p53, and its interactions with p53 are the focus of this study (*4*, *5*).

The mdm2 gene was first detected in a screen for oncogenes present on a mouse double minute chromosome (*6*). The human homologue of the gene, Hdm2, is amplified and/or overexpressed in a number of human tumors (*7*). Hdm2 can regulate p53 by binding to p53 and inibiting its transcriptional activity (*8*). Hdm2 also regulates p53 levels by acting as an E3 ligase to ubiquitinate p53 and, thereby, promotes p53 degradation (*9*). Thus, Hdm2 participates in a negative feedback loop that keeps p53 levels in tight check.

Nitric oxide is a ubiquitous signaling molecule with diverse and varied functions. NO[1] has been shown to be a signal that increases p53 levels in physiological and pathological situations. During neuronal differentiation of PC12 cells, NO acts as a cytostatic agent by inducing p53 activity (*10*). Similarly, in vascular smooth muscle cells, NO activates p21WAF1 through p53 (*11*). During apoptosis, NO plays a critical role in p53 induction, but the molecular mechanism of this induction is not known (*12*, *13*).

One mechanism by which nitric oxide functions in cells is through S-nitrosylation of cysteine residues. In the case of Ras, S-nitrosylation enhances its activity (*14*). For other proteins, though, S-nitrosylation is an inactivating modification. Caspase-3 is inactivated by S-nitrosylation in vivo (*15*). Multiple classes of proteins, including receptors, proteases, and transcription factors, are reportedly S-nitrosylated (*16*)

It has recently been shown that Hdm2−p53 binding is sensitive to oxidation and that the sulfhydryl-modifying agent *N*-ethylmaleimide destroys the binding capacity for binding of Hdm2 to p53 (*17*). In this study, we test whether nitric oxide also affects the ability of Hdm2 to bind p53. We find that NO reacts with Hdm2 and thereby inhibits Hdm2−p53 binding. Moreover, we demonstrate that this inhibition is reversible, consistent with nitrosylation of cysteine. Finally, we identify a cysteine residue proximal to the Hdm2−p53 binding domain that is responsible for the NO inhibition of binding.

MATERIALS AND METHODS

Reagents. Dithiothreitol (DTT), *N*-ethylmaleimide, cysteine, reduced glutathione, and tetramethylbenzidine (TMB) dihydrochloride tablets were all purchased from Sigma. DETA/NO (NOC-18) was purchased from Alexis.

GST−Hdm2. The GST−Hdm2 plasmid (amino acids 1−188) was a gift from D. Lane (*18*). Bacterial cultures were grown to an OD of 0.8. They were cooled to room temperature, induced with 1 mM IPTG, and then grown for

† This work was supported by NIH Grants NS21716, CA68426, and CA77735.

* To whom correspondence should be addressed: Room 319, 364 Plantation St., Worcester, MA 01605. E-mail: Alonzo.Ross@umassmed.edu. Phone: (508) 856-8016.

‡ Department of Biochemistry and Molecular Pharmacology.

§ Department of Cell Biology.

[1] Abbreviations: DETA/NO, (*Z*)-1-[(2-aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium, 2-diolate; DTT, dithiothreitol; NO, nitric oxide; NEM, *N*-ethylmaleimide; IPTG, isopropyl β-D-thiogalactopyranoside; PBS, phosphate-buffered saline; PBST-M, PBS-Tween and milk; SEM, standard error of the mean; TMB, tetramethylbenzidine.

10.1021/bi026262q CCC: $22.00
Published on Web 10/25/2002

4 h at 27 °C. Cells were harvested, and pellets were flash-frozen in liquid nitrogen. Pellets were resuspended in ice-cold buffer A [0.5 M NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 1 mM PMSF, 1 mM EDTA, and 10 mM 2-mercaptoethanol (pH 7.3)] and lysed twice in a French press. After centrifugation, the soluble fraction was incubated with 1 mL of glutathione−Sepharose 4B beads for 1 h at room temperature and then overnight at 4 °C. The entire mixture was then spun and washed six times with PBS, and then the beads were packed in a column. The protein was then eluted with buffer B [50 mM Tris-HCl, 10 mM reduced glutathione, 0.5 M NaCl, 1 mM EDTA, 1 mM PMSF, and 10 mM 2-mercaptoethanol (pH 8.0)]. Fractions containing the GST−Hdm2 fusion were pooled, aliquoted, and stored at −20 °C.

Preparation of p53 Protein in Sf9 Cells. Sf9 cells were infected with a His-tagged, full-length p53 baculovirus (*19*), and cells were harvested 48 h after infection. The cells were extracted at 4 °C with 1% Triton X-100, 150 mM NaCl, 50 mM Tris-HCl (pH 7.5), and 1 mM PMSF for 30 min. After centrifugation, the extract was passed through a 0.45 μm filter. The p53 protein was purified on a His-Bind Quick 900 Cartridge (Novagen). Protein expression and purity were checked by Western blotting with a monoclonal anti-six-His antibody (Clontech). Purified p53 was aliquoted and stored at −20 °C.

ELISA. The Hdm2−p53 interaction was assayed by using an ELISA similar to one described previously (*18*). EIA assay plates (Costar) were coated overnight at 4 °C with 1 μg/mL p53 in PBS. Plates were blocked with PBST-M (PBS containing 5% milk powder and 0.1% Tween 20) for 1 h at room temperature. The GST−Hdm2 fusion (2.5 μg/mL) in PBS was incubated with NO donor with or without cysteine or DTT for 0.5 h at room temperature. An equal volume of 2× PBST-M was then added to the sample and then applied to the ELISA plate for 1 h at room temperature. After three washes, wells were probed with a polyclonal anti-GST antibody (1:10000) from Sigma in PBST-M and then an HRP-linked polyclonal anti-rabbit antibody (1:1500) from Amersham in PBST-M. Substrate development was performed with TMB tablets (0.1 mg/mL) in 0.05 M phosphate-citrate buffer (pH 5.0) containing 0.006% hydrogen peroxide. After 15 min, reactions were stopped with an equal volume of 1 M phosphoric acid and read at 450 nm. All washing between steps was carried out with PBS.

Site-Directed Mutagenesis. Site-directed mutagenesis and generation of the GST−Hdm2C77A mutant plasmid were performed with the QuickChange site-directed mutagenesis kit from Stratagene following standard protocols. The mutant sequence was confirmed by automated DNA sequencing.

RESULTS

We tested whether treating Hdm2 with a nitric oxide donor would affect its binding to p53. We employed a GST−Hdm2 fusion protein that incorporates the first 188 amino acids of Hdm2 and thereby includes the p53 binding domain. With ELISAs, we confirmed earlier results which showed that the thiol-modifying agent *N*-ethylmaleimide (NEM) inhibits Hdm2−p53 binding (Figure 1). We demonstrated that nitric oxide inhibits this binding as well, using the nitric oxide donor DETA/NO (1, 5, and 10 mM) (Figure 1). This

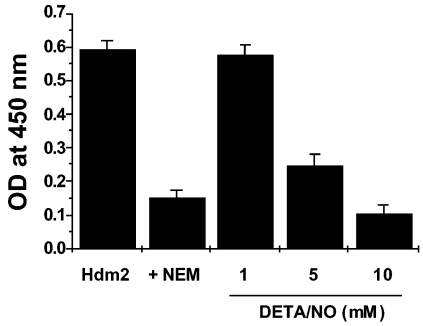

FIGURE 1: DETA/NO inhibits Hdm2−p53 binding. ELISAs were performed on p53-coated plates treated with Hdm2 (2.5 μg/mL) alone, Hdm2 and 10 mM NEM, or Hdm2 and increasing amounts of DETA/NO (millimolar). Data are presented as means ± SEM (n = 15).

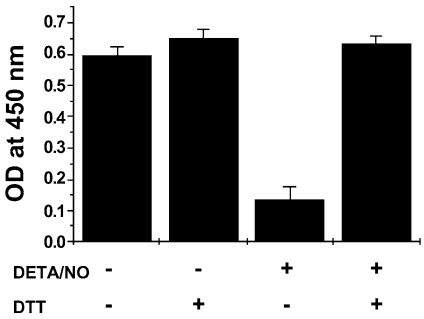

FIGURE 2: DTT blocks DETA/NO inhibition of Hdm2−p53 binding. ELISAs were performed on p53-coated plates that were presented with Hdm2 (2.5 μg/mL) alone, Hdm2 and 10 mM DTT, Hdm2 and 10 mM DETA/NO, or Hdm2, 10 mM DTT, and 10 mM DETA/NO. Data are presented as means ± SEM (n = 9).

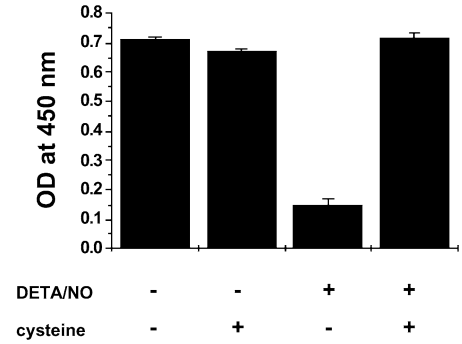

FIGURE 3: Cysteine blocks NO inhibition of Hdm2−p53 binding. ELISAs were performed on p53-coated plates treated with Hdm2 (2.5 μg/mL) alone, Hdm2 and 10 mM cysteine, Hdm2 and 10 mM DETA/NO, or Hdm2, 10 mM cysteine, and 10 mM DETA/NO. Data are presented as means ± SEM (n = 9).

inhibition is blocked by the addition of an excess of the reducing agent. When excess DTT (10 mM) is present, DETA/NO is unable to inhibit binding (Figure 2). Presumably, the excess DTT competes with Hdm2 for the nitric oxide. Moreover, addition of excess amounts of cysteine also blocked nitric oxide inhibition of Hdm2−p53 binding (Figure 3). These results are consistent with nitric oxide affecting binding by nitrosylating sulfhydryl residues.

To ensure that the effect of nitric oxide was specific for Hdm2, we performed several controls. We coated ELISA plates with the GST−Hdm2 fusion instead of p53 and tested whether the presence of nitric oxide affected the binding of the anti-GST antibody to the GST−Hdm2 protein. In these experiments, nitric oxide had no effect (data not shown). We

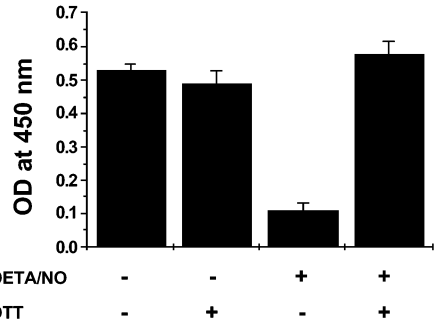

FIGURE 4: DETA/NO inhibition of Hdm2−p53 binding is reversible. ELISAs were performed on p53-coated plates. Hdm2 (2.5 μg/mL) alone or Hdm2 and 10 mM DETA/NO were incubated for 0.5 h, and then half of each sample was incubated with 10 mM DTT for 0.5 h. Data are presented as means ± SEM ($n = 9$).

also tested whether nitric oxide was modifying p53 instead of Hdm2. Plates coated with p53 were treated with DETA/NO and tested for their ability to bind Hdm2. In these experiments, DETA/NO had no effect on Hdm2−p53 binding (data not shown). From these experiments, we conclude that the effect of nitric oxide on Hdm2−p53 binding is due to a reaction of NO with Hdm2.

We tested whether the effect of nitric oxide was reversible. DETA/NO was reacted with Hdm2 for 0.5 h, and then the mixture was split in two. Half of the sample was then incubated with DTT for an additional 0.5 h, while the other half of the sample was untreated. The results from these experiments clearly indicate the inhibition by nitric oxide to be reversible, again consistent with nitrosylation of a sulfhydryl group (Figure 4).

Previously, it had been shown that the Hdm2−p53 reaction was susceptible to oxidation (*18*). This fact coupled with our results with NO led us to examine which cysteine residue is modified by NO and thereby inhibits binding. In our Hdm2 fragment, there are three cysteine residues (C2, C77, and C127). C77 seemed to be the residue most likely to be modified due to its proximity to the Hdm2 and p53 binding interface (*17*, *20*) (Figure 5). In addition, we found that this C77 is highly conserved in humans, mice, chickens, frogs, and zebrafish (Figure 5). Moreover, C77 is conserved in a related protein, MDMX (*21*). We mutated C77 to A in Hdm2 and tested the ability of this mutated protein to bind p53 and the effect of nitric oxide on this binding. As C77 is buried in a hydrophobic pocket, it was replaced with an alanine to avoid adding the hydrophilicity that the hydroxyl oxygen of a serine would. The C77A mutation did not affect normal binding when compared to the binding of the wild-type protein (Figure 6). However, when the mutant protein was treated with DETA/NO, there was no inhibition of Hdm2−p53 binding (Figure 7). Thus, the C77 to A mutation renders Hdm2 insensitive to NO inhibition and provides strong evidence that NO exerts its action through this particular cysteine residue.

DISCUSSION

We have presented a mechanism by which NO may increase p53 levels. Nitric oxide inhibits p53 binding of Hdm2, a negative regulator of p53 activity (Figure 1). This inhibition of binding would allow p53 levels to go unchecked and remain high or increase. The reducing agent DTT or the NO scavenger cysteine (Figures 2 and 3) can block this inhibition. Importantly, the inhibition of Hdm2−p53 binding is reversible, suggesting this may be a relevant form of regulation in vivo (Figure 4). Additionally, we have identified C77 in our Hdm2 fragment as the target of NO. Mutation of this residue to alanine does not inhibit normal binding to p53 (Figure 6). Nitric oxide has no effect on Hdm2C77A−p53 binding unlike wild-type Hdm2−p53 binding, which is inhibited by nitric oxide (Figure 7).

```
1   MCNTNMSVPTDGAVTTSQIPASEQETLVRPKPLLLKLLKS human 1-188
1   MCNTNMSVSTEGAASTSQIPASEQETLVRPKPLLLKLLKS mouse 1-184
1   ---------------NHISTSDQEKLVQPTPLLLSLLKS xenopus 1-107
1   MCNTEMTSLTDG----SPVSASEQEALVKPKPLLLKLLKL gallus 1-182
1   M---------ATESCLSSSQISKVDNEKLVRPKVQLKSLLED danio 1-170

41  VGAQKDTYTMKEVLFYLGQYIMTKRLYDEKQQHIVYCSND human 1-188
41  VGAQNDTYTMKEIIFYIGQYIMTKRLYDEKQQHIVYCSND mouse 1-184
25  AGAQKETFTMKEVIYHLGQYIMAKQLYDEKQQHIVHCSND xenopus 1-107
37  AGAQKDTFTMKEVIFYLGQYIMSKQLYDEKEQHIVHCAND gallus 1-182
34  AGADKDVFTMKEVMFYLGKYIMSKELYDKQQQHIVHCGED danio 1-170

81  LLGDLFGVPSFSVKEHRKIYTMIYRNLVVVNQQESSDSGT human 1-188
81  LLGDVFGVPSFSVKEHRKIYAMIYRNLVAVSQQ---DSGT mouse 1-184
65  PLGELFGVQEFSVKEPRRLYAMISRNLVSANVKESSEDI- xenopus 1-107
77  LLGDLFGVTSFSVKEHRRIYSMISRNLIAINQQDSTLAVP gallus 1-182
74  PLGAVLGVKSFSVKEPRALFALINRNLVTVKNPE---SQS danio 1-170

121 SVSENRCHLEGGSDQKDLVQELQEEKPSSSHLVSRPSTSS human 1-188
118 SLSESRRQPEGGSDLKDPLQAPPEEKPSSSDLISRLSTSS mouse 1-184
104 ------FG----------------------------------- xenopus 1-107
117 PEMMPNFGLKKKMFKRKSMQEL-EEKQTSSNATSQPTTSR gallus 1-182
111 TFSEPRSQSEPD---RGPGDTDSDSRSSTSQQ------QR danio 1-170

161 RRRAISETEENSDELSGERQRKRHKSDS              human 1-188
158 RRRSISETEENTDELPGERHRKRRRS               mouse 1-184
106 ----------------------------NV              xenopus 1-107
156 RRTHSESEENSSDDLHSDR-RKRHKSDS              gallus 1-182
142 RRRRSSDPESSSAEDESRERRKRHKSDS              danio 1-170
```

FIGURE 5: Hdm2 sequences from humans, mice, chickens, frogs, and zebrafish. The N-terminus of Hdm2 contains three cysteines (vertical boxes, C2, C77, and C127). C77 is highly conserved and is located within the p53 binding domain (horizontal box, amino acids 50−100). Sequences were aligned using the Clustal Method in MegAlign.

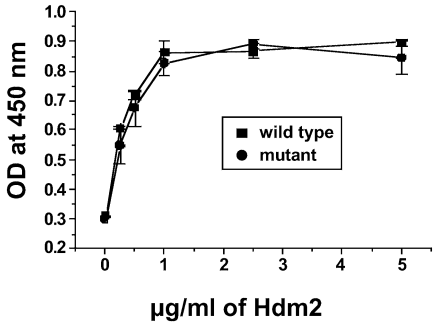

FIGURE 6: Wild-type and mutant Hdm2 bind to p53 similarly. ELISAs were performed on p53-coated plates using increasing concentrations of wild-type and mutant (C77A) Hdm2.

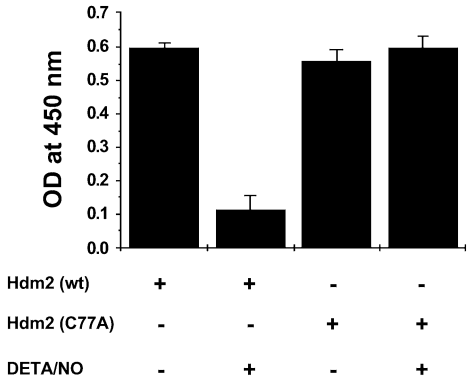

FIGURE 7: Mutant Hdm2 lacks responsiveness to DETA/NO. ELISAs were performed on p53-coated plates with either wild-type Hdm2 (2.5 μg/mL) or Hdm2 (C77A) (2.5 μg/mL) with or without 10 mM DETA/NO. Data are represented as means ± SEM ($n = 9$).

In this study, we focus on the N-terminus of Hdm2 that contains the p53 binding domain. However, Hdm2 regulates p53 by two mechanisms. In addition to its ability to bind and inactivate p53, Hdm2 also regulates p53 stability by targeting the p53 protein for proteasomal degradation (*22*, *23*). A recent report suggests that Mdm2, the murine version of Hdm2, is a RING finger-dependent ubiquitin protein ligase for itself and p53. This study identified several cysteine residues in the Mdm2 RING finger that are critical for its ability to degrade p53 (*9*). Thus, it is possible that in full-length Hdm2, these cysteines are additional targets of NO.

There are several possible products of the reaction of NO with C77. First, NO might oxidize C77. We do not think that this possibility is likely because the reaction is reversible (*24*). Second, NO might facilitate disulfide bond formation. We do not think that this is likely because C77 is buried in a hydrophobic pocket, and there are no other cysteines close by (*20*). Third, NO might S-nitrosylate C77, which we consider to be the most likely possibility.

The importance of C77 of Hdm2 is not immediately apparent as it does not participate directly in p53 binding. However, the crystal structure does show that C77 is proximal to the p53 binding pocket (Figure 8A). Specifically, C77 lies in the proximity of two residues, V75 and P91, that participate in van der Waals interactions with p53 (*20*, *25*). C77 is buried in a hydrophobic pocket directly behind the p53-interacting residues (P91, V75, V88, and L82) (Figure 8B). Reaction of NO with C77 will make this side chain both larger and more polar. One can propose two possible models. The nitrosylated C77 side chain might pop out of the hydrophobic pocket, which would distort the p53 interaction domain. Alternatively, the nitrosylated side chain might remain in the pocket, distorting the layer of p53-interacting residues, which make up much of the hydrophobic pocket. Further structural studies would be required to resolve this question.

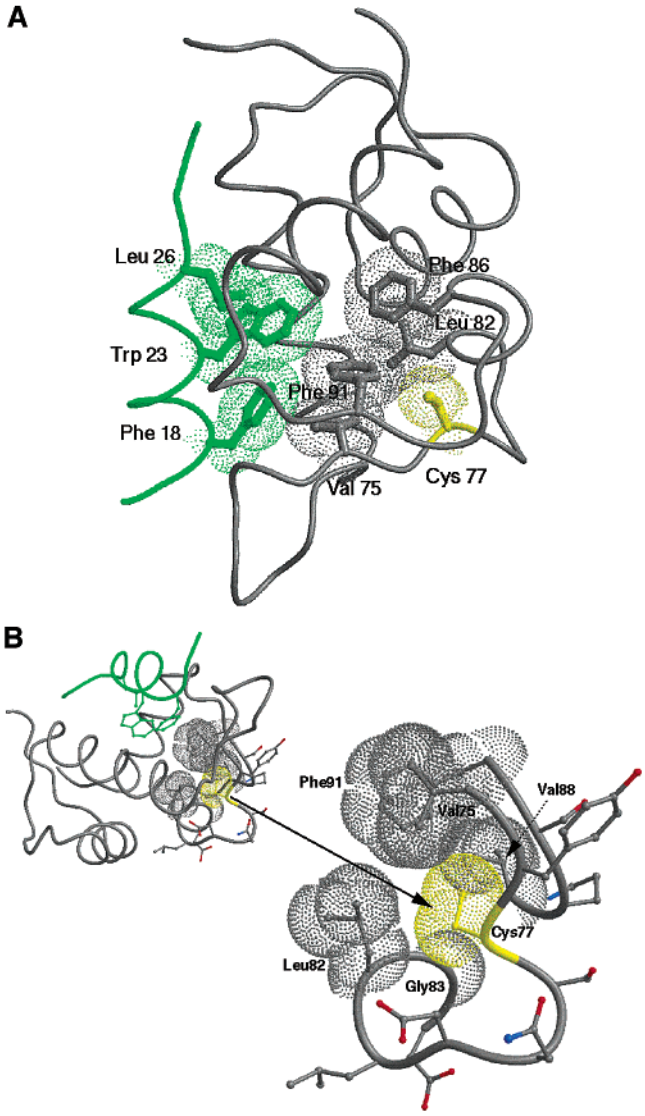

FIGURE 8: (A) Crystal structure of Hdm2 (gray) bound to a peptide from p53 (green) (*20*). C77 (yellow van der Waals surface) is located at the bottom of the hydrophobic p53 binding pocket lined by V75, L82, F86, and F91 (gray van der Waals surface). (B) In the absence of p53, NO could access and nitrosylate C77. This figure was made using the graphics display program MIDAS (*25*).

Modification of Hdm2 may be relevant to NO's action in a variety of physiological situations. Previously, we have shown that NO induces p53 accumulation during neuronal differentiation (*10*). We also know that NO acts in a cGMP-independent manner during PC12 differentiation (*26*) and (C. M. Schonhoff and A. H. Ross, unpublished findings). NO is also known to induce p53 during apoptosis. Activation of iNOS in RAW 246.7 macrophages results in the accumulation of p53. Moreover, work with NO donors showed a clear increase in p53 levels, while NO removal blocked p53 induction (*13*, *27*−*29*). Despite the clear link between NO and p53 induction during apoptosis, the molecular mechanism was not understood. In each case, neuronal

differentiation and apoptosis, NO may increase p53 activity by inactivating Hdm2.

Recently, another group has reported that p53 activation by nitric oxide involves downregulation of Mdm2 (*30*). Using NO to induce p53 levels, they noted that Mdm2 levels are decreased preceding a rise in p53 levels. Thus, it is possible that nitric oxide may regulate Hdm2 or Mdm2 by two distinct mechanisms. Nitric oxide may indirectly modulate Hdm2 levels, but it is also clear from our in vitro data that nitric oxide can directly modify Hdm2 by S-nitrosylation.

ACKNOWLEDGMENT

We are grateful to Drs. David Lane and Angelika Bottger for the Hdm2 expression plasmid. We thank Ze'ev Ronai for the p53 baculovirus.

REFERENCES

1. Harris, C. C. (1996) *J. Natl. Cancer Inst. 88*, 1442−1455.
2. Ko, L. J., and Prives, C. (1996) *Genes Dev. 10*, 1054−1072.
3. Oren, M. (1999) *J. Biol. Chem. 274*, 36031−36034.
4. Barak, Y., Juven, T., Haffner, R., and Oren, M. (1993) *EMBO J. 12*, 461−468.
5. Wu, X., Bayle, J. H., Olson, D., and Levine, A. J. (1993) *Genes Dev. 7*, 1126−1132.
6. Cahilly-Snyder, L., Yang-Feng, T., Francke, U., and George, D. L. (1987) *Somatic Cell Mol. Genet. 13*, 235−244.
7. Oliner, J. D., Kinzler, K. W., Meltzer, P. S., George, D. L., and Vogelstein, B. (1992) *Nature 358*, 80−83.
8. Momand, J., Zambetti, G. P., Olson, D. C., George, D., and Levine, A. J. (1992) *Cell 69*, 1237−1245.
9. Fang, S., Jensen, J. P., Ludwig, R. L., Vousden, K. H., and Weissman, A. M. (2000) *J. Biol. Chem. 275*, 8945−8951.
10. Poluha, W., Schonhoff, C. M., Harrington, K. S., Lachyankar, M. B., Crosbie, N. E., Bulseco, D. A., and Ross, A. H. (1997) *J. Biol. Chem. 272*, 24002−24007.
11. Ishida, A., Sasaguri, T., Miwa, Y., Kosaka, C., Taba, Y., and Abumiya, T. (1999) *Mol. Pharmacol. 56*, 938−946.
12. Brune, B., von Knethen, A., and Sandau, K. B. (1998) *Eur. J. Pharmacol. 351*, 261−272.
13. Brune, B., von Knethen, A., and Sandau, K. B. (1999) *Cell Death Differ. 6*, 969−975.
14. Lander, H. M., Hajjar, D. P., Hempstead, B. L., Mirza, U. A., Chait, B. T., Campbell, S., and Quilliam, L. A. (1997) *J. Biol. Chem. 272*, 4323−4326.
15. Mannick, J. B., Hausladen, A., Liu, L., Hess, D. T., Zeng, M., Miao, Q. X., Kane, L. S., Gow, A. J., and Stamler, J. S. (1999) *Science 284*, 651−654.
16. Stamler, J. S., Lamas, S., and Fang, F. C. (2001) *Cell 106*, 675−683.
17. Bottger, A., Bottger, V., Garcia-Echeverria, C., Chene, P., Hochkeppel, H. K., Sampson, W., Ang, K., Howard, S. F., Picksley, S. M., and Lane, D. P. (1997) *J. Mol. Biol. 269*, 744−756.
18. Bottger, V., Bottger, A., Howard, S. F., Picksley, S. M., Chene, P., Garcia-Echeverria, C., Hochkeppel, H. K., and Lane, D. P. (1996) *Oncogene 13*, 2141−2147.
19. Adler, V., Pincus, M. R., Minamoto, T., Fuchs, S. Y., Bluth, M. J., Brandt-Rauf, P. W., Friedman, F. K., Robinson, R. C., Chen, J. M., Wang, X. W., Harris, C. C., and Ronai, Z. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 1686−1691.
20. Kussie, P. H., Gorina, S., Marechal, V., Elenbaas, B., Moreau, J., Levine, A. J., and Pavletich, N. P. (1996) *Science 274*, 948−953.
21. Shvarts, A., Steegenga, W. T., Riteco, N., van Laar, T., Dekker, P., Bazuine, M., van Ham, R. C., van der Houven van Oordt, W., Hateboer, G., van der Eb, A. J., and Jochemsen, A. G. (1996) *EMBO J. 15*, 5349−5357.
22. Haupt, Y., Maya, R., Kazaz, A., and Oren, M. (1997) *Nature 387*, 296−299.
23. Kubbutat, M. H., Jones, S. N., and Vousden, K. H. (1997) *Nature 387*, 299−303.
24. Stamler, J. S., and Hausladen, A. (1998) *Nat. Struct. Biol. 5*, 247−249.
25. Ferrin, T. E., Huang, C. C., Jarvis, L. E., and Langridge, R. (1988) *J. Mol. Graphics 6*, 13−27.
26. Phung, Y. T., Bekker, J. M., Hallmark, O. G., and Black, S. M. (1999) *Brain Res. Mol. Brain Res. 64*, 165−178.
27. Messmer, U. K., and Brune, B. (1994) *Cell Signalling 6*, 17−24.
28. Messmer, U. K., and Brune, B. (1996) *Biochem. J. 319*, 299−305.
29. Messmer, U. K., and Brune, B. (1996) *Arch. Biochem. Biophys. 327*, 1−10.
30. Wang, X., Michael, D., de Murcia, G., and Oren, M. (2002) *J. Biol. Chem. 277*, 15697−15702.

BI026262Q