



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Nitric oxide causes inactivation of the low molecular weight phosphotyrosine protein phosphatase.

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Nitric oxide causes inactivation of the low molecular weight phosphotyrosine protein phosphatase / A. Caselli; G. Camici; G. Manao; G. Moneti; L. Pazzagli; G. Cappugi; G. Ramponi. - In: THE JOURNAL OF BIOLOGICAL CHEMISTRY. - ISSN 0021-9258. - STAMPA. - 269:(1994), pp. 24878-24882.

Availability:

The webpage <https://hdl.handle.net/2158/251558> of the repository was last updated on

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

La data sopra indicata si riferisce all'ultimo aggiornamento della scheda del Repository FloRe - The above-mentioned date refers to the last update of the record in the Institutional Repository FloRe

(Article begins on next page)

Nitric Oxide Causes Inactivation of the Low Molecular Weight Phosphotyrosine Protein Phosphatase*

(Received for publication, April 18, 1994, and in revised form, June 22, 1994)

Anna Caselli‡, Guido Camici‡, Giampaolo Manao‡, Gloriano Moneti§, Luigia Pazzagli‡, Gianni Cappugli‡, and Giampietro Ramponi‡¶

From the ‡Dipartimento di Scienze Biochimiche, Università di Firenze, Viale Morgagni 50, 50134 Firenze, Italy and the §Dipartimento di Farmacologia Preclinica e Clinica, Università di Firenze, 50134 Firenze, Italy

The low M_r phosphotyrosine protein phosphatase (PTPase) and *Yersinia enterocolitica* PTPase are inactivated by nitric oxide-generating compounds. Inorganic phosphate, a competitive inhibitor, protects the enzymes from inactivation, suggesting that the action of NO is directed to the active sites. Low M_r PTPase from bovine liver lost two out of eight thiol groups present in the molecule during the inactivation with sodium nitroprusside and with other NO-producing compounds. The mass spectrometric analyses of tryptic fragments of the enzyme, performed after chemical modification of the NO-unreacted thiol groups, demonstrated that NO caused the oxidation of Cys-12 and Cys-17 to form an S-S bond. A similar reaction was described previously for the reaction of NO with *N*-methyl-D-aspartate receptor. The NO-inactivated low M_r PTPase was reactivated by treating the inactive enzyme with thiol-containing reagents. Since all members of the PTPase family have the same reaction mechanism and possess a conserved active site motif that contains an essential cysteine residue, the findings on low M_r and *Yersinia* PTPases are potentially interesting for all PTPases, an enzyme class that is involved in a number of important biological processes.

Low M_r phosphotyrosine protein phosphatase (PTPase)¹ (EC 3.1.3.48) is a cytosolic enzyme that was previously known as low M_r acid phosphatase. The enzyme from bovine liver was sequenced in our laboratory (1). Although the enzyme has no general structural homology with the PTPase family enzymes (a large family that includes both receptor-type (such as leucocyte common antigen PTPase and leucocyte antigen-related PTPase) and soluble enzymes (such as human placenta PTP1B, and rat brain PTP1), we recently demonstrated that an active site motif, CXXXXXR, is common to low M_r and other PTPases (2). In addition, both low M_r PTPase and all members of the PTPase family have the same reaction mechanism. They form covalent thiol-phosphate intermediates during the catalytic process (2, 3). Isoenzyme pairs, produced through alternative

splicing of a single gene were isolated from both human erythrocytes and rat liver and then sequenced (4–6). They differ in the sole 40–73 stretch that is isoenzyme-specific.

Nitric oxide produced in biological systems has been implicated in a number of physiological processes that include smooth muscle relaxation, inhibition of platelet aggregation, neurotransmission, immune regulation, and penile erection (7, 8). Furthermore, the *S*-nitrosylation of proteins caused by NO was demonstrated (9–13). The reaction proceeds easily in physiological conditions. Taking into account that the low M_r PTPase contains two very reactive thiol groups, which are located in the active site (1), we began to study the effect of nitric oxide on this enzyme.

MATERIALS AND METHODS

All unspecified reagents were the purest commercially available. Sodium nitroprusside was from Sigma; SIN-1 was a generous gift from Cassella-AG, Frankfurt, Germany. *S*-nitrosocysteine was prepared as described by Lei *et al.* (11), and ODTB was from Fluka. Rat liver AcP1 and AcP2 isoenzymes and bovine liver PTPase were prepared as described previously (1, 6); recombinant PTPase from *Yersinia enterocolitica* (34 kDa fragment) was purchased from Boehringer Mannheim. HPLC column (Vydac protein and peptide C₁₈-column (4.6 × 250 mm)) was purchased from Vydac.

Enzyme Assay—The enzyme activity was determined using *p*-nitrophenyl phosphate (4 mM final concentration) in 0.1 M sodium acetate buffer, pH 5.5, containing 1 mM EDTA. Incubation was performed at 37 °C. The reaction was stopped with 0.1 M KOH, and the released *p*-nitrophenolate ion was measured at 400 nm ($\epsilon = 18,000 \text{ M}^{-1} \text{ cm}^{-1}$). The unit of enzyme activity is defined as the amount of enzyme that catalyzes the hydrolysis of one μmol of *p*-nitrophenyl phosphate/min.

Inactivation of PTPases with NO-producing Compounds—The low M_r PTPase (0.1 nmol) was equilibrated with 0.05 M 3,3-dimethylglutarate buffer, pH 7.0, by repeated concentration-dilution cycles using Centricon-10 microconcentrators (Amicon, Inc.). Then, it was transferred into a 0.3-ml Reacti-Vial equipped with a Mininert valve (Pierce). The volume was adjusted to 0.1 ml with the same buffer, and a nitrogen atmosphere was created by purging the vial with nitrogen gas. The valve was closed, and the needle-seal cylindrical septum was inserted in its hole. Then, the valve was opened, and 10 μl of SNP, SIN-1, or SNC solutions (freshly prepared) were added using a microsyringe (through the rubber cylindrical septum) to obtain the final desired concentration. The valve was closed, and the mixture was incubated at 25 °C. Aliquots were withdrawn (with a microsyringe through the rubber cylindrical septum) at interval times to assay the residual activity. Experiments with SNP were performed under diffused sunlight, since NO production from SNP is directly related to light exposure conditions (14). All of these experiments, i.e. those performed both in the presence and in the absence of P_i (a competitive inhibitor) as well as in the controls without SNP, were carried out contemporaneously to equilibrate the light exposure. Controls in the dark were also performed.

Inactivation Experiments with NO Gas—The low M_r PTPase (1 nmol), dissolved in 0.05 M 3,3-dimethylglutarate buffer, pH 7.0, was put into a small dialysis tube and exposed to the direct action of authentic NO gas bubbled into the dialysate (the same buffer, located in a cylindrical tube with a 2-cm diameter). Before NO treatment, the enzyme solution was deoxygenated by bubbling highly purified nitrogen gas in the dialysate. A valve that protected the dialysate from the air was

* This work was supported in part by the CNR target project on biotechnology and bioinstrumentation, by the Ministero della Università e della Ricerca Scientifica e Tecnologica, and by the Italian Association for Cancer Research (AIRC). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed.

¹ The abbreviations used are: PTPase, low M_r phosphotyrosine protein phosphatase; PTP1, rat brain PTPase; AcP1 and AcP2, rat liver low M_r PTPase isoenzymes; SIN-1, 3-morpholinodisulfonimine; SNP, sodium nitroprusside; SNC, *S*-nitrosocysteine; CF-FAB, continuous flow fast atom bombardment; ODTB, 5-(octyldithio)-2-nitrobenzoate; HPLC, high performance liquid chromatography.

TABLE I
Inactivation of some PTPases with NO or NO-generating compounds

All incubation were performed at 25 °C. The results are mean values of at least three different determinations. SNP experiments were performed under similar sunlight exposure (14).

PTPase form	Relative activity							
	0 ^a	5 ^a	10 ^a	15 ^a	20 ^a	25 ^a	30 ^a	60 ^a
Bovine (none) ^b	100		100	100	100		96	95
Bovine (none) ^c	100		100		95			
Bovine + 0.25 mM SNP ^b			43		16			
Bovine + 0.50 mM SNP ^b			26		1.1			
Bovine + 0.50 mM SNP + 20 mM P _i ^b			86		85			
Bovine + 0.50 mM SNP ^c			26		1.0			
Bovine + 0.50 mM SNP (in the dark) ^b			98		98		97	94
Bovine + 0.10 mM SNC ^b			88		72		58	
Bovine + 0.25 mM SNC ^b			58		46		37	
Bovine + 0.25 mM SNC + 20 mM P _i ^b			100		97		91	
Bovine + 0.25 mM SIN-1 ^b				27			1.5	
Bovine + 0.50 mM SIN-1 ^b				18			0.1	
Bovine + 0.25 mM SIN-1 + 20 mM P _i ^b				88			80	
Bovine + NO gas ^b			20		1.8			
Bovine + NO gas + 30 mM P _i ^b			90		85			
Rat AcP1 (none) ^b	100						100	94
Rat AcP1 + 0.25 mM SNP ^b							30	14
Rat AcP1 + 0.25 mM SNP + 20 mM P _i ^b							80	67
Rat AcP2 (none) ^b	100						96	93
Rat AcP2 + 0.25 mM SNP ^b							33	16
Rat AcP2 + 0.25 mM SNP + 20 mM P _i ^b							93	73
<i>Yersinia</i> (none) ^d	100	96	89			80		
<i>Yersinia</i> + 0.50 mM SNP ^d		30	3.7			0.4		
<i>Yersinia</i> + 0.50 mM SNP + 30 mM P _i ^d		94	65			43		

^a Incubation times in minutes.

^b 0.05 M 3,3-dimethylglutarate buffer, pH 7.0.

^c 0.1 M Tris-HCl buffer, pH 7.5.

^d 0.02 M Tris-HCl buffer, pH 7.2, containing 30% glycerol.

inserted. In addition, the surface of the enzyme solution in the dialysis tube was also maintained under a nitrogen atmosphere during the exposure to NO gas. These experiments were performed at room temperature both in the absence and in the presence of P_i. Aliquots were withdrawn (using a microsyringe through a rubber septum) at interval times to assay the activity.

Determination of Free Thiol Groups—Free thiol groups were titrated by ODTB as described by Faulstich *et al.* (15). This reagent reacts with protein-free thiols faster than Ellman's reagent; this is probably due to its lipophilic hydrocarbon chain and the absence of one negative charge that permits its access to thiol groups that normally remain undetected by the Ellman's reagent (15). Free thiols were titrated both before and after about 90% inactivation of the enzyme with SNP and SIN-1.

Enzymic Digestions, HPLC Separation, and Amino Acid Analysis—Tryptic digestion was carried out as follows. The enzyme (30 nmol), first inactivated and then treated with ODTB, was purified from low M_r reagents by repeated concentration-dilution cycles using Centricon-10 microconcentrators (Amicon, Inc.). The dilution buffer was 0.2 M ammonium bicarbonate, pH 8.5. The final volume was adjusted to 0.15 ml, and digestion with trypsin (5%, w/w) was performed by incubating the mixture at 37 °C for 2 h. Then, trypsin (5% (w/w)) was newly added, and the incubation was continued for an additional 14 h. Peptides were separated using reverse phase HPLC with a Vydac C18 column. Amino acid analysis was performed after acid hydrolysis (6 N HCl at 110 °C for 20 h) using a Carlo Erba 3A29 amino acid analyzer.

The tryptic hydrolysate was also analyzed using a capillary liquid chromatography/CF-FAB system (Kontron Instruments, Milan) with a reverse phase Hypersil C18, 3 µm, with a column 300 µm, inner diameter, × 15 cm (LC Packing, Amsterdam). The mobile phase was water/ acetonitrile containing 0.03% trifluoroacetic acid and was run at a flow rate of 4 µl/min.

Mass Spectrometry—Ion spray mass spectra were acquired on a Perkin Elmer Sciex API III mass spectrometer (Sciex Co., Thornhill, Ontario, Canada). The ion spray spectrum of the T64 peptide, in positive ion mode, was obtained under the following conditions: ion spray voltage, 5.5 kV; orifice voltage, 65 V; scan range *m/z*, 1000–2000; scan rate, 2.5 ms/atomic mass unit; resolution > 1 atomic mass unit. The sample was dissolved in methanol (10-µl loop, Rheodyne). The spectrum was acquired in MCA mode by summing 10 scans.

CF-FAB spectra were performed on a VG-7070 EQ mass spectrometer (VG Analytical, Manchester, United Kingdom). Data were acquired and processed on a Vector/two system (Teknivent corp., Maryland

Heights, MO). A laboratory-made CF-FAB probe, a µLC-500 microflow pump (Kontron, Zurich, Switzerland), and a Rheodyne (Coteti, CA) model 7520 injector with a 0.5-µl internal loop were used. The CF-FAB matrix (acetonitrile/water, 75:25, (v/v), containing 1% saturated aqueous oxalic acid and 1% 2,2'-dithiodiethanol) was carried into the mass spectrometer by a 1 m × 50 µm internal diameter deactivated fused silica capillary column at the flow rate of 4 µl/min. An Ion Tech gun (Xenon beam operated at 8 keV) was used, and the temperature of the probe tip was kept at 35 °C (scan range, *m/z*, 120–1700; scan rate 10 s/decade; resolution 1500 (10% valley definition).

RESULTS

Inactivation of Low M_r and *Yersinia enterocolitica* PTPases by Nitric Oxide—The treatment of low M_r PTPase from bovine liver with the NO-generating compounds SNP, SIN-1, and SNC causes concentration- and time-dependent loss of enzyme activity (Table I). In all cases, when P_i (a competitive inhibitor of the enzyme) was added to the incubation mixtures, strong reductions in the inactivation rates were observed. If the treatment with SNP was carried out in the dark, there was no appreciable decrease in enzyme activity during the experimental time.

SNP and SIN-1 are well known as chemical NO donors, which provide a continuous flux of NO (16). SNP releases NO spontaneously when exposed to light since a photochemical reaction is involved (14). In the dark, SNP does not release NO (14). SNC also was previously used as NO donor (11, 12). Thus, the experiments presented in Table I demonstrate that the inactivation of low M_r PTPase from bovine liver is due to the action of NO on the enzyme. The direct action of the authentic NO gas on the enzyme was tested. Table I also shows that authentic NO gas caused a time-dependent loss of enzyme activity and that P_i protected the enzyme from inactivation. Since low M_r PTPase is expressed as two different molecular forms in tissues such as human erythrocytes and rat liver (5, 6), we also performed a series of experiments with AcP1 and AcP2 rat liver isoenzymes in the presence of SNP. These isoenzymes have

common 1–39 and 74–157 sequences, whereas they differ in residues 40–73 (6). We found that the behavior of AcP1 and AcP2 is the same as that of bovine liver enzyme. This suggests that NO inactivation does not involve residues that are isoenzyme-specific. The protection of the enzyme from inactivation by P_i clearly indicates that the NO inactivation depends on the reaction of NO with some active site residues. We previously demonstrated that the binding of P_i in the active site of the enzyme involves the Cys-17–Arg-18 pair and that Cys-12 is the residue that causes the nucleophilic attack at the substrate phosphorus, producing the covalent cysteinyl-phosphate intermediate during the catalytic process (2).

The behavior of the PTPase from *Yersinia enterocolitica* in the presence of SNP was also tested. Table I reports the results obtained by incubating *Yersinia* PTPase with SNP under light exposure. It can be seen that this PTPase also lost its activity during incubation with SNP. P_i also protects this enzyme from inactivation. Considering that *Y. enterocolitica* and all other PTPases contain a conserved active site motif CXXXXXR (Cys and Arg are essential residues), we thought that our findings relative to low M_r PTPases (and *Yersinia* PTPase) might be intrinsic features of all members of the PTPase family.

Reversibility of Low M_r PTPase Inactivation—After inactivation with 0.25 mM SNC, bovine liver enzyme was treated with 5 mM 2-mercaptoethanol and incubated overnight at 4 °C. When the enzyme activity was then determined, about 75% of the initial activity was recovered. Similar results were obtained using SNP-inactivated enzyme. These findings demonstrate that inactivation of the low M_r PTPase by NO is a reversible process.

The Target of NO Action on Low M_r PTPase—Free thiol groups were titrated both before and after inactivation of bovine liver low M_r PTPase with SNP and SIN-1. We found that only 5.7 (with SNP) and 6.1 (with SIN-1) free thiol groups/enzyme molecule were titrated after enzyme inactivation, whereas 7.8 free thiol groups were titrated in the untreated enzyme. Thus, these results suggest that 2 cysteine residues are involved in the enzyme inactivation by NO.

The following strategy was employed to identify the site(s) implicated in the enzyme inactivation. Bovine liver enzyme, 30 nmol in 0.8 ml of 0.1 M Tris-HCl buffer, pH 7.5, was treated with 0.5 mM SNP. The mixture was incubated at room temperature under a nitrogen atmosphere, and aliquots were withdrawn to follow the decrease in activity. At 90% inactivation, the mixture was treated with 5 mM (final concentration) ODTB. Then, the protein was purified from low M_r reagents by repeated concentration-dilution cycles using Centricon-10 microconcentrators (Amicon, Inc.). During these cycles, the original buffer was replaced with 0.2 M ammonium bicarbonate buffer, pH 8.5, to perform trypsin hydrolysis.

Fig. 1 reports the HPLC separation of tryptic peptides. The amino acid analysis of all peptides was performed in order to identify the cysteine-containing peptides. In a number of cases, particularly for high M_r fragments, the N-terminal amino acid sequence was determined using a Milligen protein sequencer. Fragments containing all 8 cysteines of the enzyme molecule were obtained (T42, T44, T51, T54, T64). Peptide T64 includes uncleaved peptide bonds that are the target of trypsin. The inability of trypsin to cleave some peptide bonds in the chemically modified protein was likely due to the hindrance caused by the three large hydrophobic chains S–S linked to Cys-145, Cys-148, and Cys-149 (see below). The peptides T42, T44, T51, and T54 were analyzed using FAB mass spectrometry, whereas T64 was analyzed by electrospray mass spectrometry. Fig. 2 shows the mass spectra of all cysteine-containing peptides. The molecular weights found for peptides T44, T51, and T54 were

```

                20                      40
Ac-AEQVTKSVLFVCLGNICRSPFAEAVFRKLVTDQNISDNWV
                T42

                60                      80
IDSGAVSDWNVGRSPNPRAVSCLRNHGINTAHKARQVTKE
                T51

                100                     120
DFVTFDYILCMDESNLRLDNKSNQVKNCRAKIELLGSYD
                T54                      T44

                140
PQQLIIEDPYGNDADFETVYQQCVRCRAFLEKVR
                T64
  
```

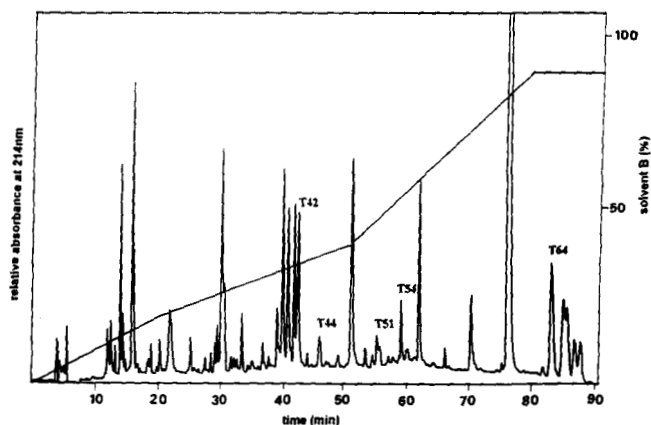


Fig. 1. HPLC separation of tryptic peptides from SNP-inactivated and *n*-octane-1-thiol-modified low M_r PTPase from bovine liver (bottom). A Vydac protein and peptide C18 column, 4.5 × 250 mm, was used. Solvent A was 10 mM trifluoroacetic acid in water, and solvent B was 10 mM trifluoroacetic acid in acetonitrile. The flow rate was 0.8 ml/min. Top, the amino acid sequence of the enzyme. The underlined sequence indicates cysteine-containing peptides that were selected for mass spectrometric analyses.

535, 792, and 1615, respectively; the calculated values of these peptides S–S-linked to *n*-octane-1-thiol are 535.25, 791.44, and 1614.73, respectively. All of these findings agree with the hypothesis that each cysteine has been modified by ODTB. The molecular weight found for peptide T64 is 4518 (the calculated value of this peptide with all three thiols S–S-linked to *n*-octane-1-thiol is 4518.24), suggesting that all 3 cysteines have been modified by ODTB. These results demonstrate that Cys-62 (contained in T51), Cys-90 (contained in T56), Cys-109 (contained in T44), and Cys-145, -148, and -149 (all contained in T69) are not involved in the SNP-inactivation of the low M_r PTPase since they remained unaltered during the enzyme inactivation. On the other hand, the M_r of 1321 found for T42 clearly demonstrates that Cys-12 and Cys-17 were not modified by ODTB after enzyme inactivation by SNP (the calculated M_r for this peptide with free thiols is 1322.7). The M_r value found for Cys-12–Cys-17-containing peptide (T42) agrees well with the hypothesis that these cysteines are linked together with an S–S bond. In fact, neither Cys-12 nor Cys-17 was *S*-nitrosylated or in free thiol forms. This finding is similar to that described for the *N*-methyl-D-aspartate receptor, where 2 vicinal cysteines reacted first with NO to form *S*-nitrosothiols and then formed an S–S bond (11). Cys-12 and Cys-17 are probably very near each other, since they are located in the active site of the enzyme (2). These results also agree with the finding described above concerning the protection from NO inactivation given by the competitive inhibitor P_i . Additional experiments with micro-HPLC-FAB were performed. Using this technique, some additional low M_r peptides that contain cysteine residues were found in the tryptic hydrolysate, whereas the T64-peptide was

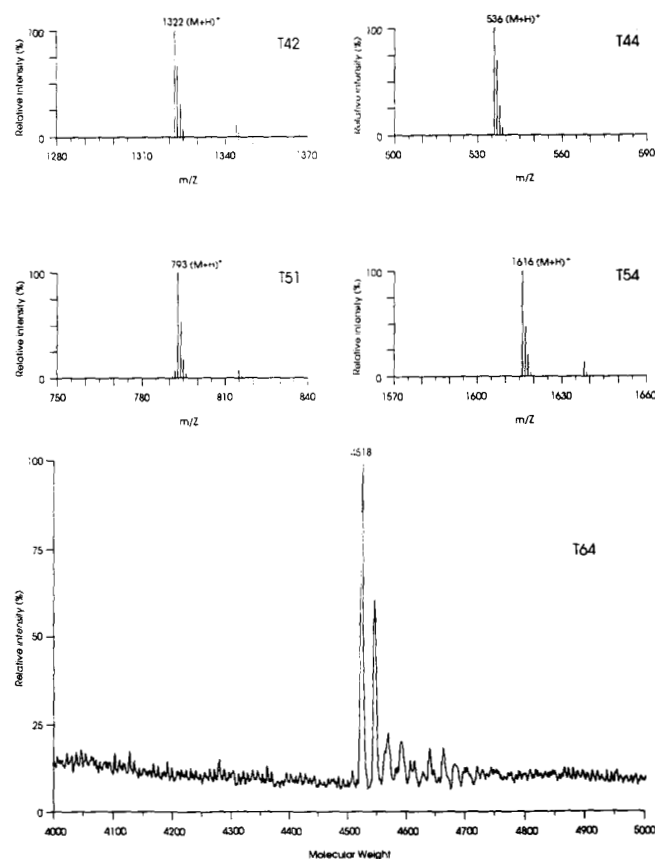


FIG. 2. Mass spectra of the cysteine-containing tryptic peptides from SNP-inactivated and *n*-octane-1-thiol-modified low M_r PTPase from bovine liver. The mass spectra of the peptides T42, T44, T51, and T54 were acquired by FAB mass spectrometry, whereas the peptide T64 was produced through a computer elaboration of the spectrum acquired by electrospray mass spectrometry. $(M+H)^+$ indicates the protonated molecular ion.

not revealed since its mass was not in the range of FAB mass acquisition spectra. The obtained results (not reported) agree well with the findings reported above and confirm the formation of an S-S bridge between Cys-12 and Cys-17 during the inactivation of the low M_r PTPase caused by nitric oxide-generating compounds.

DISCUSSION

In the few last years, it has been shown that NO is involved in several biological processes including those cited in the introduction of this paper (7–13). In a number of cases, NO produces its biological effects through the stimulation of soluble guanylate cyclase, a heme-containing enzyme. Furthermore, in the case of immunological activation, NO produced by activated macrophages acts as a cytotoxic molecule for invading intracellular microorganisms and tumor cells (17). Recently, various authors have suggested a different action of NO through its direct reaction with protein thiol groups (9–13). There are a number of cases in which enzymes or receptor proteins modulate their biological activities through the S-nitrosylation of exposed and very reactive cysteine residues. In the case of glyceraldehyde-3-phosphate dehydrogenase (9, 10), the inactivation of the enzyme by *in vivo* induction of nitric-oxide synthase, which is ultimately due to the S-ADP-ribosylation of the 4 active site cysteines in the enzyme tetramer, is preceded by the S-nitrosylation of these cysteines. Furthermore, in the case of the N-methyl-D-aspartate receptor (11), two vicinal thiols in the receptor protein were first S-nitrosylated by NO and then oxidized to form an S-S bridge. In addition, in the case of the

tissue-type plasminogen activator, S-nitrosylation by NO causes the protein to exhibit new functions (13). Finally, Gopalakrishna *et al.* (12) have recently reported that protein kinase C is reversibly and, in some cases, irreversibly inactivated by NO-generating substances as well as by activation of nitric-oxide synthase in cell cultures. They suggest that this is a new type of regulation of this important enzyme.

Our findings demonstrate that NO causes the inactivation of two members of the PTPase family. PTPases are involved in a number of biological processes such as the transmission of mitotic signals at different levels of the mitotic activation cascade. A number of oncogene proteins are mutated forms of growth factor receptors and possess unregulated tyrosine protein kinase activity; PTPases counteract their action, and some authors consider PTPases as antioncogenes (18, 19). Some aspects of cell cycle control are exerted by PTPase, as in the case of the *cdc25* gene product that dephosphorylates $p34^{cdc2}$ and causes the entry of the cell in mitosis. All PTPases have the common active site motif CXXXXXR and the same reaction mechanism (2). They form a thiol-phosphate covalent intermediate during the attack of substrate phosphorus by an essential cysteine in the active site. Although the low M_r PTPase was first studied as an acid phosphatase, its reaction mechanism is the same as that of both receptor-like and soluble PTPases and different from that of acid phosphatases (Ref. 2 and this work). Furthermore, the enzyme possesses the conserved active site motif CXXXXXR, common to all PTPases, in the 12–18 sequences of all enzyme forms sequenced up to now (20). Recently, bovine liver low M_r PTPase has been crystallized by Su *et al.* (21). The x-ray crystallographic analysis performed by the same laboratories² produced a crystal structure that shows a picture displaying some active site features common to those of phosphotyrosine protein 1B from human placenta. This crystal structure, recently reported by Barford *et al.* (22), is the first PTPase structure to be determined. Low M_r PTPase possesses specific phosphatase activity against tyrosine-phosphorylated proteins (23–27). The overexpression of this particular PTPase caused a decrease both in the growth rate and the incorporation of radioactive thymidine in normal and in transformed animal cells as well as a reduced ability of transformed cells to grow in soft agar (28). In our opinion, inactivation by NO may be a general feature of PTPase family members since they all have a very reactive cysteine in the active site.

Acknowledgments—We thank the Centro di Spettrometria di Massa of the Medical School of the University of Florence and the Centro di Studio per le Macromolecole Stereoordinate ed Otticamente Attive of the Italian CNR, Pisa, Italy. We thank Dr. A. Raffaelli (Pisa, Italy) for the acquisition and elaboration of electrospray mass spectra and Dr. G. Pieraccini (Florence, Italy) for FAB mass spectrometry analyses.

Note Added in Proof—Following the submission of the revised form of this manuscript, the crystal structures of low M_r (Su, X. D., Taddei, N., Stefani, M., Ramponi, G., and Nordlund, P. (1994) *Nature* **370**, 575–578) and *Yersinia* (Stuckey, J. A., Shubert, H. L., Fauman, E. B., Zhang, Z. Y., Dixon, J. E., and Saper, M. A. (1994) *Nature* **370**, 571–575) PTPases have been published.

REFERENCES

- Camici, G., Manao, G., Cappugi, G., Modesti, A., Stefani, M., and Ramponi, G. (1989) *J. Biol. Chem.* **264**, 2560–2567.
- Cirri, P., Chiarugi, P., Camici, G., Manao, G., Raugeri, G., Cappugi, G., and Ramponi, G. (1993) *Eur. J. Biochem.* **214**, 647–657.
- Brautigan, D. L. (1992) *Biochim. Biophys. Acta* **1114**, 63–77.
- Manao, G., Camici, G., Cappugi, G., Tremori, E., Pazzagli, L., and Ramponi, G. (1991) *Proceedings of 6th National Meeting "Proteine '91", Trieste, May 22–24*, p. 96.
- Dissing, J., Johnsen, A. H., and Sensabaugh, G. F. (1991) *J. Biol. Chem.* **266**, 20619–20625.
- Manao, G., Pazzagli, L., Cirri, P., Caselli, A., Camici, G., Cappugi, G., Saeed, A., and Ramponi, G. (1992) *J. Protein Chem.* **11**, 333–345.

² P. Nordlund, personal communication.

7. Moncada, S., Palmer, R. M. J., and Higgs, E. A. (1991) *Pharmacol. Rev.* **43**, 109–142
8. Stamler, J. S., Singel, D. J., and Loscalzo, J. (1992) *Science* **258**, 1898–1902
9. Molina y Vedia, L., McDonald, B., Reep, B., Brüne, B., Di Silvio, M., Billiar, T. R., and Lapetina, E. G. (1992) *J. Biol. Chem.* **267**, 24929–24932
10. Kots, A. Y., Skurat, A. V., Sergienko, E. A., Bulargina, T. V., and Severin, E. S. (1992) *FEBS Lett.* **300**, 9–12
11. Lei, S. Z., Pan, Z. H., Aggarwal, S. K., Chen, H. S. V., Hartman, J., Sucher, N. J., and Lipton, S. A. (1992) *Neuron* **8**, 1087–1099
12. Gopalakrishna, R., Chen, Z. H., and Gundimeda, U. (1993) *J. Biol. Chem.* **268**, 27180–27185
13. Stamler, J. S., Simon, D. I., Jarak, O., Osborne, J. A., Francis, S., Mullins, M., Singel, D., and Loscalzo, J. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 8087–8091
14. Bates, J. N., Baker, M. T., Guerra, R., Jr., and Harrison, D. G. (1991) *Biochem. Pharmacol.* **42**, (suppl.) 157–165
15. Faulstich, H., Tews, P., and Heintz, D. (1993) *Anal. Biochem.* **208**, 357–362
16. Lepoivre, M., Fieschi, F., Coves, J., Thelander, L., and Fontecave, M. (1991) *Biochem. Biophys. Res. Commun.* **179**, 442–448
17. Natan, C. (1992) *FASEB J.* **6**, 3051–3064
18. Hunter, T. (1989) *Cell* **58**, 1013–1016
19. Charbonneau, H., and Tonks, N. K. (1992) *Annu. Rev. Cell Biol.* **8**, 463–493
20. Caselli, A., Pazzagli, L., Paoli, P., Manao, G., Camici, G., Cappugi, G., and Ramponi, G. (1994) *J. Protein Chem.* **13**, 107–115
21. Su, X.-D., Agango, E. G., Taddei, N., Bucciantini, M., Stefani, M., Ramponi, G., and Nordlund, P. (1994) *FEBS Lett.* **343**, 107–108
22. Barford, D., Flint, A. J., and Tonks, N. K. (1994) *Science* **263**, 1397–1404
23. Chernoff, J., and Lee, H. C. (1985) *Arch. Biochem. Biophys.* **240**, 135–145
24. Ramponi, G., Manao, G., Camici, G., Cappugi, G., Ruggiero, M., and Bottaro, D. P. (1989) *FEBS Lett.* **250**, 469–473
25. Boivin, P., and Galand, C. (1986) *Biochim. Biophys. Res. Commun.* **134**, 557–564
26. Waheed, A., Laidler, P. M., Wo, Y. Y. P., and Van Etten, R. L. (1988) *Biochemistry* **27**, 4265–4273
27. Shekels, L. L., Smith, A. J., Van Etten, R. L., and Bernlohr, D. A. (1992) *Protein Sci.* **1**, 710–721
28. Ruggiero, M., Pazzagli, C., Rigacci, S., Magnelli, L., Raugi, G., Berti, A., Chiarugi, V. P., Pierce, J. H., Camici, G., and Ramponi, G. (1993) *FEBS Lett.* **326**, 294–298