Ligand-selective Modulation of the Permeability Transition Pore by Arginine Modification

OPPOSING EFFECTS OF p-HYDROXYPHENYLGLYOXAL AND PHENYLGLYOXAL*

Received for publication, August 9, 2001, and in revised form, October 19, 2001
Published, JBC Papers in Press, November 6, 2001, DOI 10.1074/jbc.M107645200

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Chemical modification of mitochondria with the arginine-specific reagents phenylglyoxal (PGO) and 2,3-butanedione (BAD) decreases the Ca\(^{2+}\) sensitivity of the permeability transition pore (PTP) and stabilizes it in the closed conformation (Eriksson, O., Fontaine, E., and Bernardi, P. (1998) J. Biol. Chem. 273, 12669–12674). Unexpectedly, modification of mitochondria with the arginine-specific reagent p-hydroxyphenylglyoxal (OH-PGO) resulted instead in PTP opening. Sequential modification with OH-PGO and PGO (or BAD) revealed that the effects on the PTP depended on the order of the additions. PTP opening was observed when OH-PGO preceded, and PTP closing was observed when OH-PGO followed, the addition of PGO (or BAD). The differential effects of OH-PGO and PGO on the PTP open probability (i) were not modified by the conformation-specific ligands of the adenine nucleotide translocase bongkrekate and atractylate; and (ii) were also observed in deenergized mitochondria, indicating that the effect is exerted directly on the PTP. OH-PGO dramatically sensitized PTP opening, which was triggered by depolarization even in the presence of EGTA. These data show that arginine modification modulates the PTP conformation in a ligand-selective fashion and suggest that the effects of OH-PGO, PGO, and BAD are mediated by the same arginine residues. We analyzed the structure of the arginine adducts by matrix-assisted laser desorption ionization and time-of-flight mass spectrometry using a test peptide and N-acetylariginine. The results indicate that both OH-PGO and PGO react with arginine at a stoichiometry of 2:1 and form stable adducts that may be feasible to identify the PTP at the molecular level.

The mitochondrial permeability transition is due to the opening of a large channel, the permeability transition pore (PTP), that permits diffusion of solutes with a molecular mass < 1500 Da across the inner mitochondrial membrane (1). Opening of the PTP may result in mitochondrial depolarization, swelling, and rupture of the outer membrane causing release of proteins from the intermembrane compartment. These events are under intense investigation in studies of the signal transduction of apoptosis, where the PTP may integrate several stimuli converging on mitochondria (2). Studies on isolated mitochondria have shown that the PTP is controlled by the membrane potential as well as by several ligands. High ΔΨ, CsA, ADP, H\(^{+}\), and BKA stabilize the closed conformation of the PTP, whereas low ΔΨ, intramitochondrial Ca\(^{2+}\), P\(_i\), and CATR promote the open conformation (1).

The molecular machinery of the PTP is not understood in great detail. It has been proposed that the PTP is composed of the ANT, the outer membrane VDAC, and mitochondrial cyclophilin (CyP-D), which by interactions with proteins of the Bcl-2 family would assemble to a pore-forming complex (3). This model has grown out of the findings that the ANT as well as the Bcl-2 proapoptotic homologue Bax behave as ion channels in model membranes (4, 5) and that the ANT inhibitors BKA and CATR affect the PTP conformation (6). Direct evidence as to the molecular identity of the PTP is still lacking, and presently the study of the role of specific proteins in the permeability transition is hampered by the lack of proper tools.

The characterization of functionally important amino acids residues by the use of amino acid-specific covalent reagents has provided important clues on the mechanism of the PTP. Covalent modification has revealed the role of (i) one or more histidines that react with diethylpyrocarbonate and may be involved in CyP-D binding and pH sensing (7, 8); (ii) at least two cysteines controlling the PTP conformation by thiol-disulfide interconversions (9); and (iii) arginine residues that react with PGO and BAD favoring the closed conformation of the PTP (10). To investigate further PTP modulation by arginine reagents, we have characterized the effects of the arginine-specific reagent OH-PGO (11).

We show that, in contrast with PGO, and despite their structural similarity, OH-PGO strongly promoted the open conformation of the PTP. The inducing effect of OH-PGO (i) was prevented by pretreatment with PGO, suggesting that the two reagents are reacting with one or more of the same arginine residues; (ii) was strongly favored by mitochondrial de-energization, indicating that arginine reagents affect the PTP voltage dependence (10) in a ligand-dependent way; and (iii) was not affected by the conformation-specific ligands of the ANT, BKA, ene glycol; PGO, phenylglyoxal; VDAC, outer membrane anion channel; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone.

* This work was supported by Grants from the Sigrid Juselius Foundation, The Finnish Medical Society, the Magnus Ehrnrooth Foundation, and the Perklin Memorial Foundation. 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.

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§ The abbreviations used are: PTP, permeability transition pore; ANT, adenine nucleotide translocator; BAD, 2,3-butanedione; BKA, bongkrekic acid; CATR, carboxyatractyloside; CsA, cyclosporin A; ΔΨ, transmembrane electrical potential difference; CyP-D, mitochondrial cyclophilin; MALDI-TOF, matrix-assisted laser desorption ionization and time-of-flight; OH-PGO, p-hydroxyphenylglyoxal; PEG, polyethyl-
and CATR. To clarify the nature of the adduct(s) of PGO and OH-PGO with arginine, we have analyzed their structures by MALDI-TOF (matrix-assisted laser desorption ionization and time-of-flight) mass spectrometry after reaction with a test peptide or with N-acetylarginine. Data showed that each reagent formed one major stable product with arginine at a stoichiometry of 2:1. Taken together, these results offer new insights into PTP modulation by arginine modification and should help to identify components of the PTP by chemical modification. A portion of these results has been presented in abstract form (12).

**EXPERIMENTAL PROCEDURES**

**Chemical Modification**—Rat liver mitochondria were prepared from male Wistar rats and preincubated with PGO or BAD as previously described (10). Preincubation of mitochondria with OH-PGO was performed at 1 mg of protein/ml in modification medium containing 250 mM sucrose, 10 mM succinate, 100 mM EGTA, 100 mM Hepes-KOH, pH 8.0, for 15 min at room temperature. The modification reaction was terminated by adjusting the pH to 6.8 with Hepes and cooling to 4 °C, followed by sedimentation of mitochondria by centrifugation at 8000 × g for 10 min. The pellet was resuspended at 1 mg of protein/ml in 50 mM sucrose, 10 mM succinate, 100 mM EGTA, 100 mM bovine serum albumin, 3 mM rotenone, and 10 mM Tris-HCl, pH 7.4. The centrifugation was repeated once whereupon mitochondria were resuspended at 50 mg of protein/ml in 250 mM sucrose, 10 mM succinate, 100 mM EGTA, 3 mM rotenone, and 10 mM Tris-HCl, pH 7.4. Control mitochondria were preincubated similarly, but OH-PGO was omitted. Unless otherwise stated, experiments were carried out in standard assay medium containing 250 mM sucrose, 5 mM succinate, 5 mM Pi-Tris, 3 mM P, Tris, 3 mM rotenone, and 10 mM Hepes-Tris, pH 7.4.

For sequential modification experiments, mitochondria were incubated at 1 mg of protein/ml in modification medium containing 2 mM OH-PGO for 15 min at room temperature. Excess reagent was removed by centrifugation for 10 min at 8000 × g whereupon mitochondria were resuspended in modification medium containing either 2 mM PGO or 2 mM BAD for 15 min at room temperature. The modification reaction was terminated, and mitochondria were washed twice by centrifugation as described above. Alternatively, the reagents were used in reverse order, i.e. incubation of mitochondria was performed first in modification medium containing PGO or BAD followed, after centrifugation, by incubation in OH-PGO-containing medium. The medium was supplemented with 10 mM borate when BAD was used.

**PTP Assays**—PTP opening was assayed as the mitochondrial Ca-sensitive Ca2+ release and/or swelling. Medium [Ca2+] was measured with the indicator dye ariens aceto III as the absorbance difference at the wavelength couple 665–685 nm, whereas swelling was monitored as the decrease of absorbance at 540 nm (10). PTP closing was measured as shrinkage of mitochondria essentially as described previously (Ref. 10, see Fig. 14). To achieve shrinkage, mitochondria were resuspended at 0.3 mg of protein/ml in a medium containing 30 mM sucrose, 0.5 mM Ca2+−, 10 mM Hepes-KOH, pH 7.8, at room temperature to induce the permeability transition. The absorbance of the suspension was monitored at 540 nm, and the incubation was continued until the absorbance stabilized at about 50% of the initial value (i.e. until all mitochondria had undergone permeability transition), which took 30–40 min. The arginine reagent to be tested was then added to the mitochondrial suspension, and this time was taken as time, t = 0 min. At the indicated intervals 4% polyethylene glycol (PEG) 7000 was then added to induce shrinkage of mitochondria with an open PTP. Shrinkage was completed in less than 1 min following addition of PEG. Because the absorbance of the mitochondrial suspension following permeability transition was not exactly identical for all preparations, the absorbance measured after completed shrinkage (A) was normalized by setting the absorbance preceding PEG addition (A0) to zero and the absorbance following addition of PEG and shrinkage at t = 0 min (Amax) to one unit using the formula, Anorm = (A − A0)/(Amax − A0).

**Analytical Determinations**—For MALDI-TOF mass spectrometry 100 μM of the peptide eFWPRPPQIPP or 1 mM N-acetylarginine was allowed to react with 10 mM PGO or OH-PGO in 10 mM Hepes-KOH, pH 8.5, for 60 min at room temperature. The reaction was stopped by addition of 0.1% trifluoroacetic acid. For desalting, the reaction mixture was equilibrated in a Zip Tip C18 silica bead microcolumn (Millipore), which was then washed repeatedly with 0.1% trifluoroacetic acid. Adsorbed compounds were eluted with acetonitrile, and the obtained eluate was mixed at a ratio of 1:1 with either saturated a-cyano-4-hydroxy-6-cinnamic acid in acetonitrile/0.1% trifluoroacetic acid 2:1 (for the test peptide) or 2% 2,5-dihydroxybenzoic acid in 10% ethanol (for N-acetylarginine). 0.5 μl of this solution was applied on the target. Reflectron MALDI-TOF mass spectra were recorded using a Bruker Biflex III spectrometer. For calibration, the peaks of a-cyano-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid, and of peptides of known mass were used. The peptide was selected because of its size, being optimal for MALDI-TOF mass spectrometry, and because it contained one single arginine and no primary amines that could conceivably give rise to minor side-products.

For pH titration of the arginine adduct 1 mM N-acetylarginine was allowed to react with 10 mM OH-PGO in 10 mM Hepes-KOH, pH 7.5, as above (to quench excess reagent, 7 mg/ml polyarginine (average mw 110 kDa) was added). The mixture was incubated for 30 min after the arginine adduct was separated on a PD-10 Sephadex column (Amersham Biosciences, Inc.). The pH of the phenol OH of the adduct was found to be 6.9 as determined by absorbance at λ = 340 nm (11) following addition of aliquots of HCl or KOH.

The total mitochondrial Ca2+ content of freshly isolated mitochondria was 8.4 ± 0.7 nmol/mg of protein (mean ± S.E., n = 5) as analyzed by atomic absorption spectrometry. This corresponds to a free Ca2+ of about 5 μM (14).

CaS was a gift from Novartis, OH-PGO was purchased from Pierce, carboxyatractyloside was from Calbiochem, and all other chemicals were from Sigma Chemical Co.

**RESULTS**

Freshly isolated rat liver mitochondria were preincubated for 15 min with 2 mM OH-PGO followed by removal of excess reagent by centrifugation. Mitochondria were then resuspended in standard assay medium containing succinate as the substrate, and 20 μl Ca2+ was added (Fig. 1). Control mitochondria readily accumulated and retained Ca2+ (Fig. 1A, trace a) and maintained a constant volume (panel B, trace a), indicating that these conditions were insufficient to open the PTP. Mitochondria pretreated with OH-PGO released Ca2+ soon after it had been taken up (panel A, trace b) and underwent rapid swelling (panel B, trace b); and both processes were fully prevented by the addition of 1 μM CsA (panels A and B, traces c), indicating that the PTP was the main pathway for Ca2+ release and sucrose uptake. Panel C depicts the concentration dependence of PTP induction by OH-PGO, from which a Kp of 0.3 mM OH-PGO could be estimated. These findings are of interest, because our previous studies have shown that arginine modification by PGO and BAD causes strong inhibition of the PTP (10). Thus, the present results suggest that the effect of arginine reagents on the PTP depends on the nature of the adducts rather than on the modification of arginine(s) as such.

We next investigated whether reaction with PGO, which inhibits the PTP, would prevent the PTP-inducing effects of OH-PGO, and if, conversely, OH-PGO would prevent the inhibitory effects of PGO. Mitochondria were incubated either (i) with 2 mM PGO followed by 2 mM OH-PGO for 15 min, excess reagent being removed after each incubation, or (ii) with the same reagents but in the opposite order. Mitochondria were incubated in the standard assay medium containing succinate as the substrate and 20 μl Ca2+ was added to study the response of the PTP. The results depicted in Fig. 2 show that mitochondria incubated first with OH-PGO failed to accumulate Ca2+ (Fig. 2A, trace a) and underwent large amplitude swelling (panel B, trace a). These results were due to PTP opening, because the addition of 1 μM CsA fully restored Ca2+ uptake and prevented swelling (results not shown). In contrast, when the incubation was carried out with PGO first, mitochondria accumulated and retained Ca2+ (panel A, trace b), and they did not undergo swelling (panel B, trace b), indicating that the PTP remained closed. These results show that the response of the PTP to modification by PGO and OH-PGO depends on the order of addition of the reagents. Similar results were obtained when PGO was substituted by BAD (not shown).
The assays used to study the PTP in these protocols depend on Ca\(^{2+}\)/H\(_{10545}\) uptake, which in turn relies on substrate transport and respiration. It was therefore important to exclude the possibility that the effects of OH-PGO were exerted at levels that may indirectly affect the PTP under these experimental conditions. We have previously shown that PGO induces closing of the PTP in fully de-energized mitochondria, a condition where reaction with effectors that modulate the PTP indirectly can be easily excluded (10). We have used this method to investigate whether the effects of OH-PGO were directly related to the PTP. In keeping with our previous results (10), the experiments of Fig. 3 show that the addition of 0.5 mM Ca\(^{2+}\)/H\(_{11001}\) readily opened the PTP \((a, c)\) and that PTP closing was achieved by the addition of ADP plus CsA \((b)\). Incubation of mitochondria with 2 mM PGO resulted in PTP closing with an apparent \(t_{1/2}\) of 8 min for the reaction \((c)\). As expected, incubation of mitochondria with 2 mM OH-PGO had minor effects on the already open PTP \((d)\), yet incubation of mitochondria with 2 mM PGO plus 2 mM OH-PGO significantly decreased the rate of PTP closing as compared with that observed with 2 mM PGO alone \((e)\), compare with \(c\). Because OH-PGO induced 50% inhibition of the PGO-induced PTP closing, it can be estimated that the rate constants for modification by PGO and by OH-PGO were approximately equal. These results demonstrate that OH-PGO antagonized the effects of PGO even under conditions where a contribution from electron or substrate transport can be ruled out, and strongly suggest that modification by OH-PGO and PGO directly affects the conformation of the PTP at the same site.

The ANT contains several conserved arginine residues that constitute potential targets for OH-PGO. The permeability transition is inhibited by BKA, which binds to the ANT at the matrix side thereby locking the carrier in “m”-conformation,
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while it is promoted by CATR, which binds to the ANT at the cytosolic side thereby locking it in the “c”-conformation (6). It was thus of interest to study whether the reactivity of the PTP-arginine(s) was affected by conformation-selective ANT ligands. The results presented in Fig. 4 show that, at concentrations sufficient to completely inhibit adenine nucleotide transport by the ANT (15), neither BKA nor CATR had any significant effect on the rate of PGO-induced PTP closing (closed symbols), and neither compound had significant effects on the PTP in control mitochondria that had undergone the permeability transition (open symbols). Likewise, ATP (1 mM) and ADP (1 mM) had no effects on the rate of PGO-induced PTP closing (not shown). These results indicate that arginine modification of the PTP was insensitive to the BKA- and CATR-induced conformational changes of the ANT. It appears unlikely that the arginine of interest was located on the ANT binding sites for BKA or CATR.

In the course of the experiments with OH-POG we observed that it was necessary to supplement the medium with an oxidizable substrate during and after incubation with OH-POG to prevent swelling of the mitochondria prior to the addition of Ca2+. This observation suggested that energization might affect modification of the PTP by OH-POG. To explore this possibility, freshly isolated mitochondria were energized with succinate (Fig. 5, traces a and c) or de-energized with myxothiazole and oligomycin (trace b), followed by the addition of 2 mM OH-POG directly to the cuvette. It can be seen that the addition of OH-POG to energized mitochondria had no effect on mitochondrial absorbance indicating that the PTP remained closed throughout the experiment (trace a). However, in de-energized mitochondria the addition of OH-POG readily induced swelling (trace b), which was completely prevented by 1 μM CsA (not shown). Remarkably, addition of FCCP to energized mitochondria induced swelling (trace c), whereas FCCP had no effects when added to untreated mitochondria (results not shown). These findings demonstrate that PTP opening by OH-POG is favored by mitochondrial de-energization. It should be emphasized that no Ca2+ was added in these experiments, and that endogenous Ca2+ was thus sufficient to induce the permeability transition in de-energized mitochondria treated with OH-POG. Increasing the concentration of EGTA had no effect on rate of OH-POG-induced swelling. These experiments suggest that OH-POG dramatically increases the sensitivity of the PTP to Ca2+ at low membrane potential. In contrast, we found that PGO-induced PTP closing was similar regardless of whether mitochondria were energized or de-energized during modification (results not shown).

As a first step to understand the mechanism by which arginine modification modulates the PTP, it was necessary to analyze the structure of the arginine adducts. For this purpose we elected to study the arginine adducts produced by reaction of OH-POG, PGO, and BAD with the test peptide pEWPRPQIPP. The crude reaction mixture was subjected to analysis by MALDI-TOF mass spectrometry and the region between 1050 and 1450 m/z of the resulting mass spectra are shown in Fig. 6. In the absence of reagents, the test peptide gave rise to a single peak corresponding to its monoisotopic mass (M + H+) of 1101.6 Da (trace a). The intensity of this peak decreased markedly following reaction with PGO or OH-POG, with two new peaks appearing at m/z 1351.6 (trace b) and m/z 1383.4 (trace c), respectively. On the other hand, the BAD adduct appeared to be unstable under the conditions of sample preparation, because no reaction products of the test peptide and BAD could be detected (not shown). None of the arginine reagents alone gave

Fig. 3. Time dependence of PGO-induced PTP closing and its inhibition by OH-POG. Rat liver mitochondria that had undergone permeability transition were incubated with PTP ligands or arginine reagents, added at t = 0 min. Closing of the PTP was assessed by shrinkage induced by addition of 4% PEG 7000. Absorbance was normalized as described under “Experimental Procedures.” The ligand/reagents used were: no addition (a), 0.5 mM ADP and 1 μM CsA (b), 2 mM PGO (c), 2 mM OH-POG (d), 2 mM PGO and 2 mM OH-POG (e). Values are expressed as mean ± S.E. obtained for five mitochondrial preparations.

Fig. 4. Independence of arginine modification by PGO on the ANT conformation. Closing of the PTP by PGO was assessed as in Fig. 3 in the presence of ligands of the adenine nucleotide translocator. The incubation medium was supplemented with: 10 μM bongkrekic acid (open squares), 100 μM carboxyatractylodiside (open circles), 10 μM bongkrekic acid and 2 mM PGO (closed squares), 100 μM carboxyatractylodiside and 2 mM PGO (closed circles). For comparison, the values from Fig. 3, for 2 mM PGO (a) and for no addition (b) are drawn as dashed traces.

Fig. 5. Opening of the PTP by de-energization in mitochondria modified with OH-POG. Rat liver mitochondria were suspended at 0.3 mg of protein/ml in a medium containing 250 mM sucrose, 30 mM succinate, 0.5 mM FAD, 100 μM EGTA, 3 μM rotenone, and 10 mM Hepes-KOH, pH 8.0. Swelling was measured as in Fig. 1. The addition of 2 mM OH-POG (traces a–c) and 0.2 mM FCCP (trace c) is indicated with arrows. In trace b, the medium was supplemented with 1 mM myxothiazole and 1 μM oligomycin while succinate was omitted.
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In this study we have investigated chemical modification of mitochondria by the arginine-specific reagent OH-PGO. Incubation of mitochondria with OH-PGO induced a chemical modification that increased the sensitivity of the PTP to Ca$^{2+}$ and to depolarization. This effect appears to result from a direct modification of the PTP and to be mediated by one or more of the arginines that react with OH-PGO and BAD. This interpretation is supported by the antagonism between PGO and OH-PGO. For this purpose we studied sequential modification experiments based on the rationale that, if the reagents modify one or more of the same arginines, the effect of the reagent added first should prevail. If, on the other hand, the reagents modify separate arginines the outcome should be independent of the order of addition of the reagents, because both arginines would be modified anyway. Our results indicate that the effect of the reagents was critically dependent on the order of addition, suggesting that the same site was being modified (Fig. 2). Binding to the same site is also supported by the chemical similarity of PGO and OH-PGO, because it appears extremely unlikely that any arginine would exhibit high reactivity toward one of the reagents while being completely inert with the other.

To clarify the mechanism by which arginine modification by OH-PGO affects the PTP conformation, it was essential to exclude indirect effects on the PTP. For this purpose we studied chemical modification under conditions where the PTP is independent of other mitochondrial functions such as substrate transport, respiration, and membrane potential. This can be achieved by measuring PTP closing in mitochondria that have already undergone the permeability transition. Such mitochondria are by definition de-energized, and their matrix content is similar to the suspension buffer regarding the composition of molecular species smaller than 1500 Da. Obviously, because the modification by OH-PGO could not be measured as PTP...
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closing, we investigated the effect of OH-PGO on the rate of PGO-induced PTP closing, which proceeded as an apparent first order reaction with a $t_{1/2}$ of 8 min. This value compares well with the rate of PGO modification as assayed by Ca$^{2+}$ transport (17). In the presence of OH-PGO the rate was reduced by $\sim$50% as estimated by the fraction of mitochondria with PTP in open conformation, suggesting that the rate constants for modification with PGO and OH-PGO were approximately equal (Fig. 3). These results demonstrate that the effect of arginine reagents on the PTP are independent of substrate transport, respiration, and membrane potential, thus suggesting that the effect on PTP conformation was due to modification of an arginine located on the PTP.

Much evidence shows that the ANT is implicated in PTP function, although its exact role in the molecular machinery of the PTP remains debated (1). It is possible that the ANT itself constitutes the pore-forming component (21), but it is also conceivable that the ANT, being the most abundant inner membrane protein, exerts an indirect, conformation-dependent effect on the PTP by influencing the surface potential (22, 23). To gain further insight into the link, if any, between the PTP-reactive arginines and the ANT we have studied the relationship between accessibility of the arginine to chemical modification and ANT conformation. The results indicated that arginine modification was independent of the BKA- and CATR-induced conformational changes of the ANT, and it appears unlikely that the arginine or arginines of interest were located on the binding sites of BKA and CATR, because that should have led to protection against chemical modification. Such protecting effects of BKA and CATR have indeed been observed with chemical modification of their binding sites by thiol- and lysine-specific reagents (24, 25). Thus, in agreement with our previous findings (10), the present study provided no evidence for a direct link between the OH-PGO-reactive arginines and the ANT.

To analyze the structure of the arginine adducts formed by modification, we used N-acetylagarginine and an octapeptide containing one arginine residue in a non-terminal position. MALDI-TOF mass spectrometry analysis of the reaction products between the test substances and PGO or OH-PGO shows that modification yielded one major reaction product resulting from the addition of two molecules of the reagent to one arginine. The mass of the PGO adduct corresponds to one of the structures proposed by Takahashi (16). It is thus clear that the dramatic difference in the effects of PGO and OH-PGO on the PTP can be traced to relatively subtle differences in the structure of their adducts. This in turn allows for some predictions regarding the role of the arginine of interest in the PTP. It appears unlikely that the arginine(s) is(are) located on a protein-protein interaction site, because the bulky adduct of both PGO and OH-PGO would tend to interfere with such interactions and should lead to a similar effect on the PTP conformation. For similar reasons, it is unlikely that the arginine is located in an anion binding site. Irrespective of the detailed mechanism, however, our findings are fully consistent with a critical role of the arginine residue(s) in PTP voltage sensing, where small differences in the physical properties of the adduct, such as charge, hydrophobicity, polarity, and bulkiness, could be expected to exert a large influence on the voltage-dependent changes of PTP conformation. Indeed, the low $pK_a$ of the phenol OH groups of OH-PGO (11) and its arginine adduct, carrying about 1.5 negative charges compared with the PGO adduct at neutral pH, suggests that the difference in effect of the compounds can be traced to a difference in their charge state.

The finding that the adducts of PGO and OH-PGO can readily be detected by MALDI-TOF mass spectrometry may prove useful for probing the PTP at molecular level. We are currently performing experiments to detect PGO and OH-PGO adducts on VDAC and CyP-D and to test whether PGO and OH-PGO can be used to predictably modulate PTP function in intact cells.

Acknowledgment—We thank Kaja Niva for excellent technical assistance.

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J. Biol. Chem. 2002, 277:937-942. doi: 10.1074/jbc.M107610200 originally published online November 6, 2001

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