Role of Histidines Identified by Mutagenesis in the NADPH Oxidase-associated H⁺ Channel*

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The efflux of protons through a H⁺ channel acts as the charge compensation pathway for the electrogenic generation of superoxide (O₂⁻) by human neutrophil NADPH oxidase. It has previously been shown that the N-terminal 230 amino acids of the product of the X-linked chronic granulomatous gene gp91phox contain all that is required for it to function as the arachidonate-activatable, NADPH oxidase-associated H⁺ channel (Henderson, L. M., Thomas, S., Banting, G., and Chappell, J. B. (1997) Biochem. J. 325, 701–705). To identify functionally important amino acids, Chinese hamster ovary (CHO) cell lines were constructed that expressed point mutations in gp91phox. No H⁺ flux was observed in CHO cell lines expressing the N-terminal gp91phox mutants H111L, H115L, and H119L, or H115L, or H115K. Partial retention of H⁺ channel function was, however, observed in the H115D CHO cell line. The addition of arachidonic acid to R91E,R92E CHO cells elicited a full H⁺ channel response. The buffering capacity and response of 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein to H⁺ were the same in all cell lines. Therefore, it can be concluded that His-115 is important to the ability of gp91phox to function as the NADPH oxidase-associated H⁺ channel and that the mechanism of H⁺ conduction involves protonation and deprotonation of an amino acid with an appropriate pK value.

An integral membrane component of the NADPH oxidase, gp91phox (phagocyte oxidase), has previously been demonstrated to function as the oxidase-associated H⁺ channel (1). The NADPH oxidase of phagocytes (neutrophils, monocytes, macrophages, and eosinophils) generates superoxide (O₂⁻) as part of the cellular immune response of the body. Electrons, donated by cytosolic NADPH, are utilized by the oxidase to perform the single electron reduction of O₂ to O₂⁻ (an oxygen free radical) on the external face of the membrane. The activity of the NADPH oxidase has been shown to be electrogenic (2, 3) and is associated with a rapid depolarization of the membrane potential (ΔΨ = −60 to −15 mV) (2) and a slight fall in pH (4). It has previously been shown that an efflux of H⁺ ions (oxidation of NADPH) through a channel provides the necessary charge compensation for the activity of the oxidase and prevents a large and rapid fall in pH (2, 4). This H⁺ conduction pathway is termed the NADPH oxidase-associated H⁺ channel (5). The pH of unactivated phagocytes, like that of most eu karyotic cells, is between pH 7.3 and 7.4. Therefore, the membrane of phagocytes is relatively impermeable to H⁺ ions, i.e. the channel is normally closed. Henderson and Chappell (5) have previously shown that, like the oxidase itself, the channel is opened following the addition of arachidonic acid (AA).1 In the presence of AA, the direction of H⁺ flux is dictated by the proton-motive force (Δp (ΔΨ − RT ln[H⁺]/[F−H⁺])).

Patients with chronic granulomatous disease are highly susceptible to infection. This has been demonstrated to be due to an inherited inability of their phagocytes to generate O₂⁻ (6). Analysis of the genetic lesions found in chronic granulomatous disease patients has demonstrated that the NADPH oxidase is composed of at least four proteins: a heteromeric, integral membrane cytochrome b₅₅₈ (gp91phox and p22phox) and two cytosolic proteins, p47phox and p67phox (6). In recent years, additional proteins have been proposed to contribute to the functioning of the oxidase: a small GTP-binding protein, Rac2 (7, 8), and an additional cytosolic protein, p40phox (9), with sequence similarities to both p47phox and p67phox (9, 10). Assembly of an active oxidase involves the translocation of the cytosolic proteins to the membrane, where they interact with the cytochrome b₅₅₈, forming a functional oxidase.

Through the use of Epstein-Barr virus-transformed B lymphocyte cell lines from chronic granulomatous disease patients and the construction of a stable CHO cell line, Henderson et al. (1) have demonstrated that gp91phox, the product of the X-linked chronic granulomatous disease gene (11), functions as the AA-activatable, NADPH oxidase-associated H⁺ channel. The hydropathy plot for gp91phox (11, 12) suggests that the protein contains multiple (four to six) transmembrane domains located at the N terminus and a large hydrophilic C-terminal domain that contains the predicted FAD- and NADPH-binding sites and is therefore assumed to be on the cytosolic side of the membrane. Henderson et al. (12) have previously demonstrated that the N-terminal 230 amino acids of gp91phox (gp91N) contains all that is required for the protein to function as the NADPH oxidase-associated H⁺ channel.

The mutagenesis of amino acids, both man-made and inherited, has been used to investigate and identify functionally important amino acid residues in a range of proteins and multiprotein complexes, including ion transporters, carriers, and channels. For example, the selectivity of the cardiac L-type Ca²⁺ channel has been reported to be altered from Ca²⁺ to Na⁺ by the mutation of two Glu residues to either Gln or Lys in the predicted mouth of the channel (13). The flux of H⁺ ions associated with the activity of the NADH:NADPH transhydrogenase is absent when His-91 is changed to Ser, Thr, or Cys (14). In this paper, the measurement of the AA-activated H⁺ flux in CHO cell lines expressing point mutations in gp91phox identi-

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1 The abbreviations used are: AA, arachidonic acid (sodium salt); CHO, Chinese hamster ovary; CCCP, carbonyl cyanide m-chlorophenyl-hydrazone; BCECF, 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; PCR, polymerase chain reaction; HA, hemagglutinin.
flies His-115 as an amino acid important to the functioning of the NADPH oxidase-associated H⁺ channel.

**EXPERIMENTAL PROCEDURES**

**Materials**—The following were obtained from Sigma (Poole, Dorset, United Kingdom), and stock solutions were prepared as indicated: arachidonate (sodium salt; 10 and 1 mg in 50% EtOH, stored under nitrogen at −20 °C), valinomycin (0.4 mg in EtOH), nigericin (1.5 mg in MeOH), CCCP (8 and 0.8 mg in EtOH, pH adjusted with NaOH), penicillin, streptomycin, trypsin (2.5 mg/ml of phosphate-buffered saline), and hygromycin B (100 mg/ml of sterile H₂O). Ham’s F-12 nutrient mixture with GLUTAMAXI, 10% fetal calf serum. The various cell lines were grown in a Heraeus incubator at 37 °C. The cell lines were harvested using a flow cytometer, and the cell lines were maintained in a Flow Laboratory flow hood, and the cell lines were maintained in growth media. The cell lines were constructed by Dr. Karla Biberne-Kinkade in the laboratory of Dr. Mary Phox. The various cell lines were maintained in growth media with 10% hygromycin B (100 mg/ml of sterile H₂O). Ham’s F-12 nutrient mixture with GLUTAMAXI and fetal calf serum were obtained from Life Technologies Inc. and BCCCF acetylomethyl ester was obtained from Molecular Probes, Inc. (Eugene, Oregon), and a stock solution of 1 mg was prepared in dimethyl sulfoxide dried by freeze-thawing. The ECL Western blot detection kit and HyperpH paper were obtained from Amersham Life Sciences Ltd. (Buckinghamshire, UK). The horseradish peroxidase-conjugated goat anti-mouse antibody, horseradish peroxidase-conjugated avidin, and the biotinylated SDS-polycrylamide gel electrophoresis molecular mass standards were obtained from Bio-Rad Laboratories Ltd. (Hemel, Hemipeda, UK). The compositions of the salt solutions were as follows: 150 mM NaCl, 1 mM KCl, 1 mM Hepes/Tris, and 5.5 mM glucose (pH 7.4) (Na⁺ medium) and 150 mM KCl, 5 mM Hepes/Tris, and 5.5 mM glucose (pH 7.4) (K⁺ medium).

**Mutagenesis of the N-Terminal Fragment of gp91phox**—Plasmids containing the point mutations of the N terminus of amino acids 1–230 of gp91phox were constructed by insertion following amplification in two stages, using overlapping PCR products. Synthetic mutant oligonucleotides together with oligonucleotide primers for the 5’- and 3’-ends of the N-terminal fragment of gp91phox (12) were used to generate two overlapping PCR products (nucleotides 1–352 and 336–690). Following purification from agarose gel, the two fragments were annealed and joined by extension from the region of overlap before final PCR amplification using the 5’- and 3’-end primers (12). The PCR products were inserted as a HindIII/SacII fragment into the plasmid pCMUV (12), generating CHO-N3Leu (mutant) plasmid. pCMUV contains a sequence encoding a tandem copy of the hemagglutinin (HA) epitope (YPYDVPDYAG). gp91N (mutant) HA was excised as a HindIII/BamHI fragment from pCMUV/dgp91N (mutant) HA and inserted downstream of the metallothionin promoter in the multiple cloning site of the plasmid pMEP4 (Invitrogen). gp91phox was generated in pMEP4 (mutant) HA. The PCR products for the H115D and H115K mutants were inserted directly into pMEP4 (mutant) HA. The synthetic mutant oligonucleotides used were as follows: T GCG ATT ccc ACC ATT ACC ATT GCA eT ccc ACC ATT CTA ATT CTA ATT CAA GAT TTT, C AAT GGT AAG TGC (H115D); T GCG ATT ccc ACC ATT ACC ATT GCA CAT CTA ATT CTA ATT CAA GAT TTT, C AAT GGT AAG TGC (H115K).

**Construction of CHO Cell Lines**—Stable CHO cell lines expressing the mutants of the N-terminal fragment of gp91phox were established following transfection by electroporation (230 V, 960 microfarads) of the pMEP4 constructs and selection with 100 µg/ml hygromycin B, as described previously (1). cDNA for gp91phox R91E,R92E was constructed by Dr. Karla Biberne-Kinkade in the laboratory of Dr. Mary Phox. The expression of this protein was followed using a Flow Laboratory flow hood, and the cell lines were grown in a Heraeus incubator at 37 °C. The various cell lines were maintained in Ham’s F-12 nutrient mixture with GLUTAMAXI, 10% fetal calf serum, 50 units/ml penicillin, and 50 µg/ml streptomycin. The medium was replaced every 3–5 days, and the cells were divided (one-half) once a week following trypsinization. The expression of the mutant proteins was induced by exposure of the cell lines to 10 µM Cd²⁺ for 24 h, as described previously (1, 16).

**Western Blotting**—The expression in the CHO cell lines of the N-terminal fragment of gp91phox containing the His-to-Leu mutations (3 His residues to 3 Leu residues and His to Leu) was examined by Western blotting using anti-HA epitope monoclonal antibody (16). The cells were treated with and without 10 µM Cd²⁺ for 24 h prior to being collected by trypsinization and washed twice in phosphate-buffered saline (800 g for 10 min). The proteins (10 µg/lane) were solubilized in sample buffer (20% (v/v) glycerol, 4% (w/v) SDS, 62.5 mM Tris-HCl (pH 6.8), 10% (v/v) mercaptoethanol, and 4.5% (v/v) saturated bromphenol blue) and separated by electrophoresis on a discontinuous SDS-polyacrylamide gel (4% stacking gel (pH 6.8) and 10% separating gel (pH 8.3)) using 0.1% (w/v) Tris, 1.44% (w/v) glycine, and 20% (v/v) methanol as transfer buffer. The membrane was probed with anti-HA monoclonal antibody (primary antibody) for 1 h. The presence of the primary antibody was detected following the addition of horseradish peroxidase-conjugated anti-mouse antibody (secondary antibody; 1:1), using the Western blot ECL detection kit and HyperpH paper (17). Biotinylated standards were included to enable calibration of the Western blot for molecular mass.

**Immunostaining**—Immunocytochemical techniques in combination with confocal optical scanning microscopy were used to examine the expression and cellular localization of the N-terminal fragment of gp91phox containing the various mutated amino acids as described previously (1, 12).

**Measurement of Intracellular pH**—The activity of the arachidonate-activable, NADPH oxidase-associated H⁺ channel was assessed as a change in pH, in response to the addition of AA as described previously (1, 12). The detection of pH changes was calibrated using an HCl addition to cells in high K⁺ medium in the presence of 10 µM nigericin (18, 19). The capacity of the different cell lines to buffer H⁺ ions was monitored as the fall in pH, following the addition of NaAc (26 mM).

**RESULTS AND DISCUSSION**

To understand the mechanism(s) by which the N-terminal 230 amino acids of gp91phox act as a pathway for the conduction of H⁺ ions, I attempted to identify regions and individual amino acids that are required for function. The amino acid sequence of gp91phox does not hold a high degree of similarity to any other protein, and the very high degree of homology between these species (20–23) makes it nearly impossible to identify conserved and, by implication, important residues. However, amino acids implicated in H⁺ conduction by other types of ion transporter have been described. The conduction through F₀ of the F₀F₁-ATPase has been described as requiring an acidic residue (Asp or Glu) located within a hydroporphic sequence of amino acids. Similarly, an Asp residue has been implicated in H⁺ transfer in cytochrome b₅₆₉ ubiquinol oxidase from Escherichia coli (24). The conduction of H⁺ ions into the endosome by the M2 coat protein of the influenza A virus is important to the uncoating of the virus and its subsequent infection of the cell. Within the single transmembrane domain of this protein, Ser-31 and His-37 have been demonstrated to be important in pH-dependent activation and the conduction of H⁺ ions (25–28). Similarly, βHis-91 (His-664 in the bovine enzyme) plays an important role in the proton pumping associated with the activity of the nicotinamide-nucleotide transhydrogenase from mitochondria (14). Within the N-terminal fragment of gp91phox, no candidate acidic residue located in hydrophobic sequence could be identified; however, three histidines (residues 111, 115, and 119) predicted to be contained within a transmembrane domain were identified. I therefore investigated the role of these three histidine residues in the functioning of the NADPH oxidase-associated H⁺ channel.

**Cellular Expression of N-Terminal Mutants**—A CHO cell line was constructed in which His-111, His-115, and His-119 where all mutated to Leu (CHO-N3Leu). The expression of the protein was driven by an inducible metallothionine promoter (11, 12) with HA epitope and was selected at the C-terminal end of the protein to assist determination of expression and cellular localization (12). As shown in Fig. 1a, anti-HA antibody detected a prominent broad band running at an apparent meridian molecular mass of 37.5 kDa in CHO-N3Leu cells treated with Cd²⁺. That this expression is regulated is demonstrated by the lower intensity of this protein band detected in CHO-N3Leu cells in the
Histidines in gp91phox Required for NADPH Oxidase H⁺ Channel

Fig. 1. Expression of the mutated N-terminal fragment of gp91phox in CHO cells. The CHO cell lines need to be treated with 10 μM Cd²⁺ for 24 h to induce expression of the protein from the metallothionein-sensitive promoter in pMEP4. a, Western blot. CHO-N3Leu cells treated for 24 h without (panel i) and with (panel ii) Cd²⁺ were separated on a 10% SDS-polyacrylamide gel (10 μg/lane). The expression of the mutated protein was probed with anti-HA monoclonal antibody and detected using horseradish peroxidase-coupled goat anti-mouse antibody. b, immunostaining. CHO-N3Leu cells were grown on glass coverslips, immunostained with anti-HA antibody, and imaged on a Bio-Rad 600 confocal microscope as described under “Experimental Procedures.” The diameter of the cells in each case is 15–20 μm. Panel i, CHO-N3Leu cells + 10 μM Cd²⁺ for 24 h; panel ii, CHO-N3Leu cells without Cd²⁺; panel iii, untransfected CHO cells.

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cells was assessed. Following the addition of AA to CHO-NAsp cells, a fall in pH<sub>i</sub> was observed (Fig. 3a), indicative of an AA-activated influx of H<sup>+</sup> ions. The extent of the acidification observed in CHO-NAsp cells (Fig. 3a) was greater than that observed in either CHO-N3Leu or in CHO-NLeu cells (Fig. 2, b and d), but smaller than that observed in cells expressing the non-mutated N-terminal fragment (Fig. 2a). The full pH<sub>i</sub> response was observed following the addition of CCCP (Fig. 3a). Therefore, the N-terminal fragment in which Asp is substituted for His-115 results in a protein that is partially capable of functioning as a H<sup>+</sup> conduction pathway. From the failure to observe a similar AA-induced acidification in CHO-NLys cells (His to Lys) (Fig. 3b), it can be concluded that the substitution of histidine with an alternative positively charged amino acid is insufficient to facilitate H<sup>+</sup> conduction. Therefore, the retention of activity observed in CHO-NAsp cells (Fig. 3a) is probably determined by the pK value and not by the charge or form of the amino acid side chain.

An efflux of H<sup>+</sup> ions (rise in pH<sub>i</sub>) following the addition of AA has previously been reported for both CHO-91 cells (expressing full-length gp91<sup>phox</sup>) and CHO-N cells suspended in K<sup>+</sup> medium in the presence of arachidonate-activated influx of H<sup>+</sup> ions. The extent of the acidification observed in CHO-NAsp cells (Fig. 3a) was greater than that observed in either CHO-N3Leu or in CHO-NLeu cells (Fig. 2, b and d), but smaller than that observed in cells expressing the non-mutated N-terminal fragment (Fig. 2a). The full pH<sub>i</sub> response was observed following the addition of CCCP (Fig. 3a). Therefore, the N-terminal fragment in which Asp is substituted for His-115 results in a protein that is partially capable of functioning as a H<sup>+</sup> conduction pathway. From the failure to observe a similar AA-induced acidification in CHO-NLys cells (His to Lys) (Fig. 3b), it can be concluded that the substitution of histidine with an alternative positively charged amino acid is insufficient to facilitate H<sup>+</sup> conduction. Therefore, the retention of activity observed in CHO-NAsp cells (Fig. 3a) is probably determined by the pK value and not by the charge or form of the amino acid side chain.

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CHO-N cells. Fig. 4a shows the response of BCECF-loaded CHO-NLys cells to changes in pH. The plot of the change in BCECF fluorescence versus pH (Fig. 4b) for all four cell lines is very similar. All cell lines were observed to have an initial pH similar to that shown for CHO-NLys cells: pH 7.32 (Fig. 4a). Therefore, the failure to observe a response (Figs. 2 and 3) was not due to the acidity of the resting pH or to alterations in the response of BCECF to pH.

Buffering Capacity—An increased capacity of CHO-N3Leu, CHO-NLeu, and CHO-NLys cells to buffer H ions could result in failure to observe H flux, i.e. AA induces an influx of H ions, but the cellular cytosol buffering obscures the change in pH. As shown in Fig. 5, CHO-NLeu, CHO-NLys, CHO-NAsp, and CHO-N cells all showed a similar fall in pH in response to the addition of NaAc. Therefore, they all have a similar buffering capacity.

R91E,R92E Mutation—The mutations introduced above have altered amino acid charge. Therefore, I investigated whether the change of charge alone is sufficient to alter the ability of gp91phox to conduct H ions. A CHO cell line with both Arg-91 and Arg-92 mutated to Glu was constructed (CHORR). The addition of AA to CHORR cells suspended in Na medium resulted in a large acidification of pH (Fig. 6a), which was comparable to that observed for cells expressing non-mutated gp91phox (Fig. 2a) (1, 12). As efflux of H ions was observed in cells suspended in K medium, the H channel is functional in both directions (Fig. 6b). Therefore, despite the major alteration in the charge of the amino acid side chains, there is no major change in the ability of gp91phox to act as a H conduction pathway.

I have demonstrated that mutation of His-111, His-115, and His-119 to Leu and to Lys significantly reduced the ability of the N-terminal fragment of gp91phox to function as the AA-activable H channel. In contrast, gp91phox in which His-115 was replaced with Asp retained some H conduction. That gp91phox R91E,R92E retained a fully function H channel is indicative that not all alterations in amino acid charge alter function. Therefore, it can be concluded that His-115 is important to the ability of gp91phox to function as the AA-activable, NADPH oxidase-associated H channel.
CONCLUSIONS

Henderson et al. (1, 12) have previously presented evidence that gp91<sub>phox</sub> functions as the arachidonate-activable, NADPH oxidase-associated H<sup>+</sup> channel and that all that is required for it to perform this function is contained within the first 230 amino acids, a region of the protein that includes the predicted

**FIG. 4.** Calibration of BCECF response in CHO cell lines. For cells suspended in K<sup>+</sup> medium in the presence of nigericin (Nig; 10 μM), pH<sub>i</sub> = pH<sub>o</sub>. a, fluorescence response of BCECF-loaded CHO-NLys cells (treated for 24 h with 10 μM Cd<sup>2+</sup>) to changes in pH<sub>i</sub> following addition of HCl. b, a graph of the change in fluorescence versus pH<sub>i</sub> (calibration curve) for BCECF loaded into CHO-N (small squares), CHO-NLeu (large squares), CHO-NAsp (stars), and CHO-NLys (diamonds) cells. All cell lines were treated for 24 h with 10 μM Cd<sup>2+</sup> prior to the assay.

**FIG. 5.** Buffering capacity of CHO cell lines. The response of pH<sub>i</sub> to the addition of NaAc was recorded in BCECF-loaded CHO-NLeu (a), CHO-NAsp (b), CHO-N (c), and CHO-NLys (d) cells suspended in Na<sup>+</sup> medium. All cell lines were treated for 24 h with 10 μM Cd<sup>2+</sup> prior to the assay.
transmembrane domains. In this paper, I have demonstrated that to function as a H⁺ conduction pathway, the N-terminal fragment of gp91<sub>phox</sub> requires His-115. The expression of the protein(s) in response to Cd²⁺ and their observed cellular location at or within the plasma membrane strongly suggest that the mutations introduced in this paper do not drastically alter the folding and three-dimensional conformation of the protein(s), although this possibility cannot be completely eliminated. The identification, in this study, of a histidine residue as being important to the conduction of H⁺ ions across a membrane is in keeping with similar roles for histidine, previously described, in the mitochondrial transhydrogenase (14) and in the M2 coat protein of the influenza virus (25–28). Aspartic acid, but not lysine or leucine, could partially substitute for histidine and allowed gp91<sub>phox</sub> to function as a H⁺ conduction pathway. The pK of the carboxyl group of aspartic acid is closer to that of the imidazole ring of histidine than the amine group of the side chain of lysine, suggesting that function is dependent on an amino acid side chain with an appropriate pK rather than its net charge. This suggests that the mechanism of H⁺ ion flux through gp91<sub>phox</sub> may involve a cycle of protonation/deprotonation with His-115 being exposed alternatively to the interior and exterior faces of the membrane.

The voltage sensor (S4) of the Shaker K⁺ channel contains seven positively charge residues that are believed to initiate opening of the channel as a result of voltage-dependent movement of S4. The mutation of Arg-365 and/or Arg-368 to histidine, in a non-conducting, non-inactivating version of the Shaker K⁺ channel has recently been reported to result in a channel that conducts H⁺ ions in response to repeating cycles of opening/closing, gating current (29). The ability to conduct H⁺ ions was suggested to be due to the movement of the voltage sensor, exposing the histidine residues alternatively to opposite sides of the membrane and facilitating the movement of H⁺ ions down the pH gradient.

The assay used in this study for the AA-activable, NADPH oxidase-associated H⁺ conductance is activated by AA. The similarities between the previously described phagocyte voltage-activated and the AA-activable, NADPH oxidase-associated H⁺ conductances (i.e., identification in phagocytes and the activation by AA) are intriguing. In neutrophils, the activation of the NADPH oxidase is associated with a depolarization of the membrane potential from ~60 mV to ~15 to ~10 mV (2, 4) and with a slight fall in pH (0.1 pH unit). Under physiological conditions, it is unlikely that the membrane potential of active neutrophils obtains a positive value. Therefore, in vivo opening of the H⁺ conductance probably results from a combination of the depolarization of the membrane potential and the action of AA.

It has previously been proposed that gp91<sub>phox</sub> functions as the flavocytochrome of the NADPH oxidase (38). As the predicted binding sites for both FAD and NADPH are located in the hydrophilic cytosolic C-terminal domain, there is no conflict with the role proposed in this study for the N-terminal hydrophobic domain as a H⁺ conduction pathway. It has been suggested that the NADPH oxidase may contain more than one heme moiety (39), but currently, their location within the enzyme complex remains unknown. However, like the proton-translocating cytochrome complexes of the mitochondria, cytochrome b<sub>558</sub> (i.e., gp91<sub>phox</sub> + p22<sub>phox</sub>) has been reported to have a pH-dependent midpoint potential (40). This suggests a close association between the cytochrome heme moiety and the pathway for the translocation of H⁺ ions. This is therefore in agreement with the proposed roles for gp91<sub>phox</sub> as the cytochrome b and as the NADPH oxidase-associated H⁺ channel.

In this paper, I have exploited CHO cell lines to investigate
the involvement of amino acids in the ability of gp91phox to function as a H+ conduction pathway and have identified His-115 as being functionally important. The ability of Asp to substitute in part for His and the lack of inhibition in CHORR cells suggest that the mutations exert their effects on the H+ conduction pathway directly rather than via changes in the conformation of the protein. A number of different currents have been described that are activated or enhanced by the presence of arachidonate, e.g. N-methyl-D-aspartic acid receptor current (41), outward rectifier K+ channel current in smooth muscle (42, 43), and K+ currents in hippocampus (44). Sequence comparisons with gp91phox may assist in the identification of region(s) necessary for and important in the AA activation.

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