Respiratory Burst Oxidase and Three of Four Oxidase-related Polypeptides Are Associated with the Cytoskeleton of Human Neutrophils

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Abstract

Resting and phorbol-activated human neutrophils were separated by treatment with Triton X-100 into detergent-extractable and cytoskeleton fractions. Respiratory burst oxidase activity was restricted entirely to the cytoskeleton. The cytoskeleton also contained \( \approx 15\% \) of the neutrophil cytochrome b\(_{558}\), an oxidase-associated heme protein, as well as most of the oxidase-related cytosolic polypeptide p\(_{67x}\). In contrast, the components of the oxidase-associated phosphoprotein family p47\(^{\dagger\dagger}\) were found almost exclusively in the detergent extract, suggesting that p47\(^{\dagger\dagger}\) is needed for oxidase activation but not for \( \mathrm{O}_2 \) production by the activated oxidase. Activation of the oxidase had no apparent effect on the distribution of any of these species between the cytoskeleton and the detergent extract. Our results support earlier studies implying that the cytoskeleton participates in an important way in regulating the activity of the \( \mathrm{O}_2 \)-forming respiratory burst oxidase of neutrophils. (J. Clin. Invest. 1991. 87:1345–1351.) Key words: neutrophil • cytoskeleton • respiratory burst oxidase

Introduction

The exposure of neutrophils to appropriate stimuli activates a metabolic pathway known as the "respiratory burst" whose purpose is the generation of microbicidal oxidants through the partial reduction of oxygen (1). The key to this metabolic pathway is the respiratory burst oxidase, a membrane-bound enzyme that catalyzes the one-electron reduction of oxygen to \( \mathrm{O}_2^\cdot \) at the expense of NADPH: \( \mathrm{O}_2 + \text{NADPH} \rightarrow \mathrm{O}_2^\cdot + \text{NADP}^+ + \text{H}^+ \). The respiratory burst oxidase is dormant in resting neutrophils, but acquires catalytic activity when the cells are incubated with any of a wide variety of stimuli. The \( \mathrm{O}_2^\cdot \) produced by this oxidase is the precursor of the very complex mixture of microbicidal oxidants generated by activated neutrophils during the respiratory burst.

Recent observations have furnished strong indications that the respiratory burst oxidase and its activating apparatus may be associated in some manner with the neutrophil cytoskeleton, a series of filament networks that define the shape and organelle distribution of the neutrophil and are indispensable for its motor function. Inhibitor studies have suggested that the actin microfilament network in particular plays an important role in regulating the activity of the respiratory burst oxidase. The effect of several microfilament-disrupting agents, including the cytochalasins (2) and the Clostridium botulinum C2 iota toxin (3), on the production of \( \mathrm{O}_2^\cdot \) in response to certain stimuli, most notably the N-formylated chemotactic peptides, clearly implies some sort of relationship between the cytoskeleton and the respiratory burst oxidase. Jesaitis and his associates have published several studies suggesting that this relationship is mediated through effects of the cytoskeleton on the traffic of N-formylated peptide receptors (2), but a more direct connection between the oxidase and the cytoskeleton was not ruled out. Recently, Nathan has found that the delayed but protracted respiratory burst elicited from surface-adherent neutrophils by exposure to tumor necrosis factor can be abolished by dihydroxytocolalasin B (4), again implicating the cytoskeleton in the regulation of the respiratory burst.

We have now used more direct methods to examine the association between the respiratory burst oxidase and the neutrophil actin microfilament network. Our experiments were based on the results with microfilament-depolymerizing agents described above, as well as on earlier work with neutrophil phosphoproteins (5–9). Our findings provide further support for a relationship between the actin microfilament system and the respiratory burst oxidase, suggesting that the microfilaments are connected to the oxidase not only functionally, but physically as well.

Methods

Materials. \(^{32}\)P-labelled phosphoric acid (carrier free in 0.02 M HCl) was obtained from ICN Radiochemicals, Div. Radiochemicals, Inc., Irvine, CA. Protease inhibitors (EDTA, diisopropyl fluorophosphosphate [DFP], leupeptin, and pepstatin), phorbol myristate acetate (PMA), mercaptoethanol, \( N, N', N'' \)-tetramethyl-4-diamino, DNAse I (type II), bovine pancreas), dihydroxytocolalasin B, cytochrome c (horse heart type VI), superoxide dismutase (bovine erythrocyte), dimethyl sulfoxide, pretained fumarase (used as a 48K marker for the second dimension of two-dimensional gel electrophoresis) and Triton X-100 were purchased from Sigma Chemical Co., St. Louis, MO. Ampholines and Ficoll-Hypaque were obtained from Pharmacia Fine Chemicals, Piscataway, NJ.

1. Abbreviations used in this paper: CGD, chronic granulomatous disease; DFP, diisopropyl fluorophosphatase.
Assay of neutrophil cytoskeletons and detergent extracts for cytochrome \( b_{558} \). Neutrophils (10^6 cells in 1 ml) were prepared in incubation buffer and (where indicated) activated with PMA (final concentration 0.2 \( \mu \)g/ml) exactly as described elsewhere (6). Incubations were terminated after 2 min with 10 ml ice-cold termination buffer supplemented with 10 mM EDTA. The cells were isolated by centrifugation (110 g, 4°C, 10 min), suspended in 5 ml inhibitor buffer containing 10 mM EDTA, incubated at least 20 min at 4°C, and resolated by centrifugation. Cytoskeletons were then prepared by suspending the cells in 5 ml Triton inhibitor buffer and extracting for 30 min on ice. A 1-ml portion of the whole cell lysate was reserved for the determination of cytochrome \( b_{558} \). Detergent extract and cytoskeleton were then separated by centrifugation of the remaining whole cell lysate (15 min, 4°C, Eppendorf). Detergent extract left in the cytoskeleton as initially prepared was removed by suspending the cytoskeleton in 4 ml Triton inhibitor buffer and centrifuging again. The supernatant from this second extraction (designated "residual extract") was reserved for assay of cytochrome \( b_{558} \). The firm pellet of washed cytoskeleton was suspended by trituration for 5–15 min at room temperature in 1 ml Triton inhibitor buffer containing 2 mg/ml DFP-treated DNase (DNase I in buffer containing 0.5 mM DFP [added as 10 mM DFP in dry ethylene glycol]) and 2 mM CaCl_2 to depolymerize F-actin (14). The amount of cytochrome \( b_{558} \) in each sample (i.e., whole neutrophil lysate, initial detergent extract, residual extract, and washed cytoskeleton) was determined by dithionite difference spectroscopy as described elsewhere (15), using a recording spectrophotometer (860; Kontron Elektron GmbH, Zurich, Switzerland) and measuring absorbances between 410 and 440 nm at 0.5-nm intervals.

Analysis of protein phosphorylation in neutrophil cytoskeletons and detergent extracts. Neutrophils were prepared, loaded with \( ^{32} \)P, (0.4 mCi/10^6 cells), and activated with PMA (final concentration 0.2 \( \mu \)g/ml) exactly as described elsewhere (6). Incubations were terminated after 2 min (unless otherwise indicated) with 1 ml ice-cold termination buffer supplemented with 10 mM EDTA, and the cells isolated by centrifugation (Eppendorf microfuge [15,600 g], 10 min). The neutrophils were then resuspended in 1 ml inhibitor buffer containing 10 mM EDTA, incubated at least 20 min at 0°C, and resolated by centrifugation. Cytoskeletons were then prepared by suspending neutrophils in Triton inhibitor buffer at a concentration of 2 \( \times \)10^7 cells/ml and incubating at 0°C for 30 min. The detergent extract was separated from the nonextractable cytoskeleton by centrifugation as described above. Proteins in the detergent extract were precipitated by the method of Wessel and Flugé (16), dried in air, and dissolved in 0.1 ml lysis buffer. The cytoskeleton was washed once with 0.25 ml Triton inhibitor buffer, supplemented by sonication at 0°C (model W-220F; Heat Systems-Ultrasonics, Inc., Plainview, NY, microtip, using multiple brief pulses) in 25 \( \mu \)l sonication buffer, then treated to hydrolyze DNA by incubating for 5 min at 37°C after adding 2 \( \mu \)l DFP-treated DNase. The suspended cytoskeleton was then dissolved by gentle warming after adding 25 mg solid urea followed by 50 \( \mu \)l lysis buffer. After centrifugation (1 min, Eppendorf microfuge), the solutions of cytoskeletal and detergent-soluble proteins were subjected to two-dimensional gel electrophoresis as described elsewhere (6) except that pH 2–4 amphotolines were replaced by pH 5–7 amphotolines. In preparing each gel, the entire quantity of cytoskeleton or extract solution was used as sample; all gels from a given experiment were subjected to autoradiography for the same length of time.

Immunoblotting. Immunoblots of gp91phox and p22phox were prepared by electrophoretic transfer of proteins from SDS-polyacrylamide 7–18% gradient gels to nitrocellulose as previously described (17, 18). The nitrocellulose transfers were incubated for at least 2 h in blocking buffer (10% goat serum, 3% BSA, 0.5 M NaCl, and 10 mM Heps, pH 7.4) and then probed for 3 h with 1/1,000 dilutions of affinity-purified polyclonal rabbit anti-p22phox or a polyclonal antibody raised in rabbits against a peptide corresponding to a portion of the gp91phox polypeptide chain in Dulbecco's PBS containing 3% normal goat serum, 1% BSA, and 0.2% Tween 20. The blots were then rinsed with wash buffer (0.25 M NaCl, 0.2% Tween 20, 10 mM Heps, pH 7.4); incubated for 1

Table I. Buffers

<table>
<thead>
<tr>
<th>Name</th>
<th>Composition</th>
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<tbody>
<tr>
<td>Incubation buffer</td>
<td>Heps/Tris (20 mM each) pH 7.4 containing 0.12 M NaCl, 5 mM KCl, 10 mM glucose, 0.25 mM CaCl_2, and 1 mM MgCl_2</td>
</tr>
<tr>
<td>Termination buffer</td>
<td>Heps/Tris (20 mM each) pH 7.4 containing 0.12 M NaCl, 5 mM KCl, and 10 mM glucose</td>
</tr>
<tr>
<td>Inhibitor buffer (3)</td>
<td>Termination buffer supplemented with protease inhibitors consisting of 0.5 mM DFP (added as a 10 mM solution in dry ethylene glycol), 20 mM leupeptin, and 18 mM pepstatin (final concentrations)</td>
</tr>
<tr>
<td>Triton inhibitor buffer</td>
<td>Inhibitor buffer: 5% (wt/vol) aqueous Triton X-100 = 4.1 (vol/vol)</td>
</tr>
<tr>
<td>Lysis buffer</td>
<td>Freshly prepared 9.5 M urea containing 2% (wt/vol) Nonidet P-40, 1.6% (vol/vol) pH 5–7 amphotolines, 0.4% (vol/vol) pH 3.5–10 amphotolines, and 5% (vol/vol) mercaptoethanol</td>
</tr>
<tr>
<td>Sonication buffer</td>
<td>Tris (10 mM) pH 7.4 containing 5 mM MgCl_2</td>
</tr>
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h with 1 μg/ml alkaline phosphatase conjugated goat anti-rabbit IgG (Bio-Rad) in Dulbecco's PBS containing 3% goat serum, 1% BSA, and 0.2% Tween 20; washed again; and developed using the Bio-Rad alkaline phosphatase conjugation substrate kit according to the manufacturer's directions. The stained blots were scanned using a soft laser densitometer (Zeineh, Fullerton, CA), and the data from six separate experiments averaged to accurately quantitate the relative distribution of cytochrome. (Blotting conditions were designed for optimal blocking of nonspecific proteins, but several bands were present on the gp91phox blot that were not eliminated by competition with excess synthetic peptide under conditions that eliminated the gp91phox band [data not shown]).

The cytosolic oxidase components p47phox and p67phox were also located by immunoblotting. For this purpose, antibodies were raised against the peptides C1C1C1C1FLKRKLASAV and C1C1C1C1FTDLESTR-REV, the 10 COOH-terminal residues of which are identical to the COOH-terminal decapeptides of p47phox and p67phox, respectively. Both peptides were coupled to rabbit serum albumin and also to ovalbumin (Cappel Laboratories, Cochranville, PA) using standard techniques (19), and the conjugates were used to raise polyclonal antisera in rabbits (20). The resulting antisera were screened for reactivity with the oxidase components by immunoblotting against normal neutrophil cytosol, and for specificity by immunoblotting against cytosols from patients with chronic granulomatous disease (CGD) whose neutrophils lacked either p47phox or p67phox (data not shown). For electrophoresis, cytoskeletons were dissolved in 25 μl inhibitor buffer, digested for 5 min at 37°C with DFP-treated DNase (1 mg/ml), then supplemented with 25 μl 2x sample buffer, while detergent extract proteins were precipitated as described (16) and dissolved in 25 μl inhibitor buffer plus 25 μl 2x sample buffer. Protein solutions were then applied to 10% polyacrylamide gels and subjected to electrophoresis as described by Laemmli (21). The separated proteins were transferred to nitrocellulose at 50 V for 2 h at 4°C. The nitrocellulose blots were blocked for 1 h with Blotto (22), incubated overnight with antibody (1/10,000 in Blotto) at 4°C, then washed five times with Blotto. Visualization was accomplished by incubating the blots at room temperature for 3 h with alkaline phosphatase conjugated to goat anti–rabbit IgG, washing five times with Blotto, and developing the color with the Bio-Rad alkaline phosphatase conjugation substrate kit as described above.

Protein concentrations in cytoskeleton and detergent extracts. Protein concentrations were determined by the Bradford method (23) using a kit purchased from Bio-Rad. Concentrations of protein in detergent extracts and cytoskeleton were 153.7±5.1 and 116.3±3.8 μg/10^7 cells, respectively (n = 6).

**Results**

*Both the respiratory burst oxidase and cytochrome b₅₉₉ are associated with the neutrophil cytoskeleton.* Earlier evidence supporting a relationship between the neutrophil cytoskeleton and the respiratory burst oxidase depended on the demonstration that oxidase activity in whole cells was altered by agents known to inhibit the formation of actin microfilaments. We sought to obtain more direct evidence for such a relationship by measuring respiratory burst oxidase activity in cytoskeleton preparations obtained from PMA-activated neutrophils. For our studies, cytoskeleton was prepared by extracting the cells with nonionic detergents using techniques similar to those employed in other laboratories (10–12). We found that the treatment of whole activated neutrophils with Triton X-100 brought most of the proteins into solution, but left behind an insoluble residue of detergent-resistant cytoskeleton. By two-dimensional gel electrophoresis, this insoluble residue was found to contain three major components; a protein that from its prominence and its location on the gel is probably actin, and two basic proteins, one that migrated at Mᵣ ≈ 60K and the other at Mᵣ ≈ 90K, together with a large number of minor components at low concentration (Fig. 1). The composition of the cytoskeleton fraction from our experiments was similar to that reported by Yassin et al. (11), except that they observed only one major basic component, which migrated at Mᵣ ≈ 60K.

Neutrophils treated with PMA are known to make large quantities of O₂ through the activation of the respiratory burst oxidase. Table II shows the distribution of the oxidase between the cytoskeleton and soluble fractions of PMA-treated neutrophils extracted with Triton X-100. The results indicate that when the cytoskeleton fraction was separated from the detergent-solubilized material by centrifugation, > 95% of the detectable O₂-forming activity remained with the cytoskeleton. Even if it is assumed that the loss in oxidase activity that occurred during the separation procedure (compare row 1 with row 2 plus row 3 of Table II) was sustained entirely by a hypothetical detergent-soluble oxidase pool, cytoskeleton oxidase still would represent a third of the total oxidase activity found in the original Triton-solubilized preparation (Table II, row 2). It appears therefore that much, and probably most, of the O₂-forming activity in PMA-activated neutrophils is associated with the cytoskeleton.

Closely associated with the respiratory burst oxidase is a heme protein known as cytochrome b₅₉₉ that is present only in phagocytes (1) and B lymphocytes (18, 24) and is missing in two of the four currently recognized types of CGD, an inherited condition caused by a heterogeneous group of molecular abnormalities affecting the respiratory burst oxidase or its activating

**Figure 1.** Composition of the detergent-resistant (cytoskeleton) and detergent-extractable fractions from phorbol-activated human neutrophils as determined by two-dimensional gel electrophoresis. Ten million (10⁷) neutrophils were used for this experiment. The preparation and extraction of the neutrophils and the electrophoresis of the extractable and cytoskeleton fractions were carried out as described in Methods. The proteins were visualized with Coomassie blue.
Table II. Distribution of Respiratory Burst Oxidase between the Cytoskeleton and the Detergent-soluble Extract of Activated Human Neutrophils

<table>
<thead>
<tr>
<th>Material</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/10 min/10^6 cell equivalent</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Triton-treated cells</td>
<td>10.6</td>
<td>11.7</td>
<td>(100)</td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>2.9</td>
<td>4.1</td>
<td>31.4</td>
</tr>
<tr>
<td>Detergent extract</td>
<td>0.1</td>
<td>0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Cytoskeleton + detergent extract</td>
<td>3.7</td>
<td>4.6</td>
<td>37.2</td>
</tr>
</tbody>
</table>

Experiments were carried out as described in Methods using two different neutrophil preparations. The controls for these experiments (row 1) are the Triton-treated cells that were centrifuged and immediately resuspended (see text). This procedure caused the samples to lose 50.1% (Experiment 1) and 39.7% (Experiment 2) of their original O_2-forming activity as measured in uncentrifuged samples, emphasizing the liability of the respiratory burst oxidase in the buffers employed for the preparation of cytoskeletons. * Average.

Table III. Distribution of Cytochrome b_558 between the Cytoskeleton and the Detergent-soluble Extract of Resting and Activated Human Neutrophils

<table>
<thead>
<tr>
<th>Cytochrome b_558</th>
<th>Sample</th>
<th>Resting</th>
<th>Activated</th>
<th>Resting</th>
<th>Activated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole neutrophil suspension</td>
<td>81.6</td>
<td>63.2</td>
<td>89.6</td>
<td>79.2</td>
<td></td>
</tr>
<tr>
<td>Initial extract</td>
<td>84.9</td>
<td>75.0</td>
<td>75.5</td>
<td>82.1</td>
<td></td>
</tr>
<tr>
<td>Residual extract</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>11.7</td>
<td>12.6</td>
<td>10.2</td>
<td>16.0</td>
<td></td>
</tr>
</tbody>
</table>

Percentage of total

| Cytoskeleton | 12.1 | 14.3 | 11.9 | 16.3 |

Experiments were conducted as described in Methods using two different neutrophil preparations. Total recoveries of cytochrome b_558 in the subcellular fractions averaged 111% and 131% for resting and activated cells, respectively. ND, not detected.

Figure 2. Association of the 91K (left) and 22K (right) subunits of cytochrome b_558 with the neutrophil cytoskeleton as determined by immunoblotting. Each track contained 10^6 cell equivalent of cytoskeleton (skl) or detergent extract (sup) from resting (rest) or PMA-activated (PMA) normal human neutrophils. Fractions were prepared and immunoblotting was performed as described in Methods. Similar results were obtained in a second experiment using neutrophils from a different subject. The proportions of cytochrome b_558 associated with the cytoskeletons from resting and activated neutrophils were 10.2±2.8 and 16.0±3.0% (mean±SD), respectively, as determined from scans of the autoradiograms of the 22K subunits (Zeineh 10/2D laser scanner).
free of p47\textsuperscript{phox} was obtained by immunoblotting. In preparations obtained from both resting and activated neutrophils, immunoreactive p47\textsuperscript{phox} was restricted almost entirely to the detergent extract (Fig. 4); comparison with standards (shown in the two right-hand tracks of the blot) suggests that the cytoskeleton contained < 5% of the total amount of p47\textsuperscript{phox} in the cell. These observations further indicate that to the extent that the cells contained unphosphorylated p47\textsuperscript{phox}, this unphosphorylated material was also separate from the cytoskeleton.

A 67K protein, p67\textsuperscript{phox}, has been identified as a fourth polypeptide component of the respiratory burst oxidase (30). Immunoblots with an antibody that recognizes this polypeptide have shown that in both resting and activated neutrophils, almost all the detectable p67\textsuperscript{phox} is associated with the cytoskeleton (Fig. 5). The fraction of p67\textsuperscript{phox} associated with the cytoskeleton does not seem to depend in a major way on the state of activation of the cell.

Discussion

As discussed in the introduction, the finding that the activity of the respiratory burst oxidase is related to the neutrophil cytoskeleton has been anticipated by earlier work from other groups.\textsuperscript{2} We have now found that the respiratory burst oxidase appears to be attached to the cytoskeleton, as are three of the four oxidase polypeptides discovered through studies with neutrophils from patients with CGD (31, 32). These findings constitute perhaps the most straightforward evidence to date in favor of a role for the cytoskeleton in the respiratory burst. The interaction between the oxidase and the cytoskeleton is probably the factor that allows the oxidase to be activated locally rather than globally in response to particulate stimuli (33). Other functions of the cytoskeleton-oxidase interaction, including a possible role in priming and deactivation, remain to be determined.

These experiments show that in resting neutrophils \textasciitilde 15% of the cytochrome b\textsubscript{558} is found in the cytoskeleton. This figure does not change during the activation of the oxidase, indicating that oxidase activation is not accompanied by the transfer of

\textsuperscript{2} While this manuscript was under review, Quinn et al. reported the association of oxidase activity and cytochrome b\textsubscript{558} with the membrane cytoskeleton of human neutrophils (31). These investigators found that all the oxidase activity was associated with the membrane cytoskeleton, and that cytochrome b\textsubscript{558} was distributed evenly between the membrane cytoskeleton and the detergent extract.

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cytochrome b$_{595}$ from other parts of the cell to the site of the active enzyme (i.e., the cytoskeleton). It seems likely from these observations that only a small fraction of the neutrophil cytochrome b$_{595}$ is associated with the oxidase, and that this fraction is already in place in the resting cell. These observations and the known distribution of cytochrome b$_{595}$ between the plasma membrane (∼15%) and the specific granules (∼85%) suggest that it is the cytochrome in the plasma membranes that is related to the oxidase. These considerations raise a question as to what the function of the specific granule cytochrome b$_{595}$ might be.

The foregoing results also indicate that whereas the O$_2$-forming activity from activated neutrophils is restricted almost entirely to the cytoskeleton, the p47$^{pho}$ family is found exclusively in the detergent-soluble supernatant. O$_2$$^*$ production in the cytoskeleton therefore takes place in the absence of p47$^{pho}$. It appears that p47$^{pho}$, while necessary for the activation of the respiratory burst oxidase, is not a component of the enzyme per se. Conversely, the cytoskeletal location of p67$^{pho}$ is compatible with the idea that this polypeptide is a constituent of the oxidase itself. In this connection, it is of interest that oxidase purified from activated neutrophil membranes (34) contained a 67K component, as well as a ∼48K component that from the present work is probably not p47$^{pho}$ but may be an oxidase-associated flavoprotein (35), and a ∼32K component that could represent the NADPH-binding subunit of the respiratory burst oxidase (36).

The presence in neutrophils of a 47K phosphorylated protein has also been reported by Pontremoli et al. (8). From our observations, it appears unlikely that this phosphoprotein is a member of the oxidase-related phosphoprotein family, because in their experiments the unphosphorylated protein was associated with the cytoskeleton, while in our experiments p47$^{pho}$ was always found in the detergent-soluble fraction.

Acknowledgments

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References


