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The Activity of Leukocyte NADPH Oxidase: Regulation by p47^{PHOX} Cysteine and Serine Residues

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ABSTRACT

The leukocyte NADPH oxidase is regulated chiefly by phosphorylation of the serines of p47^{PHOX}, one of its cytosolic subunits. Its activity is also regulated, however, by the four cysteines of the same subunit, as indicated by the replacement of those cysteines by alanines. Antioxid. Redox Signal. 4, 35–38.

INTRODUCTION

THE LEUKOCYTE NADPH OXIDASE is an enzyme found in neutrophils, monocytes, and eosinophils that catalyzes the production of superoxide (O_2^-) from NADPH and oxygen (4). It is a complex enzyme with a number of subunits. In the resting cell plasma membrane is a 91-kDa glycoprotein, gp91^{PHOX} (6, 19), which contains the electron transport components of the oxidase (18)—flavin adenine dinucleotide, heme, and a putative binding site for NADPH—and a 22-kDa subunit, p22^{PHOX} (7), which is thought to bind to a cytosolic subunit when the oxidase is activated. The resting cytosol contains four subunits important for oxidase activity: a 47-kDa subunit named p47^{PHOX} (15) that becomes extensively phosphorylated when the oxidase is activated; a 67k-Da subunit, p67^{PHOX} (14), which is absolutely essential for O_2^- production, but whose mechanism of action is mysterious; p40^{PHOX} (21), whose role in oxidase function is unclear (some experiments suggest that p40^{PHOX} promotes oxidase activity, whereas others suggest that it inhibits oxidase activity), and Rac, a small GTPase that is necessary for oxidase activity (1, 2). p47^{PHOX}, p67^{PHOX}, and p40^{PHOX} exist in the cytosol as a complex (10, 17).

The oxidase is activated by the exposure of the cells to any of a large number of stimuli, some particulate, such as bacteria (5) and opsonized zymosan (20), and others soluble, such as phorbol myristate acetate (3) and *N*-formyl-met-leu-phe (16). When the cell is stimulated, the cytosolic components join the oxidase components in the plasma membrane to assemble the active oxidase. The transporter is thought to be p47^{PHOX}, which when phosphorylated carries the remaining members of the cytosolic complex to the membrane. Rac appears to migrate separately, although there is some evidence that p47^{PHOX} is necessary for the migration of Rac to the membrane (8).

PHOSPHORYLATION OF p47^{PHOX}

The phosphorylation of p47^{PHOX} has been studied extensively. Initial studies showed that the only phosphorylated amino acids

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were serines, and that phosphorylation was restricted to the terminal quarter of the molecule, from S303 to V390. When single serines were replaced with alanines, the only amino acid that affected the activity of the oxidase was S379, whose conversion to A379 all but abolished O_2^- production by EBV-transformed p47^{PHOX}-deficient B-lymphoblasts (9). Whether S379 is phosphorylated at all is an open question. If it is, only a very tiny fraction of that serine is phosphorylated.

Mutations of pairs of serines gave a much clearer picture. The conversion of S303 plus S304 to A303 plus A304 reduced oxidase activity to <20% of normal (12). Activity was restored when those two serines were converted to glutamates and, surprisingly, to lysines. It may be that the phosphorylated forms of S303 and S304 pick up a cation that forms a bridge with another part of the p47^{PHOX} molecule, and that lysine mimicks that cation.

Even more interesting is the pair S359-S370 (13). Conversion of this pair to A359-A370 not only greatly reduces oxidase activity, but abolishes phosphorylation both in whole cells

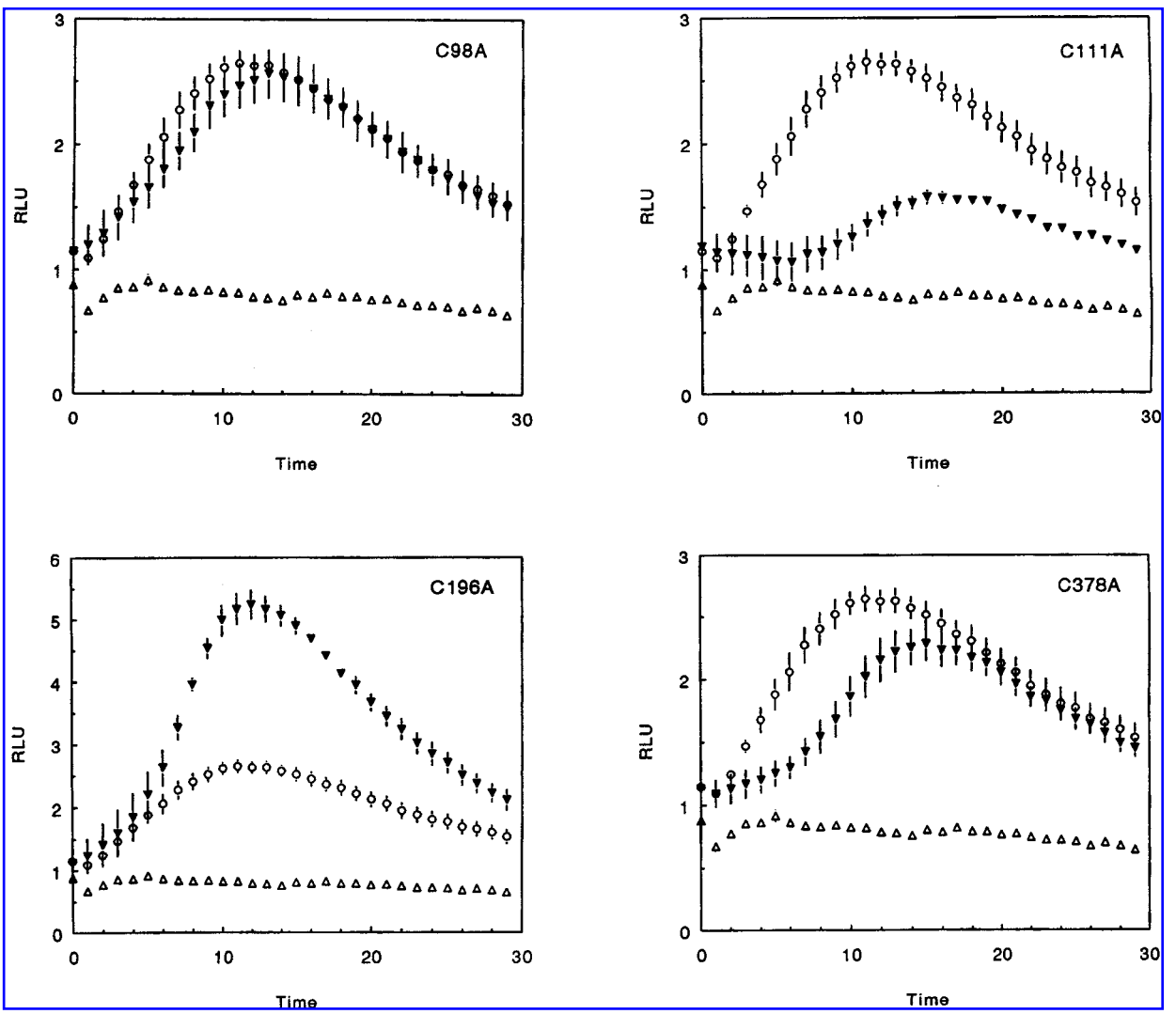

FIG. 1. O_2^- production by p47^{PHOX}-deficient B lymphoblasts expressing cysteine-to-alanine mutants of recombinant p47^{PHOX}. O_2^- production by the transfected cells was measured by chemiluminescence. The results represent means ± SE of three separate transfections. ○, wild-type p47^{PHOX}, ▼, cysteine-to-alanine mutant; Δ, empty expression vector. The location of the mutation is indicated in the upper right corner of each panel. RLU, relative light units. Reprinted with permission from Academic Press.

and in the cell-free system. Furthermore, the transfer of the mutated p47^{PHOX} to the membrane in response to phorbol myristate acetate does not take place. Replacement of S359 and S370 allows phosphorylation to take place and transfer of the mutated p47^{PHOX} to the membrane, but there is still no oxidase activity.

In summary, the experiments with single mutations show activity (except for S379), whereas the experiments with the multiple mutations indicate that phosphorylation of S359 or S370 comes first, and phosphorylation of S303 or S304 follows.

THE CYSTEINE RESIDUES OF p47^{PHOX}

p47^{PHOX} contains four cysteine residues, at positions 98, 111, 196, and 378. Mutagenesis experiments suggested that three of these four cysteines were involved in redox regulation of the activity of p47^{PHOX} (11). The experiments involved mutating each of the cysteines one at a time to alanines, then examining the activities of the mutant p47^{PHOX} species in EBV-transformed p47^{PHOX}-deficient B-lymphoblasts and in a cell-free system using the cytosols from these lymphoblasts.

The most striking finding was made with the mutant C196A (Fig. 1). Activity with this mutant was three to four times the activity with the wild-type protein. This increase in activity was apparent with both whole cells and the cell-free system. As cysteine is larger than alanine, it is unlikely that the difference in activity between the mutant and the wild type was due to steric hindrance. Cysteine does have the capacity to make hydrogen bonds, but they are extremely weak and are unlikely to account for the difference between the mutant and the wild type. It seems most likely that the difference reflects a redox effect, perhaps a disulfide bond, that is lost when C196 is changed to A196.

Interesting results were also obtained with C111A and C378A (Fig. 1). In this case, the results had less to do with the amount of O_2^- (although C111A produced considerably less O_2^- than wild type) than with the timing of O_2^- production by the mutant cells. With both mutants, there was a several minute delay before O_2^- began to appear. Furthermore, the length of the delay was similar for both mutants. This similarity raises the possibility of an interaction between C111 and C378, perhaps a redox interaction that modulates the activity of the oxidase. In this regard, it is of interest that C378 resides in the sequence CSE, which has been shown to occur in another oxidant-activated enzyme, namely AP-1 from yeast.

ABBREVIATIONS

O_2^-, superoxide; PHOX, phagocyte oxidase.

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