Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 825–829, February 1989
Biochemistry

Cytosolic components of the respiratory burst oxidase: Resolution of four components, two of which are missing in complementing types of chronic granulomatous disease

(NADPH oxidase/neutrophil/phagocyte/superoxide)

JOHN T. CURNUTTE†, PATRICIA J. SCOTT, AND LAURA A. MAYO

Department of Basic and Clinical Research, BCR-7, Research Institute of Scripps Clinic, La Jolla, CA 92037

Communicated by Ernest Beutler, October 31, 1988

ABSTRACT **The respiratory burst oxidase of neutrophils can be activated in a cell-free system in which plasma membranes, cytosol, Mg^{2+}, and a membrane-perturbing detergent, such as arachidonate or sodium dodecyl sulfate, are all required. Using the technique of preparative isoelectric focusing, the cytosol factor required for oxidase activation was resolved into four components termed C1–C4 with respective pI values of approximately 3.1, 6.0, 7.0, and 9.5. Individually, these components were incapable of activating the oxidase and could only be detected in the presence of suboptimal amounts of normal cytosol that served to supply at least a limited amount of each of the required components. Attempts to activate the oxidase with a combination of the four components failed, suggesting that there might be a yet undetected fifth cytosolic component. Patients with autosomal recessive cytochrome *b*-positive chronic granulomatous disease (type II CGD) are severely deficient in cytosol factor activity. When added to cytosol samples from two patients with this form of CGD, component C4 restored the ability of each patient's cytosol to activate dormant oxidase. None of the other three cytosol factor components (C1–C3) was effective in this regard, a finding supported by the direct demonstration that these three components were present in normal amounts in this type of CGD. A different form of type II CGD was identified in a third patient on the basis of complementation studies in which the patient's cytosol was able to activate the oxidase in the cell-free system when mixed with cytosol from one of the first two patients. The defect in this third patient's cytosol could be partially corrected by component C2, but not component C4, obtained from normal cytosol. These findings indicate that the role of cytosol in the activation of the respiratory burst oxidase is more complex than previously appreciated in that at least four cytosolic components appear to be required. Defects in two of these components have now been identified and appear to be responsible for two biochemically distinct forms of CGD.**

Neutrophils and other professional phagocytic cells are endowed with the capacity to generate extremely reactive derivatives of oxygen for the purpose of destroying virulent microbes (1). The precursor of this family of antimicrobial oxidants is superoxide (O_2^-), an oxygen radical produced by the following reaction:

$$2\ O_2 + NADPH \rightarrow 2\ O_2^- + NADP^+ + H^+ \qquad [1]$$

The enzyme responsible for O_2^- production is the respiratory burst oxidase, a membrane-bound flavoprotein that is dormant in the unstimulated cell but that becomes catalytically active when the phagocyte is stimulated (2, 3). The toxic nature of O_2^- and its derivatives necessitates that the phagocyte carefully regulate the production of these compounds in such a way that they are produced only when absolutely necessary.

The biochemical mechanism by which the respiratory burst oxidase is regulated remained obscure until the development of cell-free systems in which the oxidase could be activated under carefully controlled conditions (4–7). Studies of this system yielded the unexpected finding that both the cytosol and the plasma membrane fractions were required for oxidase activation. Furthermore, both Mg^{2+} and a membrane-perturbing agent, such as arachidonic acid or sodium dodecyl sulfate (SDS) (8–10), were also required. The extent of activation of the respiratory burst oxidase in the cell-free system is such that the rate of O_2^- production is comparable to that observed in maximally stimulated intact neutrophils (9–11).

The cytosol factor required for oxidase activation has not been purified to homogeneity nor has its identity been established. In our laboratory, the cytosol factor activity was found to elute with an apparent molecular mass of 240 kDa on gel filtration chromatography (9). Attempts to purify the cytosol factor beyond this stage have been unsuccessful in our hands. A possible explanation for this failure was provided by studies (11) on the kinetics of activation of the respiratory burst oxidase in a fully soluble cell-free system. These experiments disclosed that, among other things, the dependence of oxidase activation on cytosol concentration was nearly third order. This indicated that at least three cytosol components participated in the activation of the oxidase. Cytosol factor activity might, therefore, be expected to disappear with multiple purification steps since the necessary components could eventually be resolved from each other. In light of these considerations, a screening assay for detecting putative cytosol factor components was developed. Using this system, we have now identified four such components.

The physiologic relevance of the cell-free activation system has been underscored by studies on patients with chronic granulomatous disease (CGD), a group of inherited disorders characterized by recurrent infections because of the failure of the phagocytes to undergo a respiratory burst (3). Defects in either the membranes or the cytosol have been identified in all CGD patients thus far analyzed with the cell-free system (9, 10, 12). Membrane abnormalities have been detected in patients with either the classic form of CGD [X chromosome-linked cytochrome *b*-negative CGD (Scripps classification type I) (13)] or the rare autosomal recessive cytochrome *b*-negative form of the disease (type III) (9, 14). The molecular basis of the membrane defect in type I CGD is a mutation affecting the large subunit of cytochrome *b* (15). In type III CGD, the cytochrome *b* is also undetectable, presumably due

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: SDS, sodium dodecyl sulfate; CGD, chronic granulomatous disease; cell eq, cell equivalents.

†To whom reprint requests should be addressed.

to a mutation affecting the small subunit of the cytochrome (16). In contrast to these two forms of CGD, patients with autosomal recessive cytochrome *b*-positive CGD (type II) have normal membrane activity but severely deficient cytosol factor activity. In light of the (at least) three cytosol factor components predicted by the kinetic model, complementary forms of cytosol factor deficiency should exist, each characterized by an abnormality of a different cytosol component. Although our previous study (12) on a group of seven type II patients failed to reveal complementing defective cytosols, this report describes two female CGD patients whose cytosol abnormalities are complementary. Furthermore, the defect in each is reconstituted with a different cytosol factor component.

MATERIALS AND METHODS

Patients. Three patients with autosomal recessive cytochrome *b*-positive CGD [type II CGD (Scripps' classification) (13)] were studied, each of whom has a severe deficiency of cytosol factor activity. Patient J.C. is an 18-year-old white female who has suffered from *Nocardia* pneumonia, *Legionella* pneumonia, and recurrent skin infections as described in a case report (17). Patient J.G. is a 31-year-old white male who has had *Staphylococcus aureus* hepatic abscesses and recurrent skin infections. Both of these patients have been reported (12) by this laboratory to have normal levels of neutrophil cytochrome *b* but severe deficiency of cytosol factor activity with levels <2% of normal. J.C. and J.G. appear to share the same cytosol defect based on complementation studies in which mixtures of their cytosols failed to activate the respiratory burst oxidase in the cell-free system. At the time of this study, both patients were healthy.

Patient I.P. is a 4-year-old Hispanic female with a history of recurrent cutaneous abscesses and one episode of *Aspergillus fumigatus* pneumonia, the latter occurring at the time of this study. This patient has not been reported previously. Her neutrophils generated negligible O_2^- (<0.1% of control) nor did they become stained on the nitroblue tetrazolium slide test after stimulation with phorbol 12-myristate 13-acetate as described (9, 12). The cytochrome *b* content of her neutrophils was normal with 98.8 pmol of cytochrome *b* per 10^7 cells compared to a control value (based on 13 normal individuals) of 81.6 ± 14.4 pmol per 10^7 cells (mean ± SD), measured as described (14). In the cell-free system for activation of the respiratory burst oxidase, her membrane fraction was normal while her cytosol factor activity was severely deficient. Her cytosol factor defect appeared to be complementary to that present in the other two CGD patients studied (J.C. and J.G.).

Preparation of Neutrophils and Neutrophil Subcellular Fractions. Neutrophils from both normal subjects and CGD patients J.C. and J.G. were obtained by leukapheresis after obtaining informed consent. Dexamethasone (4-mg doses 12 and 2 hr before the start of leukapheresis) was given to normal donors to augment neutrophil yields; this treatment did not alter the properties of subcellular fractions prepared from the cells. Purification of neutrophils was subsequently carried out as described (9), except that dextran sedimentation was omitted. Neutrophil yields were 0.9–2.2 × 10^{10} cells per donor. Neutrophils from CGD patient I.P. were prepared from whole blood obtained by venipuncture (after informed consent) as described (9). Cytosol and deoxycholate-solubilized membranes were then prepared as described (10, 11). Protein concentrations of these fractions were reported (11) to be as follows: for cytosol, 250 ± 16 μg per 10^7 cell equivalents (cell eq) (mean ± SEM) and for deoxycholate-solubilized membranes, 25.7 ± 1.4 μg per 10^7 cell eq (mean ± SEM). The volumes of cytosol preparations were always adjusted so they contained 9 × 10^7 cell eq per ml.

Isoelectric Focusing. Preparative isoelectric focusing was performed using a Rotofor cylindrical focusing chamber (Bio-Rad) with a capacity of 30–55 ml. Cytosol (30–50 ml) was dialyzed at 6°C against four changes with a 1–hr incubation after each of 2.5 liters of 0.1× buffer A (1× buffer A = 100 mM KCl/3 mM NaCl/3.5 mM $MgCl_2$/10 mM Pipes, pH 7.25) using an agitating multiple dialysis chamber equipped with M_r 3500 cut-off membranes (Spectrum Medical Industries). Immediately after dialysis, pH 3–10 ampholytes (Biolyte 3/10, Bio-Rad) were added to the cytosol to 2% (vol/vol). This material was then loaded into the Rotofor chamber thermostatted at 4°C, equilibrated to temperature for 20 min, and then focused at 12 W constant power for 4–5 hr with a model 3000 Xi power supply (Bio-Rad). The total contents of the focusing chamber were collected into 20 2-ml fractions. Each fraction was analyzed for pH, supplemented with NaCl (to 0.5 M) to dissociate the ampholytes from protein, incubated for 30 min at room temperature, and then dialyzed at 6°C overnight against two changes of 0.5× buffer A and two changes of 1× buffer A by using the dialysis apparatus described above. The pH of each fraction was then measured to confirm equilibration to pH 7.25. Fractions were stored at 4°C and analyzed for cytosol factor activity within 72 hr.

Cell-Free Activation of the Respiratory Burst Oxidase (NADPH Oxidase). Activation of the dormant respiratory burst oxidase in a cell-free system by SDS in the presence of cytosol (or partially purified cytosol factor components) was monitored by measuring O_2^- production by the activated oxidase. O_2^- generation was determined by a continuous assay in which superoxide dismutase-inhibitable cytochrome *c* reduction was followed with time at 25°C in a double-beam spectrophotometer at 550 nm by a modification of a published method (10). Assay mixtures contained (final concentration) 0.15 mM cytochrome *c*, 6.5 mM $MgCl_2$, 87 mM KCl, 2.6 mM NaCl, 8.7 mM Pipes (pH 7.3), 0.27 mM ATP, 0.33 mM EGTA, 0.16 mM NADPH, and 0.04 mM SDS, plus 0.9 × 10^7 cell eq of cytosol and 1.55 × 10^6 cell eq of membranes solubilized in deoxycholate (0.94 mM) in 375 μl. The reference cuvette also received 22.5 μg of superoxide dismutase. All components except NADPH were mixed in the cuvette and allowed to stand for 3.5 min to allow activation of the oxidase by SDS. Reactions were then started with NADPH and followed at 550 nm on a Uvikon 810 dual-beam recording spectrophotometer (Kontron, Zürich). Data were collected on a strip chart recorder and the maximal velocity of the reaction, which occurred during the first 20 sec, was used to calculate the oxidase activity.

For all of the experiments involving cytosol factor components partially purified by isoelectric focusing, O_2^- production was measured by a slight modification of the method just described. To facilitate the screening of a large number of samples, eight pairs of reactions (with and without superoxide dismutase) were run simultaneously in 96-well plates with absorbance changes at 550 nm monitored automatically every 5 sec with a V_{max} kinetic microplate reader (Molecular Devices, Palo Alto, CA). The constituents of each assay mixture were the same as described above except that the reaction volume was 250 μl and contained 1.05 × 10^6 cell eq of deoxycholate-solubilized membranes, 133–160 μl of each fraction from the isoelectric focusing, and cytosol (0.9 × 10^6 cell eq of normal cytosol or 3.4 × 10^6 cell eq of CGD cytosol). Reactions were initiated by the simultaneous addition of SDS to all of the wells after a 1-min preincubation at room temperature. The maximum rate of absorbance change was determined by computer using enzyme kinetic software [Softmax (release 2.0), Molecular Devices] capable of calculating the first derivative of the time course. For any given sample, the difference between the maximal rates of cytochrome *c* reduction in the absence and presence of superoxide dismutase was taken as the rate of O_2^--dependent

cytochrome *c* reduction. The molar amounts of O_2^- generated were then determined using an extinction coefficient for reduced minus oxidized cytochrome *c* of 20.5 mmol/cm^2, a value determined experimentally for the 550 ± 1-nm filter used in the kinetic microplate reader and the path length of the 250-μl reaction mixture.

RESULTS

Resolution of Four Components of Cytosol Factor by Preparative Isoelectric Focusing. Preparative isoelectric focusing was chosen as a method to separate cytosol factor components because of the resolving power of this technique as well as the commercial availability of a high-capacity focusing apparatus. Fig. 1 shows the results of a representative experiment. The pH gradient ranged between pH 1.5 and pH 12 with the bulk of the cytosol protein focusing in the pH 6–7 range (Fig. 1*A*). Each of the 20 fractions collected was first tested for its ability to activate dormant oxidase in the cell-free system with SDS. None of the fractions exhibited activity under these conditions (Fig. 1*B*, open circles), suggesting that the cytosol factor may have been resolved into components that were individually incapable of activating the oxidase. To test this possibility, each fraction was then assayed in the presence of a suboptimal or threshold concentration of cytosol. The rationale for this approach was that a low concentration of normal cytosol in each reaction mixture would provide at least a small amount of each of the putative cytosol components required for oxidase activation. If a given fraction contained at least one of the cytosol factor components, it would then enrich the reaction mixture in that particular component and enhance the extent of oxidase activation. The sensitivity of this assay was optimized by exploiting the exponential relationship between oxidase activation and cytosol concentration and adjusting the latter to a point where O_2^- production was just beginning to increase abruptly. Using this method, four cytosol factor components were detected whose peak activities focused in the following pH ranges: component C1 (pI 2.5–3.7), C2 (pI 5.7–6.2), C3 (pI 6.8–7.3), and C4 (pI 8.5–10.5). Components C1–C3 were consistently seen in repeated experiments whereas component C4 was usually, but not always, detected. Fig. 1*B* shows a representative experiment in which only the first three components were seen. The failure to see component C4 consistently may be due to its relative excess compared to the other three components in certain cytosol preparations. Under these conditions, fractions containing component C4 would not be expected to augment oxidase activation.

Experiments were then performed to determine whether any of these four components could reconstitute the ability of type II CGD cytosol to activate the respiratory burst oxidase. Cytosol from either of two patients with type II CGD (patients J.C. and J.G.) was substituted for normal cytosol in the screening assay described above. Since type II CGD cytosol is nearly devoid of cytosol factor activity, it could be incorporated into the assay at a concentration higher than that used for normal cytosol and thereby increase the sensitivity of the assay for the missing component(s). As shown in Fig. 1*C*, component C4 (pI 8.7) partially restored the ability of each patient's cytosol to activate dormant oxidase whereas components C1–C3 were ineffective. This finding suggests that patients J.C. and J.G. share a defect involving component C4 in which this protein is either missing or nonfunctional. The failure of their cytosols to complement each other is consistent with this conclusion. It is of note that while component C4 was inconsistently detected when normal cytosol was used in the screening assay, it was always seen with cytosol from either J.C. or J.G. (e.g., the experiment in Fig. 1 *B* and *C*).

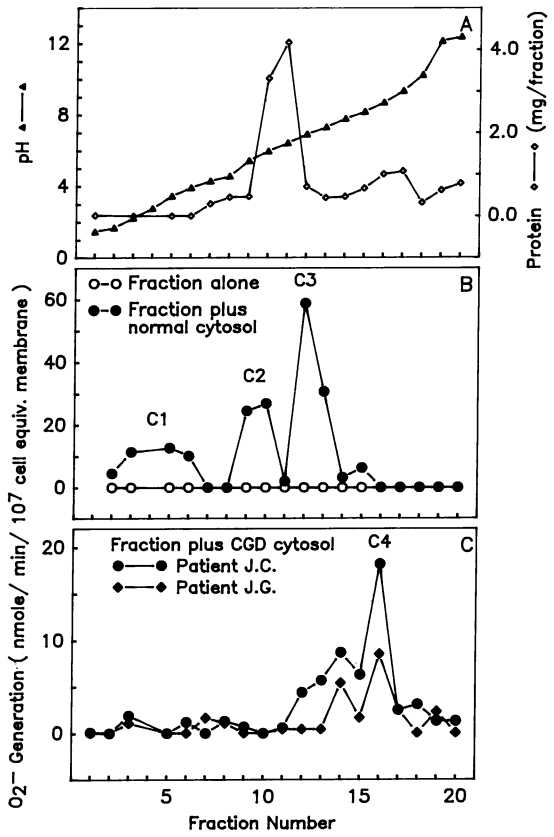

FIG. 1. Resolution of cytosol factor components by preparative isoelectric focusing. Normal cytosol (49.6 ml) in 0.1× buffer A (4.5 × 10^9 cell eq of cytosol = 99.2 mg of protein) was focused for a total of 3200 V·hr. (*A*) pH gradient obtained (▲) and distribution of recovered soluble protein (◇). (*B*) Each fraction (160 μl) was assayed for its ability to activate dormant respiratory burst oxidase in the fully soluble cell-free activation system in the absence (○) or presence (●) of a suboptimal amount of normal unfractionated cytosol (10 μl per 250-μl reaction mixture). This amount of cytosol was capable of activating dormant oxidase to the extent of 6.7 nmol of O_2^- per min per 10^7 cell eq of membrane in this experiment. This background rate was subtracted from that obtained in the presence of each of the fractions. (*C*) Similar assay of oxidase activation was performed except that 38 μl of cytosol from CGD patient J.C. (●) or J.G. (◆) was included in each assay mixture instead of normal cytosol. This quantity of CGD cytosol was derived from 3.3 × 10^6 neutrophils. Protein that precipitated during the focusing was inactive in each of the cell-free activation assays. The recovery of soluble protein was 15% of that applied to the focusing chamber. Recovery of cytosol factor activity could not be accurately calculated because the dependence of oxidase activation on cytosol concentration was nearly third order. A representative experiment is shown from a total of seven experiments, each performed with a different preparation of normal cytosol. In the experiment shown, the four cytosol components focused with the following pI values: C1, 3.5; C2, 5.7; C3, 6.9; and C4, 8.7. In the experiment shown, fraction 4 was lost during dialysis.

Studies were then performed to see whether a combination of components C1–C4 was sufficient to activate the oxidase in the absence of supplemental cytosol. Component C1–C4 mixtures from three separate isoelectric focusing experiments all failed to activate the respiratory burst oxidase. As a control, all 20 fractions in each of these experiments were combined after removal of ampholytes (which are inhibitory) by dialysis and were found to have little or no activity. These results suggest that either (*i*) there is a fifth component that is lost during fractionation or dialysis or (*ii*) there are only four components, but optimal conditions for recombining them have yet to be established.

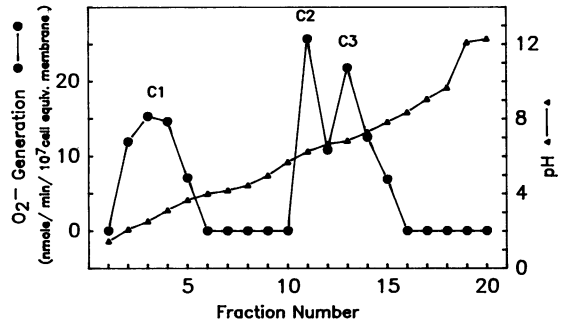

FIG. 2. Isoelectric focusing of cytosol obtained from a patient with type II CGD. The experiment was performed as described in Fig. 1 except that 28 ml of cytosol (58 mg of protein) from CGD patient J.C. was focused for a total of 2900 V·hr and then assayed in the cell-free activation system in the presence of 10 μl of normal cytosol (which activated oxidase to the extent of 7.1 nmol of O_2^- per min per 10^7 cell eq of membrane). As in Fig. 1, this background rate was subtracted from that obtained in the presence of each of the fractions. In this experiment, the cytosol components focused at the following pI values: C1, 3.0; C2, 6.2; and C3, 6.8. The experiment was performed just once due to the large amount of patient cytosol required. The recovery of soluble protein from the isoelectric focusing was 20%.

Determination of the Cytosol Factor Components Present in Type II CGD. The results from Fig. 1*C* suggest that only cytosol factor component C4 is deficient in the two type II CGD patients studied in that experiment. The implication is that the other three cytosol factor components should be normal. This was verified in an experiment in which cytosol from patient J.C. was subjected to preparative isoelectric focusing by the same method used with the normal cytosol in Fig. 1. Each fraction was then assayed in the presence of a suboptimal amount of normal cytosol. As shown in Fig. 2, the cytosol from this patient contained component C1 (pI 3.0), component C2 (pI 6.2), and component C3 (pI 6.8). Component C4, as expected, was not detected. These results indicate that in this form of CGD, three of the four identified cytosol factor components are normal.

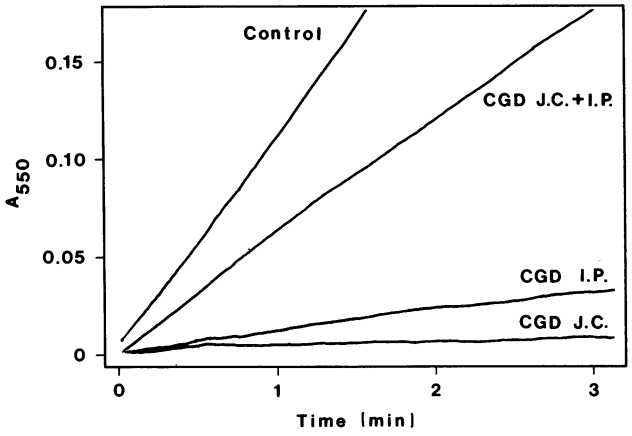

FIG. 3. Complementation of the cytosol factor defects in two patients with type II CGD. O_2^- production, here shown as the superoxide dismutase-inhibitable change in absorbance at 550 nm (A_{550}), was assayed. For tracing CGD J.C.+I.P., 5×10^6 cell eq of cytosol from each of the two CGD patients J.C. and I.P. were combined in the reaction mixture, while for the other tracings, 10^7 cell eq of cytosol were present in each reaction. The figure shows tracings of the spectrophotometric data. The rates of O_2^- production (nmol of O_2^- per min per 10^7 cell eq of membrane) were as follows: control, 18.2; CGD J.C.+I.P., 8.9; CGD I.P., 1.9; and CGD J.C., 0.3. A representative set of tracings is shown from an experiment performed in triplicate with the following number of cytosol preparations: control, 2; CGD J.C., 1; and CGD I.P., 2.

Complementation Studies. As discussed above, kinetic analysis of respiratory burst oxidase activation in the cell-free system suggested that at least three components of the cytosol are required (11). Thus, there may be other forms of type II CGD besides the one that affects patients J.C. and J.G. Mixing experiments with cytosol samples from our first seven patients failed (12) to disclose complementary forms of cytosol factor deficiency. However, we have discovered a type II CGD patient (I.P.) whose cytosol does complement that of patient J.C. Fig. 3 shows the results of complementation studies in which the oxidase-activating capacity of mixtures of the two defective cytosols was examined. In an assay mixture containing equal volumes of the two different cytosols, the observed oxidase activity was 8.9 nmol of O_2^- per min per 10^7 cell eq. This rate was nearly nine times greater than that predicted if the activities in the two cytosols were simply additive and was similar to the rate obtained with a comparable quantity of normal cytosol (18.2 nmol of O_2^- per min per 10^7 cell eq of membranes).

From the complementation experiment just discussed, it can be inferred that different cytosol factor components should be deficient in patients J.C. and I.P. This prediction was tested directly. Cytosol from a normal donor was fractionated by preparative isoelectric focusing, and in this instance, all four cytosol factor components were identified when screened with a low concentration of normal cytosol (Fig. 4 *Upper*). The isoelectric focusing in this particular experiment was better than usual and may have resolved component C2, usually seen as a single peak between pH 5.7 and 6.2, into two separate peaks at pI 5.7 and 6.1. It is not

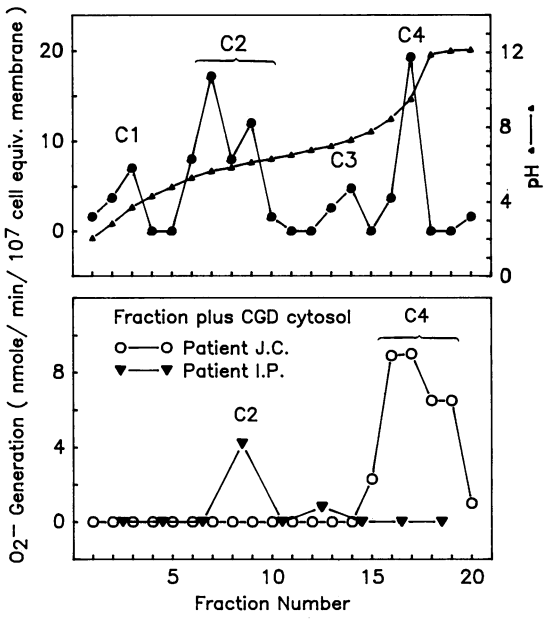

FIG. 4. Reconstitution of cytosol factor activity in complementary type II CGD patients by various cytosol components. The experiment was performed as described in Fig. 1 except that 39 ml of normal cytosol (80 mg of protein) was focused for a total of 5400 V·hr. (*Upper*) The pH gradient (▲) and the ability of each fraction to activate the respiratory burst oxidase in the presence of a suboptimal amount of normal unfractionated cytosol (10 μl per reaction mixture) (●) are shown. (*Lower*) Each fraction (113 μl) was added to 37 μl of cytosol from patient J.C. (○). Due to the small amount of cytosol available from patient I.P., 66.5 μl of fractions 2–19 were added in pairs to 37 μl of I.P. cytosol (▼). The experiment was performed once although the O_2^- rates in the presence of normal cytosol (●) or J.C. cytosol (○) are representative of measurements performed in triplicate. The rates in the presence of I.P. cytosol were measured once. In this experiment, the cytosol components focused at the following pI values: C1, 3.7; C2, 5.7-6.2; C3, 7.3; and C4, 9.5.

clear whether these two peaks represent distinct cytosol factor components. Each of the isoelectric focusing fractions was assayed in the presence of CGD cytosol from either patient J.C. or I.P. for its ability to reconstitute the oxidase-activating capacity of the defective cytosol. A result similar to the one obtained in Fig. 1*C* was obtained with cytosol from patient J.C. Component C4 (pI 8.5–12) was able to augment substantially the ability of her cytosol to activate dormant oxidase. In contrast, these same fractions were unable to correct the defect in cytosol from I.P. Instead, the cytosol factor component C2 (pI 6.1) was capable of partially reconstituting cytosol factor activity in her cytosol. These results are consistent with the complementation studies shown in Fig. 3 and indicate that separate and distinct cytosol factor components are defective in these two patients.

DISCUSSION

The experiments presented above indicate that the cytosol factor required for respiratory burst oxidase activation is, in fact, composed of at least four components that can be resolved by isoelectric focusing. It is possible that there may be a fifth component yet to be identified given the inability of the combination of the four known components to activate the respiratory burst oxidase. As discussed above, however, the inability to reconstitute oxidase-activating capacity in the combined peaks could also be due to nonoptimal mixing conditions.

The resolution of cytosol factor activity into multiple components is consistent with a report from this laboratory (11) regarding the kinetics of activation of the respiratory burst oxidase in the cell-free system. The kinetic data obtained in those experiments could be explained by the following scheme:

$$M + S \underset{Mg^{2+}}{\rightleftharpoons} M{\cdot}S \xrightarrow[SDS,\ Mg^{2+}]{\text{slow}} [M{\cdot}S]^* + C_\alpha \rightleftharpoons [M{\cdot}S]^*C_\alpha + C_\beta \rightleftharpoons [M{\cdot}S]^*C_\alpha C_\beta \quad [2]$$

where M is the membrane-associated component(s) of the resting oxidase, S is a cytosolic component that forms a preactivation complex with the membrane (M·S), [M·S]* is the activated form of that complex that is generated through the action of the detergent (SDS) and Mg^{2+}, C_α and C_β are the cytosolic proteins (possibly identical) that convert the activated membrane complex into catalytically active forms of the oxidase, and [M·S]*C_α and [M·S]*$C_\alpha C_\beta$ are the high- and low-K_m forms of the active oxidase, respectively. Thus, three cytosolic factors are required by this model for oxidase activation — S, C_α, and C_β. The model does not preclude the involvement of other cytosolic proteins since each of these kinetic components could be comprised of two or more subunits (e.g., S could be an S_1S_2 dimer). Under the conditions of isoelectric focusing, some of these complexes could dissociate. We have not assigned a kinetic function to any of the four cytosol factor components thus far identified.

The biochemical defect in two of the three CGD patients studied (J.C. and J.G.) appears to be confined to cytosol factor component C4. In the one patient (J.C.) whose cytosol was tested directly, components C1–C3 appeared to be normal (Fig. 2). While the identity of component C4 has not been established, it is possible that it may be closely related to a family of 48-kDa phosphoproteins that have been implicated in the activation of the respiratory burst in human neutrophils (18–20). It is believed that a highly basic (i.e., pI > 10) 48-kDa precursor protein undergoes a series of six phosphorylations in response to neutrophil stimulation that results in a family of phosphoproteins with pI values of ≈10, 9.5, 8.7, 7.8, 7.3, and 6.8 (21, 22). These phosphoproteins appear to participate in a yet undefined way in the activation of the oxidase. Two lines of evidence support the hypothesis that component C4 and the 48-kDa protein(s) may be identical. First, most patients with type II CGD, including patients J.C. and J.G. (21), appear to be missing all of the members of the 48-kDa family of phosphoproteins. Second, the isoelectric point of component C4 is similar to that of the postulated unphosphorylated precursor of the 48-kDa phosphoproteins (22). Since the cytosol preparations used in all of the experiments in this paper were derived from unstimulated neutrophils, the unphosphorylated (basic) precursor would be expected to be the predominant 48-kDa protein. Moreover, in unstimulated neutrophils there appear to be several 48-kDa phosphoproteins that vary in pI value from ≈10.5 to 8.0 (see figure 1 in ref. 21). This is similar to the pH range of fractions capable of partially correcting the defect in the cytosols from patients J.C. and J.G. (Figs. 1*C* and 4 *Lower*).

We thank Susan Burke for her outstanding help in the preparation of this manuscript and Dr. Robert Smith for his help in composing the isoelectric focusing figures. We also thank Drs. Myron I. Liebhaber, Robert L. Roberts, John F. Bastian, Stanley A. Schwartz, and John Casey for kindly allowing us to obtain blood samples from their patients. Most of all, we are indebted to the three patients we studied without whose cooperation much of this research could not have been performed. This work was supported in part by U.S. Public Health Service Grant AI-24838 (J.T.C.). J.T.C. is an Established Investigator of the American Heart Association.

1. Babior, B. M. (1978) *N. Engl. J. Med.* **298,** 659–668.
2. Glass, G. A., DeLisle, D. M., DeTogni, P., Gabig, T. G., Magee, B. H., Markert, M. & Babior, B. M. (1986) *J. Biol. Chem.* **261,** 13247–13251.
3. Curnutte, J. T. & Babior, B. M. (1987) *Adv. Hum. Genet.* **16,** 229–297.
4. Bromberg, Y. & Pick, E. (1984) *Cell. Immunol.* **88,** 213–221.
5. Curnutte, J. T. (1985) *J. Clin. Invest.* **75,** 1740–1743.
6. McPhail, L. C., Shirley, P. S., Clayton, C. C. & Snyderman, R. (1985) *J. Clin. Invest.* **75,** 1735–1739.
7. Heyneman, R. A. & Vercauteren, R. E. (1984) *J. Leuk. Biol.* **36,** 751–759.
8. Bromberg, Y. & Pick, E. (1985) *J. Biol. Chem.* **260,** 13539–13545.
9. Curnutte, J. T., Kuver, R. & Scott, P. J. (1987) *J. Biol. Chem.* **262,** 5563–5569.
10. Curnutte, J. T., Kuver, R. & Babior, B. M. (1987) *J. Biol. Chem.* **262,** 6450–6452.
11. Babior, B. M., Kuver, R. & Curnutte, J. T. (1988) *J. Biol. Chem.* **263,** 1713–1718.
12. Curnutte, J. T., Berkow, R. L., Roberts, R. L., Shurin, S. B. & Scott, P. J. (1988) *J. Clin. Invest.* **81,** 606–610.
13. Curnutte, J. T. (1988) in *Hematology/Oncology Clinics of North America*, ed. Curnutte, J. T. (Saunders, Philadelphia), Vol. 2, pp. 241–252.
14. Weening, R. S., Corbeel, L., de Boer, M., Lutter, R., van Zwieten, R., Hamers, M. N. & Roos, D. (1985) *J. Clin. Invest.* **75,** 915–920.
15. Dinauer, M. C., Orkin, S. H., Brown, R., Jesaitis, A. J. & Parkos, C. A. (1987) *Nature (London)* **327,** 717–720.
16. Parkos, C., Dinauer, M., Jesaitis, A., Orkin, S. & Curnutte, J. (1987) *Blood* **70,** 93a (abstr.).
17. Peerless, A. G., Liebhaber, M., Anderson, S., Lehrer, R. I. & Stiehm, E. R. (1985) *J. Pediatr.* **106,** 783–785.
18. Hayakawa, T., Suzuki, K., Suzuki, S., Andrews, P. C. & Babior, B. M. (1986) *J. Biol. Chem.* **261,** 9109–9115.
19. Heyworth, P. G. & Segal, A. W. (1986) *Biochem. J.* **239,** 723–731.
20. Caldwell, S. E., McCall, C. E., Hendricks, C. L., Leone, P. A., Bass, D. A. & McPhail, L. C. (1988) *J. Clin. Invest.* **81,** 1485–1496.
21. Okamura, N., Curnutte, J. T., Roberts, R. L. & Babior, B. M. (1988) *J. Biol. Chem.* **263,** 6777–6782.
22. Okamura, N., Malawista, S. E., Roberts, R. L., Rosen, H., Ochs, H. D., Babior, B. M. & Curnutte, J. T. (1988) *Blood* **72,** 811–816.