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Biochemistry

Primary structure and unique expression of the 22-kilodalton light chain of human neutrophil cytochrome *b*

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ABSTRACT **Cytochrome *b* comprising 91-kDa and 22-kDa subunits is a critical component of the membrane-bound oxidase of phagocytes that generates superoxide. This important microbicidal system is impaired in inherited disorders known as chronic granulomatous disease (CGD). Previously we determined the sequence of the larger subunit from the cDNA of the *CGD* gene, the X chromosome locus affected in "X-linked" CGD. To complete the primary structure of the cytochrome *b* and to assess expression of the smaller subunit, we isolated cDNA clones for the 22-kDa polypeptide by immunoscreening and confirmed their authenticity by direct N-terminal protein sequencing. Although the deduced amino acid sequence of the 22-kDa subunit is not overtly similar to other known cytochromes, we observed a 31-amino acid stretch of 39% identity with polypeptide I of mitochondrial cytochrome *c* oxidase centered on a potential heme-coordinating histidine. Similarities in the hydropathy profiles and spacing of histidines of the 22-kDa protein and myoglobin suggest structural motifs in common with other heme-containing proteins that are not readily revealed by primary amino acid sequences. Although RNA for the larger subunit has been found only in cells of the phagocytic lineage, stable RNA encoding the 22-kDa subunit was observed in all cell types. However, the stable 22-kDa protein was detected only in phagocytic cells that were expressing the larger subunit RNA. This observation suggests that the large subunit may play a role in regulating the assembly of the heterodimeric cytochrome *b*.**

Phagocytic cells (neutrophils, macrophages, and eosinophils) are central to the host defense against invading microbes (1, 2). Upon stimulation with a variety of particulate or soluble agents, a latent NADPH-oxidase system is activated and produces large quantities of superoxide (3–5). This radical is converted to toxic oxygen derivatives important for microbicidal activity and inflammatory tissue injury (1, 2, 6). The protein components of the oxidase have been incompletely defined, but evidence suggests that it is a nonmitochondrial, plasma membrane-bound electron transport system that includes a *b*-type cytochrome with an unusually low redox potential and probably a flavoprotein (4, 5, 7). Compelling evidence for the functional importance of the cytochrome *b* is provided by studies of the genetic disorder X chromosome-linked chronic granulomatous disease (X-CGD) (7), which results from mutations in a gene now known to encode one component of the cytochrome (refs. 8 and 9 and see below). This disorder is characterized by the inability of phagocytes to produce superoxide (7). Consequently, affected individuals lack an essential host microbicidal system and develop recurrent, severe bacterial and fungal infections.

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Cytochrome *b* purified from neutrophil membranes appears to be a heterodimer of a glycosylated 91-kDa heavy chain and a nonglycosylated 22-kDa light chain (10–12). The 91-kDa subunit is encoded by a gene designated *CGD*, residing at chromosomal position Xp21, which originally was identified on the basis of genetic linkage without reference to a specific protein product (8). Antisera generated to either a synthetic peptide predicted from the cDNA or to a fusion protein produced in *E. coli* recognized the 91-kDa component of purified cytochrome *b* (9). Furthermore, direct N-terminal amino acid sequencing confirmed the nature of the predicted protein (13). Since the sequence of the 91-kDa component demonstrated no significant similarity to known cytochromes at the primary amino acid sequence level (8), the relative function of the two subunits, the location of the heme prosthetic group in the heterodimer, and the nature of the association between the subunits remain unknown. Further understanding of the structure of the cytochrome *b* heterodimer requires characterization of the smaller subunit.

Here we report the isolation and nucleotide sequence of cDNA clones encoding the 22-kDa subunit,§ and confirmation of the deduced N-terminal amino acids by direct sequencing of the 22-kDa subunit. Comparison of the primary structure of the two subunits with known cytochromes suggests that the cytochrome *b* is, indeed, an atypical cytochrome species. In contrast to the mRNA of the *CGD* gene encoding the 91-kDa subunit, which is highly regulated in a lineage-specific manner (8), the transcript for the 22-kDa subunit appears to be expressed constitutively in a variety of cell types, even though the polypeptide itself appears to be present in stable form only within phagocytic cells.

METHODS

Isolation and Sequencing of cDNA Clones. Bacteriophage cDNA libraries in the λ phage vectors λgt11 and λgt10, constructed with mRNA derived from human promyeleocytic leukemia HL60 cells, have been described (8). λgt11 recombinants were screened essentially as described (14) by using the IgG fraction of a polyclonal antiserum directed to the 22-kDa polypeptide (10). To obtain a full-length cDNA clone, the *Eco*RI insert of clone 12 (see *Results*) was radiolabeled by random priming (15) and used to screen the λgt10 library by plaque hybridization (16, 17). Nucleotide sequences subcloned in phage M13 derivatives were determined by the dideoxynucleotide chain-termination method (18, 19).

Purification of Neutrophil Cytochrome *b*, Amino Acid Sequencing, and Immunoblot Analysis. Neutrophil cytochrome

Abbreviations: CGD, chronic granulomatous disease; X-CGD, X chromosome-linked CGD.

§The sequence reported in this paper is being deposited in the EMBL/GenBank database (Intelligenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03774).

b was purified by the procedure of Parkos *et al.* (10). Final purification for protein sequencing was achieved by one-dimensional $NaDodSO_4$/PAGE and electroelution onto a poly(vinylidene difluoride) membrane essentially as described by Matsudaira (20). Sequence analysis (20) of the 22-kDa band was performed with an Applied Biosystems (Foster City, CA) model 470 sequenator equipped with on-line phenylthiohydantoin analysis using the program O3RPTH and reverse-phase HPLC on a Brownlee C_{18} column.

Electrophoretic immunoblots were performed as described (9–11).

RNA Blot Hybridization and S1 Nuclease Protection Analysis. Total cellular RNA was isolated from cultured cells as described (7) and subjected to blot analysis (17). For S1 nuclease protection assays, the cDNA insert from clone 17 was digested with *Bst*EII, 5′-end-labeled with γ-ATP and polynucleotide kinase, and hybridized to total cellular RNA samples under conditions as described (21). RNA·DNA hybrids were digested with 750 units of S1 nuclease per ml and electrophoresed under denaturing conditions in urea/acrylamide gels.

Expression of 22-kDa cDNA in COS Cells. For transient expression of the cDNA encoding the 22-kDa subunit, the *Eco*RI sites at the ends of clone 17 were converted to *Hin*dIII sites with synthetic linkers, and the fragment was recloned in pSV-HdIII (22). Monkey kidney COS cells were transfected by the procedure of Chen and Okayama (23) and harvested for immunoblots 48 hr after removal of the calcium phosphate precipitate from the cells.

Computer Analyses. Computer analysis of the primary structure was performed on a Digital Equipment Corporation VAX computer utilizing the software available from the University of Wisconsin Genetics Computer Group (UWGCG) and the Protein Identification Resource of the Division of Research Resources of the National Institutes of Health. Sequence similarities with proteins listed in the National Biomedical Research Foundation data base were analyzed with FAST-P. Hydropathy analyses and plots were obtained from application of CHOUFASMAN and PEPPLOT (UWGCG) programs. These programs apply the Kyte and Doolittle method (24) to calculate a hydrophobicity index for each amino acid residue averaged over a window of nine residues.

RESULTS

Isolation of cDNA for the 22-kDa Subunit of Neutrophil Cytochrome *b*. Polyclonal antiserum directed to the 22-kDa subunit of the cytochrome *b* purified from human neutrophils (10) was used to screen a λgt11 expression vector library (8) prepared from dimethylformamide-treated HL60 cells. Initial screening of 700,000 recombinants identified 26 positive clones. All except 1 clone displayed high-stringency cross-hybridization. A 680-base-pair *Eco*RI fragment of clone 12 was used to isolate a full-length cDNA (clone 17) from the λgt10 library.

Fig. 1 displays the nucleotide sequence of the putative cDNA for the 22-kDa subunit and the predicted amino acid sequence. The sequence of 680 nucleotides [excluding the poly(A) tract] reveals a potential open reading frame extending from the ATG at nucleotide positions 29–31 to a termination codon (TGA) at 614–616. A consensus polyadenylylation signal (25) (AATAAA) is present at positions 659–664. The predicted primary translation product of 195 amino acids has an estimated M_r of 20,961. The deduced primary amino acid sequence is notable for its relatively high proline content (10%). Of the 20 predicted proline residues, 17 lie within the C-terminal 63 amino acids. The directly determined N-terminal sequence of 25 amino acids obtained from the purified 22-kDa polypeptide (Fig. 2) established that the cDNA isolated by antibody screening is derived from the mRNA that encodes the 22-kDa subunit.

Structural Features of the 22-kDa Subunit of Neutrophil Cytochrome *b*. Because the 22-kDa subunit of the cytochrome *b* is a candidate for the heme-bearing subunit of the hetero-

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                                        30                                  60
  G CAG TGT CCC AGC CGG GTT CGT GTC GCC ATG GGG CAG ATC GAG TGG GCC ATG TGG GCC AAC GAG CAG GCG CTG GCG
                                        Met Gly Gln Ile Glu Trp Ala Met Trp Ala Asn Glu Gln Ala Leu Ala

                 90                                 120                 150
TCC GGC CTG ATC CTC ATC ACC GGG GGC ATC GTG GCC ACA GCT GGG CGC TTC ACC CAG TGG TAC TTT GGT GCC TAC TCC
Ser Gly Leu Ile Leu Ile Thr Gly Gly Ile Val Ala Thr Ala Gly Arg Phe Thr Gln Trp Tyr Phe Gly Ala Tyr Ser

                       180                                 210
ATT GTG GCG GGC GTG TTT GTG TGC CTG CTG GAG TAC CCC CGG GGG AAG AGG AAG AAG GGC TCC ACC ATG GAG CGC TGG
Ile Val Ala Gly Val Phe Val Cys Leu Leu Glu Tyr Pro Arg Gly Lys Arg Lys Lys Gly Ser Thr Met Glu Arg Trp

      240                                 270                 300
GGA CAG AAG CAC ATG ACC GCC GTG GTG AAG CTG TTC GGG CCC TTT ACC AGG AAT TAC TAT GTT CGG GCC GTC CTG CAT
Gly Gln Lys His Met Thr Ala Val Val Lys Leu Phe Gly Pro Phe Thr Arg Asn Tyr Tyr Val Arg Ala Val Leu His

                       300                                 330
CTC CTG CTC TCG GTG CCC GCC GGC TTC CTG CTG GCC ACC ATC CTT GGG ACC GCC TGC CTG GCC ATT GCG AGC GGC ATC
Leu Leu Leu Ser Val Pro Ala Gly Phe Leu Leu Ala Thr Ile Leu Gly Thr Ala Cys Leu Ala Ile Ala Ser Gly Ile

                                  420                 450
TAC CTA CTG GCG GCT GTG CGT GGC GAG CAG TGG ACG CCC ATC GAG CCC AAG CCC CGG GAG CGG CCG CAG ATC GGA GGC
Tyr Leu Leu Ala Ala Val Arg Gly Glu Gln Trp Thr Pro Ile Glu Pro Lys Pro Arg Glu Arg Pro Gln Ile Gly Gly

                 480                                 510                 540
ACC ATC AAG CAG CCG CCC AGC AAC CCC CCG CCG CGG CCC CCG GCC GAG GCC CGC AAG AAG CCC AGC GAG GAG GAG GCT
Thr Ile Lys Gln Pro Pro Ser Asn Pro Pro Pro Arg Pro Pro Ala Glu Ala Arg Lys Lys Pro Ser Glu Glu Glu Ala

                            570                                 600
GCG GCG GCG GCG GGG GGA CCC CCG GGA GGT CCC CAG GTC AAC CCC ATC CCG GTG ACC GAC GAG GTC GTG TGA CCT CGC
Ala Ala Ala Ala Gly Gly Pro Pro Gly Gly Pro Gln Val Asn Pro Ile Pro Val Thr Asp Glu Val Val END

      630                                 660
CCC GGA CCT GCC CTC CCA CCA GGT GCA CCC ACC TGC AAT AAA CGC AGC GAA GGC CGG GAA A...
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FIG. 1. Nucleotide sequence of cDNA for the 22-kDa subunit of the neutrophil cytochrome *b* and its deduced amino acid sequence.

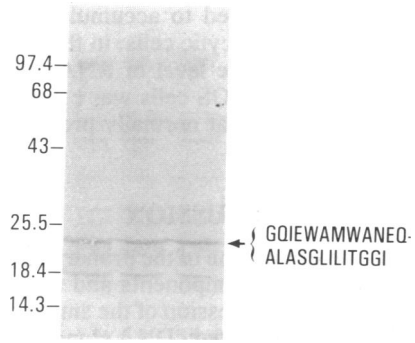

FIG. 2. Determination of the NH_2-terminal sequence of the 22-kDa light chain of purified neutrophil cytochrome *b*. Highly purified cytochrome *b* (*ca.* 2 nmol) was concentrated and subjected to NaDodSO$_4$/PAGE on a 12–17% polyacrylamide gradient gel. Proteins were then electroblotted onto a poly(vinylidene difluoride) membrane stained with Coomassie blue G-250 (20). The 22-kDa bands to the left of the arrow were then excised and placed in an Applied Biosystems model 470 sequenator for analysis. The sequence (30-pmol signal) of the 25 NH_2-terminal amino acids in the single-letter code is shown to the right of the 22-kDa band.

dimeric cytochrome *b* (9, 10), we considered features of its predicted primary and secondary structures that might be shared with other heme-containing proteins. Although a computer search (26) revealed no overt similarities of the deduced 22-kDa protein to known protein sequences, a potentially significant similarity to at least one known cytochrome was observed. A 31-residue region containing His-94 was 39% identical to a corresponding histidine-bearing region of polypeptide I of mitochondrial cytochrome *c* oxidase (Fig. 3). The important features of this comparison are the alignment of 12 residues of identity and 6 conservative substitutions. Consistent with the lack of close similarity to other cytochrome species, immunoblot analysis of bovine cytochrome-b_5, bovine adrenal chromaffin granule cytochrome b_{561}, and *b* cytochromes of bovine mitochondrial complexes II and III revealed no reactivity with the antibody to the 22-kDa subunit (not shown).

Secondary structure predictions by the method of Chou and Fasman (28) suggest that the polypeptide is highly flexible, particularly near its C terminus and contains at least three α-helical regions. A hydropathy analysis of the predicted 22-kDa protein (Fig. 4) predicts three or four significant hydrophobic domains that could serve as membrane-anchorage regions of the protein consistent with the hydrophobic nature (V_{bar} = 0.80–0.82 ml/g) of the cytochrome *b* heterodimer (10). The overall hydropathy plot of the 22-kDa protein superficially resembles that of myoglobin (Fig. 4), with a greatly increased hydrophobic environment for one of the potential Fe coordination sites of the heme at His-94. This histidine residue of the 22-kDa polypeptide aligns exactly

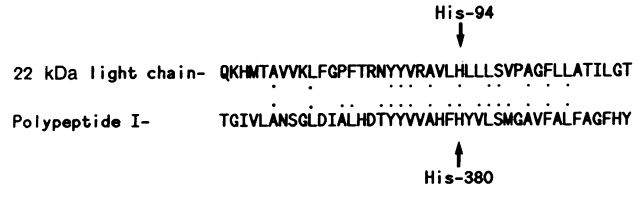

FIG. 3. Comparison of amino acid sequences of the 22-kDa polypeptide with a histidine-bearing segment of polypeptide I of mitochondrial cytochrome *c* oxidase (27). Identical residues are denoted with double dots, and conservative substitutions, with single dots. This similarity generates a score of 39 using FAST-P (26), which is 3 SDs displaced from the mean score of 20.2 derived from analysis of 890 sequences of the National Biomedical Research Foundation data base.

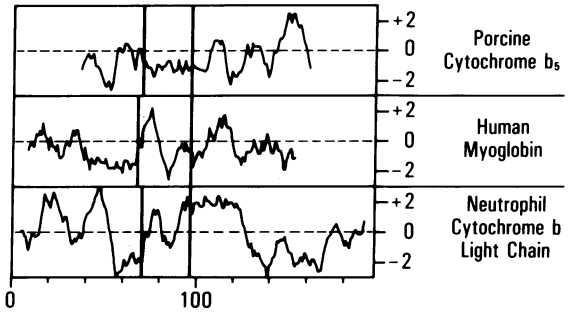

FIG. 4. Hydropathy analysis of porcine cytochrome b_5, human myoglobin, and the 22-kDa light chain of human neutrophil cytochrome *b*. The full-length sequences of human myoglobin and porcine cytochrome b_5 were obtained from the University of Wisconsin Computer Group access to the National Biomedical Research Foundation. These sequences and that determined herein for the 22-kDa light chain of human neutrophil cytochrome *b* were compared in hydrophobicity as a function of amino acid residue number. Calculations for hydropathy were based on the method of Kyte and Doolittle (24). Alignment of the sequences is at the Fe-coordinating histidines distal to the amino terminus on myoglobin and cytochrome b_5 and His-94 on the neutrophil cytochrome *b* light chain.

with an iron-coordinating histidine of myoglobin. In addition, the heme-coordinating histidines in myoglobin and cytochrome b_5 of endoplasmic reticulum are separated by 29 and 24 residues, respectively, a distance similar to that between the only two histidines in the 22-kDa protein. If, indeed, a heme group is coordinated by His-72 and His-94 of the 22-kDa protein, it might be buried within a hydrophobic environment that would facilitate rapid electron transfer (29).

Expression of the 22-kDa Protein and Its RNA Transcript. In an effort to understand how synthesis of the cytochrome *b* heterodimer is regulated, we examined the distribution of the mRNA encoding the 22-kDa subunit in a variety of cell types. The expression of stable transcripts was assessed by RNA blot analysis of uninduced and dimethylformamide-induced HL60 cells and several cell lines derived from nonphagocytic cells, including HeLa, HepG2 (hepatic), K562 (erythroleukemic), cultured human endothelial cells, and Epstein–Barr virus-transformed B cells. A single species of ≈0.8 kb was detected in all cells. Examples are shown in Fig. 5.

The presence of RNA encoding the 22-kDa subunit in nonphagocytic cells was unexpected in that the cytochrome *b* is present in its assembled, stable form only within phagocytic cells. The steady-state levels of the specific RNA in the various cell types were comparable. Although the level in HL60 cells increased somewhat upon induction of differentiation, the constitutive level in HepG2 and K562 cells was at least equivalent or slightly greater. The striking induction of mRNA specific for the 91-kDa subunit in HL60 cells upon dimethylformamide treatment and its absence in nonphagocytic cells are also shown in Fig. 5. From these data we can conclude confidently that the mRNAs encoding the cytochrome b subunits are regulated independently.

To examine the nature of the RNA transcripts found in HeLa and HepG2 cells, S1 nuclease protection analysis was performed with a 600-base-pair 5′-end-labeled fragment that spans virtually the entire predicted open-reading frame (see *Methods*). The S1 probe fragment was completely protected by RNAs isolated from HL60, HeLa, and HepG2 cells (not shown). We deduce, therefore, that the stable RNA found in nonphagocytic cells is indistinguishable from that seen in HL60 cells and does not represent a similar, and perhaps alternatively spliced, version that might encode a different polypeptide.

In view of the unexpected distribution of the RNA encoding the 22-kDa cytochrome *b* subunit, we examined cells for

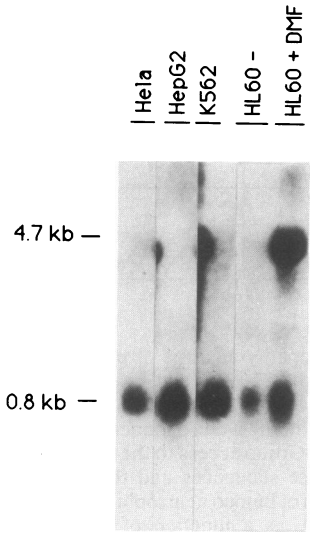

FIG. 5. Expression of cytochrome *b* subunit RNAs in various cell types. Total cellular RNAs (5 μg each) from the indicated cell lines were subjected to blot analysis. The nitrocellulose filter was hybridized simultaneously to cDNAs for the large subunit [marked 4.7 kb (91 kDa)] and for the light chain [marked 0.8 kb (22 kDa)]. RNAs were prepared from control HL60 cells ($HL60^-$) and from cells treated with dimethylformamide for 5 days ($HL60^+$). The density to the left side of the lane containing the K562 RNA resulted from strong hybridization in an adjacent lane of the RNA blot.

the presence of the 22-kDa protein by immunoblot analysis. The 22-kDa polypeptide was detected in induced HL60 cells, where the 91-kDa transcript and its protein product are expressed (ref. 8, and unpublished data), but was absent or barely detectable in HepG2 cells, neutrophils from X-CGD patients (Fig. 6) or K562 and HeLa cells (not shown). Therefore, although nonphagocytic cells contain RNA for the 22-kDa subunit in an abundance similar to that of granulocytic HL60 cells, stable polypeptide is virtually absent. When full-length cDNA encoding the 22-kDa subunit was transfected into monkey kidney COS cells under the control of the simian virus 40 early promoter in a pSV vector (22), high

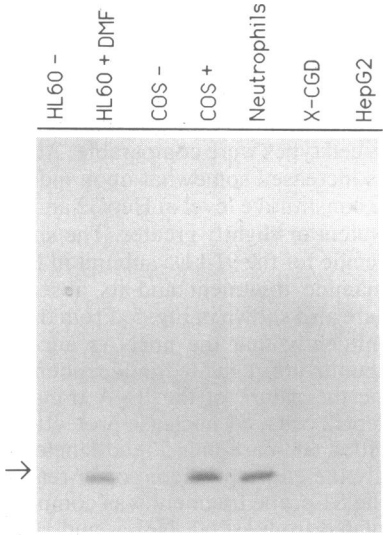

FIG. 6. Immunoblot analysis of the 22-kDa (arrow) protein in total cell extracts. Cells of the indicated sources were solubilized as described (10) and electrophoresed in a 10.8% polyacrylamide gel for immunoblot analysis with polyclonal antisera to the 22-kDa subunit. COS^-, control COS cells; COS^+, cells transfected with 22-kDa cDNA expressed from a simian virus 40 promoter; X-CGD, neutrophils of a classic X-CGD patient.

levels of RNA expression led to accumulation of protein, comparable to that in phagocytic cells, in the absence of the 91-kDa subunit (Fig. 6). The level of RNA for the 22-kDa subunit in the transfected COS cells was estimated to be at least 20- to 50-fold above that normally present in HL60 or HepG2 cells (not shown).

DISCUSSION

To complete the determination of the primary structure of the neutrophil cytochrome *b* components and acquire reagents useful for assessing the expression of the smaller subunit, we have isolated and characterized cDNA clones for the 22-kDa polypeptide. The identity of cDNA clones selected by immunoscreening was confirmed by directly determined N-terminal amino acid sequences of purified 22-kDa protein. One notable characteristic of the deduced sequence is its high proline content, an observation previously made for a bovine neutrophil cytochrome *b* preparation of 11–14 kDa (30). The absence of a cleavable signal peptide suggests that targeting of the 22-kDa subunit to the membrane is mediated by internal signal sequences or by its interaction with the 91-kDa glycoprotein subunit of the cytochrome. Although the deduced 22-kDa protein sequence reveals no striking similarity to known proteins, one exception of possible significance was identified. A functionally interesting stretch of the 22-kDa chain containing a histidine (His-94) resembles a region of the heme-bearing subunit (polypeptide I) of the oxygen-reactive mitochondrial cytochrome *c* oxidase (Fig. 3). Furthermore, the similarities of the hydropathy profiles of the 22-kDa chain and myoglobin and the similar spacing of histidines in myoglobin, cytochrome b_5, and the 22-kDa subunit may suggest structural motifs in common with other heme-containing proteins that are not readily revealed at the primary amino acid sequence level.

Previously, we had noted that the 91-kDa X chromosome-derived component also does not display significant similarities to known cytochromes (8). In view of this and the apparent distant relationship of the 22-kDa subunit to any known heme-containing proteins, it is not possible to conclude how the heme prosthetic group(s) in the heterodimer contacts the subunits. The heme prosthetic group could be coordinated with residues of the light chain, the heavy chain, or both. The experimentally determined molecular weight of the protein portion of the detergent-solubilized cytochrome *b* is 100–127 kDa, which indicates that it is a heterodimer or a complex of one 91-kDa and two 22-kDa subunits (10, 11). The theoretical specific heme content, if there were only one heme per cytochrome complex, should be 7.9–10 nmol/mg of protein. However, the measured heme content of the purified cytochrome *b* is 20–30 nmol/mg of protein (10, 30) depending on the value used for the extinction coefficient (29.3–21.6 $mM^{-1} \cdot cm^{-1}$; refs. 31 and 32). Hence, it is possible that more than one heme is present per cytochrome *b*. In that histidine residues at positions 100, 110, and 117 and at positions 207, 208, and 220 of the 91-kDa subunit are spaced similarly to the heme-coordinating histidines-93 and -97 and histidines-182 and -196 of the mitochondrial cytochrome of complex III (33), it is conceivable that a heme prosthetic group could be carried by the 91-kDa subunit alone. Overall, the apparent distant relationship of its subunits to known cytochromes suggests the neutrophil cytochrome *b* is, indeed, an unusual cytochrome species.

The constitutive expression of RNA encoding the small subunit in a variety of cell types is surprising and stands in marked contrast to the lineage-specific expression of the transcript for the 91-kDa subunit, which is derived from the *CGD* gene (8). The latter RNA is detected only in mature phagocytic cells with a functional NADPH-oxidase and cytochrome *b* spectrum and at a low level in Epstein–Barr

virus-transformed B cells, which are thought to express the oxidase system weakly (34). Because patients with X-CGD, who lack the 91-kDa subunit because of a lesion in *CGD* (8, 9), also lack the 22-kDa subunit (refs. 10 and 12; Fig. 6), we have suggested that the small subunit may be unstable in cells in the absence of the larger component of the heterodimer (9). We may speculate that mutations leading to a deficiency of the 22-kDa polypeptide underlie the rare, autosomally inherited, cytochrome-*b*-negative form of CGD (7) in which both subunits are also absent in affected phagocytes (35).

A model in which the 91-kDa subunit stabilizes the light chain is most consistent with the virtual absence of the 22-kDa subunit in nonphagocytic cells (Fig. 6), although a block in mRNA translation has not been formally excluded. Biosynthetic studies have shown rapid degradation of unassembled polypeptide components in several different multimeric membrane protein complexes, such as the acetylcholine receptor (36) and the B-cell and T-cell antigen receptors (37, 38). Since there does not appear to be marked regulation of the RNA for the 22-kDa subunit in cells, the steady-state level of the 22-kDa polypeptide in phagocytic cells may then be determined in large part through production of the 91-kDa subunit. This situation is distinctly unusual. Although mRNAs occasionally may be expressed in stable form without the appearance of their stable protein products (39), this has been described only in instances in which the cell is ultimately destined to use the encoded protein at a later stage of development or maturation.

Although the primary amino acid sequences of the two cytochrome *b* subunits are now complete from cloned cDNAs, several aspects pertinent to the structure and regulation of the heterodimer are as yet unresolved. The topologic organization of the subunits, the domains involved in their interaction, and the location and identity of the axial ligands of the heme prosthetic group(s) in the heterodimer remain to be defined. Finally, the structural basis for participation of the cytochrome *b* in the generation of superoxide anion is unknown.

Note in Proof. Sequencing of genomic DNA of a normal individual revealed a potential polymorphism at nucleotide 243 (C → T), which results in an amino acid change from His-72 (observed in two independent cDNA clones) to Tyr-72. Thus, the presence of two histidines in the 22-kDa polypeptide may not be invariant.

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