**Staphylococcus aureus** Coenzyme A Disulfide Reductase, a New Subfamily of Pyridine Nucleotide-Disulfide Oxidoreductase

**SEQUENCE, EXPRESSION, AND ANALYSIS OF cdr**

(Received for publication, June 20, 1997, and in revised form, September 26, 1997)

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The **cdr** gene encoding coenzyme A disulfide reductase (CoADR) from *Staphylococcus aureus* 8325-4 was cloned, sequenced, and overexpressed. The gene encodes a 438-amino acid polypeptide that has a calculated molecular weight of 49,200 and sequence similarity to the pyridine nucleotide-disulfide oxidoreductase family of flavoenzymes. The deduced primary structure contains consensus sequences for flavin adenine dinucleotide and NADPH-binding regions but lacks the catalytic disulfide signature sequence typical of the glutathione reductase family of disulfide reductases. The active site region of CoADR has only a single cysteine residue that is similar to that in the conserved SFXXC active site motif of NADH oxidase and NADH peroxidase from *Enterococcus faecalis*. CoADR is the first disulfide reductase reported having this active site region, and sequence comparisons of CoADR to representative members of the pyridine nucleotide-disulfide reductase superfamily placed CoADR in a distinct subfamily. CoADR was overexpressed in *Escherichia coli* using the **p**ET expression system, and 5–10 mg of fully active recombinant enzyme were recovered per liter of *E. coli* cells.

We are investigating thiol chemistry and biology in Gram-positive pathogenic bacteria and are interested in how the thiol metabolisms of these organisms compare with that of their human hosts. The human pathogen *Staphylococcus aureus* does not utilize the thiol/disulfide redox system based on glutathione and glutathione reductase (GSR) found in eukaryotes and Gram-negative bacteria (1, 2). Instead, *S. aureus* appears to use a redox system based on CoA and coenzyme A disulfide reductase (CoADR) (22). CoADR is a dimeric flavoprotein that specifically catalyzes the NADPH dependent reduction of oxidized CoA (Equation 1).

\[
\text{CoASSCoa} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{CoASH} + \text{NADP}^+ \quad (\text{Eq. 1})
\]

This work was supported by funding from Abbott Laboratories. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the **GenBank** (EBI Data Bank with accession numbers) **AF041467**.

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1. The abbreviations used are: GSR, glutathione reductase; CoADR, coenzyme A disulfide reductase; NOX, NADH oxidase; NFX, NADH peroxidase; FAD, flavin adenine dinucleotide; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; bp, base pair(s).

**EXPERIMENTAL PROCEDURES**

**Strains and Media**—*S. aureus* 8325-4 was the source of CoADR and genomic DNA (from John Iandolo, Kansas State University, Manhattan, KS). *E. coli* DH5α was from Life Technologies, Inc. (Burlington, ON, Canada), strain BL21 (DE3) and plasmid pET22B (+) were from Novagen (Madison, WI). *S. aureus* was grown in tryptic soy broth (Difco Laboratories, Detroit, MI) at 37 °C. Isolation of *S. aureus* hydrogenase. In addition, the disparate disulfide specificities of CoADR and its presumed human counterpart, GSR, identified this enzyme as a possible target for the design of selective inhibitors that would interrupt the thiol metabolism of *S. aureus* and function as anti-staphylococcal agents. To better compare CoADR to human GSR and to facilitate the recovery of large amounts of recombinant enzyme for structural and functional studies, we have cloned and expressed the **cdr** gene in *Escherichia coli*. Our analysis of the CoADR primary structure reveals striking features that distinguish this enzyme not only from GSR but from other members of the pyridine nucleotide-disulfide oxidoreductase family. This analysis also highlights regions of the enzyme that likely mediate disulfide specificity and provokes questions as to the catalytic mechanism of this enzyme and to the function of homologous enzymes.
Laboratory on an ABI 476A Protein Sequencer. CoADR was purified from S. aureus as described previously (22).

Identification of a DNA Fragment Encoding the N terminus of CoADR—The gene encoding CoADR was identified and cloned using a reverse genetic strategy. Purified CoADR (5 μg) was cleaved with cyanogen bromide as described (6), and the resulting fragments were separated by SDS-PAGE, blotted onto a polyvinylidene difluoride membrane, and stained with Coomassie Blue. Protein bands corresponding to native CoADR and a 35-kDa CNBr cleavage product were excised and sequenced. Degenerate oligonucleotide primers (SD111 and 113; see Table 1), designed from the N-terminal and internal sequences (see Table 1), were used to amplify the N-terminal of the CoADR gene using the PCR. The PCR was performed in PCR buffer (Life Technologies, Inc.) containing S. aureus genomic DNA (10 ng), primers SD111 and SD113 (100 pmol each), MgCl₂ (2.4 mM), tetramethylammonium chloride (60 mM), and dNTPs (0.25 mM each). The reaction was incubated at 95 (30 s), 47 (30 s), and 72 °C (30 s) for 30 cycles. The 600-bp product was ligated directly with the pCRII cloning vector (Invitrogen) and electroporated into E. coli DH5α. The fragment was entirely sequenced from the resulting plasmid, pCR:CDR.

Cloning and Sequencing of the Gene Encoding CoADR—The cloned PCR fragment encoding the N-terminal of CoADR was excised from pCR:CDR by digestion with EcoRI and purified by agarose gel electrophoresis. The excised fragment was labeled with digoxigenin and used to probe Southern blots of S. aureus genomic DNA digested with various restriction enzymes (4). A single 3.8-kilobase pair HindIII fragment that hybridized to the probe under stringent conditions (68 °C, 0.1 SSC buffer containing 0.1% SDS) was cloned from a size selected library (3–5 kilobase pairs) into plasmid pUC18. The resulting plasmid, pUCdR4, was identified by colony hybridization to the PCR fragment and purified. Both strands of the gene encoding CoADR were then sequenced by primer walking. The open reading frame encoding CoADR was compared with a nonredundant protein data base using BLASTP and was aligned to individual members of the pyridine nucleotide-disulfide oxidoreductase family using the GAP algorithm of the Genetics Computer Group software package (Madison, WI). CoADR was then compared by multiple sequence alignment to representative members of this superfamily using the CLUSTAL algorithm of the Genetic Data Environment software package, version 2.2, using a PAM 100 weight-matrix, a k topole of 2, and a fixed and floating gap penalty of 10. TERMINATOR (Genetics Computer Group) was used to identify possible stem loop structures functioning as transcriptional stop signals.

Heterologous Overexpression of cdr in E. coli—The structural gene for CoADR was overexpressed in E. coli using the pET expression system (7, 8). The open reading frame encoding CoADR was amplified by the PCR using primers SD301 and SD302 (see Table 1), which were designed to create Ndel and HindIII sites at the 5′ and 3′ termini of the CoADR gene. The amplified fragment was digested with Ndel and HindIII, purified by agarose gel electrophoresis, and ligated with pET22B(+) (Novagen, Madison WI) digested with the same two enzymes and purified. The resulting plasmid, pXCDR (expression of CoADR; see Fig. 3A), was sequenced to confirm the presence of cdr and transformed into the expression strain BL21 (DE3). For overexpression, TB (10 ml) containing ampicillin (400 μg/ml) was inoculated with a fresh colony of BL21 (DE3)pXCDR and shaken overnight. Cells were harvested by centrifugation, washed twice in TB (10 ml), and used as an inoculum for TB (1 liter) containing ampicillin (400 μg/ml), and incubated with shaking at 37 °C until it reached an A₆₀₀ of 1.2. Isopropyl-β-D-thiogalactoside (to 1 mM) was added, and incubation was continued for 3 h. The cells were harvested, and the recombinant enzyme was purified as described for native CoADR (22) except that lysozyme (0.2 mg/ml) was used in place of lysostaphin in cell disruption. The purity of the enzyme was assessed by SDS-PAGE and staining with Coomassie Blue. The specific activity was measured spectrophotometrically by following the oxidation of NADPH (22). GSR activity of the final sample was measured to confirm that no E. coli GSR had been copurified.

RESULTS

Isolation and Nucleotide Sequence Analysis of cdr—The cdr gene encoding CoADR was identified and cloned using protein sequencing and a reverse genetic strategy. The N-terminal amino acid sequences of CoADR and a 35-kDa CNBr fragment of CoADR are shown in Table I. Degenerate oligonucleotides (Table I) were designed to encode these peptides and used in the PCR to amplify the N-terminal portion of the CoADR gene. This fragment was used as a probe in Southern blots to identify a single HindIII fragment carrying the entire CoADR gene. The structure of the 3.8-kilobase pair HindIII fragment containing cdr and carried in the plasmid pUCdR4 is depicted in Fig. 1A. The complete nucleotide sequence of cdr, the deduced amino acid sequence of CoADR, and the 5′ and 3′ flanking sequences are shown in Fig. 1B. The 1317-bp gene encodes a 438-amino acid polypeptide having a calculated molecular weight of 49,200. The gene contains 35% guanosine and cytosine (G+C) nucleotides consistent with that of S. aureus (3). The N-terminal and internal peptides (the residues with asterisks in Fig. 1B) sequenced from the native and CNBr digested protein are both encoded within this polypeptide, and the sequence of the PCR fragment used as probe (the underlined region in Fig. 1B) is present within the nucleotide sequence. A putative ribosome-binding site (3) was tentatively identified 9 bp upstream from the start codon (ATG). A putative transcriptional start site recently mapped by primer extension3 is located 23 bases upstream from the ATG start codon. The structural gene for CoADR is preceded by another open reading frame (ORF1 in Fig. 1A) that terminates 50 bp upstream from the CoADR start codon. There was no evidence for stem loop structures that might serve as factor independent transcriptional terminators.

Sequence Comparisons—The sequence of CoADR shared greatest similarity to members of the pyridine nucleotide-disulfide oxidoreductase superfamily (P(N) = 10⁻⁶–10⁻³⁰). Members of this superfamily are characterized by a conserved arrangement of functional domains: an N-terminal ADP-binding motif, GXXGXXG, for binding the nucleotide moiety of FAD, followed by a catalytic region having a pair of redox active cysteines, CXXXXC, another ADP-binding motif for the binding

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2 http://www.ncbi.nlm.nih.gov.

3 M. K. Pope, personal communication.
FIG. 1. Structure of cdr. A, structure of the 3.8-kilobase pair HindIII fragment from the S. aureus genome harboring the cdr gene. cdr is preceded upstream by an open reading frame (ORF1) of unidentified function. The dotted arrow indicates a transcript that was recently identified.3 B, DNA sequence of the cdr gene and the deduced amino acid sequence of CoADR. The 438-amino acid polypeptide is encoded by a 1317-bp open reading frame. The tentative ribosome-binding site (51–57) and promoter regions are shaded. The underlined fragment (69–673) represents the PCR fragment used as a probe, and the peptides marked with asterisks are those sequenced from the native and CNBr-generated fragment. The only two cysteine residues are underlined, and all amino acid residues showing homology to conserved regions of flavoprotein disulfide reductases are shown in bold. Cys42 is proposed to be the sole catalytic cysteine functioning similarly to Cys42 of the NADH oxidase from E. faecalis.
of the pyridine nucleotide, and a flavin-binding region. A pair-
wise comparison revealed that CoADR was 20–30% identical
and 40–50% similar to representative members of this super-
family (Table II) and that sites of similarity were concentrated
in areas corresponding to the FAD and pyridine nucleotide-
binding motifs. However, an active site disulfide motif was
missing from the sequence. Instead, CoADR has a stretch of
amino acids containing a single cysteine residue that is similar
to that of the conserved active site signature sequence SFXXC

**Table II**

Sequence identity (similarity) of CoADR to members of the pyridine
nucleotide-disulfide reductase family

| Enzyme Identity (similarity) | %          |
|-----------------------------|------------|
| E. faecalis NPX             | 24 (50)    |
| E. faecalis NOX             | 26 (53)    |
| Streptococcus mutans NOX    | 29 (53)    |
| M. jannaschii NOX           | 28 (53)    |
| Mycoplasma genitalium NOX   | 28 (53)    |
| Mycoplasma pneumonia NOX    | 22 (50)    |
| Hemophilus influenzae GSR   | 23 (51)    |
| Pseudomonas aeruginosa GSR  | 22 (47)    |
| E. coli GSR                 | 21 (45)    |
| Human erythrocyte GSR       | 22 (47)    |
| Trypanosoma cruzi TSR       | 23 (48)    |
| S. aureus MER               | 19 (40)    |
| S. aureus DLD               | 21 (44)    |
| E. coli DLD                 | 24 (53)    |
| B. subtilis DLD             | 24 (47)    |
| E. coli TRXB                | 26 (50)    |
| Streptomyces coelicolor TRXB| 18 (43)    |

**FIG. 2.** Primary sequence alignment of CoADR with *E. coli* (ecgor) and human erythrocyte (hsgor) glutathione reductase (A) and
the NADH oxidase (efnox) and NADH peroxidase (epfpx) from *E. faecalis* (B). The primary structures were aligned using the CLUSTAL
algorithm of the Genetic Data Environment software package, version 2.2, using a PAM 100 weighting matrix, a k topple of 2, and a fixed
and floating gap penalty of 10.

Overproduction of CoADR in *E. coli*—To overcome the
difficulty of isolating CoADR from *S. aureus*, the
cdr gene was cloned into the vector pET22B(+) and overexpressed in
*E. coli*. Extracts of BL21 (DE3) cells harboring pXCDR (Fig. 4A) and
induced with isopropyl-

β-D-thiogalactoside contained signifi-
cant CoADR activity. CoADR was purified from the soluble
fraction at 5–10 mg/liter of culture to greater than 99% purity
according to SDS-PAGE and Coomassie Blue staining (Fig. 4B).

The specific activity (4,600 units/mg) and kinetic parameters
(1.9 ± 0.5 μM, K_{cat} = 14 ± 3 μM,
and k_{cat} = 1100 ± 200 s^{-1}) of the recombinant enzyme were
indistinguishable from that of CoADR isolated from *S. aureus*
(Ref. 22 and Tables I and II). The purified CoADR sample had

of NADH oxidase (NOX) and NADH peroxidase (NPX) from
*Enterococcus faecalis* (Fig. 2A), enzymes considered to be mem-
mbers of the pyridine nucleotide-disulfide reductase superfamily.
Furthermore, a multiple sequence alignment of CoADR to
members of the superfamily places CoADR in a new subfamily
of disulfide reductase that is separate from GSR, dihydrolipo-
amide dehydrogenase, thioredoxin reductase, and mercuric re-
ductase and that more closely resembles NPX and NOX (Fig.
3). Sequence alignments of CoADR with NOX and NPX are
shown in Fig. 2A and with GSR from *E. coli* and human erythro-
cyte in Fig. 2B. It is clear from these comparisons that the
CoADR sequence is divergent from both classes of enzyme but
shares the catalytic region of the NPX and NOX subfamily and
sequences typical of NADPH as opposed to NADH specific
enzymes with the GSR subfamily. The upstream open reading
frame, ORF1, which was also compared with the protein data
base using BLASTP, encodes a homolog to a family of “hypo-
thetical proteins” identified in the DNA sequences of a variety
of microorganisms. The closest member of that family was the
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protein from *Bacillus subtilis*, P(N) 10 2 (GenBank accession number for the
*Bacillus* homolog is Z79580).
no detectable GSR activity, and the N-terminal sequence of the recombinant enzyme was identical to that of native CoADR purified from *S. aureus* (Table I).

**DISCUSSION**

The *cdr* gene of *S. aureus* encodes a polypeptide having a calculated molecular weight of 49,200 that is consistent with a subunit *M*₉ of 49,000 and native *M*₉ of 90,000 ± 10,000 estimated previously (22). The gene contains a putative ribosome-binding site (Fig. 1B) and putative transcriptional start site. The deduced primary structure of CoADR is similar to that of the pyridine nucleotide disulfide oxidoreductase superfamily and contains a catalytic region, two FAD-binding regions, and a single NADPH-binding region in appropriate arrangement. The most striking feature of the CoADR primary structure is its lack of a conserved pair of catalytic cysteine residues. CoADR instead has a region containing a single cysteine residue (Cys⁴³) that is similar to the active site cysteine (Cys⁴⁰) of the NPX and NOX from *E. faecalis*. These enzymes are members of the pyridine nucleotide-disulfide reductase superfamily, but their unique active sites have thus far distinguished them from enzymes that actually reduce disulfide bonds (9). Claiborne and co-workers have extensively characterized NPX and demonstrated that Cys⁴² forms a stabilized sulfenic acid intermediate during the catalytic reduction of H₂O₂ by this enzyme (10, 11).

Their analysis of the x-ray crystal structure of NPX further showed that Cys⁴² is structurally and functionally similar to Cys⁶¹, the charge transfer cysteine, of human erythrocyte GSR (12, 13). Cys⁴⁰ of CoADR likely participates in substrate reduction as well but in a manner that more closely resembles the reduction of O₂ and H₂O₂ by NOX and NPX than the reduction of disulfides by GSR. Indeed, we find that during catalysis CoADR forms a stable mixed disulfide intermediate with CoA. These findings are discussed in detail in the accompanying paper (22).
following the central glycine in CoADR is alanine, a residue that could not functionally replace the conserved histidine (His10) that is believed to stabilize the sulfenic acid that forms at Cys42 during catalysis by these enzymes (11, 13, 15). CoADR has two cysteine residues, one in the proposed active site region (Cys43) and the other in the center of the N-terminal ββ motif (Cys46). Thiol titrations of CoADR suggest that one of these activities. We have shown that CoADR has only minimal O2 or question whether NPX, NOX, and CoADR share catalytic ac-
The similarity of CoADR to NPX and NOX also provokes the
The true function of the protein encoded by this gene?
what is the oxidized enzyme (22). Residue 16 of NPX is buried in the
core of the FAD-binding region. Cys46 of CoADR would be
buried in the analogous region of CoADR and is thus likely the
cysteine that is recalci
tant to oxidation. In catalysis by GSR a C-terminal histidine residue, His467 of the human erythrocyte
enzyme, acts as general base in the activation of the inter-
change cysteine; however, no analogous residue in CoADR is
obvious. Another ββ ADP-binding fold starts at Lys150 in
CoADR and presumably binds the ADP moiety of NADPH. The
replacement of the terminal glycine in the GXGXXG motif with
serine is common among enzymes that bind NADPH rather than
NADH (14). The bulkier serine residue is proposed to
expand the fold to sterically permit the 2′ phosphate into the
binding cleft. An arginine residue (Arg279) just following the
terminal β sheet likely binds the 2′ phosphate of NADPH. Both
NPX and NOX, which use NADH as cofactor, maintain the
terminal glycine residue and have a conserved aspartate follow-
ning the terminal β sheet that is believed to form a hydrogen
bond with the 2′-hydroxyl of the ribityl moiety (9, 14, 16).
CoADR has an additional fingerprint region (residues 267–277)
that likely participates in the binding of the flavin moiety of
FAD. The terminal aspartate of this region (Asp277) is well
conserved and in other enzymes is known to interact with the
3′ hydroxyl of the FAD ribityl moiety (17–19).

The structural differences between CoADR and human
GSR that result in the distinct disulfide specificities of these
enzymes are of particular interest. In the absence of tertiary
structural information for CoADR, a comparison of the x-ray
structures for NPX and human GSR was made instead.
Although the overall fold of these two enzymes are quite simi-
lar, the region following the cysteine redox centers are of
dramatically different structure. In human GSR this region
(residues 63–122) comprises two long α-helices connected by
a loop region (19), whereas in NPX this region (residues
43–68) comprises several short stretches of helix and coil (12,
13, 20). Because these regions in both enzymes span the
substrate-binding pocket, it is possible that the analogous
region in CoADR (residues 44–70) may participate in the
specificity for CoA disulfide. Future structure-function studies
of the recombinant enzyme will be necessary to address
these speculations.

CoADR is the first disulfide reductase reported to have a
SFXXC active site motif and represents a new subfamily of
disulfide reductase (Fig. 3). This finding implies that proteins
that are identified as similar to NPX and NOX due to the
presence of the SFXXC motif may not reduce H2O2 or O2 but
instead reduce disulfide bonds. For example, in the sequence of
Methanococcus jannaschii (21) a gene was identified as encoding
an NADH oxidase. This gene has the SFXXC motif but
shares greater sequence similarity to CoADR than to NOX (Fig.
3) and lacks a residue similar to the conserved His10 of
NOX. What is the true function of the protein encoded by this gene?

The similarity of CoADR to NPX and NOX also provokes the
question whether NPX, NOX, and CoADR share catalytic ac-
tivities. We have shown that CoADR has only minimal O2 or
H2O2 reductase activity, <0.1% that of its CoA disulfide reduc-
tase activity (see Table II of Ref. 22), but can NPX or NOX
reduce CoA disulfide? E. faecalis NPX was shown to have no
disulfide reductase activity using the substrates lipoamide,
glutathione disulfide, and DTNB, however, CoADR activity
was never measured. NOX was recently shown to have no
detectable CoADR activity.4

Previous purifications of CoADR yielded approximately 200 µg of enzyme from 10L of S. aureus cells. This level was
sufficient for an initial characterization of the enzyme but has
hampered more detailed structure/function investigations.
The ability to rapidly purify milligram quantities of enzyme will
now permit a structural analysis of CoADR. Protein engineer-
ing studies aimed at the identification of those residues within
CoADR that mediate substrate recognition and turnover are
now underway.

Low molecular weight thiols play diverse and important
roles in aerobic organisms. CoADR was originally identified
as an enzyme central to the thiol metabolism of the human
pathogen S. aureus and as a potential point of intervention in
the treatment of staphylococcal infections (22). The cdr
sequence has further confirmed that CoADR is structurally
divergent from other disulfide reductases, and recent inves-
tigations show that these enzymes reduce disulfide bonds by
different chemical mechanisms. A key question now is
whether CoADR is essential for S. aureus pathogenicity.
The molecular genetics of S. aureus are well established and
provide a means of testing this possibility directly. The path-
ogenicity of a mutant strain of S. aureus in which cdr has
been disrupted can be compared with that of an otherwise
isogenic organism. Such a study would provide insight as to
the role thiols play during infection and would provide the
first direct test of whether interrupting the thiol metabolism
of a pathogen could curtail virulence.

Acknowledgments—We thank Chris Radomski and Barbara Waters
of Terragen Diversity, Inc. for technical support in the sequencing of the
cdr gene and Yossi Av-Gay for tutorials in using the Genetic Data
Environment software.

REFERENCES
1. Fahey, R. C., Brown, W. C., Adams, W. B., and Wersham, M. B. (1978)
J. Bacteriol. 133, 1126–1129
2. Newton, G. L., Arnold, K., Price, M. C., Sherrill, C., del Cardayre, S. B.,
Aharonomitzi, Y., Cohen, G., Davies, J., Fahey, R. C., and Davis, C. (1996)
J. Bacteriol. 178, 1990–1995
3. Novick, R. P. (1991) Methods Enzymol. 204, 587–636
4. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A
Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring
Harbor, NY
5. Asnuel, P. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith,
J. A., and Struhl, K. (1998) Current Protocols in Molecular Biology, John
Wiley & Sons, Inc., New York
6. Matsudaira, P. (1990) Methods Enzymol. 182, 602–613
7. Studier, F. W., and Moffatt, B. A. (1986) J. Mol. Biol. 189, 113–130
8. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990)
Methods Enzymol. 185, 60–89
9. Ross, R. P., and Claiborne, A. (1992) J. Mol. Biol. 227, 658–671
10. Claiborne, A., Ross, R. P., and Parsonage, D. (1992) Trends Biochem. Sci. 17,
183–186
11. Claiborne, A., Miller, H., Parsonage, D., and Ross, R. P. (1993) FASEB J. 7,
1483–1490
12. Stehlé, T., Claiborne, A., and Schulz, G. E. (1993) Eur. J. Biochem. 211,
221–228
13. Yeh, J. I., Claiborne, A., and Hol, W. G. (1996) Biochemistry 35, 9951–9957
14. Bellamacina, C. R. (1996) FASEB J. 10, 1257–1269
15. Crane, E. J., III, Parsonage, D., and Claiborne, A. (1996) Biochemistry 35,
3280–3287
16. Ross, R. P., and Claiborne, A. (1991) J. Mol. Biol. 221, 857–871
17. Schreuder, H. A., van der Laan, J. M., Hol, W. G. J., and Drenth, J. (1988)
J. Mol. Biol. 199, 637–648
18. Schierbeek, A. J., Swarte, M. B. A., Dijkstra, B. W., Vriend, G., Read, R. J., Hol,
W. G. J., Drenth, J., and Betzel, C. (1989) J. Mol. Biol. 206, 365–379
19. Karplus, P. A., and Shulz, G. E. (1987) J. Mol. Biol. 195, 701–729
20. Mande, S. S., Parsonage, D., Claiborne, A., and Hol, W. G. (1995) Biochemistry
34, 6985–6992
21. Bult, C. J., et al. (1996) Science 273, 1058–1073
22. del Cardayre, S. B., Stock, K. P., Newton, G. L., Fahey, R. C., and Davies, J. E.
(1998) J. Biol. Chem. 273, 5744–5751

4 A. Claiborne, personal communication.