The Smallest Membrane Anchoring Subunit (QPs3) of Bovine Heart Mitochondrial Succinate-Ubiquinone Reductase

CLONING, SEQUENCING, TOPOLOGY, AND Q-BINDING DOMAIN*

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The cDNA encoding the smallest membrane-anchoring subunit (QPs3) of bovine heart mitochondrial succinate-ubiquinone reductase was cloned and sequenced. This cDNA is 1330 base pairs long with an open reading frame of 474 base pairs that encodes the 103 amino acid residues of mature QPs3 and a 55-amino acid residue presequence. The cDNA insert has an 820-base pair long 3’-untranslated region, including a poly(A) tail. The molecular mass of QPs3, deduced from the nucleotide sequence, is 10,989 Da. QPs3 is a very hydrophobic protein; the hydrophathy plot of the amino acid sequence reveals three transmembrane helices. Previous photoaffinity labeling studies of succinate-ubiquinone reductase, using [3H]azido-Q derivatives (10), identified QPs3 as one of the putative Q-binding proteins in this reductase. An azido-Q-linked peptide with a retention time of 66 min is obtained by high performance liquid chromatography of the chymotryptic digest of carboxymethylated and succinylated [3H]azido-Q-labeled QPs3 purified from labeled succinate-ubiquinone reductase by a procedure involving phenyl-Sepharose 4B column chromatography, preparative SDS-polyacrylamide gel electrophoresis, and acetone precipitation. The amino acid sequence of this peptide is NH2-L-N-P-C-S-A-M-D-Y-C-

Bovine heart mitochondrial succinate-ubiquinone (Q)1 reduc-
tase, also known as Complex II (1), catalyzes electron transfer from succinate to Q. Succinate-Q reductase is composed of two parts, soluble succinate dehydrogenase and a membrane anchoring protein fraction (QPs). Succinate dehydrogenase contains two protein subunits, a 70-kDa flavoprotein (FP) with a covalently linked flavin adenine dinucleotide and a 27-kDa iron-sulfur protein (IP) with three iron-sulfur clusters (2Fe-2S, 3Fe-4S, and 4Fe-4S) (2, 3). The amino acid sequences of FP and IP have been determined by peptide (4) and nucleotide (5) sequencing.

Reconstitutively active QPs has been isolated and characterized (6–8). Purified QPs shows two protein bands (6–8) in the SDS-PAGE system of Weber and Osborn (9) and three (10) in the high resolution SDS-PAGE system of Schägger et al. (11). These three subunits, with apparent molecular masses of 14, 11, and 9 kDa, are named QPs1, QPs2, and QPs3, respectively. The function of QPs is to provide membrane docking for succinate dehydrogenase and the Q-binding sites for succinate-Q reductase. Soluble succinate dehydrogenase catalyzes electron transfer from succinate to redox dyes, such as phenazine methosulfate and ferricyanide (12), but it cannot catalyze the 2-thienyll trifluoroaceton (TTFA)-sensitive electron transfer from succinate to Q. Addition of QPs to soluble succinate dehydrogenase converts succinate dehydrogenase to the membrane-bound, TTFA-sensitive succinate-Q reductase (13) with detectable ubisemiquinone radicals (14).

Participation of QPs in the Q-binding of succinate-Q reduc-
tase was further established by photoaffinity labeling studies with [3H]azido-Q derivatives (10). When a succinate-free, partially Q-deficient succinate-Q reductase is treated with [3H]azido-Q derivatives in the dark followed by illumination with a long wavelength UV light, about 50% of bound Q is located on QPs1 and the other 50% is equally distributed between QPs2 and QPs3 (10). The Q-binding domain in the proposed model of QPs1, based on the deduced amino acid sequence (15, 16), is located in a loop connecting helices 2 and 3 of QPs3, in mitoplasts and submitochondrial particles. The ubiquinone-binding domain in the proposed model of QPs3 is probably located at the end of transmembrane helix 1 toward the C-side of the mitochon-
drial inner membrane.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EMBL Data Bank with accession number(s) U50897.
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The abbreviations used are: Q, ubiquinone; DCPIP, dichlorophenol-indophenol; TTFA, 2-thenoyltrifluoroacetone; PAGE, polyacrylamide gel electrophoresis; RACE, rapid amplification of cDNA ends; [3H]azido-Q, 3-azido-2-methyl-5-methoxy[3H]-6-decyl-1,4-benzoquinone; FP, flavoprotein; IP, iron-sulfur protein; HPLC, high performance liquid chroma-
mography; PCR, polymerase chain reaction; bp, base pair(s); SMP, submitochondrial particles.

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Mitochondrial Succinate-Ubiquinone Reductase

QPs2 or QPs3. However, identification of the Q-binding domains in QPs2 or QPs3 requires knowledge of the amino acid sequences of these two subunits. Herein, we report cloning and nucleotide sequencing the cDNA encoding QPs3, the immunological determination of the topology of QPs3 in the inner mitochondrial membrane using monospecific polyclonal antibodies against two synthetic peptides corresponding to residues 1–14 and 55–66, isolating and sequencing an azido-Q-linked peptide from labeled QPs3, and the localization of the Q-binding domain in the proposed model of QPs3. The identity of QPs2 is also discussed.

EXPERIMENTAL PROCEDURES

Materials—Bovine serum albumin, dichlorophenolindophenol (DCPIP), Triton X-100, sodium cholate, TFTA, and phenyl-Sepharose CL-4B, were obtained from Sigma. Protein A-horseradish peroxidase conjugate, protein molecular weight standards, SDS, acrylamide, urea, and DEAE Affi-Gel blue were from Bio-Rad. Tolymerase was from Promega. TA cloning kit was from Invitrogen. The bovine heart cDNA library constructed in the Uni-ZAP XR vector was from Stratagene. Insta-gel liquid scintillation mixture was from Packard Instrument Co. Oligonucleotides and peptides were synthesized by the DNA/Protein Conjugate Facility of Thomas State University. Nitrocellulose membranes were from Schleicher & Schuell. All other chemicals were of the highest purity commercially available.

The ubiquinone derivatives, 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (Q10C10), and 3-azido-2-methyl-5-methoxy- and 3-azido-2-methyl-5-methoxy(3H)-6-decyl-1,4-benzoquinone (azido-Q and 3H-azido-Q, respectively) were synthesized according to previously reported methods (17). Calcium phosphate was prepared according to Jenner (18) and mixed respectively) were synthesized according to previously reported methods (17). Calcium phosphate was prepared according to Jenner (18) and mixed according to previously reported methods (17). Calcium phosphate was prepared according to Jenner (18).

Enzyme Preparation and Assays—Intact bovine heart mitochondria (19), mitoplasts (20), sub mitochondrial particles (21), and succinate-Q reductase (22) were prepared and assayed as previously reported. Succinate-Q reductase was assayed at room temperature, for its ability to catalyze TTFA-Q reductase (DCPIP) reduction by succinate using a Shimadzu UV-2100PC. The reaction mixture (1 ml) contains 40 μmol of DCPIP, 100 μmol of sodium potassium phosphate buffer, pH 7.4, 20 μmol of succinate, 10 nmol of EDTA, 25 nmol of Q10C10, and 0.01% of Triton X-100. The reduction of DCPIP was followed by measuring the absorption decrease at 600 nm, using a millimolar extinction coefficient of 21 nmol mm-1 cm-1. The concentration of TTFA used was 10 μM.

DNA Sequencing—This was done at the Core Facility of Oklahoma State University with an automatic DNA sequencer from Applied Biosystems, model 373A.

Purification and Partial NH2-terminal Amino Acid Sequencing of QPs3—Isolated succinate-Q reductase was diluted to 2 mg/ml with 50 mM Tris-Cl, pH 7.4, containing 0.2% sodium cholate. The solution was stirred at room temperature for 30 min and applied to a phenyl-Sepharose CL-4B column equilibrated with 50 mM Tris-Cl, pH 7.4, containing 0.2% sodium cholate. The column was, in sequence, washed with 50 mM Tris-Cl, pH 7.4, containing 0.2% sodium cholate; 50 mM Tris-Cl, pH 7.4, containing 2% sodium cholate; 50 mM Tris-Cl, pH 7.4, containing 2% sodium cholate and 4 mM urea; and 50 mM Tris-Cl, pH 7.4, containing 2% sodium cholate. QPs was eluted from the column with 50 mM Tris-Cl, pH 7.4, containing 0.15% SDS.

Pure QPs3 was obtained from QPs by preparative SDS-PAGE essentially according to the previously reported method (10) except that the gels were pre-run for 10 h at 45 V with an anode buffer containing 0.1 mM Tris-Cl, pH 8.9, 0.1 mM sodium thioglycollate and a cathode buffer containing 2% Tris, 0.1 mM Tricine, 0.1% SDS, and 0.1 mM sodium thioglycollate. The gel-eluted QPs3 protein was concentrated by membrane filtration, using centricon-10, to a protein concentration of 2 mg/ml and precipitated with cold acetone (–20 °C). These precipitates were washed with 50% acetone, dried under argon, and subjected to NH2-terminal sequence analysis. These analyses were done at the Molecular Biology Resource Facility, Saint Francis Hospital of Tulsa Medical Research Institute, University of Oklahoma Health Sciences Center under the supervision of Dr. Ken Jackson.

Preparation of the Reductive Carboxymethylated, Succinylated, [3H]Azido-Q-labeled QPs3—Purified succinate-Q reductase was photoaffinity labeled with [3H]Azido-Q derivative as reported previously.

RESULTS AND DISCUSSION

Isolation of the cDNA Encoding QPs3 from the Beef Heart cDNA Library in aZAP by PCR Cloning—Since the amino acid sequences of the membrane-anchoring subunits in succinate-Q reductase from different species show little conservation (25), we did not use the homology probing strategy to obtain the cDNA for QPs3. The availability of anti-QPs3 antibodies in our laboratory together with our previous success in immunological screening of a beef heart cDNA expression library in αglt11 to obtain cDNAs for the Rieske iron sulfur protein (26), the QPc 9.5 kDa (27) of ubiquinol-cytochrome c reductase, and the QPs1 (15) of succinate-Q reductase encouraged us to use the immunological screening method to isolate the cDNA for QPs3. However, no positive result was obtained. This failure to obtain cDNA for QPs3 by the immunological screening method is probably due to the low titer of antibodies against QPs3 rather than the lack of QPs3 cDNA in the cDNA library used because we also failed to obtain a positive clone from other beef heart cDNA libraries, such as αZAP (from Stratagene). In an effort to obtain high titer anti-QPs3 antibodies, we added several more booster injections to rabbits and tried to raise antibodies in chickens. However, both attempts failed.

It was reported (28) that specific cDNA inserts are obtained from the αZAP library screened in αglt11 by PCR amplification using synthetic guessmers. The design of synthetic guessmers requires knowledge of a partial amino acid sequence of the target protein. When purified QPs3 (Fig. 1A, lane 6) was subjected to protein sequencing, 43 residues from the NH2-terminus were obtained, NHHGSKMAASLHWTD-ERQSVVLLGLIPAAPYLNPCSAMYDLSYATL. This enabled
us to use the PCR cloning method to isolate the QPs3 cDNA from a beef heart cDNA library in λZAP. A 110-bp cDNA fragment was amplified from a beef heart cDNA library in λZAP (4 × 10⁶ plaque-forming unit) by PCR using two synthetic guessmers, 5'-GCTGCTCTCCTACGTGGAG-3' (sense primer) and 5'-TCAAGCCAGGAGGATGCTCA-3' (antisense primer). The sense primer represents the guessed sequence for residues 41–35 and 31.15 min, respectively, on an HPLC chromatogram. Although the connecting peptide (X), corresponding to residues 21–27 (sense primer), and 5'-TTGCTCCTGGGCCTAAT-3' (antisense primer), were synthesized and used with the primers of vector ZAP, T7 and T3, respectively, in the subsequent PCR reactions to yield 3'- and 5'-RACE products. These two PCR products were cloned into the PCR II vector (TA cloning kit) and sequenced. The DNA sequence of this 110-bp PCR product translated to match the amino acid residues between Trp10 and Met35 of the chemically determined partial NH₂-terminal sequence of QPs3.

Based on the nucleotide sequence for residues 10–35 of QPs3, two gene specific primers, 5'-TTTGGTCTCCGGGGACTAATCTC-3', corresponding to residues 21–27 (sense primer), and 5'-AGGAGGAAACTGTAACAC-3', corresponding to residues 22–17 (antisense primer), were synthesized and used with the primers of vector ZAP, T7 and T3, respectively, in the subsequent PCR reactions to yield 3'- and 5'-RACE products. These two PCR products were cloned into the PCR II vector and sequenced. The 3'-RACE product is confirmed by matching the deduced amino acid sequence for residues 21–42 of QPs3 with chemically determined partial NH₂-terminal amino acid sequence, and residues 59–66 and 44–49 with chymotryptic peptides of QPs3 with retention times of 18.96 and 31.15 min, respectively, on an HPLC chromatogram. Although the 5'- and 3'-RACE products can be joined together by PCR using a marathon cDNA amplification method (29), no effort was made to obtain a combined RACE product in this investigation. However, for future structure-function studies of QPs3, we have obtained a 331-bp BamHI-EcoRI cDNA fragment encoding mature QPs3 protein by PCR amplification from the beef heart cDNA library in λZAP using two primers, GGATCCTCTTGGTTCAG (sense primer) and GAATTCCAAAAGGTCCAGAGC (antisense), and cloned into a PCR vector.

Sequence Analysis of QPs3—Fig. 2 shows the nucleotide sequence and the deduced amino acid sequence of QPs3. The QPs3 cDNA is 1330 base pairs long with an open reading frame of 474 base pairs that encodes 158 amino acid residues, of which 103, starting with serine, belong to mature QPs3 and 55, starting with methionine, constitute an NH₂-terminal presequence. In addition, the cDNA has 820 nucleotides of 3' non-coding sequence and contains a poly(A) tail. Horseradish peroxidase conjugate was used as second antibody.
jugates, prepared from antibodies against synthetic peptides corresponding to residues 1–14 and 55–66 of QPs3, in mitoplasts, submitochondrial particles (SMP), and alkali-treated submitochondrial particles. In this model, QPs3 has three transmembrane helices corresponding to residues 15–34 (helix I), 37–56 (helix II), and 67–89 (helix III). The NH2-terminal region, residues 1–14, and the loop connecting helices II and III, residues 57–66, are extruded from the M-side of the inner mitochondrial membrane. The loop connecting helices I and II, residues 36–37, and the COOH-terminal region, residues 90–103, are on the C-side of the membrane.

The sidedness of the membrane in this model was determined immunologically with Fab'-horseradish peroxidase conjugates prepared from anti-QPs3, anti-NH2-terminal peptide (residues 1–14), and anti-connecting peptide (residues 57–66) antibodies in bovine heart mitoplasts (digitonin-treated intact mitochondria), SMP (reverse orientation), and alkaline-treated SMP (SMP devoid of succinate dehydrogenase). The peroxidase activity assays of these three particles are shown in Fig. 5. Since the peroxidase activity observed with preimmune Fab'-horseradish peroxidase-treated preparations is assumed to be due to nonspecific binding, it is subtracted from that of the anti-QPs3, anti-NH2-terminal peptide, or anti-connecting peptide Fab'-horseradish peroxidase-treated preparations. The intactness of mitoplasts and submitochondrial particle preparations was established by the absence and presence of rotenone-sensitive NADH-Q reductase activity.

When mitoplasts and SMP preparations were treated with anti-QPs3 Fab' fragment-peroxidase conjugates, peroxidase activity was detected in both preparations. The slightly higher activity in treated SMP suggests that QPs3 is a transmembrane protein with slightly more mass exposed on the matrix side of the membrane. When an alkali-treated SMP preparation is treated with anti-QPs3 Fab' fragment-horseradish per-
containing 0.25 M sucrose. This procedure was repeated three more
evident from the following observations. (i) When an elec-
tected because the QPs3 sequence is contained in QPs2. This is
shown). This immunocross-reaction of QPs2 and QPs3 is ex-
Fig. 1). Antibodies against QPs2 also react with QPs3 (data not
activities indicated are after subtracting the control activity.
when mitoplasts and SMP preparations are treated with
Modification of isolated azido-Q labeled QPs3 by reductive car-
urea in the digestion mixture does not increase proteolysis.
should be mentioned that during the course of immuno-
ological studies of QPs3 we observed that antibodies against
QPs3, the NH₂-terminal, and the connecting peptides cross-
react with QPs2 (Fig. 1, B-D, lanes 3 and 5). They do not react
with QPs1 or proteins in ubiquinol-cytochrome c reductase (see
Fig. 1). Antibodies against QPs2 also react with QPs3 (data not
shown). This immunocross-reaction of QPs2 and QPs3 is ex-
pected because the QPs3 sequence is contained in QPs2. This is
evident from the following observations. (i) When an elec-
phoretically pure QPs2 preparation (Fig. 1A, lane 5) is sub-
jected to protein sequencing, a major peptide with a partial
NH₂-terminal amino acid sequence of Ser-Pro-Ser-His-His-Ser-
Gly-Ser-Lys-Ala- is obtained. This sequence contains five
amino acid residues from the COOH terminus of the pre-
sequence and five amino acid residues from the NH₂ terminus of
the proposed structure of QPs3 is to isolate an [³H]azido-
Q-binding role for QPs3 is to isolate an [³H]azido-
Q-purified peptide from [³H]azido-Q labeled QPs3 obtained from
[³H]azido-Q-labeled succinate-Q reductase.
[³H]azido-Q-labeled QPs3 is isolated from [³H]azido-Q la-
abeled succinate-Q reductase by a procedure involving phenyl-
Sepharose CL-4B column chromatography, preparative SDS-
PAGE, electrophoretic elution of proteins from gel slices, and
aceton precipitation. The use of a hydrophobic column, phenyl-
Sepharose CL-4B, and elution with different detergents results in
the isolation of [³H]azido-Q-labeled QPs from [³H]azido-Q-labeled succinate-Q reductase. Since the FP and IP subunits of succinate
dehydrogenase are less hydrophobic than those of QPs subunits,
they are eluted with detergents having less hydrophobicity than
that used for eluting QPs. This column chromatographic step also
removes most of the non-protein bound [³H]azido-Q from QPs. Pure
[³H]azido-Q-labeled QPs3 is isolated from the labeled QPs by pre-
parative SDS-PAGE using high resolution gel system in the pres-
ence of 8 M urea. The use of preparative SDS-PAGE not only
separates QPs3 from other QPs subunits, it also further removes
non-protein bound azido-Q addscts from QPs3. QPs3 in gel slices is
eluted by electrophoretic eluting. The SDS present in the eluted
protein solution was removed by 50% aceton precipitation.
Although the isolated [³H]azido-Q-labeled QPs3 is pure and
free of free azido-Q, it is highly aggregated and resistant to
proteolytic enzyme digestion. Inclusion of 0.1% SDS and 2 M
urea in the digestion mixture does not increase proteolysis.
Modification of isolated azido-Q labeled QPs3 by reductive car-
boxymethylation followed by succinylation renders the protein
susceptible to chymotrypsin digestion. Reductive carboxym-
ethylated and succinylated QPs3 is not completely soluble in
aqueous solution; the solution becomes clear only after chymo-
trypsin digestion. A similar situation was observed with azido-Q labeled-cytochrome b (23).
Fig. 6 shows the radioactivity distribution among the chy-
motryptic peptides of QPs3 separated by HPLC. Most of the
radioactivity was found in fraction 66 (P-66). It should be
mentioned that the HPLC chromatograms and radioactivity
distribution of the chymotryptic peptides of QPs2 are identical
to those of QPs3 (data not shown).
When P-66 from QPs3 was sequenced, a partial NH₂-termi-
nal sequence of Leu-Asn-Pro-Cys-Ser-Ala-Met-Asp-Tyr, corre-
sponding to residues 29–37 in QPs3, is obtained. An identical
sequence is obtained for the radioactivity containing fraction from
QPs2. Thus the Q-binding domain in the proposed structure of
Mitochondrial Succinate-Ubiquinone Reductase

The finding that the Q-binding domains in QPs3 and QPs1 of beef heart mitochondrial succinate-Q reductase are on opposite sides of the membrane is in line with a two-Q binding site hypothesis formulated by inhibitor studies of this enzyme complex (41). The presence of two quinone-binding sites in E. coli fumarate reductase are incorporated into a proposed mechanism of Q reduction in photoreaction centers (42, 43). Glu-29, Ala-32, His-82, Trp-86 of FrdC and His-80 of FrdD are considered participants in a Q₆-type site, and FrdD Phe-57, Glu-59, and Ser-60 in an apolar QA-type site (39). According to the proposed structure of E. coli FrdC and FrdD, the Q₆-type site is located at the cytoplasmic side and the QA-type site at periplasmic side. If this reasoning is applied to beef heart mitochondrial succinate-Q reductase, the Q₆-binding domain identified in QPs1 (10) would be the Q₆-type site and the domain in QPs3 is the QA-type site. More detailed information on Q-binding must await determination of the three-dimensional structure of succinate-Q reductase.

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REFERENCES
1. Ziegler, D. M., and Doeg, K. A. (1962) Arch. Biochem. Biophys. 97, 41–50
2. Hederstedt, L., and Ohnishi, T. (1992) in Molecular Mechanism in Bioenergetics (Ernster, L., ed) pp. 163–198, Elsevier Science Publishing Co. Inc., New York
3. Ackrell, B. A. C., Johnson, M., Gunsalus, R. P., and Cecchini, G. (1992) in Chemistry and Biochemistry of Flavoenzymes (Muller, F., ed) Vol. III, pp. 229–297, CRC Press, London
4. Yao, Y., Wakabayashi, S., Matsuda, S., Matsubara, H., Yu, L., and Yu, C. A. (1986) in Iron-Sulfur Protein Research (Matsubara, H., Katsube, T., and Wada, K., eds) pp. 215–222, Karger, Basel
5. Hirawake, H., Takamiya, S., Ma, Y-C., Aoki, T., Sekimizu, K., Kojima, S., and Kita, K. (1996) Biochim. Biophys. Acta 1276, 1–5
6. Wood, D., Darlison, M. G., Wilde, R., and Guest, J. R. (1984) Biochem. J. 222, 519–534
7. Cole, S. T. (1982) Eur. J. Biochem. 122, 479–484
8. Nakamura, K., Yamaki, M., Sarada, M., Nakayama, S., Vihat, C. R. T. Gennis, R. B., Nakayashiki, T., Inokuchi, H., Kojima, S., and Kita, K. (1996) Biochim. Biophys. Acta 1365, 1–5
9. Weber, K., and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412
10. Lee, G. Y., He, D-Y., Yu, L., and Yu, C. A. (1995) J. Biol. Chem. 270, 6193–6198
11. Schägger, H., Link, T. A., Engel, W. D., and von Jagow, G. (1986) Methods Enzymol. 126, 224–257
12. King, T. E. (1966) Methods Enzymol. 10, 322–331
13. Yu, L., Xu, J-X., Haley, P. E., and Yu, C. A. (1987) J. Biol. Chem. 262, 1137–1143
14. Yu, L., Wei, Y-Y., Usui, S., and Yu, C. A. (1992) J. Biol. Chem. 267, 24508–24515
15. Ackrell, B. A. C., Johnson, M., Gunsalus, R. P., and Cecchini, G. (1992) Arch. Biochem. Biophys. 299, 61–66
16. Yu, L., Yang, F-D., and Yu, C. A. (1985) J. Biol. Chem. 260, 963–973
17. Ackrell, B. A. C., Johnson, M., Gunsalus, R. P., and Cecchini, G. (1992) in Bioenergetics (Ernster, L., ed) pp. 163–198, Elsevier Science Publishing Co. Inc., New York
18. Jenner, E. L. (June 5, 1973) U. S. Patent 3737516
19. Harmon, H. J., and Crane, P. L. (1976) Biochim. Biophys. Acta 440, 45–58
20. Greenawalt, J. W. (1974) Methods Enzymol. 31, 310–330
21. Harmon, H. J. (1982) J. Bioenerg. Biomembr. 14, 377–386
22. Yu, L., and Yu, C. A. (1983) J. Biol. Chem. 258, 2018–2023
23. He, D-Y., Yu, L., and Yu, C. A. (1994) J. Biol. Chem. 269, 2292–2298
24. Usui, S., Yu, L., and Yu, C. A. (1990) Biochim. Biophys. Acta 1057, 215–222
25. Hagerhall, C., and Hederstedt, L. (1996) FEBS Lett. 389, 25–31
26. Yu, L., Deng, K-P., and Yu, C. A. (1995) J. Biol. Chem. 270, 25634–25638
27. Friedman, K. D., Rosen, N. L., Newman, P. J., and Montgomery, R. (1988) Nucelic Acids Res. 16, 8718
28. CLONTECH (1995) Clontech Techniques, Vol. 10, pp. 4–8, Clontech Laboratory Inc., Palo Alto, CA
29. Hearn, E. C., and Van Loo, A. P. (1986) Trends Biochem. Sci. 11, 204–207
30. Hendrick, P. J., Hodges, P. J., and Rosenberg, L. E. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 4056–4060
31. Hendrick, P. J., Hodges, P. J., and Rosenberg, L. E. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 12086–12091
32. Horwich, A. L., Kalousek, F., Fenton, W. A., Pollock, R. A., and Rosenberg, L. E. (1993) Cell 44, 451–459
33. Chu, T. W., Grant, P. M., and Strauss, A. W. (1987) J. Biol. Chem. 262, 12866–12911
34. Boles, B. L., and Lemire, B. D. (1994) J. Biol. Chem. 269, 6543–6549
35. Saruta, F., Hirawake, H., Takamiya, S., Ma, Y-C., Aoki, T., Sekimizu, K., Kojima, S., and Kita, K. (1996) Biochim. Biophys. Acta 1365, 1–5
36. Wood, D., Darlison, M. G., Wilde, R., and Guest, J. R. (1984) Biochem. J. 222, 519–534
37. Cole, S. T. (1982) Eur. J. Biochem. 122, 479–484
38. Nakamura, K., Yamaki, M., Sarada, M., Nakayama, S., Vihat, C. R. T. Gennis, R. B., Nakayashiki, T., Inokuchi, H., Kojima, S., and Kita, K. (1996) J. Biol. Chem. 271, 521–527
39. Westenberg, D. J., Gunsalus, R. P., Ackrell, B. A. C., Sices, H., and Cecchini, G. (1993) J. Biol. Chem. 268, 815–822
40. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
41. Tanaka, J., Sato, T., and Kita, K. (1995) Biochim. Biophys. Acta 1276, 1–5
42. Michel, H., Epp, O., and Deisenhofer, J. (1986) EMBO J. 5, 2445–2451