Menaquinone as Well as Ubiquinone as a Bound Quinone Crucial for Catalytic Activity and Intramolecular Electron Transfer in Escherichia coli Membrane-bound Glucose Dehydrogenase*

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Escherichia coli membrane-bound glucose dehydrogenase (mGDH), which is one of quinoproteins containing pyrroloquinoline quinone (PQQ) as a coenzyme, is a good model for elucidating the function of bound quinone inside primary dehydrogenases in respiratory chains. Enzymatic analysis of purified mGDH from cells defective in synthesis of ubiquinone (UQ) and/or menaquinone (MQ) revealed that Q-free mGDH has very low levels of activity of glucose dehydrogenase and UQ2 reductase compared with those of UQ-bearing mGDH, and both activities were significantly increased by reconstitution with UQ. On the other hand, MQ-bearing mGDH retains both catalytic abilities at the same levels as those of UQ-bearing mGDH. A radiolytically generated hydrated electron reacted with the bound MQ to form a semiquinone anion radical with an absorption maximum at 400 nm. Subsequently, decay of the absorbance at 400 nm was accompanied by an increase in the absorbance at 380 nm with a first order rate constant of 5.7 × 10^3 s^-1. This indicated that an intramolecular electron transfer from the bound MQ to the PQQ occurred. EPR analysis revealed that characteristics of the semiquinone radical of bound MQ are similar to those of the semiquinone radical of bound UQ and indicated an electron flow from PQQ to MQ as in the case of UQ. Taken together, the results suggest that MQ is incorporated into the same pocket as that for UQ to perform a function almost equivalent to that of UQ and that bound quinone is involved at least partially in the catalytic reaction and primarily in the intramolecular electron transfer of mGDH.

Facultative anaerobic bacteria and lower eukaryotes are known to have capabilities to adapt to environmental changes for survival. One such capability is acquired by synthesis of both UQ and MQ. Some respiratory components that intrinsically interact with Q are capable of catalyzing redox reactions with both UQ and MQ (7–11). Such interactions may be important in physiological and evolutionary aspects in addition to enzymatic function. DsbB, which is involved in the formation of disulfide bonds in Escherichia coli extracytoplasmic space, and fumarate reductase differentially employ Q under aerobic and anaerobic conditions (7–9). The respiratory oxidation in E. coli of α-glycero-phosphate and D-lactate is promoted by both UQ and MQ, whereas NADH oxidase and succinate oxidase require only UQ for activity (12).

E. coli membrane-bound mGDH, which is known as a quinoprotein, catalyzes the oxidation of D-glucose to D-gluconate at the periplasmic side to feed electrons to ubiquinol oxidase via UQ in the respiratory chain (13–15). Topological analysis of mGDH revealed a unique structure consisting of an N-terminal hydrophobic domain with five membrane-spanning segments and a large C-terminal domain residing in the periplasm (15), which contains PQQ as a coenzyme and Ca^2+ - or Mg^2+ -binding sites in a superbarrel structure, conserved in quinoproteins (15–19). Although its tertiary structure has not been determined, the arrangement of amino acid residues around PQQ has been modeled (19) and has been confirmed by results of several experiments with site-directed amino acid substitutions (20–24). The substitution of either Lys-493, which is modeled to hydrogen-bond to an orthoquinone portion of PQQ as a vital part for the catalytic reaction, or Asp-466, which is located close to that portion and functions in extraction of a proton from menaquinone; KPB, potassium phosphate buffer; DM, N-dodecyl-β-D-maltoside; PMS, phenazine methosulfate; eaq-, hydrated electron; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; HPLC, high pressure liquid chromatography.
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substrate glucose, has a severe effect on the catalytic ability of the enzyme (24, 25).

Although a large number of primary dehydrogenases have been discovered, little is known about their intramolecular electron transfer pathways. Among them, E. coli mGDH has been relatively well studied. It has been demonstrated that mGDH has two UQ-binding sites, one (Q1) for bound UQ and the other (Q2) for bulk UQ (26), which is near the membrane surface rather than in the hydrophobic interior (27), and that intramolecular electron transfer following the catalytic reaction occurs from PQHQ2 directly to UQ in the Q1 site or via bound UQ. Our recent studies revealed that εmax produced by pulse radiolysis caused a rapid reduction of bound UQ followed by intramolecular electron transfer to PQQ in mGDH and suggested that the two redox centers are closely located at a distance of 11–13 Å (28) and that Asp-466 and Lys-493 are involved in proton donation to the semiquinone anion radical of bound UQ and in electron transfer from bound UQ to PQQ, respectively (25). However, no evidence for the interaction with MQ has so far been provided in mGDH.

E. coli mGDH has been shown to be expressed under both aerobic and anaerobic conditions, although the expression level under the latter condition is relatively low (29). mGDH is a good model for primary dehydrogenases in terms of its occurrence as a single protein and as an apo-protein (30), which allows study with both forms of apo- and holo-enzymes (31). This model might provide valuable information on the physiological function of bound Q. In this work, we provided the first evidence that the primary dehydrogenase in respiratory chains utilizes both MQ and UQ as a bound Q and suggest that bound MQ occurs in a fashion similar to that of bound UQ in the mGDH molecule and functions as an electron acceptor from PQQ. We also present the data here for the first time to suggest the requirement of bound Q for catalytic reaction in quinoprotein dehydrogenases.

EXPERIMENTAL PROCEDURES

Materials—UQn was kindly provided by Eizai Co., Ltd. (Japan). All other chemicals were of analytical grade and were purchased from commercial sources.

Bacterial Strains and Plasmid—E. coli YU423 (Δ(ftsH ptsI crr) galP::Tn10 gcd::cm) (30), AN384 (ubiA420 menA401), AN385 (ubiA420), and AN386 (menA401) strains (12) were used as host bacteria, and the plasmid used was pUCGCD1 bearing the wild-type mGDH gene (gcd) (29).

Purification of mGDHs—YU423, AN384, AN385, or AN386 cells harboring wild-type pUCGCD1 were grown in LB medium (1% Bacto-tryptone, 0.5% yeast extract, and 0.5% NaCl) containing ampicillin (50 µg/ml) for 12 h at 30 °C under aeration conditions (200 rpm) except for AN384 and AN385 cells, which were grown under static conditions. Cultivation of AN384 cells was carried out as described by Takahashi et al. (7). Preparation of membrane fractions was done as described previously (15). Purification of mGDH from membrane fractions was performed at 4 °C by the modified procedure with two column chromatographies of DEAE-Toyopearl (Toyoda Soda) and ceramic hydroxyapatite (Bio-Rad) as described by Elias et al. (26). Furthermore, to improve the recovery of UQ-containing mGDH, we avoided using chloride-containing buffer and Triton X-100, and dialysis after the first column chromatography was eliminated. Membrane fractions (~10 mg/ml of protein) were treated with 10 mM KPB (pH 7.0) containing 0.04% DM and centrifuged at 86,000 × g for 90 min, and the resultant membrane fractions were subjected to solubilization for 60 min in the presence of 100 mM KPB (pH 7.0) containing 0.2% DM. The suspension was centrifuged at 86,000 × g for 90 min, and the supernatant obtained was dialyzed against 10 mM KPB (pH 7.0) without DM. The dialysate was applied onto a DEAE-Toyopearl column (1-ml bed volume/~10 mg of protein) equilibrated with 10 mM KPB (pH 7.0) containing 0.1% DM. The column was washed with 10 bed volumes of the same buffer and successively with 10 bed volumes of 10 mM KPB (pH 7.0) containing 0.02% DM and 10 bed volumes of 130 mM KPB (pH 7.0) containing 0.02% DM. Active fractions eluted were pooled and directly applied onto a ceramic hydroxyapatite column (1-ml bed volume/~5 mg of protein) equilibrated with 10 mM KPB (pH 7.0) containing 0.02% DM. The column was washed with 10 bed volumes of 200 mM KPB (pH 7.0) containing 0.02% DM. mGDH was eluted by a linear gradient composed of 10 bed volumes of 200 mM KPB (pH 7.0) containing 0.02% DM and 10 bed volumes of 1 M KPB (pH 7.0) containing 0.02% DM. Active fractions at ~700 mM KPB were pooled and dialyzed against 10 mM KPB (pH 7.0). Active fractions pooled after the ceramic hydroxyapatite column were concentrated by a small DEAE-Toyopearl column (1-ml bed volume/~10 mg of protein), in which the enzyme adsorbed was eluted with a small volume of 150 mM KPB (pH 7.0) containing 0.1% DM. These concentrated materials were found to have homogeneity of ~95%, judging from SDS–7% polyacrylamide gel electrophoresis, and were used as purified mGDHs.

Measurement of Enzyme Activities—Protein content was determined according to the Dulley and Grieve method (32) using bovine serum albumin as a standard. Holo-enzyme formation was performed by incubating purified mGDH in 10 mM 3-(N-morpholino) propanesulfonic acid (pH 7.0) containing 30 µM PQQ and 0.1 mM MgCl2 for 30 min at 25 °C (24). Using the holo-enzyme thus prepared, the following enzyme activities were measured. PMS reductase activity was measured spectrophotometrically (U-2000A, Hitachi) with PMS and 2,4-dichlorophenol indophenol as an electron mediator and acceptor, respectively, as described previously (13, 15). UQ2 reductase activity was also measured spectrophotometrically in the presence of 0.025% Tween 20 (24).

Determination of Q Content in mGDHs—Three nmol of purified mGDH was treated with 10 volumes of 100% ethanol in the presence of 3 nmol of UQ, as an internal standard and incubated for 30 min with mild shaking at 30 °C. The solution was centrifuged at 14,000 rpm for 10 min to remove denatured protein molecules. The supernatant was mixed with 2.5 volumes of n-hexane for 1 min. The upper phase was collected and dried, and the residue was then resolved in 0.2 ml of HPLC solvent (ethanol/methanol, 3:1 v/v). In previous experiments, MQ was not detected in samples from ubiA cells (26), which may be due to the different solvent composition. The resolved materials
were subjected to reverse-phase HPLC using a Zorbas ODS column (Mitsui-toatsu, Japan) at a flow rate of 0.8 ml/min. The elution was monitored at 278 nm by using an SPD-M6A photodiode array detector (Shimadzu, Japan). UQ from purified UQ-mGDH samples was identified by comparison of its migration with those of standard UQs (UQ8 and UQ6), and the content was estimated from the ratio of the peak area to that of the internal standard UQ8. The content of MQ from purified MQ samples was determined by correction, with the ratio of the extinction coefficient of MQ to that of UQ, of the values estimated similarly by comparison with that of internal standard UQ8. The extinction coefficients of 17.5 and 14.9 mM−1 cm−1 were used for MQ and UQ, respectively.

MALDI-TOF Mass Spectrometry — Compounds eluted from reverse-phase HPLC were collected, dried, and resolved in 0.1 ml of 100% ethanol as described previously (26). The resolved materials were placed on an analyzing plate. The molecular masses of these compounds were determined on a Voyager MALDI-TOF mass spectrometry device (Perspective Biosystems). The spectra obtained by this process were analyzed using Micromass software.

Reconstitution of Q-free mGDH with UQ6 — Purified Q-free mGDH after ceramic hydroxyapatite column chromatography was dialyzed against 10 mM KPB (pH 7.0) and incubated for 30 min with different concentrations of UQ1, dissolved in 0.5% formic acid. The UQ6-mGDH samples was identified by comparison of its migration with the same conditions as those described by Elias et al. (26), except that PQP was not added for detection of the semiquinone radical of bound UQ. An equimolar concentration of ferricyanide was added for oxidation of both apo- and holomGDH. In all cases the EPR signals obtained were normalized in their intensity against that of the wild-type mGDH for comparison, taking account of the protein concentration.

RESULTS

Existence of MQ-bearing mGDH — mGDH from cells grown under aerobic conditions has been shown to bear UQ as a bound Q, in close proximity to PQP in its molecule, which has been proposed to function in accepting electrons from reduced PQP following the catalytic reaction and donating them to bulk UQ (25, 26 and 28). mGDH is also expressed under anaerobic conditions, although the expression level is relatively low (29). To assess whether mGDH can cope with MQ as a bound Q, mGDH was expressed in the ubiA mutant defective in UQ biosynthesis and in the ubiA menA double mutant defective in biosynthesis of both UQ and MQ as well as in the menA mutant and purified by two column chromatographies. The elution patterns in both chromatographies of mGDH from ubiA and ubiA menA cells were found to be nearly the same as those from menA and wild-type cells. Identification of bound Q was examined by reverse-phase HPLC with extracted samples from each purified mGDH (Fig. 1). A peak at 17 min was detected in mGDH purified from menA mutant cells in complete agreement with that of authentic MQ8. However, a peak at 20 min, but not at 17 min, was detected in mGDH from ubiA mutant cells. Mass spectrometric analysis established the identities of the peak at 20 min as that of MQ8 (7). The Q contents in both purified mGDHs from menA and ubiA cells were found to be almost identical (0.9 ± 0.03 mol/mol of mGDH). Purified mGDHs from menA and ubiA cells are thus designated as UQ-mGDH and MQ-mGDH, respectively. On the other hand, no peak was observed in mGDH from the double mutant cells, which is thus Q-free mGDH.
Enzymatic Comparison of Q-free and MQ-bearing mGDHs with UQ-bearing mGDH

Enzymatic activity of purified mGDH is generally evaluated either with PMS or UQn with a short isoprenoid chain as an electron acceptor following glucose oxidation (15, 24). Functional activities of purified MQ-mGDH and Q-free mGDH were thus compared with those of UQ-mGDH from menA mutant cells. Both PMS reductase activity as glucose dehydrogenase activity and UQ2 reductase activity of MQ-mGDH were found to be equivalent to those of UQ-mGDH (Table 1). The latter activity reflects the total ability of catalytic reaction and the successive intramolecular electron transfer from PQQ to UQ2 at the QII site. The Q-free enzyme, however, exhibited only 18% of the dehydrogenase activity and 6% of the UQ2 reductase activity of UQ-bearing mGDHs.

It has been reported that UQ1 incorporated into the Q-free DsbB functions in a manner similar to that of bound Q (8). We thus examined the effect of external addition of UQ1 on Q-free mGDH activity. The Q-free enzyme was incubated for 30 min in the presence of different concentrations of UQ1 and successively subjected to the formation of holo-enzyme and assay. As a result, UQ1 dose-dependent PMS reductase activity of the enzyme was obtained, which showed a significant increase in the activity, ~180% of PMS reductase activity of the original Q-free enzyme as shown in Fig. 2. A similar level of increase in UQ2 reductase activity was observed in the presence of UQ1.

However, no such effect of UQ1 was observed for MQ- or UQ-mGDH. These results strongly suggest that MQ is capable of being incorporated into the mGDH molecule to act as UQ does and that bound Q is crucial not only for the intramolecular electron transfer but also for the catalytic reaction.

Pulse Radiolysis of UQ- and MQ-bearing mGDHs

Pulse radiolysis is a powerful tool for investigating electron transfer within a protein, often allowing an electron to be introduced...
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radicals in a *E. coli* quinol fumarate reductase variant (9). These results indicate that a portion of the UQ or MQ molecules borne in mGDH are in the semiquinone radical state, and the rest are in the fully reduced state in addition to the oxidized state as discussed previously for bound UQ (25).

**Bound MQ as an Electron Acceptor for Reduced PQQ**—Reduced PQQ, PQQH₂, has been proposed to be formed in the catalytic reaction cycle (39, 40). A large amount of the semiquinone radical of PQQ, probably PQQH⁺, has been detected after the addition of a substrate glucose to holo-mGDH, indicating an electron transfer from PQQH₂ to bound UQ (26). To determine whether such an intrinsically important intramolecular electron transfer occurs in MQ-mGDH, we performed EPR experiments (Fig. 5). After the addition of PQQ in the absence of glucose, PQQ radicals giving small but intensified sharp signals compared with the signals from bound Qs were detected both in UQ-mGDH and MQ-mGDH. The electrons to form PQQ radicals are assumed to come from bound UQ or MQ in semiquinone-radical or reduced forms. Although the sharp signal comes from the mixture of PQQ radical and bound Q radical, contribution of the latter may be relatively small (Fig. 4). Interestingly, when glucose was added, the sharp signal in MQ-mGDH significantly increased in intensity as in the case of UQ-mGDH, although the intensity of the signal from MQ-mGDH was ~2.5-fold higher than that in UQ-mGDH, probably because the MQ radical was more stable than the UQ radical because of the resonance stabilization of the π-radical. These results clearly indicate that the bound MQ is able to act as an electron acceptor for reduced PQQ like bound UQ and that the intramolecular electron transfer occurs from reduced PQQ to MQ in mGDH.

**EPR of UQ- and MQ-bearing mGDHs**—The spectrum of the semiquinone radical of bound UQ in mGDH has successfully been observed in a PQQ-free background by EPR (25), which was not plainly observed in PQQ-bearing mGDH because of overlapping with a PQQ semiquinone radical (26). The characteristics of the spectrum appear to consist of a broad signal with the peak-to-peak line width of nearly 100 G and a small signal at γ = 2.004 (at ~3400 G) with that of nearly 10 G (Ref. 25 and Fig. 4A). The latter signal was intensified by the addition of ferricyanide, a single electron acceptor (Fig. 4B), and diminished by borohydride, a reducing agent (data not shown). Both the broad and sharp signals were absent from Q-free mGDH (Fig. 4, E and F). EPR measurements on the PQQ-free apo-enzyme of MQ-mGDH also yielded similar spectra. In this case, however, the intensity of the sharp signal of bound MQ was relatively large and was further intensified by the addition of ferricyanide (Fig. 4, C and D). Detailed comparison of Fig. 4 (B and D) revealed that the line shape of the former signal was the Gaussian type, whereas that of the latter was the Lorentzian type (see Fig. 6). This is consistent with results reported for MQ and UQ.

In our previous study on wild-type mGDH, a radiolytically generated e⁻ Q reacted predominantly with bound UQ to form a UQ semiquinone neutral radical, and intramolecular electron transfer from UQ semiquinone to PQQ subsequently occurred (28). Pulse radiolysis analysis was performed to determine whether such an electron transfer occurs in MQ-holo-mGDH. Pulse radiolysis of MQ-mGDH causes an increase in the absorbance in the visible region. The kinetic difference spectrum 10 μs after pulse radiolysis (Fig. 3A) shows that the reaction intermediate has an absorption maximum at 400 nm. The transient spectrum shown in Fig. 3A is similar to that of the semiquinone anion radical upon pulse radiolysis of free naphthoquinone in aqueous solution (38). This indicates that e⁻ Q reacts with MQ bound to the enzyme to form an MQ anionic semiquinone. Subsequently, a decay in the absorbance at 400 nm was observed on a millisecond time scale (Fig. 3C), indicating reoxidation of the MQ semiquinone radical. Concomitantly, an absorbance increase at 380 nm appeared 500 μs after pulse radiolysis (Fig. 3, A and B). A similar absorbance change was observed after pulse radiolysis of UQ-bound mGDH (28). Therefore, we concluded that the MQ bound by mGDH is first reduced and that this is followed by reoxidation of the MQ semiquinone radical and reduction of PQQ. The intramolecular electron transfer from MQ to PQQ was calculated to be 5.7 × 10⁸ s⁻¹.

**FIGURE 4. EPR spectra of native and oxidized PQQ-free apo-mGDHs.** Spectra of UQ-native (A) and oxidized (B), MQ-native (C) and oxidized (D) and Q-free native (E) and oxidized (F) forms of apo-mGDHs are shown. Oxidation was done by the addition of an equimolar concentration of ferricyanide. Protein solutions were 100 μM in 200 μl of 10 mM KPB (pH 7.0) containing 0.1% DM. EPR conditions: microwave frequency, 9.35 GHz; field modulation frequency and amplitude, 100 kHz and 10 G, respectively; microwave power, 0.6 milliwatt; averaged scans, 10 times; and temperature, 20 K.
It has been proposed that *E. coli* mGDH possesses two UQ-binding sites in the molecule, one for a tightly bound UQ and the other for bulk UQ (26). UQ in the former, which is called Q₁, exists very close to PQQ and acts as an electron acceptor for reduced PQQ (25, 28). In this study, we showed that MQ is incorporated as a bound Q into mGDH from the *ubiA* mutant strain and has a function equivalent to that of bound UQ. Puriﬁed MQ-mGDH exhibits levels of activities of glucose dehydrogenase and UQ₂ reductase similar to those of UQ-mGDH. Moreover, bound MQ is suggested by EPR experiments to function in accepting electrons from reduced PQQ following glucose oxidation as bound UQ does. Therefore, bound MQ as well as bound UQ are thought to similarly mediate the intramolecular electron transfer in mGDH.

A previous study showed that bound UQ flanking PQQ interacts with Asp-466 and Lys-493 (25), which have been proposed to be involved in catalytic reaction and in hydrogen bonding with PQQ, respectively (19). This evidence allows us to speculate that bound Q plays a vital role in the catalytic reaction of mGDH. Further evidence supporting this speculation was provided by this study. Q-free mGDH was found to exhibit very low levels of glucose dehydrogenase and UQ₂ reductase activities, and successive reconstitution experiments with UQ₆ or UQ₈ was also attempted but without success. Although the reason for the low reconstitution efﬁciency is not clear, it might be due to oxidative inﬂuence on mGDH protein because of the lack of Q in the membrane. However, we could not detect any difference by Western blot with an anti-mGDH antibody in expression levels of Q-free and UQ- and MQ-mGDHs in membrane fractions. Taken together, we provided the data suggesting a critical requirement of bound Q for the catalytic reaction of mGDH. Electron extraction by bound Q from reduced PQQ may promote the process to the next catalytic reaction. NADH-quinone oxidoreductase from *Saccharomyces cerevisiae* has been demonstrated to bear a catalytic Q-binding site, but the contribution of Q to the catalytic activity appears to be relatively low (41).

Further detailed characterization of bound UQ in mGDH suggests that bound UQ accepts hydrated electrons and immediately passes them to PQQ and that puriﬁed mGDH molecules contain one of three different states of the semiquinone radical, reduced, and oxidized bound UQ (25, 28). We thus examined bound MQ in mGDH by pulse radiolysis and EPR analyses. Comparison with UQ-mGDH revealed the following minor but distinctive features of MQ-mGDH. First, an anionic semiquinone radical of MQ was detected, whereas a neutral one of UQ was observed when pulse radiolysis was applied to PQQ-containing mGDH. Second, electron transfer from the semiquinone form of MQ to PQQ seems to be faster than that of UQ, which may be due to the lack of protonation to the reduced MQ by $e_{aq}$. Third, the content of the reduced form of bound MQ seems to be higher than that of bound UQ in puriﬁed samples.
because oxidation by the equimolar ferricyanide provides more radicals in MQ-mGDH than in UQ-mGDH.

All of the data presented of the two Q-mGDHs, including enzymatic characterization, acceptability of $e_{aq}$ and further electron transfer to PQQ, intramolecular electron transfer from reduced PQQ to bound Q and general features of EPR spectra of bound Q allow us to conclude that bound MQ is located in the same site (Q site) as is bound UQ. Previously, an anionic semiquinone radical was formed by D354N mutation, which may alter the microenvironment around Asp-466 (25).

From these data and the distinctive features of the two Qs, we speculate that MQ is situated in a different spatial arrangement from that of UQ in the Q site, which may prevent the protonation of MQ semiquinone radical. Notably, the E. coli wild-type quinol fumarate reductase, which bears a single exchangeable Q-site where either UQ or MQ can bind, is unable to stabilize semiquinone radicals of both UQ and MQ, but rather a mutation is required for the stabilization (9). Under general growth conditions in which no PQQ is supplied because of an inherent lack of PQQ-synthesizing genes in E. coli (30), bound Q may accept electrons from reduced bulk Q, which might contribute to scavenging extra electrons in the membrane.

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REFERENCES

1. Collins, M. D., and Jones, D. (1981) Microbiol. Rev. 45, 316–354
2. Ingledew, W. J., and Poole, R. K. (1984) Microbiol. Rev. 48, 222–271
3. Soballe, B., and Poole, R. K. (2000) Microbiology 146, 787–796
4. Suvarna, K., Stevenson, D., Meganathan, R., and Huds, P. E. S. (1998) J. Bacteriol. 180, 2782–2787
5. Megnathan, R. (2001) Vitam. Horm. 61, 173–218
6. Bekker, M., Kramer, G., Hartog, A. F., Wagner, M. J., de Koster, C. G., and van den Heuvel, J. H., and Cecchini, G. (2006) Trans. Faraday Soc. 102, 11145–11146
7. Kobayashi, K., Mustafa, G., Tagawa, S., and Yamada, M. (2005) J. Biol. Chem. 280, 43536–43541
8. Kobayashi, K., Tagawa, S., and Yamada, M. (2001) J. Biol. Chem. 276, 48356–48361
9. Kobayashi, K., Salleh, R. A., and Anthony, C. (1999) Biochim. Bioch. Acta 1412, 29–36
10. Kobayashi, K., Tagawa, S., and Yamada, M. (2004) Biochim. Biophys. Acta 11145–11146
11. Kobayashi, K., Tagawa, S., Daff, S., Sugi, S., and Shimizu, T. (2001) J. Biol. Chem. 276, 39864–39871
12. Kobayashi, K., and Tagawa, S. (2003) J. Am. Chem. Soc. 125, 10213–10218
13. Duine, J. A., Frank, J., and van Zeeland, J. K. (1979) FEMS. Lett. 108, 443–446
14. Ameyama, M., Matsushita, K., Ohno, Y., Shinagawa, E., and Adachi, O. (1981) FEMS. Lett. 130, 179–183
15. Hommes, R. W. J., Postma, P., O. M., Tempest, D. W., Dokter, P., and Duine, J. A. (1984) FEMS. Microbiol. Lett. 24, 329–333
16. Cozier, G. E., Salleh, R. A., and Anthony, C. (1999) Biochim. Bioch. Acta 1412, 29–36
17. Kobayashi, K., Mustafa, G., Tagawa, S., and Yamada, M. (2005) J. Biol. Chem. 280, 22215–22221
18. Kobayashi, K., Tagawa, S., Miyoshi, H., and Adachi, O. (1985) Agric. Biol. Chem. 49, 1227–1231
19. Kobayashi, K., Konish, T., and Tagawa, S. (1997) Biochim. Biophys. Acta 13611–13616
20. Suzuki, S., Kohzuma, T., Deligeer Yamaguchi, K., Nakamura, N., Shidara, S., Kobayashi, K., and Tagawa, S. (1994) J. Am. Chem. Soc. 116, 11145–11146
21. Yamada, M., Inbe, H., Takahashi, Y., Toyama, H., Matsushita, K., and Adachi, O. (1985) J. Biol. Chem. 260, 679–685
22. Kobayashi, K., and Tagawa, S. (2001) Trans. Faraday Soc. 69, 814–825
23. Anthony, C., Ghosh, M., and Blake, C. C. F. (1994) Biochim. J. 304, 665–674
24. Yamada, M., Elias, M. D., Matsushita, K., Migita, C. T., and Adachi, O. (2003) Biochim. Biophys. Acta 1647, 185–192
25. Ameyama, M., Takahashi, Y., Ohno, Y., Shinagawa, E., and Adachi, O. (1981) FEBS. Lett. 130, 179–183
26. Hommes, R. W. J., Postma, P., O. M., Tempest, D. W., Dokter, P., and Duine, J. A. (1984) FEMS. Microbiol. Lett. 24, 329–333
27. Kobayashi, K., Mustafa, G., Tagawa, S., and Yamada, M. (2000) J. Biol. Chem. 275, 7321–7326
28. Kobayashi, K., Mustafa, G., Tagawa, S., Miyoshi, H., and Adachi, O. (1985) J. Biol. Chem. 260, 26812–26817
29. Kobayashi, K., Tagawa, S., Miyoshi, H., and Adachi, O. (1985) J. Biol. Chem. 266, 10213–10218
30. Kobayashi, K., Tagawa, S., Daff, S., Sugi, S., and Shimizu, T. (2001) J. Biol. Chem. 276, 39864–39871
31. Kobayashi, K., and Tagawa, S. (2003) J. Am. Chem. Soc. 125, 10213–10218
32. Kobayashi, K., and Tagawa, S. (1994) J. Am. Chem. Soc. 116, 11145–11146
33. Kobayashi, K., and Tagawa, S. (2001) J. Biol. Chem. 276, 39864–39871
34. Kobayashi, K., and Tagawa, S. (2000) J. Biol. Chem. 275, 26082–26088
35. Kobayashi, K., and Tagawa, S. (1994) J. Am. Chem. Soc. 116, 11145–11146
36. Kobayashi, K., and Tagawa, S. (2003) J. Am. Chem. Soc. 125, 10213–10218
37. Kobayashi, K., and Tagawa, S. (1994) J. Am. Chem. Soc. 116, 11145–11146
38. Kobayashi, K., and Tagawa, S. (2001) J. Biol. Chem. 276, 39864–39871
39. Kobayashi, K., and Tagawa, S. (2003) J. Am. Chem. Soc. 125, 10213–10218
40. Kobayashi, K., and Tagawa, S. (1994) J. Am. Chem. Soc. 116, 11145–11146
41. Kobayashi, K., and Tagawa, S. (2001) J. Biol. Chem. 276, 39864–39871
42. Kobayashi, K., and Tagawa, S. (2003) J. Am. Chem. Soc. 125, 10213–10218