The \textit{gld} genes encoding adenosylcobalamin-dependent glycerol dehydrase of \textit{Klebsiella pneumoniae} were cloned by cross-hybridization with a DNA fragment of \textit{K. oxytoca} diol dehydrase genes. Since the \textit{Escherichia coli} clones isolated did not show appreciable enzyme activity, plasmids for high level expression of cloned genes were constructed. The enzyme expressed in \textit{E. coli} was indistinguishable from the wild-type glycerol dehydrase of \textit{K. pneumoniae} by the criteria of polyacrylamide gel electrophoretic, immunochemical, and catalytic properties. It was also shown that the recombinant functional enzyme consists of \textit{M}, 61,000; \textit{M}, 22,000; and 16,000 subunits. Sequence analysis of the genes revealed four open reading frames separated by 2–12 bases. The sequential three open reading frames frames from the first to the third (\textit{gldA}, \textit{gldB}, and \textit{gldC} genes) encoded polypeptides of 553, 194, and 141 amino acid residues with predicted molecular weights of 60,659(\alpha), 21,355(\beta), and 16,104(\gamma), respectively. High level expression of these three genes in \textit{E. coli} produced more than 14-fold higher level of fully active apoenzyme than that in \textit{K. pneumoniae}. It was thus concluded that these are the genes encoding the subunits of glycerol dehydrase. The deduced amino acid sequences of the three subunits were 71, 58, and 54\% identical with those of the \alpha, \beta, and \gamma subunits of diol dehydrase, respectively, but failed to show any apparent homology with other proteins.

The \textit{gld} genes encoding adenosylcobalamin-dependent glycerol dehydrase of \textit{Klebsiella pneumoniae} were cloned by cross-hybridization with a DNA fragment of \textit{K. oxytoca} diol dehydrase genes. Since the \textit{Escherichia coli} clones isolated did not show appreciable enzyme activity, plasmids for high level expression of cloned genes were constructed. The enzyme expressed in \textit{E. coli} was indistinguishable from the wild-type glycerol dehydrase of \textit{K. pneumoniae} by the criteria of polyacrylamide gel electrophoretic, immunochemical, and catalytic properties. It was also shown that the recombinant functional enzyme consists of \textit{M}, 61,000; \textit{M}, 22,000; and 16,000 subunits. Sequence analysis of the genes revealed four open reading frames separated by 2–12 bases. The sequential three open reading frames frames from the first to the third (\textit{gldA}, \textit{gldB}, and \textit{gldC} genes) encoded polypeptides of 553, 194, and 141 amino acid residues with predicted molecular weights of 60,659(\alpha), 21,355(\beta), and 16,104(\gamma), respectively. High level expression of these three genes in \textit{E. coli} produced more than 14-fold higher level of fully active apoenzyme than that in \textit{K. pneumoniae}. It was thus concluded that these are the genes encoding the subunits of glycerol dehydrase. The deduced amino acid sequences of the three subunits were 71, 58, and 54\% identical with those of the \alpha, \beta, and \gamma subunits of diol dehydrase, respectively, but failed to show any apparent homology with other proteins.

**Glycerol dehydrase (glycerol hydro-lyase, EC 4.2.1.30)** and diol dehydrase (\textit{M},1,2-propanediol hydro-lyase, EC 4.2.1.28) are enzymes that catalyze the AdoCbl-dependent conversion of 1,2-diols to the corresponding deoxy aldehydes (1, 2). They are formed by some genera of \\\textit{Enterobacteriaceae}, such as \textit{Klebsiella} and \textit{Citrobacter}, when the bacteria grow anaerobically in a medium containing glycerol and 1,2-propanediol, respectively, and participate in the fermentation of these substrates (3, 4). The enzymic characteristics of these enzymes have been extensively studied. It has been shown that these enzymes are similar in molecular weights and substrate spectra and dissociation into two dissimilar protein components but are different in immunochemical reactivity toward anti-diol dehydrase antiserum, monovalent cation selectivity patterns, affinity for AdoCbl, and substrate specificity (for review, see Ref. 4).

In the previous paper (5), we have reported cloning and sequence analysis of the \textit{pdd} genes encoding the three subunits of diol dehydrase of \textit{Klebsiella oxytoca} (formerly \textit{Klebsiella pneumoniae} and \textit{Aerobacter aerogenes}) ATCC 8724. Since glycerol dehydrase and diol dehydrase are mutually related enzymes, it seems of much help in deducing the functional sites of these AdoCbl-requiring enzymes to elucidate the similarity and difference between these enzymes at a molecular level. We attempted to isolate the genes encoding glycerol dehydrase whose amino acid sequences have not yet been reported. Tong et al. (6) reported that \textit{Escherichia coli} harboring a plasmid containing the \textit{dha} region of \textit{K. pneumoniae} showed very low glycerol dehydrase activity. Daniel and Gottschalk (7) found the growth temperature-dependent activity of glycerol dehydrase in \textit{E. coli} expressing the \textit{Citrobacter freundii} \textit{dha} regulon.

This article describes cloning and sequence analysis of the \textit{gld} genes encoding glycerol dehydrase of \textit{K. pneumoniae} (formerly \textit{A. aerogenes}) ATCC 25955. High level expression systems for the glycerol dehydrase genes in \textit{E. coli} are also reported here.

**EXPERIMENTAL PROCEDURES**

\textbf{Materials—}\textit{[^13]C]DTPaS (specific activity, 1500 Ci/mmol)} was purchased from DuPont NEN and Amersham Corp. \\textit{Crystalline AdoCbl} was a gift from Eizai, Co., Ltd., Tokyo, Japan. \\textbf{DNA Manipulations—}Standard recombinant DNA techniques were performed as described by Sambrook et al. (8). Genomic DNA from \textit{K. pneumoniae} was isolated according to the method of Marmur (9). \\textbf{Construction and Screening of the K. oxytoca Genomic DNA Library—}Genomic DNA library of \textit{K. pneumoniae} ATCC 25955 was constructed as described previously (5). Transformation of \textit{E. coli} JM109 was performed by the electroporation method using BRL Gene porator as described by Dower et al. (10). Screening procedures were carried out with a DIG labeling kit (Boehringer Mannheim) using Millipore HATF nitrocellulose filters. The 1,6-kb \textit{PstI} fragment of plasmid \textit{pUCD11}, which covers most of \textit{pddA} gene encoding the \textit{a} subunit of \textit{K. oxytoca} diol dehydrase (5), was used as a probe. Hybridization was carried out at 55°C. Positive clones were isolated and characterized by restriction endonuclease mapping.

\textbf{Nucleotide Sequencing—}\textit{The plasmid recovered from one of the weakly hybridizing \textit{E. coli} clones was designated \textit{pUCGD25} and used for analysis of the nucleotide sequence. Restriction fragments of \textit{pUCGD25} were subcloned into \textit{pUC118} or \textit{pUC119} (11), and the template single-stranded DNAs were prepared from them. DNA sequencing was performed by the dideoxyribonucleotide chain termination method of Sanger et al. (12) using a Sequencing Pro kit (Toyobo Co., Osaka, Japan). To resolve compression artifacts, urea-acrylamide gel contain-
Construction of Expression Plasmid for Glycerol Dehydrase Genes—
The DNA fragment encoding the N-terminal region of the 61,000 enzyme activity was measured with homogenates using 1,2-propanediol as substrate. Addition of 1% Brij 35 to homogenates did not affect the glycerol dehydrating activity to 1,2-propanediol-dehydrating activity.

Enzyme and Protein Assays—
Homogenates and cell-free extracts prepared by sonication were assayed for glycerol dehydrase activity by the 3-methyl-2-benzothiazolinone hydrazone method (14). Since glycerol serves as both substrate and suicide inactivator for glycerol dehydrase, 1,2-propanediol was used as a substrate for routine assay of the enzyme activity. One unit of glycerol dehydrase is defined as the amount of enzyme that catalyzes the formation of 1 μmol of propionaldehyde/min at 37 °C. Protein was assayed by the method of Lowry et al. (15) with crystalline bovine serum albumin as a standard. Specific activity is expressed as units/mg protein.

Polyacrylamide Gel Electrophoresis and Activity Staining of Glycerol Dehydrase—Polyacrylamide gel electrophoresis of cell-free extracts was performed under nondenaturing conditions as described by Davis (16) in the presence of 0.1 M 1,2-propanediol or under denaturing conditions as described by Laemmli (17). Protein staining was carried out with Coomassie Brilliant Blue G-250. Activity staining for glycerol dehydrase was performed as described previously for diol dehydrase (5).

RESULTS
Cloning of the Glycerol Dehydrase Genes—Genomic DNA from K. pneumoniae ATCC 25955 was digested partially with restriction enzyme Sau3AI, and resulting 6–20-kb fragments were inserted into the BamHI site of plasmid pUC119 (11). E. coli JM109 was transformed with the plasmids. Ampicillin-resistant transformants were screened by using the 1.6-kb PstI fragment of pUCDD11 that carries most of the gene encoding the α subunit of K. oxytoca diol dehydrase (5). Thirteen strongly hybridizing and 14 weakly hybridizing E. coli clones were isolated from 5 × 10⁸ transformants. Southern blot analysis of the plasmids from hybridization-positive clones with the probe revealed that plasmids from the former 13 clones possessed 1.2-kb and/or 0.4-kb PstI fragments and plasmids from 12 of the latter 14 clones possessed a 0.8-kb PstI fragment.

When grown in a glycerol medium or a glycerol/1,2-propanediol medium (18), five of the nine strongly hybridizing clones examined showed definite 1,2-propanediol-dehydrating activity, irrespective of aeration during cultivation. In contrast, none of the weakly hybridizing clones showed significant 1,2-propanediol-dehydrating activity (<0.01 unit/mg protein) irrespective of growth conditions. Since K. pneumoniae produces both diol dehydrase and glycerol dehydrase, it may be possible that the plasmids from the strongly hybridizing clones contain genes encoding diol dehydrase, whereas plasmids from the weakly hybridizing clones contain genes encoding glycerol dehydrase.

Characterization of the Glycerol Dehydrase Genes by High Level Expression—Further characterization of the glycerol de-
hydade genes in the insert DNA fragment of a weakly hybridizing clone was attempted by high level expression. pUCGD25, a plasmid recovered from one of such clones, was analyzed for expressing plasmids. Cell-free extracts of E. coli BL21(DE3)/pLysS carrying pRK172(GD accessory 0.8-kb clonewas attempted by high level expression pUCGD25, and pRK172(GD accessory 0.8-kb were electrophoresed on 7% polyacrylamide gel under non-denaturing conditions. Resulting gel was subjected to protein staining (A) or activity staining (B). Experimental details are described in the text. BPB, bromphenol blue.

Identification as Glycerol Dehydrase and Characterization of the Expressed Gene Products—Upon polyacrylamide gel electrophoresis under non-denaturing conditions in the presence of 1,2-propanediol (19), cell-free extracts of the recombinant E. coli strains carrying plasmid pUSI2E(GDEcoRI) or pRK172(GDEcoRI), as described previously (5), which contained the DNA region from this ORF to the EcoRI site downstream of tac and T7 promoters, respectively.

Both E. coli JM109 carrying pUSI2E(GDEcoRI) and E. coli BL21(DE3)/pLysS carrying pRK172(GDEcoRI) exhibited 1,2-propanediol-dehydrating activity when cultured in LB + 1,2-propanediol medium in the presence of isopropyl-1-thio-β-D-galactopyranoside. Specific activity of the homogenates was 15 and 8.3 units/mg protein, respectively, which was 12 and 6 times higher than that of cell-free extract of K. pneumoniae ATCC 25955 grown on glycerol (Table I). It is therefore evident that the insert DNA fragment includes genes for functional dehydrase.

Fig. 2. Expression of glycerol dehydrase in E. coli JM109 carrying expression plasmids. Cell-free extracts of K. pneumoniae grown on glycerol, E. coli JM109 carrying pUSI2E, pUSI2E(GDEcoRI), E. coli BL21(DE3)/pLysS carrying pRK172(GDEcoRI), and E. coli JM109 carrying pUSI2E/DD were electrophoresed on 7% polyacrylamide gel under non-denaturing conditions. Resulting gel was subjected to protein staining (A) or activity staining (B). Experimental details are described in the text. BPB, bromphenol blue.

Fig. 3. SDS-polyacrylamide gel electrophoresis of homogenates of E. coli carrying expression plasmids. Cell-free extracts of E. coli JM109 carrying pUSI2E, pUSI2E(GDEcoRI), E. coli BL21(DE3)/pLysS carrying pRK172(GDEcoRI), and E. coli JM109 carrying pUSI2E/DD were electrophoresed on 11% SDS-polyacrylamide gel. Resulting gel was subjected to protein staining. Experimental details are described in the text. Molecular weight markers, Sigma Dalton Mark VII-L. BPB, bromphenol blue.

Slowly, the substrate specificity expressed by a ratio of glycerol-dehydrating activity to 1,2-propanediol-dehydrating activity (G/P ratio) was also shown in Table I. Cell-free extract from E. coli JM109 carrying pUSI2E(GDEcoRI) showed a G/P ratio of 3.0 in the 1-min assay and 0.6 in the 10-min assay. These values coincided well with those for glycerol dehydrase of K. pneumoniae but not with those for diol dehydrase (3, 18). Furthermore, the 1,2-propanediol-dehydrating activity in the extract was not immunoprecipitated by rabbit antiserum against diol dehydrase of K. oxytoca (18) (data not shown). From these results, it was concluded that the dehydrase formed in E. coli carrying pUSI2E(GDEcoRI) or pRK172(GDEcoRI) is identical to the wild-type glycerol dehydrase of K. pneumoniae.

When analyzed by SDS-polyacrylamide gel electrophoresis, the cell-free extract of E. coli carrying pUSI2E(GDEcoRI) or pRK172(GDEcoRI) contained three thick protein bands with M, of 61,000, 22,000, and 16,000, but the extract of E. coli carrying expression vector pUSI2E (control) did not (Fig. 3). Recombinant glycerol dehydrase expressed in E. coli carrying pUSI2E(GDEcoRI) was further characterized by two-dimensional gel electrophoresis, i.e. polyacrylamide gel electrophoresis in the presence of 1,2-propanediol (non-denaturing condi-

Fig. 4. Two-dimensional gel electrophoresis of cell-free extract of E. coli carrying an expression plasmid. Cell-free extract of E. coli JM109 carrying pUSI2E(GDEcoRI) was electrophoresed on 7% polyacrylamide gel under non-denaturing conditions (first dimension, from left to right) and then on 11% SDS-polyacrylamide gel under denaturing conditions (second dimension, from top to bottom). BPB, bromphenol blue.
tions) followed by SDS-polyacrylamide gel electrophoresis (denaturing conditions). As shown in Fig. 4, the functional glycerol dehydrase that migrated as a single band under non-denaturing conditions in the presence of the substrate (marked with an arrowhead on the top) then dissociated into the three polypeptides with \( M_r \) of 61,000, 22,000, and 16,000 upon SDS-polyacrylamide gel electrophoresis. Thus, it is evident that glycerol dehydrase apoenzyme is composed of the 61,000, 22,000, and 16,000 subunits, which were designated \( a \), \( b \), and \( g \) subunits, respectively.

Sequence Analysis and Identification of the Glycerol Dehydrase Genes—As glycerol dehydrase genes were located in the DNA region from ORF1 to the \( \text{EcoRI} \) site, the region was subjected to nucleotide sequence analysis according to the strategy shown in Fig. 1. As summarized in Figs. 1 and 5, there existed four successive ORFs (ORF1 to ORF4). ORF1 to ORF4 were separated by 2 to 12 bases, respectively. Shine-Dalgarno sequences were found 6 to 11 bases upstream of the putative initiation codon (ATG) (GTG for ORF2) for each ORF. ORF1 to ORF4 encode polypeptides of 555, 194, 141, and 607 amino acid residues with predicted molecular weights of 60,659, 21,355, 16,104, and 63,594, respectively. The first three predicted molecular weights coincided well with \( M_r \) of the \( a \), \( b \), and \( g \) subunits of glycerol dehydrase, respectively, suggesting that ORF1 to ORF3 are the genes encoding subunits of glycerol dehydrase. To confirm this, we constructed expression plasmid pUSI2E(GD) that contained ORF1 to ORF3 but lacked ORF4 except for the first 24 bases. The homogenate of \( \text{E. coli} \) JM109 carrying this plasmid exhibited glycerol dehydrase activity of 18 units/mg protein, which was slightly higher than that of \( \text{E. coli} \) JM109 carrying pUSI2E(GD \( \text{EcoRI} \)) (Table I). The enzyme expressed with pUSI2E(GD) was indistinguishable from that expressed with pUSI2E(GD \( \text{EcoRI} \)) in the following properties, electrophoretic mobilities (Figs. 2 and 3), the reactivity with Fixture a...
anti-diol dehydrase antiserum, and a G/P ratio in 1- and 10-min assays (Table I). These results indicate that inclusion of the first three ORFs (ORF1-ORF3) encoding 61,659, 21,355, and 16,104 polypeptides in an expression plasmid was sufficient to form high levels of functional glycerol dehydrase. It is evident that the polypeptide encoded by ORF4 is not a subunit of glycerol dehydrase. No additionalsubunits were required for activity, because specific activity of the recombinant glycerol dehydrase purified from E. coli carrying pUSI2E(GD) was essentially the same as that reported (20) with the enzyme purified from K. pneumoniae (data not shown). From all the results presented in this paper, it was concluded that ORF1, ORF2, and ORF3 are the genes encoding the α, β, and γ subunits of glycerol dehydrase, respectively. These were designated gldA, gldB, and gldC genes, respectively. The amino acid sequences of the α, β, and γ subunits of glycerol dehydrase deduced from the nucleotide sequences of gldA, gldB, and gldC genes, respectively, are shown in Fig. 5.

Sequence Homologies—The deduced amino acid sequences of the subunits of glycerol dehydrase were compared with those of the corresponding subunits of K. pneumoniae glycerol dehydrase (20) with the enzyme purified from E. coli carrying pUSI2E(GD) was essentially the same as that reported (20) with the enzyme purified from K. pneumoniae (data not shown). From all the results presented in this paper, it was concluded that ORF1, ORF2, and ORF3 are the genes encoding the α, β, and γ subunits of glycerol dehydrase, respectively. These were designated gldA, gldB, and gldC genes, respectively. The amino acid sequences of the α, β, and γ subunits of glycerol dehydrase deduced from the nucleotide sequences of gldA, gldB, and gldC genes, respectively, are shown in Fig. 5.

**Fig. 6. Alignment of amino acid sequences of the subunits of glycerol dehydrase with those of diol dehydrase.** A, α subunit; B, β subunit; C, γ subunit. KpnGDα-γ, α-γ subunits of K. pneumoniae glycerol dehydrase; KoxDDα-γ, α-γ subunits of K. oxytoca diol dehydrase. Identical amino acids are shown with asterisks and similar amino acids with dots. Gaps are shown as dashes.

Glycerol dehydrase has been reported to dissociate into components A and B upon chromatography on DEAE-cellulose or Sephadex G-100 in the absence of substrate (20, 24). Neither component alone is active, but enzymatic activity is restored when both are combined. The molecular weight of components A and B determined by gel filtration are 22,000 and 189,000, respectively. We confirmed that recombinant glycerol dehydrase was also separated into components A and B, which were composed of the 22,000 (β) subunit and the 61,000 (α) and 16,000 (γ) subunits, respectively (data not shown). The nucleotide sequences of the K. pneumoniae dha regulon have been submitted to GenBank (Accession U30903). According to the data, the genes corresponding to gldA, gldC, and ORF4 in this paper have been assigned as genes encoding a medium subunit, a small subunit, and a large subunit of glycerol dehydrase, respectively. In clear contrast to this, however, we reached the conclusion that the polypeptide encoded by ORF4 is not a subunit of glycerol dehydrase. Functions of the product of ORF4 are under current investigation.

**DISCUSSION**

Glycerol dehydrase has been reported to dissociate into components A and B upon chromatography on DEAE-cellulose or Sephadex G-100 in the absence of substrate (20, 24). Neither component alone is active, but enzymatic activity is restored when both are combined. The molecular weight of components A and B determined by gel filtration are 22,000 and 189,000, respectively. We confirmed that recombinant glycerol dehydrase was also separated into components A and B, which were composed of the 22,000 (β) subunit and the 61,000 (α) and 16,000 (γ) subunits, respectively (data not shown). The nucleotide sequences of the K. pneumoniae dha regulon have been submitted to GenBank (Accession U30903). According to the data, the genes corresponding to gldA, gldC, and ORF4 in this paper have been assigned as genes encoding a medium subunit, a small subunit, and a large subunit of glycerol dehydrase, respectively. In clear contrast to this, however, we reached the conclusion that the polypeptide encoded by ORF4 is not a subunit of glycerol dehydrase. Functions of the product of ORF4 are under current investigation.

The homology search revealed other highly homologous genes, i.e. the C. freundii dhaB, dhaC, and dhaE genes that lie...
in an opposite direction in the region upstream of the 1,3-propanediol dehydrogenase gene (GenBank; Accession U08771). The deduced amino acid sequences of C. freundii dhaB, dhaC, and dhaE genes were 94, 89, and 86% identical to those of K. pneumoniae gldA, gldB and gldC genes, respectively. Since C. freundii is known to produce glycerol dehydrase (3), the dhaB, dhaC, and dhaE genes of C. freundii can be regarded as the genes encoding the α, β, and γ subunits of glycerol dehydrase of this bacterium.

Comparison of the deduced amino acid sequences of the α, β, and γ subunits of glycerol dehydrase with those of the α, β, and γ subunits of diol dehydrase showed a conspicuous homology between them (Fig. 6). This indicates that these dehydrases are evolutionarily related. Alignment of the amino acid sequences of the subunits of glycerol dehydrase with those of diol dehydrase indicated that the α subunit may be divided into the three regions. The middle region (amino acid residues 121–406 in the glycerol dehydrase α subunit) showed the highest regional homology (83% between the enzymes), whereas the amino-terminal (residues 1–120) and carboxyl-terminal (residues 407–555) regions are relatively divergent (regional homology 57 and 59%, respectively) (Fig. 6). In β subunit, a highly homologous region existed in the middle of the β subunit (residues 90–143 in the glycerol dehydrase β subunit, regional homology 91%), although homologies in the rest of the β and γ subunits were lower than 60%. Amino acid deletions were observed in the amino-terminal region of the β and γ subunits of glycerol dehydrase. From these regional homologies, it seems likely that the α subunit and the middle region of the β subunit is structurally and functionally important for both enzymes in common.

The deduced amino acid sequences of the glycerol dehydrase subunits did not show significant homology to the other proteins listed in the PIR and SWISSPROT data bases when analyzed using FASTA program (25). Recent x-ray crystallographic analyses of the Cbl-binding domain of E. coli methionine synthase (26) and Propionibacterium shermanii methylmalonyl-CoA mutase (27) revealed that Cbl is bound to these enzymes with the 5,6-dimethylbenzimidazole moiety displaced by an imidazole of the histidine residue. The sequence DXHXXG, which contains this histidine residue, is reported to be conserved in these and some of the other cobalamin-dependent enzymes (26). However, this motif was not found in either diol dehydrase (5) or glycerol dehydrase, suggesting that Cbl is bound to these dehydrases in a different manner.

Acknowledgments—Computer analysis of the amino acid sequences was carried out using programs and data bases in DNA Data Bank of Japan (DDBJ), Mishima, Japan. We thank Yukiko Kurimoto for her assistance in manuscript preparation.

REFERENCES

1. Lee, H. A., Jr., and Ables, R. H. (1963) J. Biol. Chem. 238, 2367–2373
2. Toraya, T., Shirakashi, T., Kosuga, T., and Fukui, S. (1976) Biochem. Biophys. Res. Commun. 69, 475–480
3. Toraya, T., Kuno, S., and Fukui, S. (1980) J. Bacteriol. 141, 1439–1442
4. Toraya, T. (1994) Metal Ions Biol. Syst. 30, 217–254
5. Tobimatsu, T., Hara, T., Sakaguchi, M., Kishimoto, Y., Wada, Y., Isoda, M., Sakai, T., and Toraya, T. (1995) J. Biol. Chem. 270, 7142–7148
6. Tong, I.-T., Liao, H. H., and Cameron, D. C. (1994) Appl. Environ. Microbiol. 57, 3541–3546
7. Daniel, R., and Gottschalk, G. (1992) FEBS Microbiol. Lett. 100, 281–286
8. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
9. Marmur, J. (1961) J. Mol. Biol. 3, 208–218
10. Dower, W. J., Miller, J. F., and Ragsdale, C. W. (1988) Nucleic Acids Res. 16, 6127–6145
11. Vieira, J., and Messing, J. (1987) Methods Enzymol. 153, 3–11
12. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
13. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
14. Toraya, T., Unhia, K., Fukui, S., and Hogenkamp, H. P. C. (1977) J. Biol. Chem. 252, 963–970
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
16. Davis, B. J. (1966) Ann. N. Y. Acad. Sci. 121, 404–427
17. Laemmli, U. K. (1970) Nature 277, 680–686
18. Toraya, T., and Fukui, S. (1977) Eur. J. Biochem. 76, 285–289
19. Poznanskaja, A. A., Tanizawa, K., Sada, K., Toraya, T., and Fukui, S. (1979) Arch. Biochem. Biophys. 194, 379–386
20. Stromski, A., Pawelkiewicz, J., and Johnson, B. C. (1974) Arch. Biochem. Biophys. 162, 321–330
21. Dayhoff, M. O., Schwartz, R. M., and Orcutt, B. C. (1978) in Atlas of Protein Sequence and Structure (Dayhoff, M. O., ed) Vol. 5, Suppl. 3, pp. 345–352, National Biochemical Research Foundation, Silver Spring, MD
22. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
23. Chou, P. Y., and Fasman, G. D. (1978) Annu. Rev. Biochem. 47, 251–276
24. Schneider, Z., Pech, K., and Pawelkiewicz, J. (1966) Arch. Biochem. Biophys. 108, 227, 6–12
25. Pearson, W. R., and Lipman, D. J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2444–2448
26. Drennan, C. L., Huang, S., Drummond, J. T., Matthews, R. G., and Ludwig, M. L. (1994) Science 266, 1669–1674
27. Mancia, F., Keep, N. H., Nakagawa, A., Leadlay, P. F., McSweeney, S., Rasmussen, B., Bisecke, P., Diat, O., and Evans, P. R. (1996) Structure 4, 339–350
Cloning, Sequencing, and High Level Expression of the Genes Encoding Adenosylcobalamin-dependent Glycerol Dehydrase of *Klebsiella pneumoniae*

Takamasa Tobimatsu, Muneaki Azuma, Hirokazu Matsubara, Hiroe Takatori, Takashi Niida, Kyouichi Nishimoto, Hideaki Satoh, Ryuji Hayashi and Tetsuo Toraya

*J. Biol. Chem.* 1996, 271:22352-22357.
doi: 10.1074/jbc.271.37.22352

Access the most updated version of this article at [http://www.jbc.org/content/271/37/22352](http://www.jbc.org/content/271/37/22352)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 26 references, 9 of which can be accessed free at [http://www.jbc.org/content/271/37/22352.full.html#ref-list-1](http://www.jbc.org/content/271/37/22352.full.html#ref-list-1)