The Essential Arginine Residue at Position 210 in the a Subunit of the Escherichia coli ATP Synthase Can Be Transferred to Position 252 with Partial Retention of Activity*

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The substitution of arginine at position 210 in the a subunit of Escherichia coli F$_{0}$F$_{1}$-ATPase by either lysine or alanine causes dominance in complementation tests with a chromosomal c subunit mutation. Reversal of dominance was achieved for the a R210K mutation but not for the a R210A mutation by the presence of an aspartic acid residue at position 50 or at position 252 in the a subunit. It was concluded that position 210 in putative helix 4 of a previously proposed model of the a subunit is close to position 252 in putative helix 5 and to position 50 in putative helix 1. The juxtaposition of residues 252 and 210 was also indicated by the observation that the double mutant a R210Q/Q252R was partially functional. A revertant of the partially functional double mutant, isolated on succinate medium, was found to contain a third mutation resulting in Pro-204 in the a subunit being replaced by threonine. That the revertant phenotype was due to the a P204T change was confirmed by site-directed mutagenesis. ATP synthesis in the revertant strain was at near normal levels as judged by growth yield experiments, but the revertant strain was unable to pump protons in response to ATP hydrolysis.

The F$_{0}$F$_{1}$-ATPase enzyme complex catalyses the terminal step in oxidative phosphorylation and photophosphorylation and is located in mitochondrial, chloroplast, and bacterial membranes. In Escherichia coli, the enzyme comprises eight nonidentical subunits, a, b, c, α, β, γ, δ, and ε, encoded by the genes uncF, E, A, D, G, H, and C, respectively (1). The a, b, and c subunits are integral membrane proteins and form the F$_{0}$ portion of the complex, which can function as a proton pore. The α, β, γ, δ, and ε subunits are peripheral membrane proteins forming the F$_{1}$-ATPase portion of the complex, which retains ATP hydrolytic activity when removed from the membrane. The a, b, and c subunits of the proton pore are present in a stoichiometry of 1:2:6–12 (2), and all are required for proton translocation. Residues essential for proton translocation have been found in the a and c subunits (2). It has been proposed that the proton pore involves four amino acids: Arg-210, Glu-219, and His-245 on the a subunit and Asp-61 on the c subunit (3). Of these, only Arg-210 is absolutely conserved, but all c subunits contain an acidic residue equivalent to Asp-61. In E. coli, neither Arg-210 nor Asp-61 can be substituted by other residues without complete loss of proton-coupled ATP synthesis (4–9). The remaining two residues required for proton translocation are not strictly conserved, and some amino acid substitutions at either position retain some proton translocating activity (4–6, 10, 11).

In order to understand the mechanism of proton translocation, information on the positions of these essential residues is required. There is a considerable body of evidence indicating that the c subunit forms a helical hairpin structure, placing Asp-61 in the center of the membrane (for review, see Ref. 12). However, the structure of the a subunit is less clear, with a number of different models being proposed (2, 13–17). These models differ in the positioning, within the membrane, of the amino acid residues essential for proton translocation.

The use of site-directed mutagenesis in combination with analysis of second site revertants has proved a powerful tool in investigations of structure and function. For example, the finding that the essential aspartic acid residue in the c subunit can be moved to position 24 on the other helix and still retain some activity provides confirmation of the hairpin structure of the c subunit (18). We have previously identified amino acid changes in second-site mutants that suppress dominance of the a subunit mutant in which Arg-210 is replaced by lysine (14). Analysis of these revertants suggested that position 50 on putative helix 1 was adjacent to Arg-210 on helix 4 in the five-transmembrane helix model of the a subunit (14). In the present study, we have extended this work to show that Arg-210 is also close to position 252 on putative helix 5. Furthermore, the residues at positions 210 and 252 can be exchanged with partial retention of function.

EXPERIMENTAL PROCEDURES

Enzymes and Chemicals—All chemicals and enzymes used were of the highest quality commercially available. Oligonucleotides were synthesized by the Biomolecular Resource Facility, ANU Canberra. 35S-labeled dATP$_{5}$S was obtained from Amersham Corp.

Media and Growth of Organisms—All bacterial strains were derived from E. coli K12 and are described in Table I. The mineral salts minimal medium used and additions were as described previously (21). Cells for the preparation of membranes were grown in 10-liter fermenters as described previously (22). The minimal salts medium in the fermenters was supplemented with 5% (v/v) Luria broth (23). Turbidities of cultures were measured with a Klett-Summerson colorimeter. Growth yields were measured as turbidities after growth had ceased in minimal medium containing limiting (5 mM) glucose and supplemented with 5% Luria broth as well as specific requirements.

Preparation of Plasmid and Phage M13 DNA—Single-stranded "template" DNA was prepared as described for the oligonucleotide-directed in vitro mutagenesis system (Amersham Corp.). Replicative form DNA and plasmid DNA were prepared by the alkaline lysis method (24).

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3The abbreviation used is: dATP$_{5}$S, deoxyadenosine 5'-o-thiotriphosphate.
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| Plasmid number | Genotype | Codon change in uncB gene | Amino acid change encoded | Strain number in AN943 background | Strain number in AN727 background |
|----------------|----------|---------------------------|----------------------------|-----------------------------------|-----------------------------------|
| pAN174         | Cm'tc    | NA                        | NA                         | AN3238                            | AN2736                            |
| pAN495         | uncB_E'F'Cm'y | NA                        | WT                         | AN3252                            | AN2940                            |
| pAN766         | uncB_960'E'F'Cm'y | G(466)GC → GAC           | R210K                      | AN3296                            |                                   |
| pAN788         | uncB_960'E'F'Cm'y | C(628)GA → AAA           | R210K                      | AN3333                            |                                   |
| pAN767         | uncB_969'E'F'Cm'y | T(472)TT → GAC           | R210K                      | AN3298                            |                                   |
| pAN789         | uncB_969'E'F'Cm'y | A(475)TC → GAC           | I159D                      | AN3334                            |                                   |
| pAN790         | uncB_969'E'F'Cm'y | C(478)TG → GAC           | R210K                      | AN3333                            |                                   |
| pAN845         | uncB_713'E'F'Cm'y | A(481)TT → GAC           | I161D                      | AN3425                            |                                   |
| pAN846         | uncB_713'E'F'Cm'y | C(484)TG → GAC           | L162D                      | AN3426                            |                                   |
| pAN847         | uncB_713'E'F'Cm'y | T(487)TC → GAC           | F163C                      | AN3427                            |                                   |
| pAN848         | uncB_713'E'F'Cm'y | A(481)TT → GAC           | I161D                      | AN3425                            |                                   |
| pAN891         | uncB_730'E'F'Cm'y | A(496)TC → GAC           | K167D                      | AN3551                            |                                   |
| pAN936         | uncB_730'E'F'Cm'y | A(499)AA → GAC           | R210K                      | AN3550                            |                                   |
| pAN899         | uncB_735'E'F'Cm'y | A(502)TG → GAC           | M168D                      | AN3485                            |                                   |
| pAN892         | uncB_735'E'F'Cm'y | A(754)AA → GAC           | Q252D                      | AN3492                            |                                   |
| pAN894         | uncB_735'E'F'Cm'y | T(760)TC → GAC           | R210K                      | AN3496                            |                                   |
| pAN895         | uncB_735'E'F'Cm'y | C(628)GA → AAA           | R210K                      | AN3496                            |                                   |
| pAN939         | uncB_739'E'F'Cm'y | A(769)TG → GAC           | M257D                      | AN3553                            |                                   |
| pAN879         | uncB_719'E'F'Cm'y | A(499)AA → CAA           | K167Q                      | AN3475                            |                                   |
| pAN951         | uncB_743'E'F'Cm'y | A(505)AA → CAA           | K169Q                      | AN3569                            |                                   |
| pAN950         | uncB_743'E'F'Cm'y | C(628)GA → AAA           | R210K                      | AN3569                            |                                   |
| pAN953         | uncB_743'E'F'Cm'y | G(147)TG → GAC           | V50D                       | AN3566                            |                                   |
| pAN955         | uncB_743'E'F'Cm'y | T(487)TC → GAC           | F163C                      | AN3572                            |                                   |
| pAN1017        | uncB_752'E'F'Cm'y | C(628)GA → AAA           | R210Q                      | AN3648                            |                                   |
| pAN1042        | uncB_756'E'F'Cm'y | C(754)AA → CGT           | Q252R                      | AN3684                            |                                   |
| pAN1040        | uncB_756'E'F'Cm'y | C(754)AA → CGT           | R210Q                      | AN3677                            |                                   |
| pAN1125        | uncB_759'E'F'Cm'y | C(610)CA → ACA           | P204T                      | AN3780                            |                                   |
| pAN1155        | uncB_759'E'F'Cm'y | C(610)CA → ACA           | P204T                      | AN3841                            |                                   |

* * *

Single-stranded DNA for sequencing was prepared as described by Messing (25).

Site-directed Mutagenesis—Mutagenesis was carried out using the Amersham oligonucleotide-directed in vitro mutagenesis system. The method used was that described in Ref. 42. The oligonucleotide primers were designed to be between 17 and 25 nucleotides in length, to contain the mutant sequence approximately in the middle, and to have GC-rich N and C termini. The mutant codons are shown in Table I. Single-stranded template was derived from the replicative form of M13mp18 in which a 2.4-kilobase HindII/EcoRI fragment carrying the uncB, uncE, uncF, and uncI genes was cloned into the multiple cloning site. The presence of each mutation was confirmed by DNA sequencing using the dideoxy chain-terminating method of Sanger et al. (26). The USB T7 sequencing kit version 2.1 and 32P-labeled dATP and dCTP were used. Construction of Plasmids—A 2.2-kilobase HindII/I fragment carrying the mutated uncB gene as well as the uncE and uncF genes was subcloned from the recombinant M13mp18 replicative form into the vector pAN174, as described previously (3). The correct plasmid was identified where possible as one that conferred on a strain with a chromosomal mutation in the subunit AN1440 (uncF-469) (27) the ability to grow on succinate minimal medium. In the cases where no succinate positive colonies were obtained and it was suspected that the mutation in the uncB gene might exert a dominant effect on complementation of the uncF gene, approximately 50 colonies isolated from the
nonselective plate (glucose nutrient medium with chloramphenicol) were sectored onto succinate minimal and glucose nutrient medium. After 3 days, colonies showing no background growth on succinate but a large number of revertants were subcultured from the corresponding nutrient plate and retained for further study. In all cases, the presence of the correct insert was confirmed by restriction analysis. At least three independent isolates, with the correct restriction pattern were characterized in each case. Plasmids containing the correct insert were then used to transform strains AN727 (uncB402) and strain AN943 (uncE429). Several independent isolates from each transformation were compared for growth characteristics and growth yield on limiting glucose. One typical isolate was retained for biochemical studies. Two control plasmids were also transformed into appropriate background strains; a coupled control in which wild-type uncB, uncE and uncF genes were subcloned into pAN174 as described and an uncoupled control consisting of the vector pAN174.

Preparation of Subcellular Membrane Fractions—The growth of the cells in 10-liter fermenters, fractionation, and subsequent washing procedures have all been described previously (28).

Assays—Mg-ATPase activity was determined as described previously (29). Inhibition of membrane-bound ATPase activity was determined by assaying the membranes after dialysis to release the bound ATPase (28). The dicyclohexylcarbodiimide sensitivity of membrane bound Mg-ATPase was determined by sampling at timed intervals a reaction mixture containing membranes, reaction buffer (50 mM Tris-HCl, pH 8.0) and 12 ?g/ml dicyclohexylcarbodiimide. The reaction was stopped by adding 0.9 ml of the above reaction mixture to 100 ?l of 50% trichloroacetic acid on ice. The ATPase activity was determined by the use of King’s reagent to measure inorganic phosphate released (30). Atebrin fluorescence quenching activities were measured as described previously (21). Protein concentrations were determined using Folin’s phenol reagent with bovine serum albumin as standard (31).

RESULTS

Location of Mutations in a Subunit That Overcame Domi-

nance of the R210K Mutation—A mutant in which lysine was substituted for Arg-210 in a subunit of the E. coli ATP synthase was found previously to be unable to translocate protons. Unexpectedly, a plasmid carrying this mutation, along with wild-type genes encoding the c and b subunits, failed to complement a chromosomal c subunit mutation (14). Subsite mutations were obtained that were able to complement a chromosomal c subunit mutation, and among these was a G53D mutation (14). Further experimentation indicated that an aspartate at positions 50 or 46 had the same effect, whereas substitutions on other faces of a putative helix had little or no effect (14). It was concluded that the effect was due to a salt bridge formed between the aspartate at positions 46, 50, or 53 and the lysine at position 210 and that putative helix 1 of a previously proposed model (3) was close to helix 4. Helices 3 and 5 in addition to helix 1, flank helix 4 in the proposed model (3) and it was therefore of interest to see if aspartate residues in appropriate positions in helices 3 or 5 could overcome the dominant effect of the R210K mutation. A series of mutagenesis experiments was carried out in order to substitute aspartate residues for amino acids 252–257 in helix 5 and amino acids 156–168 in helix 3 (see Table I). Plasmids carrying these mutations along with wild-type uncE and uncF genes were transformed into a strain with a chromosomal c subunit mutation AN943 (uncE429). Substitutions in particular positions in both helices did overcome the dominant effect of the R210K mutation as judged by growth yield determinations. Helical periodicity was observed in helix 3 but not in helix 5 (Fig. 1). There were substitutions in three positions that resulted in almost complete reversal of the dominance of the R210K mutation. These positions were 163 in helix 3, and 252 and 253 in helix 5.

Aspartic acid at position 163 may form a salt bridge with Lys-167 since these two residues are one turn of a helix apart, and this interaction may be responsible for the reversal of dominance. To exclude this possibility, both Lys-167 and Lys-169 were replaced by glutamine in the presence of the R210K and F163D mutations. The K167Q/K169Q double mutation had previously shown to have no effect on ATP synthase function (4). The strain carrying the four mutations showed the same reversal of dominance as the R210K/F163D double mutant. This indicated that this phenotype was not due to a charge pair between aspartic acid at 163 and lysine at 167. It is possible, however, that an aspartate residue in any of the positions 163, 252, or 253, or in position 50, is exerting its effect directly and not via salt bridge formation with lysine at position 210.

Effects of Aspartate Residues at Positions 50, 163, and 252 on the Dominance of an R210A Mutation—The R210A mutation was constructed by site-directed mutagenesis and subcloned, along with genes encoding the wild-type c and b subunits, into the vector pAN174. This plasmid was used to transform a strain carrying a chromosomal c subunit mutation. The resultant strain was unable to grow on succinate and gave an uncoupled growth yield on a growth-limiting concentration of glucose (see Table III). Membranes prepared from the R210A mutant strain, surprisingly, appeared to have proton-leaky membranes, as judged by NADH-dependent a-tebrin fluorescence quenching activity (see Table III). The ATPase activity was the same as the coupled control.

The R210A mutation was similar to the R210K mutation in that it was found to be dominant in complementation tests with a chromosomal c subunit mutant. If the reversal of dominance by aspartate at positions 50, 163, and 252 in the R210K mutant was due to salt bridge formation with lysine, then reversal of dominance should not be achieved in the R210A mutant. Reversal of dominance did not occur in the R210A/V50D or the R210A/Q252D double mutants, but it did occur in the R210A/F163D double mutant (Table I). The F163D mutation is therefore exerting its effect directly. These results suggest that position 210 is close to positions 50 and 53 but not to position 163. Helix 4 is therefore oriented in such a way that Arg-210 is close to helix 5 rather than to helix 3. Such an orientation would also mean that Glu-219 and His-245 do not directly interact (10).

The Properties of Mutant Strains with an Arginine Residue at Position 252—If Arg-210 is close to Gin-252, then it might be possible to switch these two residues and retain function. Plasmids were therefore prepared that carried the a subunit mutations R210Q or Q252R or the double mutation R210Q/Q252R. These plasmids were used to transform a strain with a chromosomal a subunit mutation AN727 (uncB402). As expected (4), the R210Q mutant was unable to grow on succinate as sole carbon source (Fig. 2). However, both Q252R and the double
Table II

| a subunit mutation         | Growth on succinate | Growth yield |
|----------------------------|---------------------|--------------|
| Coupled control            | ++                  | 200          |
| Uncoupled control          | –                   | –            |
| R210A                      | –                   | 145          |
| R210A/V50D                 | –                   | 146          |
| R210A/Q252D                | –                   | 148          |
| R210A/F163D                | +                   | 175          |

The a subunit mutation and wild-type c subunit are plasmid-encoded. The host strain carries a chromosomal mutation in the c subunit gene (uncE429) with the remaining unc genes wild-type.

Growth yield on 5 mM glucose, expressed as Klett units.

The coupled control strain carries a plasmid encoding wild-type a and c subunits.

The uncoupled control strain carries the vector without the unc gene insert.

mutant R210Q/Q252R were able to grow on succinate (Fig. 2), although not at the wild-type rate. These results indicate that the ATP synthase is at least partially functional, and this was confirmed by the values obtained for growth yields (Table III). The Q252R mutant gave a slight increase in growth yield compared with the vector control. The R210Q mutant gave a lower growth yield than the vector control but was significantly increased when the R210Q mutation was combined with the Q252R mutation.

Membranes were prepared from each of the three strains and assayed for ATPase activity and atebrin fluorescence-quenching activity. The ATPase activities indicated that there was a somewhat reduced assembly in each of the mutant strains, with the R210Q/Q252R double mutant the most severely affected (Table III). This double mutant also gave the largest inhibition of ATPase activity when F1 was bound to the membranes (see Table III). ATP-dependent fluorescence quenching was absent in the R210Q mutant, as found previously (4), and was also essentially absent in the R210Q/Q252R double mutant (Table III). The Q252R mutant showed a low ATP-dependent atebrin fluorescence quenching activity (Table III).

Second-site Revertants of the R210Q/Q252R Double Mutant Strain—The R210Q/Q252R double mutant grows slowly on succinate media (see Fig. 2). Revertant colonies appeared at high frequency on prolonged incubation. Many such revertants were isolated, and one was found to carry the reversion on the plasmid. The plasmid-borne revertant caused wild-type growth on succinate media (see Fig. 2). The uncB gene on the revertant plasmid was sequenced and in addition to the mutations causing the R210Q and Q252R substitutions a C at position 610 was replaced by an A. This resulted in a CCA codon being changed to an ACA codon, and the proline residue at position 204 being replaced by threonine. The growth yield of the revertant strain on limiting glucose was increased compared with the parental R210Q/Q252R double mutant (Table III) to a near wild-type level. The level of ATPase activity in membrane preparations was increased compared with the parental double mutant, but this was due to less inhibition of activity on binding to the membrane (Table III), and there was essentially no ATP-dependent atebrin fluorescence quenching activity. The reversion therefore exerts its effect in the direction of ATP synthesis rather than in the direction of ATP-dependent proton pumping. However the measurement of atebrin fluorescence quenching activity was carried out using an in vitro system, and for this mutant, proton pumping activity may have been lost during the preparation of membranes. In order to confirm that the phenotype of the revertant was due to the P204T mutation (and not some other undetected change in the c or b subunit) a plasmid was constructed by site-directed mutagenesis that carried the undisrupted wild-type a subunit. The other two residues required for proton-coupled ATP synthesis. Previously, evidence had been obtained that position 210 was close to position 50 in the folded subunit structure (14). The work described here leads to the conclusion that position 210 is also close to position 252. If this is the case, then it would preclude any direct interaction between His-245 and Glu-219 (10) but would be consistent with a close interaction between Gly-218 and His-245 (32) (see Fig. 3). The evidence for the latter interaction would appear to be stronger in that the double mutant G218K/H245G is essentially wild-type (32), whereas the double mutant E219H/H245E is only marginally different in energy coupling from the H245E mutant (10). Furthermore the E219H mutant can be changed from essentially uncoupled to fully coupled by a second mutation R140H (33), which is also consistent with this face of helix 4 being close to helix 3 rather than helix 5. The positions of these residues in a five-helix model of the a subunit are shown in Fig. 3. Arg-210 is placed close to position 50 in the folded subunit structure (14). The work described here leads to the conclusion that position 210 is also close to position 252. If this is the case, then it would preclude any direct interaction between His-245 and Glu-219 (10) but would be consistent with a close interaction between Gly-218 and His-245 (32) (see Fig. 3). The evidence for the latter interaction would appear to be stronger in that the double mutant G218K/H245G is essentially wild-type (32), whereas the double mutant E219H/H245E is only marginally different in energy coupling from the H245E mutant (10). Furthermore the E219H mutant can be changed from essentially uncoupled to fully coupled by a second mutation R140H (33), which is also consistent with this face of helix 4 being close to helix 3 rather than helix 5. The positions of these residues in a five-helix model of the a subunit are shown in Fig. 3. Arg-210 is placed close to position 50 in helix 1 and position 252 in helix 5 and is also able to interact with Asp-61 in the c subunit. The other two residues required for proton translocation, Glu-219 and His-245 of the a subunit, are also placed where they can interact with the c subunit Asp-61. The a subunit helices are positioned such that Arg-210 and Glu-219 are able to interact with the Asp-61 of different c subunits.

Work described by Vik and Antonio (34) is difficult to rationalize with this model in that they have concluded that position 252 is close to 219. They found that the double mutants Q252E/E219G and Q252E/E219K were functional. However Q252E alone is also functional so it might be that lysine or glycine at position 219 can functionally replace glutamate or allow other residues to take over that role even when the wild-type residue glutamate is at position 252. The other difference between the data reported by Vik and Antonio (34) and that reported in the
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**Table III**

Properties of a subunit mutant strains

| a subunit mutation(s) on plasmid* | Growth on succinate | Growth yieldb | ATPase activityc | Atebrin fluorescence quench |
|----------------------------------|---------------------|---------------|------------------|---------------------------|
|                                  |                     |               | Native           | Stripped                  | ATP-dependent |
| Coupled control                  | ++                   | 145           | 0.4 (0.4)        | 87                        | 88           | 6          |
| R210A                            | −                    | 145           | 1.0 (1.0)        | 87                        | 40           | 70         |
| Q252R                            | +                    | 155           | 0.6             | 85                        | 90           | 10         |
| R210Q                            | −                    | 125           | 0.5             | 89                        | 85           | <1         |
| R210Q/Q252R                      | +                    | 150           | 0.2 (0.4)       | 87                        | 88           | 4          |
| R210Q/Q252R/P204T                 | ++                   | 180           | 0.3 (0.4)       | 88                        | 88           | 2          |
| P204T                            | ++                   | 200           | 1.2             | 90                        | 59           | 35         |

* The host strain carries the chromosomal mutation uncB402 in the \( \alpha \) subunit gene.

b Growth yield on 5 mM glucose, expressed as Klett units.

c ATPase activity expressed as \( \mu \text{mol/min/mg of protein} \). The ATPase activity after removal of the \( F_1 \) from the membrane, if determined, is given in parentheses.

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The present work is the effect of the replacement of Gln-252 by arginine. Their finding that the Q252R mutant was unable to grow on succinate may be due to different background strains used.

The retention of function after switching residues 210 and 252, albeit at a reduced level, is strong evidence for the juxta-position of Arg-210 and Gln-252. The result is directly analogous to that obtained by Miller et al. (18) when they demonstrated retention of function in a mutant in which the essential aspartate at position 61 in the \( \alpha \) subunit was shifted to position 24. A further similarity between the Asp-61 shift in the \( \alpha \) subunit and the Arg-210 shift in the \( \alpha \) subunit is the ability to improve function by additional mutations in helix 4 of the \( \alpha \) subunit (35). The major difference between the Asp-61 shift and the Arg-210 shift is the observation that in the latter case ATP-dependent proton pumping is lost, whereas ATP synthesis is retained. In the Asp-61 shift experiments, both of these activities were retained (18). This difference may be rationalized by a consideration of the different positions the \( \alpha \) subunit Asp-61 and the Arg-210 of the \( \alpha \) subunit occupy in the proton-translocating pathway. It would appear that the \( \alpha \) subunit Asp-61 is located at about the center of the bilayer (12) and is intimately involved in the movement of protons in both directions. Evidence has been obtained that a subunit residues Glu-219 and His-245 are also involved in proton translocation, and it has been proposed that Arg-210 interacts with the \( \alpha \) subunit Asp-61 from the opposite side of the bilayer to the residues Glu-219 and His-245 (14). The results reported in the present work would indicate that Arg-210 of the \( \alpha \) subunit is placed between the cytoplasm and the \( \alpha \) subunit Asp-61. Thus Arg-210 would normally receive protons for transfer to Asp-61 during ATP hydrolysis, but the shift of the arginine residue has disrupted the pathway, presumably within the \( \alpha \) subunit, by which the proton reaches Arg-210 from the cytoplasmic side of the membrane. This disruption would not affect ATP synthesis since the movement of protons from the periplasm to the \( \alpha \) subunit Asp-61 via His-245 and Glu-219 of the \( \alpha \) subunit would proceed normally and arginine at position 252 is still able to interact with Asp-61 of the \( \alpha \) subunit. Given that there are 6–12 \( \alpha \) subunits and one \( \alpha \) subunit and that the Asp-61 in each \( \alpha \) subunit is required for activity (36), movement of the \( \alpha \) subunit with respect to the \( \alpha \) subunits is also required for activity. If the movement is a rotation of the \( \alpha \) subunit within a ring of \( \alpha \) subunits (13), then it is reasonable that, despite the considerable shift in the position of the key arginine residue, some function could be retained provided that the new position is in the same plane of the membrane. The same argument would apply to the shift in the aspartate residue in the \( \alpha \) subunit from position 61 to position 24 (18). The concept of rotation now forms the basis of many models for the mechanism of the ATP synthase. (34, 37–41).
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The Essential Arginine Residue at Position 210 in the \( \alpha \) Subunit of the \textit{Escherichia coli} ATP Synthase Can Be Transferred to Position 252 with Partial Retention of Activity

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