ATP-Dependent Inactivation of the β-Ser339Cys Mutant F_1-ATPase from *Escherichia coli* by *N*-Ethylmaleimide†

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ABSTRACT: We introduced mutations at the highly-conserved residue Ser-339 in subunit β of *Escherichia coli* F_1-ATPase. The mutations βS339Y and βS339F abolished ATPase activity and impaired enzyme assembly. In contrast βS339C F_1 retained function to a substantial degree. *N*-Ethylmaleimide (NEM) at 0.2−0.3 mM inactivated βS339C F_1-ATPase by 80−95% in the presence of MgATP or MgADP but did not inactivate appreciably in absence of nucleotide or presence of EDTA. In absence of nucleotide, 0.7 mol of [^{14}C-NEM] was incorporated into β-subunits of 1.0 mol F_1; in presence of MgATP the amount was 1.7 mol/mol, i.e. the introduced Cys residue became more accessible to reaction in the presence of MgATP. In the X-ray structure of F_1 (Abrahams *et al.* (1994) *Nature 370,* 621−628) one of the catalytic nucleotide-binding domains is empty (on the "β_E subunit") and contains a cleft. Residue β-339 lies within this cleft; the cleft does not occur in the other two β-subunits. Our data are consistent with the conclusion that in wild-type enzyme under physiological conditions, MgATP or MgADP induce an enzyme conformation in which residue β-Ser-339 becomes more exposed, possibly similar to the situation seen in the "β_E-subunit" in the X-ray structure.

F_0F_1-ATP-synthases use an electrochemical gradient of H^+ to synthesize ATP in mitochondria, chloroplasts, and bacteria. They consist of two sectors: F_0, which is membrane-embedded and conducts protons across the membrane, and F_1, which is bound to F_0 on the membrane-surface. In *Escherichia coli* F_0 consists of three subunits (ab_2c_{10-12}) and F_1 of five different subunits ($\alpha_3\beta_3\gamma\delta\epsilon$) (Senior, 1990; Fillingame, 1990; Capaldi *et al.*, 1994). F_1 contains the catalytic nucleotide-binding sites and may be obtained in soluble purified form which hydrolyses ATP.

Recently, the X-ray structure of crystals of F_1 from bovine heart mitochondria was solved (Abrahams *et al.*, 1994). The α and β subunits are arranged in an alternating hexagon, with the γ subunit in the center. Both α and β subunits consist of three domains, namely an N-terminal domain containing six β-strands, a central nucleotide-binding domain containing alternating β-strands and α-helices and a C-terminal domain consisting predominantly of α-helices. The three noncatalytic nucleotide-binding sites are located primarily on the α subunits, with small contributions from β-subunits; the three catalytic sites are located primarily on the β-subunits, with two residues from an α-subunit also participating at each site. The crystals were obtained in the presence of sodium azide, MgAMPPNP, and MgADP, and in the absence of ATP. The three noncatalytic sites are filled with MgAMPPNP. One catalytic site contains MgAMPPNP, one contains MgADP, and the third catalytic site is empty (named "β_E subunit" by Abrahams *et al.*, 1994). In the third catalytic site the C-terminal part of the nucleotide-binding domain is shifted, opening a cleft in the binding site.

Recent data from our laboratory have established that for V_{max} rates of ATPase activity, all three catalytic sites must fill with MgATP, and that K_M(MgATP) corresponds to filling of the third site (Weber *et al.*, 1993, 1994). It is of importance to establish whether the structure seen by Abrahams *et al.* represents a form of the enzyme that occurs naturally, e.g. as part of the normal catalytic cycle. There are large differences between the binding affinities for MgAMPPNP and MgATP in F_1 as noted in Senior *et al.* (1993) and elsewhere, so that an enzyme form lacking bound MgATP might not mimic the natural form(s). Also the presence of sodium azide, which is one of the few inhibitors to give complete inactivation of F_1, may have forced the enzyme into an unusual conformation. Abrahams *et al.* suggest that the structure seen may mimic the so-called "ADP-inhibited" form of the enzyme, reported by Boyer and colleagues in mitochondrial and chloroplast F_1 (Boyer, 1993). However there is no evidence to suggest whether this form of the enzyme occurs naturally in cells either as a regulatory form or as part of the normal catalytic activity. Furthermore a stable "ADP-inhibited" form of the enzyme does not occur in the *E. coli* enzyme as prepared here and assayed under multisite V_{max} or unisite ATPase conditions (Senior *et al.*, 1992).

In the X-ray structure the region of β-strand 8 and α-helix H of the "β_E subunit" nucleotide-binding domain is open and exposed to solvent. Residue β-Ser-339 lies within the cleft which has opened in the nucleotide-binding domain, with the side-chain pointing into the cleft (Walker, J. E., Abrahams, J. P., & Leslie, A. G. W., personal communication). The cleft is not present in the other two β-subunits. In this paper we have generated mutations of residue β-Ser-339 in order to investigate whether its accessibility changes in a nucleotide-dependent manner.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The strains of *E. coli* used were the following: SWM1 (wild-type) (Rao *et al.*, 1988a); JP17 (β-subunit deletion) (Lee *et al.*, 1991); TG1rA (transformation host)(Rao *et al.*, 1988b).

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Site-Directed Mutagenesis of Residue β-Ser-339 to Cys, Phe, and Tyr. Mutagenesis was by the method of Vandeyar *et al.* (1988) using the T7-GEN mutagenesis kit (US Biochemical Corp.). The template was M13mp18 phage containing an insert (*HindIII-KpnI*) encoding the F_1 β- and ε-subunit genes (Weber *et al.*, 1993). The mutagenic oligonucleotide was 5′-C CCG CTG GAC TDC ACC AGC CGT CAA CTG GAC CC (D ≡ 33% A, G, or T), in which the underlined D generates the codons TTC (Phe), TAC (Tyr), and TGC (Cys), and the underlined A eliminates a *PvuII* site present in wild-type. Mutant phage were identified by *PvuII* digestion, followed by DNA sequencing. Three different isolates of each mutation were transferred on a *HindIII-KpnI* fragment into plasmid pUC118 and transformed into TG1rA. The plasmids from colorless colonies were isolated, tested for their *PvuII* pattern, and transformed into JP17 for expression of the mutant β-subunits and characterization of enzyme activity.

Growth of Cells, Preparation of Membrane Vesicles, and Purification of F_1. Growth yield analyses on limiting glucose (3 mM) were performed as described (Senior *et al.*, 1984). Membrane vesicles were prepared as in Senior *et al.* (1979). Soluble F_1 was released from membranes and purified as in Weber *et al.* (1992). F_1-free membranes were prepared by KSCN washings (Perlin *et al.*, 1983).

Biochemical Techniques. Steady-state ATPase activity was determined in 0.5−1 mL assay medium (50 mM $TrisSO_4$, 10 mM ATP, 4 mM $MgCl_2$, pH 8.5). F_1 concentration was 12.5 μg/mL . For K_M measurement total [ATP] was 2.5 times [Mg]. This ATP/Mg ratio is known to optimize ATPase activity and to minimize inhibition by Mg^{2+} ions (Al-Shawi *et al.*, 1988). P_i released was estimated by the method of Taussky and Shorr (1951) or van Veldhoven and Mannaerts (1987). ATP-dependent proton-pumping in membrane vesicles was assayed by acridine orange fluorescence quenching (Perlin *et al.*, 1983). Nucleotide content of F_1 preparations was assayed by the luciferin-luciferase technique as in Senior *et al.* (1992). Protein was estimated as described by Lowry *et al.* (1951) (membranes) or Bradford (1976) (soluble F_1). SDS−PAGE was performed as by Laemmli (1970).

Reaction with N-Ethylmaleimide. *N*-Ethylmaleimide (NEM) was dissolved in 10 mM Na-OAc, pH 5.0; the concentration was verified by measuring the absorption at 302 nm using an extinction coefficient of 620 M^{-1} cm^{-1}. F_1 was preequilibrated in 50 mM $TrisSO_4$, pH 8.5, 10% (v/v) glycerol, by passage through a Sephadex G-50 centrifuge column. Unless otherwise specified, F_1 (0.3 mg/mL) was incubated for 60 min with 0.3 mM NEM at room temperature, and the reaction was stopped by addition of 17-fold excess of DTT. Where required, nucleotide and $MgCl_2$ were present at a ratio of 2.5/1 (see above). The enzyme was then passed through a centrifuge column before estimation of ATPase and protein. For determination of NEM labeling stoichiometry, *N*-[1-^{14}C]-ethylmaleimide in pentane was purchased from Amersham. To prepare an aqueous stock solution 100−150 μL aliquots were added to 30 μL of 10 mM Na-AOc, pH 5.0; the pentane was evaporated with N_2 at room temperature. Reaction with F_1 was carried out as above and after addition of DTT, samples were taken for SDS−PAGE and determination of the [^{14}C]NEM concentration. The remainder was passed through centrifuge columns and radioactivity, ATPase activity, and protein concentration were determined on the eluates.

Table 1: Growth Yield in Minimal Glucose Medium

strain	A_{595}
wild-type[a]	0.79
Δ β-subunit[b]	0.45
βS339C	0.74, 0.75, 0.74[c]
βS339F	0.45, 0.45, 0.45[c]
βS339Y	0.45, 0.44, 0.44[c]

[a] Strain pDP31N/JP17 (Weber *et al.*, 1993). [b] Strain pUC118/JP17. [c] Measurements for three different isolates.

Table 2: ATPase Activity and ATP-Driven Proton Pumping in Membrane Vesicles

strain	ATPase activity (units mg^{-1})	ATP-driven proton pumping[a] (% fluor quench)	
		untreated	KSCN/F_1-treated[b]
wild-type	2.4	97	85
βS339C	2.0	92	82
βS339F	0.02	0	33
βS339Y	0.03	0	22

[a] Assayed by quench of acridine orange fluorescence. [b] Membranes depleted of F_1 by washing with KSCN and then reconstituted with wild-type F_1.

The SDS-gels were stained with Coomassie Brilliant Blue and subjected to fluorography. Then subunits α, β, γ, and δ were cut out and incubated with 300 μL of 30% H_2O_2 for 3 h at 70 °C. A 50 μL amount of sodium ascorbate (100 g/L) and 10 mL of scintillation cocktail (Ecoscint, National Diagnostics) were added, and radioactivity in each band was counted.

RESULTS

Functional Effects of Mutations βS339C, βS339F, and βS339Y in Cells. Three isolates of each mutation were transferred to plasmids and transformed into strain JP17 (β-subunit deletion strain) for expression and characterization. All three isolates of the Cys mutant grew as well as wild-type on succinate plates; the three Phe and three Tyr mutants showed no growth. Growth yields in limiting glucose medium were determined (Table 1). βS339F and βS339Y were indistinguishable from the negative control (pUC118/JP17). βS339C showed a growth yield a little lower that that of the wild-type. Therefore we may conclude that the βS339C mutation supports oxidative phosphorylation in vivo but that the βS339F and Y mutations do not.

Properties of Membranes. In agreement with the properties of the cells, isolated membranes from the βS339C mutant showed about 80% of the ATPase activity and nearly the same ATP-driven proton-pumping as wild type, while only very little activity was found with F or Y mutants (Table 2). To test whether the ATP synthase was normally-assembled in the membranes from the mutants, F_1 was removed by KSCN washes, and the membranes were reconstituted with wild type F_1. The βS339C membranes could be reconstituted to a large degree but the βS339F and βS339Y regained only limited H^+-transport ability (Table 2). It was concluded that the latter two mutations caused incorrect assembly of the ATP synthase and they were not further investigated.

Properties of βS339C F_1. The yield of purified enzyme was 0.07 mg/g wet wt of cells. On Coomassie blue-stained SDS-gels the mutant enzyme resembled wild-type except that it contained somewhat less of the δ-subunit. The βS339C

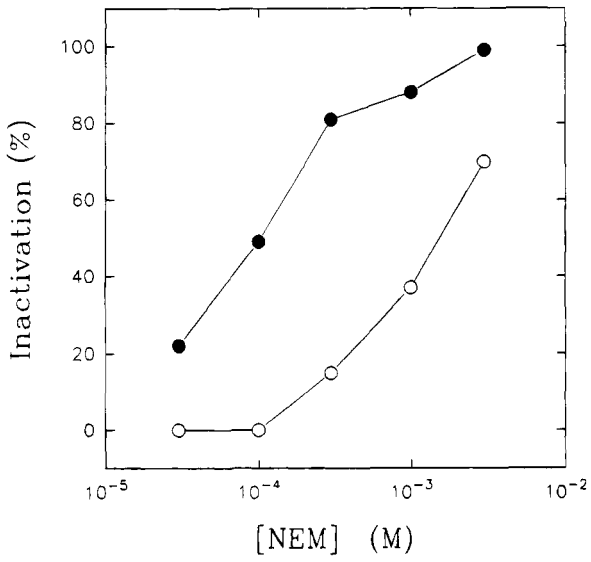

FIGURE 1: Dependence of inactivation of ATPase of βS339C F_1 on concentration of NEM. βS339C F_1 was incubated for 10 min (open circles) or 60 min (closed circles) with NEM in the presence of 10 mM ATP and 4 mM $MgCl_2$ at pH 8.5 (see Methods for further details).

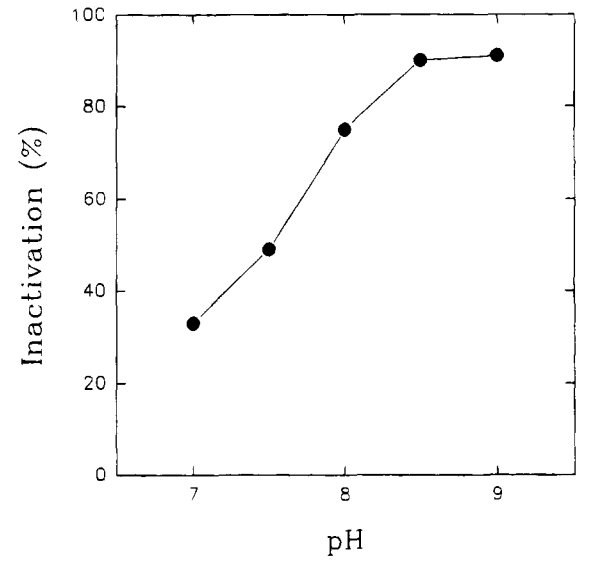

FIGURE 2: pH Dependence of inactivation of ATPase by NEM. F_1 from βS339C was incubated for 10 min with 3 mM NEM, 10 mM ATP, and 4 mM $MgCl_2$. The buffer was 50 mM $TrisSO_4$, 10% glycerol, adjusted to the required pH. Remaining ATPase activity was assayed at pH 8.5 as described in Methods.

F_1 had a K_M(MgATP) of 530 μM (wild-type run alongside = 100 μM) and a V_{max} of 13.0 units mg^{-1} (parallel wild-type = 36 units mg^{-1}). The pH-profile of ATPase activity was similar to wild type (data not shown), with maximal activity occurring at pH 9.3. The nucleotide content was assayed after preequilibration in 50 mM Tris-SO_4, pH 8.5, 10% (v/v) glycerol, by passage through a centrifuge column (as for enzyme subjected to reaction with NEM, see Methods). The βS339C F_1 contained 2.9 ATP and 0.84 ADP (mol/mol F_1). Wild-type enzyme prepared in the same way contained 3.0 ATP and 0.96 ADP (mol/mol F_1). Therefore the mutation did not significantly affect the nucleotide content.

Inhibition of ATPase Activity by NEM. A preliminary series of experiments showed that 0.3 mM NEM caused only slight (0−15%) inhibition of the ATPase activity of wild-type enzyme after a 60 min incubation at room temperature, in the presence or absence of 10 mM ATP, with either 4 mM $MgCl_2$ or 4 mM EDTA present. Similar results were obtained with the βS339C F_1 in the absence of nucleotide, with Mg or EDTA present. However in the presence of 10 mM ATP, and with 4 mM $MgCl_2$, 90−94% inactivation of βS339C F_1 ATPase activity was seen. This inactivation was dependent upon Mg because in the presence of 4 mM EDTA and absence of added Mg the inactivation in presence of ATP was only 5−14%.

Figure 1 shows the dependence of inhibition of ATPase activity on the NEM concentration in the presence of MgATP. Figure 2 shows the pH-dependence of the inactivation, from which it is clear that the reaction accelerates at alkaline pH. Figure 3 shows the time course of the NEM-inhibition. From these data, reaction conditions of 60 min at room temperature, 0.2−0.3 mM NEM, pH 8.5, were chosen for further experiments (see Methods for further details).

Dependence of Inactivation of ATPase on Nucleotides. Figure 4 (open squares) shows data for wild-type F_1 reacted with NEM in the presence of ATP. Similar results were obtained with wild-type in presence of ADP or AMPPNP.

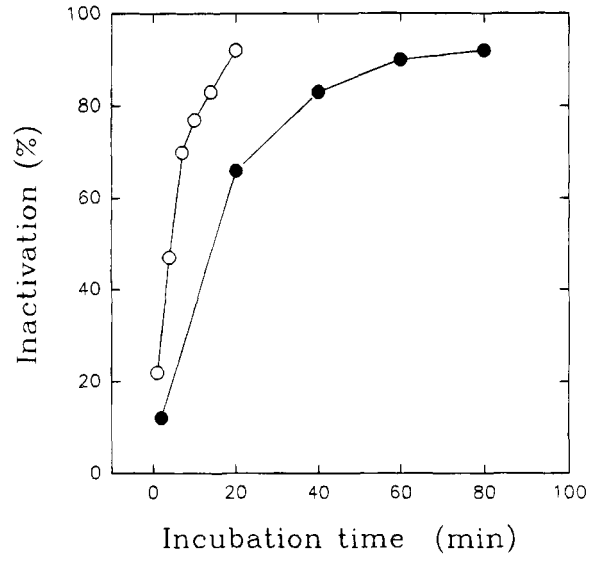

FIGURE 3: Time dependence of inactivation of ATPase by NEM. F_1 from βS339C was incubated for 10 min with 0.3 mM (closed circles or 3 mM (open circles) NEM in the presence of 10 mM ATP and 4 mM $MgCl_2$ at pH 8.5.

The solid symbols are data for βS339C F_1 reacted with NEM in presence of ATP (squares) or ADP (circles) or AMPPNP (triangles). At 10mM ATP plus 4 mM $MgCl_2$ the inhibition reached 90−95% and at 10 mM ADP plus 4 mM $MgCl_2$ it reached 75−79% (data not shown).

Stoichiometry of [^{14}C]-NEM Incorporation into F_1. In order to determine the stoichiometry of NEM incorporation, F_1 from wild-type and βS339C mutant was incubated for 60 min with [^{14}C]-NEM in the presence or absence of 10 mM ATP and 4 mM $MgCl_2$ at pH 8.5. After passing through a centrifuge column, protein concentration, ATPase activity, and enzyme-bound [^{14}C]-NEM were determined. Figure 5 shows a typical result, with the degree of inactivation of ATPase activity in the βS339C enzyme being 87% (plus ATP and $MgCl_2$) or zero (no ATP/$MgCl_2$), and that in wild-type being zero (± ATP/$MgCl_2$), in this experiment.

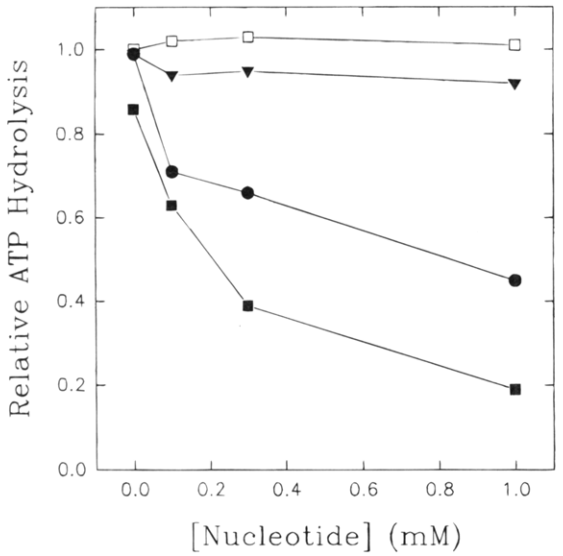

FIGURE 4: Effect of nucleotides on inactivation of ATPase by NEM. F_1 was incubated with 0.3 mM NEM for 60 min in the presence of different nucleotides and $MgCl_2$ at pH 8.5. [$MgCl_2$] was 40% of the indicated nucleotide concentration. The data are expressed as activity in presence of NEM relative to activity in absence of NEM. As noted in the text the inactivation by NEM in the absence of added nucleotide varied from 0−15%. Open squares, wild-type F_1 with ATP. Filled symbols, βS339C F_1; nucleotides were ATP (squares), ADP (circles), and AMPPNP (triangles).

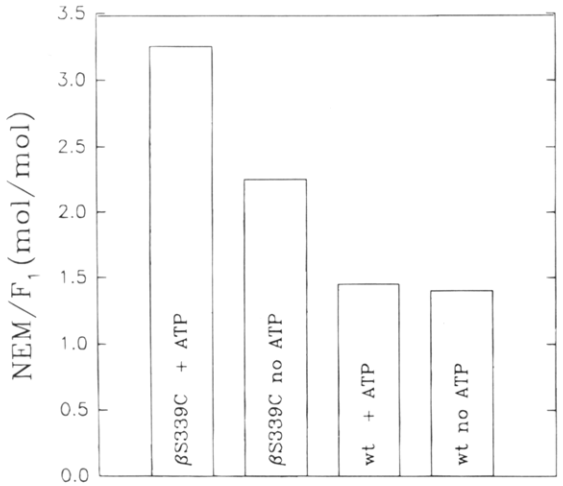

FIGURE 5: Labeling of F_1 with [^{14}C]-NEM. F_1 was incubated with 200 μM [^{14}C]-NEM in the presence or absence of 10 mM ATP plus 4 mM $MgCl_2$ for 60 min. After quenching the reaction with DTT, the enzyme was applied to a centrifuge column. Radioactivity and protein concentration were determined in the eluate.

Distribution of [^{14}C]-NEM among the Subunits of Labeled F_1. The subunits of the labeled wild-type and mutant enzymes were separated by SDS−PAGE, stained, and then subjected to fluorography. Figure 6 shows a typical experiment. The pattern of subunit labeling in wild-type enzyme was consistent with the previous report by Mendel-Hartvig and Capaldi (1991). We have assumed that the labeled band running in front of the ε-subunit (which contains no Cys) is a proteolytic fragment (δ') as determined by these workers. Labeling of the α-subunit was variable in different experiments (see Figure 7 standard deviation bars). The experiment of Figure 6 reflects this variability in lanes C and D, but as noted below, the difference was not statistically-significant. The mutant enzyme showed lower labeling of subunit δ; however, as was noted above, subunit δ is deficient in the

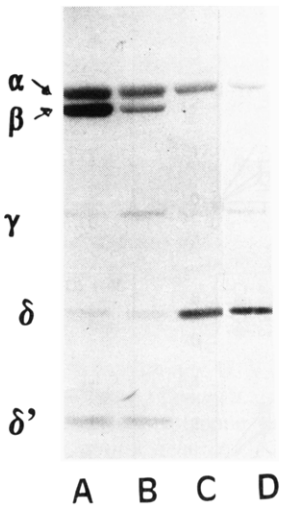

FIGURE 6: SDS−PAGE of [^{14}C]-NEM labeled F_1. F_1 was reacted with [^{14}C]NEM as in Figure 5. 10 μg of reacted protein was electrophoresed in a 12% SDS polyacrylamide gel. After staining with Coomassie Blue and destaining, the gel was soaked with Amplify (Amersham) for 40 min, dried, and fluorographed at −70 °C using Kodak X-OMAT film XR. Lane A: βS339C F_1 reacted in the presence of 10 mM ATP plus 4 mM $MgCl_2$; lane B: βS339C F_1 reacted in the absence of ATP plus $MgCl_2$; lane C: wild-type F_1 reacted in the presence of 10 mM ATP plus 4 mM $MgCl_2$; lane D: wild-type F_1 reacted in the absence of ATP plus $MgCl_2$.

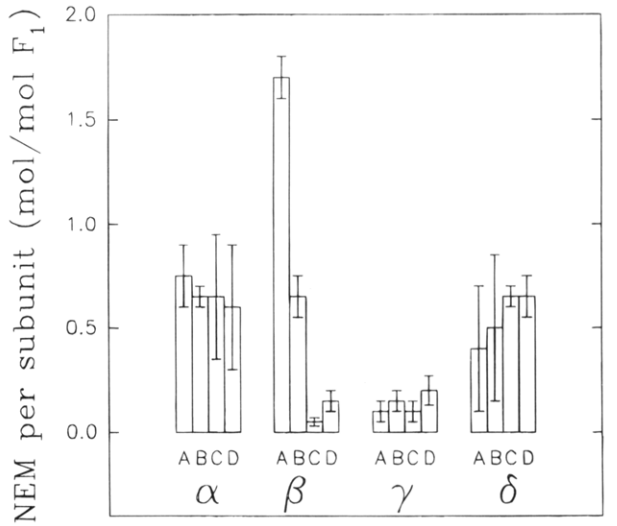

FIGURE 7: Distribution of [^{14}C]NEM in the subunits of labelled F_1. Coomassie Blue-stained subunits were cut from gels and counted as described in Methods. Averages and standard deviations (bars) from six experiments are shown. Lane A: βS339C F_1 reacted in the presence of 10 mM ATP plus 4 mM $MgCl_2$; lane B: βS339C F_1 reacted in the absence of ATP plus $MgCl_2$; lane C: wild-type F_1 reacted in the presence of 10 mM ATP plus 4 mM $MgCl_2$; lane D: wild-type F_1 reacted in the absence of ATP plus $MgCl_2$.

mutant F_1. The major difference seen between mutant and wild-type is the labeling pattern in the β subunits. While very little label is present in this subunit in wild type F_1, significant label is present in the β-subunits of the βS339C mutant. Moreover, in the mutant the labeling of β is much stronger in presence of MgATP.

In order to obtain quantitative information regarding the distribution of label among the subunits, stained gel bands were cut out, dissolved by H_2O_2, and counted. Figure 7 shows the results of six determinations with standard deviations. As indicated above, subunit δ is deficient in the βS339C mutant and also variable in amount as indicated by

the bars in Figure 7. Incorporation of [^{14}C]-NEM into subunit δ of wild type F_1 was constant, but indicated a content of only 0.65 mol/mol F_1, consistent with previous work by Ziegler *et al.* (1994). Subunit γ was labeled to only minor extent in mutant and wild-type enzymes. The incorporation of [^{14}C]-NEM into the α subunit of βS339C F_1 was about the same with and without MgATP and not significantly different from wild type F_1 when the standard deviations are compared.

Little [^{14}C]-NEM was bound to the β-subunit of wild type F_1. In the absence of MgATP, 0.7 mol of [^{14}C]-NEM was incorporated into the β subunits of 1.0 mol F_1 from βS339C, and in the presence of MgATP, 1.7 [^{14}C]-NEM (mol/mol) was incorporated into the β subunits.

DISCUSSION

We introduced a Cys residue at the highly-conserved position β-Ser-339 of *E. coli* F_1-ATPase. The homologous residue was determined from inspection of the X-ray structure to lie within the cleft in the "β_E subunit" of the bovine mitochondrial enzyme and to be accessible to solvent (Walker, J. E., Abrahams, J. P., & Leslie, A. G. W., personal communication). In contrast, in the two other β-subunits which do not contain the cleft this residue is not exposed.

We tested the accessibility of the introduced Cys residue in the βS339C F_1 by reaction with NEM and found that it appeared to react to a limited degree in the absence of MgATP and to a significantly greater degree in presence of MgATP. There was considerable inactivation of ATPase activity by NEM in the presence of MgATP (up to 95%) or MgADP (up to 79%), but not in the presence of MgAMP-PNP. In absence of Mg and presence of EDTA there was little inactivation, whether ATP and ADP were present or not, and there was also little inactivation in presence of Mg alone.

Wild type *E. coli* F_1 contains a total of 19 cysteine residues, comprised of twelve in α, three in β, two in γ, and two in δ subunit (Walker *et al.*, 1984; Stan-Lotter et al., 1986). Two of these react readily with NEM, one in the α subunits and one in subunit δ, and neither reaction was found to inactivate the enzyme (Mendel-Hartvig and Capaldi, 1991). Only low incorporation of NEM was found in the β and γ subunits by Mendel-Hartvig and Capaldi (1991). Our experiments with wild-type enzyme were fully consistent with this previous work (Figure 6).

In βS339C F_1 the labeling of the α and γ subunits was not significantly different from wild-type. The mutant enzyme appeared to contain less subunit δ than wild-type from the stained gels, and this explained the lesser incorporation of NEM into the δ in mutant as compared to wild-type. The only significant difference in NEM-labelling between wild-type and βS339C mutant was in the β-subunits and it was also clear that the degree of labeling of the mutant β was strongly affected by the presence of MgATP (Figure 7).

It was puzzling that in the absence of nucleotide there was little (0−15%) inactivation of the mutant βS339C ATPase activity by NEM, and yet about 0.7 mol NEM/mol F_1 was incorporated into the β-subunits. One possibility to explain this result could be that a fraction of the F_1 preparation is denatured from the start, and the denatured polypeptides react readily with NEM. However, one would expect in this case to see higher incorporation of NEM into other subunits, particularly subunit α. Since the other subunits behaved much like wild type, this explanation seems unlikely. Wild-type subunit β has a cysteine residue at position 137 which does not react with NEM (see above). It is also possible that this residue becomes more reactive in the βS339C mutant. However, it has been shown that both βC137S (Lee *et al.*, 1991) and βC137Y (Kironde *et al.*, 1989) mutations strongly impair ATPase activity, and so it seems unlikely that enzyme with NEM attached covalently to this cysteine should be active. Therefore at the present time this finding remains unexplained.

The α and β subunits possess extensive sequence and structural homology with each other (Walker *et al.*, 1984; Abrahams *et al.*, 1994). Residue βS339C is equivalent to residue αS373, which itself has been subjected to mutagenesis, with both αS373C and αS373F mutations having been studied extensively (Wise *et al.*, 1984; Lee *et al.*, 1992; Turina *et al.*, 1993). However from comparison of the data in these papers, it is clear that residues βS339 and αS373, and their respective C and F mutations, show different properties. This is borne out by examination of the X-ray structure which shows that their environments are not the same.

CONCLUSIONS

The data demonstrate enhanced reaction of residue β-Cys-339 in βS339C mutant F_1 with NEM in the presence of MgATP and MgADP but not of MgAMPPNP. The enhanced reaction in the presence of the substrates MgATP and MgADP was accompanied by inactivation of ATPase activity. The data are consistent with the conclusion that in wild-type enzyme under physiological conditions, MgATP or MgADP induce an enzyme conformation in which residue β-Ser-339 becomes more exposed, possibly similar to the situation seen in the "β_E-subunit" in the X-ray structure.

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REFERENCES

Abrahams, J. P., Leslie, A. G. W., Lutter, R., & Walker, J. E. (1994) *Nature 370*, 621−628.
Al-Shawi, M. K., Parsonage, D., & Senior, A. E. (1988) *J. Biol. Chem. 263*, 19633−19639.
Boyer, P. D. (1993) *Biochim. Biophys. Acta 1140*, 215−250.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248−254.
Capaldi, R. A., Aggeler, R., Turina, P., & Wilkens, S. (1994) *Trends Biochem. Sci. 19*, 284−289.
Fillingame, R. H. (1990) In *The Bacteria*, Vol. XII, pp 345−391, Academic, New York.
Kironde, F. A. S., Parsonage, D., & Senior, A. E. (1989) *Biochem. J. 259*, 421−426.
Laemmli, U. K. (1970) *Biochemistry 19*, 4620−4626.
Lee, R. S.-F., Pagan, J., Wilke-Mounts, S., & Senior, A. E. (1991) *Biochemistry 30*, 6842−6847.
Lee, R. S.-F., Wilke-Mounts, S., & Senior, A. E. (1992) *Arch. Biochem. Biophys. 297*, 334−339.
Lowry, O. H., Rosebrough, M. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265−275.
Mendel-Hartvig, J., & Capaldi, R. A. (1991) *Biochim. Biophys. Acta 1060*, 115−124.

Perlin, D. S., Cox, D. N., & Senior, A. E. (1983) *J. Biol. Chem. 258*, 9793−9800.
Rao, R., Al-Shawi, M. K., & Senior, A. E. (1988a) *J. Biol. Chem. 263*, 5569−5573.
Rao, R., Pagan, J., & Senior, A. E. (1988b) *J. Biol. Chem. 263*, 15957−15963.
Senior, A. E. (1990) *Annu. Rev. Biophys. Biophys. Chem. 19*, 7−41.
Senior, A. E., Fayle, D. R. H., Downie, J. A., Gibson, F., & Cox, G. B. (1979) *Biochem. J. 180*, 111−118.
Senior, A. E., Latchney, L. R., Ferguson, A. M., & Wise, J. G. (1984) *Arch. Biochem. Biophys. 228*, 49−53.
Senior, A. E., Lee, R. S. F., Al-Shawi, M. K., & Weber, J. (1992) *Arch. Biochem. Biophys. 297*, 340−344.
Senior, A. E., Wilke-Mounts, S., & Al-Shawi, M. K. (1993) *J. Biol. Chem. 268*, 6989−6994.
Stan-Lotter, H., Clarke, D. M., & Bragg, P. D. (1986) *FEBS Lett. 197*, 121−124.
Taussky, H. H., & Shorr, E. (1953) *J. Biol. Chem. 202*, 675−685.
Turina, P., Aggeler, R., Lee, R. S. F., Senior, A. E., & Capaldi, R. A. (1993) *J. Biol. Chem. 268*, 6978−6984.
van Veldhoven, P. P., & Mannaerts, G. P. (1987) *Anal. Biochem. 161*, 45−48.
Vandeyar, M., Weiner, M., Hutton, C., & Batt, C. (1988) *Gene 65*, 129−133.
Walker, J. E., Saraste, M., & Gay, N. J. (1984) *Biochim. Biophys. Acta 768*, 164−200.
Weber, J., Lee, R. S.-F., Grell, E., & Senior, A. E. (1992) *J. Biol. Chem. 267*, 1712−1718.
Weber, J., Wilke-Mounts, S., Lee, R. S.-F., Grell, E., & Senior, A. E. (1993) *J. Biol. Chem. 268*, 20126−20133.
Weber, J., Wilke-Mounts, S., & Senior, A. E. (1994) *J. Biol. Chem. 269*, 20462−20467.
Wise, J. G., Latchney, L. R., Ferguson, A. M., & Senior, A. E. (1984) *Biochemistry 23*, 1426−1432.
Ziegler, M., Xiao, R., & Penefsky, H. S. (1994) *J. Biol. Chem. 269*, 4233−4239.

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