THE *uncA401* MUTATION ALTERS A NUCLEOTIDE-BINDING SITE IN THE α-SUBUNIT OF THE F_1 ADENOSINE TRIPHOSPHATASE FROM *ESCHERICHIA COLI*

J. H. VERHEIJEN, P. W. POSTMA and K. VAN DAM

Laboratory of Biochemistry, B. C. P. Jansen Institute, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam, The Netherlands

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1. Introduction

A H^+-translocating ATPase-complex (F_0F_1) is present in the cytoplasmic membrane of *Escherichia coli*. This complex is involved in energy conversion and has some similarities in structure and function with the ATPase complexes from mitochondria and chloroplasts (reviewed [1,2]). The ATPase-complex of *E. coli* consists of two parts, a soluble part, F_1, and a membrane-bound part, F_0. The F_1 part has been relatively well investigated and consists of 5 types of non-identical subunits. These subunits are called α, β, γ, δ and ε in order of decreasing molecular weight. The α and β subunits are essential for enzymatic activity as was shown by a variety of methods [1,2]. One approach to study the properties of the ATPase complex and its various subunits has been the isolation of mutants with a defect in one of those subunits. This approach was initiated when an *E. coli* mutant AN120, *uncA401*, was isolated with an enzymatically inactive F_1-ATPase [3].

Many mutants with defects in different parts of the ATPase complex have been isolated since (reviewed [1]). The F_1-ATPase of AN120 was isolated in [4,5] and shown to be indistinguishable from the wild-type enzyme with respect to number and molecular weight of its subunits, binding properties of the inhibitor NbfCl and the presence of bound nucleotides. Using dissociation and reconstitution techniques, a mutation in the *uncA* gene resulted in an altered α-subunit [6]. The same was concluded using two-dimensional gel electrophoresis [7].

We have shown that the α-subunit of *E. coli* F_1 contains a nucleotide-binding site. This site is essential for enzymatic activity and can bind the ATP analogue, 8-azido-ATP [8].

Here we show that 8-azido-ATP binding to the *uncA401* mutant enzyme is significantly reduced in comparison with that to the wild-type enzyme. From our results, combined with those in [6,7] we conclude that, as a result of the *uncA401* mutation, an essential nucleotide-binding site on the α-subunit is altered.

Abbreviation: NbfCl, 4-chloro-7-nitrobenzofurazan

2. Materials and methods

2.1. *Growth of bacteria*

E. coli AN120, *uncA401, argE, thi, str*^{R} [3] and *E. coli* K-12 (λ) were grown in a 14 l New Brunswick Microferm fermentor on a minimal salt medium [8] with 5 g/l D-glucose as a carbon source. The medium was supplemented with 1 mg/l thiamine; in the case of AN120, also 15 mg/l casamino acids and 1 mM arginine were added. Cells were harvested in the early stationary phase.

2.2. *Isolation of* F_1

F_1-ATPase from K-12 (λ) was purified as in [9]. Enzymatically inactive F_1 from AN120 was purified by the same method, omitting the final gel filtration step. During purification the presence of the enzymatically inactive mutant enzyme in the column fractions was detected by Ouchterlony double-immunodiffusion against wild-type F_1 antiserum; 1 mm thick 1% (w/v) agarose gels in 50 mM Tris–HCl (pH 8.0), 2.5 mM $MgCl_2$ were used. The presence of the α, β and γ subunits in the fractions giving a positive reaction in this test was verified by gel electrophoresis in the presence of sodium dodecylsulphate.

2.3. *Miscellaneous techniques*

8-azido-ATP labeling, inhibition by 8-azido-ATP, electrophoretic techniques, gel slicing, radioactivity determination and determination of enzyme activity were done as in [8]. 8-Azido-[2-^{3}H]ATP was a gift of R. J. Wagenvoord. *E. coli* strain AN120 was obtained from M. Satre and strain K-12 (λ) from L. Heppel.

3. Results and discussion

To study the binding of 8-azido-ATP to wild-type and mutant F_1-ATPase, both enzymes were incubated with 8-azido-[2-^{3}H]ATP and illuminated as in the legend to fig.1. Wild-type F_1 was readily labeled by 8-azido-ATP (fig.1A) in agreement with [8]. In contrast almost no photolabel was bound to the mutant F_1 (fig.1B). From the data in fig.1, the amount of 8-azido-ATP bound/mol F_1 was calculated for both cases (table 1). Since the final gel filtration step was omitted in the purification procedure of the inactive mutant F_1 (see section 2), this F_1 is less pure than wild-type F_1 and contains several impurities which amount to ~25% of the protein as deduced from gel scans (fig.1B). The binding results shown in table 1 have been corrected for the presence of these impurities. It is clear that the (inactive) F_1 from AN120 binds much less azido-ATP than wild-type F_1, treated in exactly the same way in a parallel experiment. These binding experiments were repeated a number of times and always had essentially the same result. The binding to the mutant F_1 was always <10% of the binding to the wild-type F_1. The results in fig.1 and table 1 were obtained in the same experiment.

The resolution obtained by gel slicing between the α and β subunits in this experiment does not allow the calculation of the 8-azido-ATP binding to the α and β subunits separately in a reliable way. Earlier experiments [8] have shown that ~75% of the label is bound to the α subunit under those conditions. The experiments do suggest that in the case of the mutant

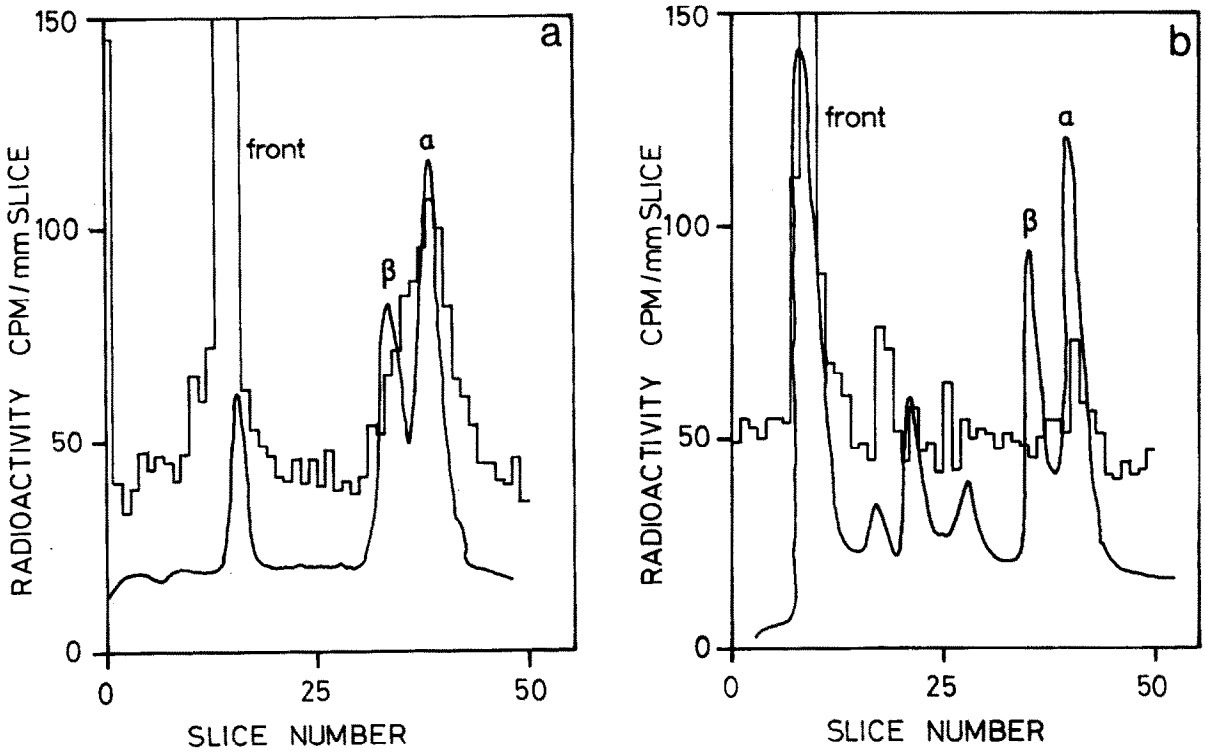

Fig.1. Photoaffinity labeling of wild-type (K-12 (λ)) and mutant (AN120)F_1. Wild-type F_1, 26 μg protein (A) or mutant F_1, 33 μg protein (B) were photolabeled with 8-azido-[2-^{3}H]ATP (14.1 mCi/mmol, 0.52 GBq/mmol) and electrophoresed as in paper [8]. The curve indicates the absorbance of the Coomassie blue-stained gels. The bars indicate the amount of radioactivity present in each gel slice, not corrected for the background.

Table 1
Photoaffinity labeling of wild-type (K 12 (λ)) and mutant (AN120)F_1

	Wild-type (K-12 (λ))F_1	mutant (AN120)F_1
8-azido-ATP bound (mol/mol F_1)	0.51	0.04
% inhibition of enzyme activity	53%	–

From fig.1, the total binding of 8-azido-ATP/mol enzyme was calculated. The results of mutant F_1 were corrected for the fact that the preparation was not entirely pure, the amount of F_1 being ~75% of the protein present, as was concluded from gel scans. ATPase activity and 8-azido-ATP binding were determined as in section 2

enzyme, azido-ATP binding to both subunits is diminished.

There are no indications that the mutant F_1 isolated by us is structurally not intact, since it behaves identically to wild-type F_1 during ion exchange chromatography and has the same binding characteristics towards NbfCl, a specific binding inhibitor [4,8]. We conclude that the inability to bind 8-azido-ATP is an intrinsic property of this mutant F_1 caused by the *uncA401* mutation. It was shown [6,7] that a mutation in the *uncA* gene leads to an altered α-subunit. We can extend the finding by concluding that as a result of the *uncA401* mutation, an essential nucleotide-binding site on the α-subunit of F_1 is changed.

Acknowledgements

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