Volume 292, number 1,2, 145–147 FEBS 10335 November 1991

ADONIS 001457939101043C

Detection and localization of the *i* protein in *Escherichia coli* cells using antibodies

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Received 11 September 1991

Using antibodies raised against the purified *i* protein, the expression of the chromosomal *uncI* gene was demonstrated. The *i* protein was identified as a component of the cytoplasmic membrane and shown to be present in preparations of F_o or F_1F_o. The protein is not associated with the F_1 moiety.

ATP synthase; F_o complex; *uncI* gene; anti-*i* antibody; *Escherichia coli*

1. INTRODUCTION

The membrane-bound ATP synthase (F_1F_o) of *Escherichia coli* is composed of two structurally and functionally distinct components [1–4]. The hydrophilic F_1 portion carries the catalytic centers of the enzyme complex and the membrane-integrated F_o part functions as a proton channel. The genes of the eight different subunits of the ATP synthase are organized in the *unc* operon together with a ninth gene, *uncI*, coding for a hydrophobic and basic protein, designated *i* protein, of so far unknown function [5,6]. Complementation studies of strains harboring Tn*10* insertions within the *unc* operon [7] and a preliminary characterization of an *uncI* deletion mutant [8] revealed that the *uncI* gene product is not essential for an active ATP synthase complex. However, in comparison to the wild-type strain, the deletion mutant showed a reduction in growth yield and ATPase activity [8]. Carrying out in vitro protein synthesis and minicell experiments the expression of the *uncI* gene was demonstrated [9]. The synthesis level of the *i* protein was found to be comparable to that of subunit *a* or *γ* [9], which is, however, in sharp contrast to the in vivo situation, since the *i* protein has so far not been detected in intact cells or in preparations of the ATP synthase complex. Furthermore, from investigations on translational initiation carried out with the individually subcloned *uncI* gene [10,11] or with *uncI::lacZ* fusions [12,13] it was concluded that the protein is either synthesized on a substoichiometric level in comparison to all other ATP synthase subunits or it is not expressed at all [12].

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Due to the expected very low synthesis level of the *i* protein in *E. coli* cells, in the present communication polyclonal antibodies were used as a tool for detection and localization of the protein.

2. MATERIALS AND METHODS

2.1. *Strains, plasmids, and growth conditions*

Cells of the *E. coli* strains JM109 [14] and CM1470 (*ΔuncI-A*) [7] (a gift of Dr. H.U. Schairer, Heidelberg, Germany) were grown on LB medium [15] or minimal medium [16] containing glucose (0.4%) and thiamine (0.5 μg/ml). In case of CM1470 the minimal medium was supplemented with asparagine (50 μg/ml) and the LB medium with 0.2% glucose. For cells bearing plasmids 50 μg/ml ampicillin were added to the media. The *E. coli* strain KY7485 (provided by Drs. R.H. Fillingame, Madison, USA, and H. Kanazawa, Okayama, Japan) carries the *unc* operon on a prophage and overproduces the ATP synthase complex several-fold after heat induction of the prophage [17]. Cells of this strain were grown on minimal medium [18] supplemented with glucose (1%), thiamine (0.5 μg/ml), arginine (84 μg/ml), and guanine (45 μg/ml) under the conditions described [17].

2.2. *Preparation of antisera and immunoblotting*

Dried portions containing 0.3–0.5 mg of *i* protein, which has been isolated by chloroform/methanol extraction from cells of JM109 bearing plasmid pBS6 [10], were dissolved in 40 μl 10% SDS overnight at room temperature. After the addition of 160 μl water the sample was emulsified with 800 μl Freund adjuvant. Immunization of a rabbit was carried out as in [19] using the same amount of *i* protein for booster injections. The antiserum taken after the third immunization was used in this study. Preadsorption of this serum was performed using everted and right-side-out vesicles of the *E. coli* strain CM1470 (*ΔuncI-A*) as described in [20]. Immunoblotting [19] and detection of antibody binding with a goat anti-(rabbit IgG) alkaline phosphatase conjugate [20] was carried out as described.

3. RESULTS AND DISCUSSION

3.1. *Specificity of the antisera*

Immunoblot analyses of membrane preparations and whole cells showed that the antisera recognized apart

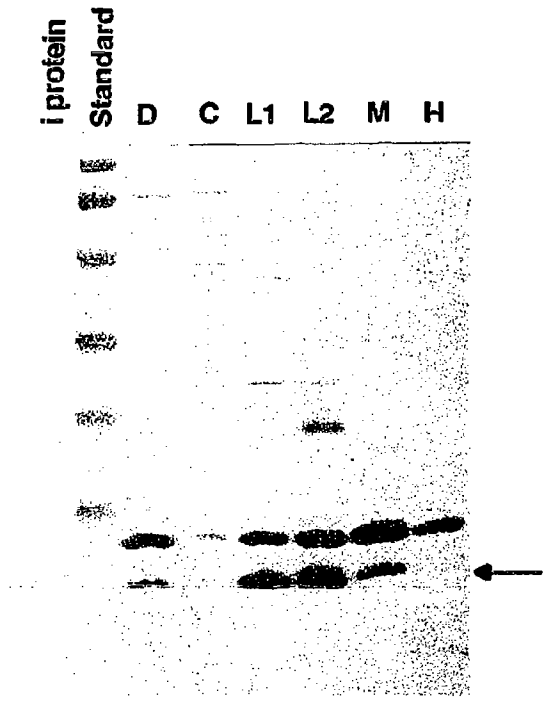

Fig. 1. Detection of the *i* protein in membrane fractions using polyclonal antibodies. Cells of the *E. coli* strain JM109 transformed with plasmid pBS21 were grown on LB medium to late logarithmic phase and a membrane separation [22] was carried out. The resulting membrane fractions were washed in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride and samples (20 μg/lane) were analysed together with prestained protein standard and *i* protein purified from cells of JM109 bearing plasmid pBS81 [10] using SDS-polyacrymalide gels containing 13% acrylamide and 6 M urea [23] followed by immunoblotting. The nitrocellulose sheet was incubated with antiserum against the *i* protein diluted 1:5000. D, cells debris; C, cytoplasmic proteins; L1 and L2, cytoplasmic membrane fraction; M, unseparated membrane fraction; H, outer membrane fraction. The *i* protein is indicated by an arrow. The proteins of the standard exhibit the apparent molecular weights of 106 000, 80 000, 49 500, 32 500, 27 500 and 18 500.

from the *i* protein a range of unidentified proteins (data not shown). In order to reduce the unspecific reactions, a preadsorption using everted and right-side-out vesicles of the *E. coli unc* deletion strain CM1470 (*ΔuncI-A*) was carried out. Most of the antibodies showing cross-reactivity were removed. Remaining unspecific reactions of the antibodies were observed in some cases with proteins exhibiting distinctly different molecular weights than the *i* protein and did therefore not interfere with the present analysis.

3.2. *Detection and localization of the* i *protein*

The expression of the *uncI* gene, the first gene of the *unc* operon of *E. coli*, has so far not been detected in wild-type cells. From the DNA sequence a hydrophobic character was deduced for the *i* protein [5,6], which was confirmed by the fact that this protein can be isolated from cells by chloroform/methanol extraction [10] like the proteolipid of the ATP synthase [21]. This feature

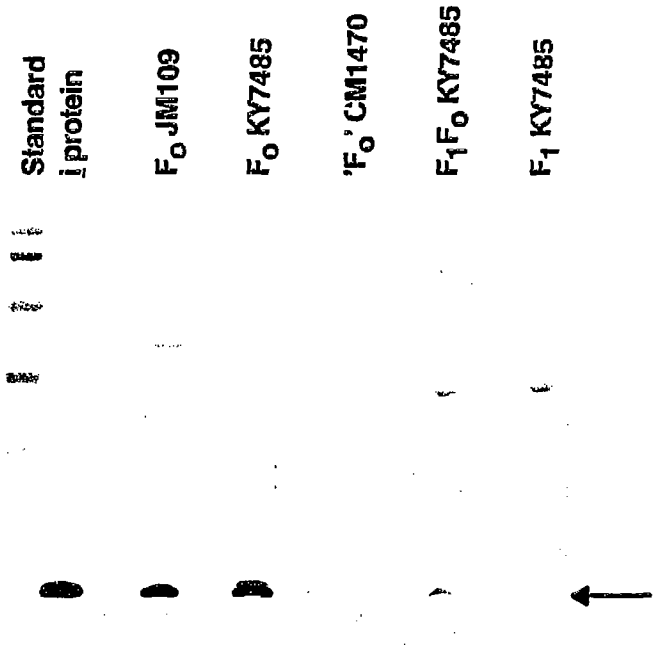

Fig. 2. Detection of the *i* protein in preparations of the ATP synthase complex using anti-*i* antibodies. Preparations of F_o [24], F_1 [25], and F_1F_o [26] were performed as described using cells of the *E. coli* strains JM109 (*unc* wild-type) and KY7485. As a control the purification procedure for the F_o complex was also applied to the *unc* deletion strain CM1470 (*ΔuncI-A*). The samples (20 μg/lane for F_o, 40 μg/lane for F_1F_o, 60 μg/lane for F_1) were analysed as described in the legend of Fig. 1. The *i* protein is indicated by an arrow.

and the tendency of association of the protein with membranes during in vitro protein synthesis [9] led to the assumption that the *i* protein could be part of the cytoplasmic membrane.

In order to clarify this point a membrane separation [22] was performed using cells of the *E. coli* strain JM109 (*unc* wild-type) with or without plasmid pBS21, which causes higher expression of the *i* protein after induction of the corresponding gene at 42°C [10]. An analysis of the different fractions obtained by gel electrophoresis and immunoblotting revealed that the *i* protein was present in fractions containing fragments of the cytoplasmic membrane, whereas no specific reaction was observed with samples of the cytoplasm or the outer membrane (Fig. 1). Thus the *i* protein was clearly identified as a component of the cytoplasmic membrane.

Due to this location an interaction with the membrane-embedded F_o part of the ATP synthase seemed to be conceivable. Analysing preparations of the ATP synthase complex by immunoblotting, it could be demonstrated that the *i* protein can be isolated from membranes together with F_1F_o or the F_o part and that it is not associated with the F_1 moiety (Fig. 2). However, it cannot be distinguished between the *i* protein being a component of the F_o part or being only cosolubilized with it under the same conditions. In this respect it is worthwhile mentioning that the *i* protein could also be solubilized in the absence of F_o subunits applying the

purification protocols of F_o or F_1F_o to cells of the *E. coli unc* deletion strain CM1470 (*ΔuncI-A*) transformed with plasmid pBS21 (data not shown). On the other hand, the presence of the *i* protein in samples of the F_o subunits *a* and *b* purified as described by Schneider and Altendorf [27] and its absence in any preparation of subunit *c* (data not shown) could be a hint for a possible association of the *i* protein with those proteins.

With the detection of the *i* protein in preparations of the cytoplasmic membrane or of the F_o part of cells from JM109 (Fig. 2) the expression of the chromosomal *uncI* gene was demonstrated for the first time. The *i* protein of the *unc* wild-type strain shows the same molecular weight as the predominant one of the two *uncI* gene products detected for F_o samples prepared from strain KY7485, which overproduces the ATP synthase complex several-fold [17]. These results are in accord with those obtained with minicells harboring plasmids which encode the *uncI* gene with its natural translational initiation region, thereby confirming the conclusions drawn with respect to the molecular weight and the N-terminal sequence of the chromosome-encoded protein [10,11]. The relatively weak immunolabeling of the *i* protein in preparations of F_o and F_1F_o compared to a labeling of the F_o subunits (data not shown) lends further support to the notion that this protein is synthesized in substroichiometric amounts [11–13].

Acknowledgements: We thank Eva-Maria Uhlemann for excellent technical assistance and Johanna Petzold for typing the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 171), the Fonds der Chemischen Industrie, and the Studienstiftung des Deutschen Volkes (fellowship to B.S.).

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