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ANTIGENIC SIMILARITY BETWEEN THE β-SUBUNITS OF THE ATPases OF A BACTERIUM, A YEAST AND A HIGHER PLANT

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Key Word Index—*Triticum aestivum*; wheat; *Saccharomyces cerevisiae*; yeast; *Escherichia coli*; bacterium; ATPase; membranes; antigenic homology; chloroplast protein.

Abstract—Antiserum raised against the β-subunit of wheat (*Triticum aestivum*) chloroplast ATPase cross-reacts with a 51 000 protein located in the membrane fraction of *Escherichia coli*. The differential solubility of this polypeptide after chloroform treatment of unc^+ and *uncD*409 strains indicates that this cross-reacting polypeptide is the bacterial β-subunit of ATPase. Thus a high degree of conservation of antigenic determinant sites exists between a bacterial β-subunit and the β-subunit of a monocot. This conservation also seems to extend to the β-subunit of mitochondrial ATPase of yeast (*Saccharomyces cerevisiae*).

INTRODUCTION

The synthesis of ATP in bacteria, chloroplast and mitochondria is driven by a transmembrane proton flux through the membrane-bound adenosine triphosphatase complex (reviewed in refs. [1–3]). The ATPase complex has an intrinsic F_0 portion involved in gating the proton flux and an extrinsic F_1 portion bearing the catalytic site for ATP synthesis or hydrolysis. The extrinsic F_1 portion of both *E. coli* and plant chloroplasts contains five types of subunit (α, β, γ, δ and ε), with different MWs. The suggested active site of the enzyme for ATP synthesis is located on the β-subunit in both chloroplasts [1, 4] and *E. coli* [3]. A comparison of the DNA sequences of the β-subunit genes from *E. coli* [5], spinach [6] and maize [7] reveals that the three proteins have strikingly similar amino acid sequences. This sequence is also shared with the bovine mitochondrial β-subunit [5].

Such a high degree of conserved sequence homology might be expected for a subunit which contains an active site. Consistent with this conclusion is the observation that regions of the β-subunit also have homology with other ATP-binding proteins [3]. In addition, immunological studies have revealed homology between the β-subunits of the dicot plant Swiss chard (*Beta vulgaris*), *E. coli* and *Saccharomyces cerevisiae* mitochondria [8]. In this report we extend these comparisons and demonstrate conservation of antigenic determinant sites in the β-subunit between the other main evolutionary division of angiosperms, namely the monocots, *E. coli* and yeast mitochondria.

RESULTS AND DISCUSSION

When total labelled proteins from *E. coli* strain W3350 were incubated with antiserum against the β-subunit of wheat chloroplasts, a single polypeptide was immunoabsorbed. This polypeptide has a MW of 51 000, which is in close agreement with the size of 50 157 predicted from DNA sequence analysis of the *E. coli* β-gene [5]. To verify further the identification of this polypeptide, the labelled *E. coli* cells were fractionated into washed membrane and soluble protein fractions. The immunologically reacting polypeptide is located primarily in the membrane fraction, although a small amount can be detected in the soluble fraction after a longer exposure of the autoradiograph (data not shown). This distribution is the anticipated result for the β-subunit which is located primarily in the membrane fraction as part of the membrane-bound ATPase complex. A similar result was obtained for the other two *E. coli* strains used.

Additional identification of the immunoabsorbed protein was obtained by making use of an unusual property of a mutant *E. coli* β-subunit. Fayle *et al.* [9] have shown that when the membranes from a strain containing a mutant β-subunit (*uncD*409 allele) are treated with chloroform, the β-subunit is not released. In contrast, the β-subunit of wild-type membranes is released by chloroform treatment. To exploit this information and to help identify the immunoprecipitated membrane proteins, washed membranes were isolated from C600 (unc^+) and AN817 (*uncD*409) and the proteins were treated with antiserum against that β-subunit. It was found that membranes for both strains contain similar amounts of the cross-reacting protein. The membranes were then treated with chloroform and the soluble protein fraction reacted with anti-wheat β-serum. We observed that in the unc^+ strain the immunoprecipitated protein is released by chloroform treatment, but in the *uncD*409 strain the protein is not released (data not shown). Thus the solubility properties after chloroform treatment of the protein immunoabsorbed by anti-wheat β-serum confirms that the *E. coli*

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protein cross-reacting with anti-wheat β-serum is the β-subunit of the membrane-bound ATPase complex. *E. coli* β-subunits therefore share antigenic homology with the β-subunits of wheat, a monocot. This structural conservation must be widely distributed in the plant kingdom because similar homology exists between a dicot and *E. coli* [8].

To extend this comparison of β-ATPase subunits to yeast, labelled proteins of yeast were incubated with anti-wheat β-serum to see if the yeast mitochondrial β-subunit would also cross-react. A single yeast polypeptide was recognized by the anti-wheat β-serum. Although we have not rigorously demonstrated that this cross-reacting protein is the yeast β-ATPase subunit, it is very likely to be so based on the previously demonstrated homology between yeast and Swiss chard β-subunits of ATPase [8].

EXPERIMENTAL

Antiserum raised against the β-subunit of ATPase from wheat (*T. aestivum*) chloroplasts was a generous gift from J. C. Gray. The *E. coli* strains used were W3350 [10], C600 [11] and AN817 (*uncD409*) [12]. The *S. cerevisiae* strain used was MC 16 [13]. For labelling *E. coli* cells with [^{35}S]methionine, the cultures were grown in M9 minimal medium [14] supplemented with 1% (w/v) methionine assay medium (Difco) and 40 μg/ml each of threonine and leucine for C600, and 40 μg/ml arginine and 10 μg/ml uracil for AN817. Cells were grown to $A_{600} = 0.5$, and 10 μCi [^{35}S]methionine was added to 1 ml of culture. After addition of the methionine, the culture was incubated for 1 hr at 37° and the cells were sonicated and then fractionated into washed membranes and soluble protein fractions [15]. The soluble protein fraction was precipitated with 5 vols. cold Me_2CO and the membrane pellet rinsed with 1 ml 80% Me_2CO. After air-drying, the Me_2CO-treated proteins were dissolved by boiling in 100 μl 2% (w/v) SDS and diluted with 1 ml 1% Triton X-100, 0.15 M NaCl, 50 mM Tris–HCl, pH 7.8, and 2 mM EDTA. Incubation with antiserum, absorption to protein A Sepharose, gel electrophoresis and fluorography were carried out as described elsewhere [16, 17]. Gels were calibrated with ^{14}C-methylated protein markers containing lysozyme (14000), carbonic anhydrase (30000), ovalbumin (46000), bovine serum albumin (69000), phosphorylase b (92000) and myosin (200000). For $CHCl_3$ solubilization of β-subunits, the method of Cox *et al.* [18] was adopted. Membrane pellets that had been washed in 10 mM Tris–H_2SO_4 (pH 7.5), 1 mM EDTA and 6 mM *p*-aminobenzamidine were resuspended in 200 μl of the same buffer, 100 μl $CHCl_3$ was added and the mixture was vortex-mixed for 30 sec. Membranes from the aq. phase were collected by centrifugation [15], and after Me_2CO treatment of supernatant and membranes as described above, they were processed for incubation with antiserum.

Yeast cells were grown in 0.67% yeast nitrogen base, 2% glucose and 20 μg/ml adenine, histidine and lysine and labelled with 10 μCi [^{35}S]methionine for 60 min. The cells were converted into protoplasts with zymolyase, and the protein was isolated and immunoprecipitated as described above for labelled *E. coli* cells.

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