DNA-dependent ATPases in Bacillus subtilis Mutants and in Competent Cells

By G. MAZZA,1* A. SIDOLI1 AND S. RIVA2

1Dipartimento di Genetica e Microbiologia, Università di Pavia, Italy
2Istituto di Genetica Biochimica ed Evoluzionistica del Consiglio Nazionale delle Ricerche, Pavia, Italy

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Three DNA-dependent ATPases (γ phosphohydrolases) can be isolated from Bacillus subtilis cells. We studied these enzymes in a number of mutants deficient in recombination or repair functions (rec, uvr) and in competent cells. The recA mutant studied had lower ATPase II activity, while competent cells had higher ATPase I activity, in comparison with the parental strain not brought to competence.

INTRODUCTION

DNA-dependent ATPases have been isolated and studied both in bacteria and in eukaryotic cells (Abdel-Monem & Hoffman-Berling, 1980; Falaschi et al., 1980; Riva & Plevani, 1980). In many cases a role for these enzymes in DNA metabolism has been demonstrated; in general, they seem to intervene in processes where mechanical work is required, i.e. duplex denaturation (Abdel-Monem et al., 1977; Abdel-Monem & Hoffman-Berling, 1980), DNA polymerase or replicative apparatus progression (Kolodner & Richardson, 1978; Kornberg, 1980; Piperno & Alberts, 1978), strand displacement and strand transfer (D-loop formation) during recombination (Cunningham et al., 1980; Radding, 1981; Shibata et al., 1981; Weinstock et al., 1979) and generation of supercoiled structures (Geider & Hoffman-Berling, 1981; Gellert, 1981; Kornberg, 1980).

Mazza & Riva (1981a, b, 1982) described the partial purification of three DNA-dependent ATPases from Bacillus subtilis. Experiments aimed at demonstrating in vitro some of the activities mentioned above have so far been unsuccessful. We thought that more information on these enzymes might be obtained by a genetical approach: by studying the ATPases in competent cells and in rec and uvr mutants, that is in systems where the recombinative functions are altered (Dubnau, 1982; Kawamura & Saito, 1982). The array of such mutants in B. subtilis (Mazza & Galizzi, 1978) is as diverse as in Escherichia coli, while little is known of the underlying biochemistry. The recE gene of B. subtilis probably codes for a 'recombination protein' of 45000 daltons and recE mutations can be complemented by the recA gene from E. coli (de Vos & Venema, 1983; de Vos et al., 1983).

METHODS

Bacterial strains, media and growth conditions. The isogenic strains of B. subtilis used are shown in Table 1. The complete genotypes of these strains, deficient in different repair and recombination functions, were described by Mazza (1982). The properties of rec and uvr mutations have been reviewed by Mazza & Galizzi (1978).

Cultures were grown in a fermenter at 37 °C in Difco antibiotic medium no. 3. Bacteria were collected by centrifugation and frozen in liquid nitrogen. Competent cells of strain PB1652 were prepared essentially as described by Stewart (1969).

ATPase assay. The standard assay was performed in 25 μl incubation mixtures containing 50 mM-potassium phosphate pH 6.2 mM-MgCl2, 1 mM-DTT, 5 mM-Na2P2O7, 5 μg bovine serum albumin, 2.7 μM bacteriophage fd single-stranded DNA, 0.338 μM-[8-3H]adenosine 5'-triphosphate (specific activity 4.3 × 107 c.p.m. μmol-1) and 0.4-4 units of enzyme. After 30 min incubation at 37 °C the reaction was stopped by chilling in ice and adding...
EDTA (10 mM final concentration). Samples of 2 μl were spotted on poly(ethyleneimine) cellulose strips (0.6 × 6 cm) and covered with 1 μl of a mixture of ATP, ADP and AMP (10 mg ml⁻¹ each). The chromatograms were developed in 1 M-formic acid/0.25 M-LiCl solution at room temperature for 15-20 min. The ADP spot (which determined. One unit of ATPase activity is defined as the amount of enzyme that hydrolyses 1 pmol ATP in 20 min under the assay conditions described above.

**ATPase purification procedure.** A sample of bacterial paste (5 g) was suspended in 20 ml cold lysis buffer (Tris/HCl pH 7.5 50 mM, MgCl₂ 10 mM, NaCl 100 mM, DTT 1 mM, glycerol 3% (w/v), sucrose 10% (w/v) containing protease inhibitors: phenylmethylsulphonylfluoride (Boehringer) 1 mM, Na₂S₂O₃ 10 mM, Pepstatin (Sigma) 1 μg ml⁻¹). After addition of 15 mg lysozyme, the suspension was kept on ice for 45 min with gentle agitation and then transferred to 37°C for a few minutes until lysis occurred, as indicated by a large increase in viscosity. The lysate was transferred to ice again and rendered 2°C. The lysate was centrifuged for 30 min at 10,000 g and the pellet discarded. PEG 6000 (10%, w/v) was added and after 2 h at 0°C the suspension was centrifuged for 30 min at 15,000 g at 0°C and the pellet discarded. The supernatant was then dialysed against two changes of 10 vol. 0.35 M-potassium phosphate (pH 7.5), 0.1 mM-EDTA, 0.5 mM-EGTA, 0.5 mM-DTT, 5% (v/v) glycerol. The dialysed suspension was filtered through a DEAE-cellulose column (20 ml) equilibrated with the dialysis buffer. The filtrate (containing DNA-dependent ATPase activity) was dialysed against 20 mM-potassium phosphate pH 7.5. Fifty fractions were collected and assayed for DNA-dependent ATPase activity. Three peaks of activity, ATPases I, II and III, were detected, in the order of elution.

**RESULTS AND DISCUSSION**

Three DNA-dependent ATPases of *B. subtilis* were eluted from a phosphocellulose column with a potassium phosphate gradient (Mazza & Riva, 1981a, b; 1982; Fig. 1a). The enzymes (ATPases I, II and III) have been further purified and their properties determined (Mazza & Riva, 1981a). They have rather high molecular weights (108,000, 115,000 and 148,000, respectively) but their subunit composition is not yet unambiguously established. We have been so far unable to demonstrate any functions for these enzymes (besides ATP hydrolysis), although we have some evidence that ATPase II might slide on single-stranded DNA during ATP hydrolysis (Mazza & Riva, 1981a, b).

ATPases I and III represent about 20% each of the total activity, while ATPase II accounts for the remaining 60% (Fig. 1a). The elution profile of the DNA-dependent ATPases is characteristic and reproducible from one experiment to another, in terms both of molarity of elution and of ATPase activity in the peaks. We therefore screened isogenic rec and uur mutants for the presence and the relative abundance of the three enzymes. Similar experiments were also done on competent cells of strain PB1652 brought to maximum competence (3% frequency of transformation to Trp⁺ at saturating DNA concentration) and used without any enrichment. The activities of the three enzymes in the peak fractions are reported in Table 1. The enzyme activities in most of the rec and uur mutants were very close to that of the parental strain, except for the case of recA where a five- to sixfold decrease in ATPase II activity was observed. This was confirmed in two separate experiments.

In the case of competent cells, a significant (about threefold) increase in ATPase I activity was observed, which was confirmed in two different experiments. Such an increase is certainly meaningful since the fraction of competent cells in a non-enriched competent culture is at most 20% (Dubnau, 1982) and the elution profile from phosphocellulose does not depend on the culture medium or the state of growth (Mazza & Riva, 1968b). Fig. 1(c) shows the elution profile of ATPases from competent cells. We have not been able to show induction of *B. subtilis* ATPases by a DNA-damaging treatment.

The enhancement of ATPase I observed in competent cells is interesting in view of the presence in these cells of specific proteins (Eisenstadt et al., 1975; Joenje & Venema, 1975) and of the reported induction of an 'SOS' repair system (Yasbin, 1977a, b) and of enzymes involved in DNA methylation and in homologous (recA-like) recombination (Ganesan, 1979). A 45000
Fig. 1. Elution profile of the three DNA-dependent ATPase activities from phosphocellulose columns. Sources: (a) parental strain PB1652; (b) PB1795 (recA1); (c) unfractionated competent cells of PB1652. ●, ATPase activity; ——, potassium phosphate concn.

**Table 1. DNA-dependent ATPase activity in mutants of Bacillus subtilis**

The ATPase activity at the peaks of elution from phosphocellulose columns (see Fig. 1) is shown.

<table>
<thead>
<tr>
<th>Bacterial strain and relevant marker</th>
<th>ATPase activity (units ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATPase I</td>
</tr>
<tr>
<td>PB1652 (parental)</td>
<td>1.2</td>
</tr>
<tr>
<td>PB1795 (recA1) Exp. I</td>
<td>1.1</td>
</tr>
<tr>
<td>Exp. II</td>
<td>1.1</td>
</tr>
<tr>
<td>PB1796 (recB2)</td>
<td>0.8</td>
</tr>
<tr>
<td>PB1797 (recD41)</td>
<td>1.9</td>
</tr>
<tr>
<td>PB1791 (recE4)</td>
<td>0.9</td>
</tr>
<tr>
<td>PB1792 (recE45)</td>
<td>1.5</td>
</tr>
<tr>
<td>PB1798 (recF33)</td>
<td>1</td>
</tr>
<tr>
<td>PB1799 (recG40)</td>
<td>0.8</td>
</tr>
<tr>
<td>PB1808 (recM13)</td>
<td>1.9</td>
</tr>
<tr>
<td>PB1802 (uvrA1)</td>
<td>1.6</td>
</tr>
<tr>
<td>PB1652 (competent cells) Exp. I</td>
<td>3.8</td>
</tr>
<tr>
<td>Exp. II</td>
<td>2.7</td>
</tr>
</tbody>
</table>

dalton ‘recombination’ protein is induced in competent cells (de Vos & Venema, 1982); however, its role has still to be fully elucidated. This protein is virtually absent from strains carrying the recE4, recA1 and recA73 mutations, and a model for the regulation of the recE protein which postulates a repressor role for the recA gene product has been proposed (de Vos &
Venema, 1983). ATPase I is present at normal levels in both recE and recA mutants (Table 1) and therefore has probably no relationship with the 40 000 dalton 'recombination protein'.

Our results with the recA mutant are also very intriguing. This mutant is defective in recombination and repair (Mazza & Galizzi, 1978) and in the induction of the 'SOS' repair system (Yasbin, 1977a, b); also, the recA gene product may regulate the Venema, 1983). If the recA gene product is a regulatory protein, the observed drop in ATPase activity might reflect an altered regulation. Our observations suggest a role (probably indirect) of ATPase II in recombination and repair.

Although the involvement of ATPases I and II in recombination and repair still remains to be demonstrated in vivo, our results indicate that many other enzymes (besides the 45 000 dalton 'recombination protein') are involved in these processes.

REFERENCES


