Protein Synthesis during Recovery of Tellurite Resistance by Heat-injured *Streptococcus faecalis*

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INTRODUCTION

Payne & Morley (1976) have shown that cells of *Streptococcus faecalis* heated at 60 °C rapidly lose resistance to potassium tellurite. Recovery of tellurite resistance occurred on subsequent incubation in heart infusion broth at 33 °C and was dependent on protein synthesis. Several organisms, including *Salmonella typhimurium*, *Staphylococcus aureus* S6 and *Vibrio parahaemolyticus*, require protein synthesis to recover from sub-lethal heating (Tomlins & Ordal, 1971; Hurst *et al.*, 1973; Emswiler *et al.*, 1976; Gomez *et al.*, 1976). However, little information is available on the extent of such protein synthesis and whether this involves many or only a few specific proteins.

Recently it has been shown that heat-injured *S. faecalis* can recover tellurite resistance in a semi-defined medium that does not support growth (Payne, 1978). This recovery system has now been used to investigate further the extent and range of proteins synthesized during recovery of tellurite resistance by heat-injured *S. faecalis*.

METHODS

Maintenance and culture of organisms. Cultures of *Streptococcus faecalis* var. *liquefaciens* EB/F/30/39 were maintained and grown as previously described (Payne & Morley, 1976). For isotope dilution experiments the cells were grown in medium containing L-[U-¹⁴C]valine at 0.02 µCi ml⁻¹. This amino acid was used to ensure that ¹⁴C was only incorporated into protein.

Heat-injury and recovery procedure. These procedures were as previously described when cells were heated for 4 min at 60 °C at densities of about 1 × 10⁹ viable units ml⁻¹ (Payne, 1978), except that cells were washed with 100 mM-K₂HPO₄/KH₂PO₄ buffer, pH 7.1. Washed cell pellets of unheated, heated, and heated cells incubated in recovery medium were stored at -20 °C prior to extraction and assay.

In experiments to follow the incorporation of ¹⁴C-labelled amino acids into protein synthesized during recovery from heat-injury, L-[U-¹⁴C]aspartate was used since preliminary experiments had shown that at least 60% of the aspartic acid content of the recovery medium was utilized during the recovery period. Although valine utilization was not so great, L-[U-¹⁴C]valine was used in confirmation experiments. Radioactive amino acid was added to the recovery medium at a final concentration of 0.02 µCi ml⁻¹.

In some experiments chloramphenicol, at a final concentration of 100 µg ml⁻¹, was added to the recovery medium to inhibit protein synthesis.

Viable counts. These were carried out as previously described (Payne & Morley, 1976).

Extraction and estimation of total cell protein. Protein was extracted from washed cells using a modification of the method of Schneider (1945). Cells were first extracted with 5 ml 5% (w/v) trichloroacetic acid (TCA) for 15 min at 0 to 2 °C, centrifuged and the supernatant was discarded. The pellet was further extracted with 5 ml 5% (w/v) TCA for 15 min at 90 °C, centrifuged and the supernatant was discarded. Protein in the pellet material was solubilized by heating for 5 min at 100 °C with 3 ml 1 M-NaOH. Duplicate 0.1 ml samples of the protein solution were removed for determination of radioactivity and the remainder was assayed for protein by a modification of the biuret method (Herbert *et al.*, 1971).

Preparation of cell-free extracts. Washed cell pellets were resuspended in 2.5 ml 100 mM-K₂HPO₄/KH₂PO₄ buffer, pH 7.1, with 3 g glass beads (0.11 mm diam.). This suspension was shaken on a Mickle disintegrator (Mickle Laboratory Engineering Co., Guildford), tuned to maximum amplitude, for 15 min at 1 °C, followed

by low-speed centrifugation (2000 *g*, 10 min, 2 °C) to remove the glass beads and then high-speed centrifugation (25000 *g*, 10 min, 2 °C) to remove cell debris. The clear supernatant was dialysed against 100 mM-K₂HPO₄/KH₂PO₄ buffer, pH 7.1, for 16 h at 1 °C and used immediately for polyacrylamide gel electrophoresis. Protein was assayed by the method of Lowry *et al.* (1951).

Polyacrylamide gel electrophoresis. Disc gel electrophoresis was based on the method of Davis & Ornstein (1961) at pH 8.5 with 7.5 % (w/v) acrylamide. Volumes of cell-free extract (containing 200 µg protein) were applied to gels with 0.05 ml bromophenol blue (0.001 %, w/v) as marker. Electrophoresis was carried out at 1 °C with a current of 5 mA per tube until the marker had travelled the length of the gel. Gels were stained with 1 % (w/v) amido black in 7 % (v/v) acetic acid and destained by washing repeatedly with 7 % acetic acid. Densitometer profiles of the stained gels were recorded with a Chromoscan (Joyce-Loebl, Gateshead, Tyne & Wear). The gels containing radioactive protein were subsequently sliced into 1 mm discs using a Mickel gel slicer and the discs were dried and prepared for radioactive counting by the method of Goodman & Matzura (1971).

Measurement of radioactivity. Radioactive protein, in NaOH solution (0.1 ml) or in dissolved gel slices, was counted in polypropylene counting vials (Packard) after adding 10 ml scintillator [toluene/Triton X-100/water (6:3:1, by vol.) containing 0.6 % (w/v) 2,5-diphenyloxazole]. Samples were allowed to stabilize in the dark at 17 °C for at least 4 h prior to counting radioactivity in a Philips model PW4510 liquid scintillation spectrometer. Counting efficiency was determined by a channels ratio technique calibrated with standard [¹⁴C]hexadecane, taking into account any quenching of the samples, and was about 70 % and 50 % for ¹⁴C in protein in NaOH solution and dissolved gel slices, respectively. The observed count was corrected for background and efficiency and expressed as d.p.m.

The proportion of cellular protein synthesized during recovery was calculated from the extent of isotope dilution using the formula:

$$\text{Protein synthesized during recovery as a percentage of the total cell protein at the end of recovery} \\ = 100 - \frac{(\text{specific activity of protein from recovered cells})}{(\text{specific activity of protein from heated cells})} \times 100$$

RESULTS AND DISCUSSION

Effect of chloramphenicol on recovery

When heat-injured cells of *S. faecalis*, which had lost resistance to potassium tellurite, were incubated for 6 h in the standard recovery medium that did not permit growth, at least 70 % of injured cells recovered (Payne, 1978). When chloramphenicol was added to this medium less than 20 % of the cells recovered. Examination of polyacrylamide gels did not reveal any differences between proteins extracted from heat-injured cells incubated in recovery medium with or without chloramphenicol.

Isotope dilution experiments showed that in the presence of chloramphenicol the amount of new protein synthesis (Table 1, expts 2, 5 and 6) was not significant within the limitations of the method. However, this does not preclude the suggestion that in the presence of the antibiotic there might have been a small amount of protein synthesis which was sufficient to account for the recovery of tellurite resistance by a small proportion of the heat-injured cells.

Extent of protein synthesis during recovery

In isotope dilution experiments the specific radioactivities of the protein fraction from cells grown in the presence of L-[U-¹⁴C]valine were similar for both heated and unheated cells (Table 1, expts 3, 4 and 5). Protein extracted from heated cells incubated in recovery medium for 6 h had a lower specific radioactivity which corresponded to about 40 % of the cell protein being synthesized during recovery. Unheated cells incubated under similar conditions appeared to have synthesized at least 50 % new protein (Table 1, expts 1 and 2).

Since the heat-injured cells were recovered in a medium that did not support growth and were therefore not dividing, experiments were carried out to determine if the protein synthesis resulted in an increase in cellular protein. Comparison of the cellular protein content, on a dry wt or total cell number basis, showed no net increase during the 6 h recovery

Table 1. *Isotope dilution of radioactive protein during recovery of tellurite resistance by heat-injured S. faecalis*

Cells were grown in heart infusion broth containing L-[U-¹⁴C]valine, heated for 4 min at 60 °C and incubated in semi-defined recovery medium at 33 °C for 6 h. Total protein was extracted from samples of unheated, heated and recovered cells and the specific radioactivity was determined.

Specific radioactivity of protein $\pm$ s.e. (d.p.m. mg ⁻¹)						
Expt	Unheated cells	Unheated cells incubated in recovery medium	Unheated cells incubated in recovery medium + chloramphenicol	Heated cells	Heated cells incubated in recovery medium	Heated cells incubated in recovery medium + chloramphenicol
1	26860 $\pm$ 800	12212 $\pm$ 508	—	—	—	—
2	21129 $\pm$ 369	4954 $\pm$ 173	20333 $\pm$ 648	—	—	—
3	27175 $\pm$ 762	—	—	27765 $\pm$ 802	18252 $\pm$ 773	—
4	29030 $\pm$ 943	—	—	26016 $\pm$ 899	14663 $\pm$ 543	—
5	21880 $\pm$ 325	—	—	22912 $\pm$ 418	11003 $\pm$ 315	21952 $\pm$ 734
6	—	—	—	17128 $\pm$ 477	10407 $\pm$ 351	15650 $\pm$ 481

period [0.40 mg protein (mg dry wt)⁻¹ and 0.34 mg protein (mg dry wt)⁻¹ for heated and recovered cells, respectively] indicating that protein synthesis was balanced by at least an equivalent amount of degradation, and therefore represented turnover. With non-growing cultures of *Escherichia coli*, Mandelstam (1958) estimated a cellular protein turnover rate of 5% h⁻¹, while Pine (1965) concluded that the value was 20% h⁻¹. Our data for *S. faecalis* indicate turnover rates of about 6% h⁻¹ and 12% h⁻¹ for heated and unheated cells, respectively.

Incorporation of ¹⁴C into protein during recovery

Polyacrylamide gel electrophoresis of the soluble proteins extracted from heat-injured cells after incubation in recovery medium containing L-[U-¹⁴C]aspartate showed that the recovery of tellurite resistance was accompanied by the incorporation of ¹⁴C into all the soluble protein bands. The profiles were similar to those obtained when unheated cells were incubated under similar conditions. Recovery experiments in which L-[U-¹⁴C]valine replaced L-[U-¹⁴C]aspartate gave essentially similar results.

These results suggest that there is no protein synthesis specific for recovery and further investigation is required to discover which of the many proteins synthesized are involved.

The protein synthesis occurring in heated cells or unheated cells incubated in recovery medium may not be required for recovery *per se* but to maintain an active system for detoxifying tellurite in unheated cells or heated cells that have recovered. However, this would not appear to be the case since, when protein synthesis is prevented by the addition of chloramphenicol or by omitting amino acids from the medium, tellurite resistance and viability of unheated cells are not affected.

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