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DSC ANALYSIS OF FOODBORNE BACTERIA

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Differential scanning calorimetry (DSC) is applicable to studying the thermal properties of bacteria when treated with heat, cold, or antibiotics. Foodborne pathogens are inactivated by heat, and denaturation transitions observed by DSC indicate potential sites of cellular injury. Ribosomes, which are the sites for messenger RNA translation, are one critical component of thermal damage as evidenced by characteristic denaturation transitions in the 66–74°C range. These transitions disappear when cells of *Clostridium perfringens* are subjected to heat, suggesting structural or conformational changes to ribosomal proteins, and when cells of *Listeria monocytogenes* are cold-shocked by refrigeration, indicating ribosomal dissociation. DSC can be used to show that refrigeration followed by heat treatment improves the killing of dangerous microorganisms.

Keywords: bacteria, Clostridium, differential scanning calorimetry, Listeria, ribosomes

Introduction

Bacterial pathogens are responsible for over 4 100 000 cases of foodborne illness in the U.S. each year [1]. Almost 250 000 of these cases result from contamination by *Clostridium perfringens* [1], a spore-forming anaerobe which may initiate spore production in response to acidic conditions in the gastrointestinal tract [2]. Illness due to *Listeria monocytogenes* is much less prevalent but far more serious, leading to 500 deaths annually [1]. These and other foodborne pathogens can be inactivated by heat or antibiotics, which alter the efficacy of protein synthesis in ribosomes. Ribosomes, which are organelles found in the cytoplasm of all cells, assemble amino acids into proteins by using the directions supplied by messenger RNA molecules [3]. In bacteria, ribosomes consist of a small subunit, 30S, and a large subunit about twice the size, 50S; these fit together to form the 70S ribosome (Fig. 1). An *E. coli* cell contains about 15 000 ribosomes, each with a molecular mass of $2.7 \cdot 10^6$ daltons, accounting for one-fourth of the total mass of the cell [4]. Stressing microorganisms at relatively high or low temperatures, known as heat-shocking or cold-shocking, decreases their thermal tolerance by impairing the operation of the 30S subunit [5]. The decrease in thermal tolerance can be quantitated by determining the microorganism's D_{60} value, which is the length of time required for the viable population to decrease tenfold at 60°C.

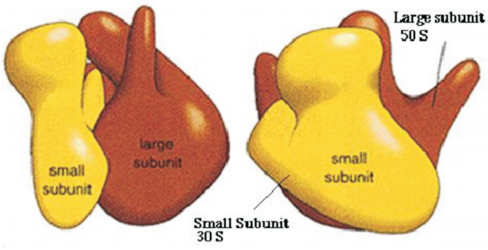

Fig. 1 Subunits of bacterial ribosomes [3]; reprinted with permission

DSC has been used by many investigators to obtain thermal properties of proteins, such as bovine serum albumin [6], and 7S (β-conglycinin) and 11S (glycinin) in soy [7]. Usually, proteins are irreversibly denatured when heated, producing an endothermal effect which disappears upon reheating. Ribosomal proteins, which comprise 35–40% of the mass of the ribosome, can be analyzed by DSC if the sample is sufficiently concentrated. As part of our Center's investigations into precooking and refrigerating food to assure its safety, two separate projects were conducted to examine cold- and heat-shocked reductions in thermal tolerance of ribosomes in *C. perfringens* and *L. monocytogenes*. In addition, *L. monocytogenes* cells were exposed to several antibiotics that bind to ribosomes in order to mimic cold-shock responses. The purpose of the present study was to determine the usefulness of DSC in examining changes in temperatures of endothermal effects of ribosomal proteins using different microorganisms and experimental conditions.

Experimental

Enterotoxin-producing strains of *C. perfringens* were grown in fluid thyoglycolate bacteriological medium.

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Ribosomes from *C. perfringens* were isolated using the procedure of Novak *et al.* [2]. Harvested vegetative cells were concentrated by centrifugation and resuspended in buffer consisting of 25 mM Tris (pH 7.5), 1 mM EDTA (pH 7.5), 5 mM β-mercaptoethanol, 6 mM $MgCl_2$, and 30 mM NH_4Cl. Cells were broken in a French pressure cell at 82.7 MPa and DNase was added. Further centrifugation at 32500× *g* produced pellets of crude ribosomes.

Whole cells of *L. monocytogenes* were concentrated by centrifugation and resuspended in buffer consisting of 10 mM Tris (pH 7.5), 6 mM $MgCl_2$, and 30 mM NH_4Cl using the procedure of Bayles *et al*. [8]. Investigations of antibiotic-treated cultures were performed after exposing cells to antibiotics for 30 min at 37°C and then centrifuging and resuspending in buffer. Antibiotics used were chloramphenicol, erythromycin, kanamycin, puromycin, rifampin, streptomycin, and tetracycline (Sigma Chemical Co., St. Louis, MO). Cold shocking was performed by incubating at the specified temperature for 3 h.

C. perfringens cells and ribosomes were analyzed using a Perkin-Elmer DSC-7 equipped with an Intercooler cooling accessory, and DSC of *L. monocytogenes* cells was performed in a Perkin-Elmer Pyris I with a liquid nitrogen cooler (Perkin-Elmer Corp., Norwalk, CT). Samples weighing approximately 12–20 mg were hermetically sealed in volatile sample pans, and the appropriate Tris buffer was used as a reference. After placing the pan in the instrument, *C. perfringens* samples were cooled to 10°C and *L. monocytogenes* samples were cooled to 0°C. After 2 min, samples were scanned to 100°C at 10°C min^{-1}, and the baseline obtained from scanning the sample a second time was subtracted, producing the final curve. At least three replicate analyses of each sample were performed. The instruments' software was used to calculated peak temperatures; areas were not calculated. Helium was used as the flow gas in both instruments, which were regularly calibrated with an indium standard.

Thermal tolerance studies and determination of D_{60} values were conducted by dilution, submerged-coil heating, plating, and enumeration as previously described by Bayles *et al*. [8].

Results and discussion

Clostridium perfringens

The endothermal effect of ribosomes isolated from *C. perfringens* vegetative cells is shown in Fig. 2a. There was an endothermal effect with a peak around 72°C, which corresponded to the 50S subunit and 70S particle [9]. A shoulder at 66–67°C was due to the 30S subunit [10]. The peak and shoulder disappeared

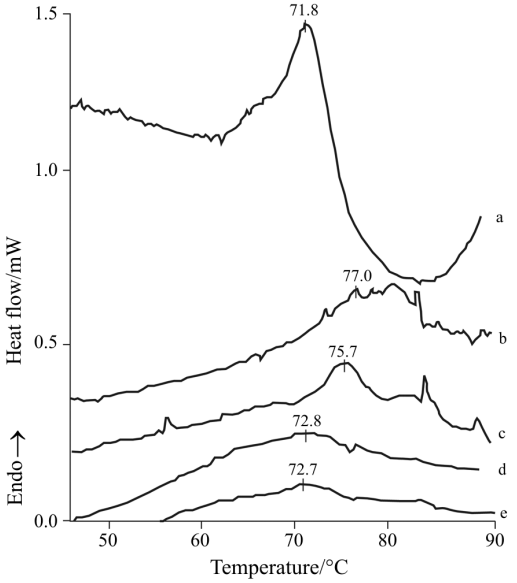

Fig. 2 DSC of *C. perfringens* vegetative cells; a – isolated ribosomal proteins; b – whole cells treated at 46°C for 60 min; c – whole cells treated at 28°C for 60 min; d – b followed by storage at 4°C for several days; e – c followed by storage at 4°C for several days

with a subsequent scan without a change in baseline, proving that the ribosomal proteins were denatured by heat. The denaturation peaks of whole cells kept at 46°C (heat-shocked) and 28°C (control) were several degrees higher (Figs 2b and c). The heat-shocked sample exhibited an increased resistance to heat, indicating that the structure or conformation of the protein was altered at elevated temperatures [2]. Additional endothermal effects were observed around 81–85°C, which have been attributed to bacterial DNA [9]. Storage at 4°C for several days, mimicking refrigeration in a supermarket or by a consumer, caused the peaks of both the heat-shocked and control samples to flatten and shift to lower temperatures (Figs 2d and e). The increased heat resistance of the heat-shocked cells was lost, supporting the theory that this resistance is transient [11]. Heat is uniformly distributed in a cell, resulting in damage to the most sensitive molecules within it. Cells may then die or recover depending on the degree of injury [12]. The results suggest that conformational changes in ribosomal proteins in response to temperature differences alter protein synthesis in *C. perfringens*, and that refrigeration will destroy this organism in food. These conformational changes, which may involve changing the shape and structure of the protein, are readily discerned by evaluation of DSC scans.

Listeria monocytogenes

The DSC curve of *L. monocytogenes* cells (Fig. 3a) exhibited melting transitions at 67.5±0.4°C, corre-

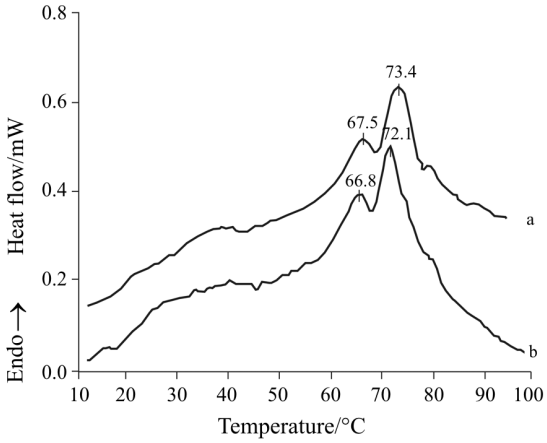

Fig. 3 DSC of *L. monocytogenes* cells; a – control grown at 37°C; b – grown at 37°C and cold shocked at 0°C for 3 h

sponding to thermal denaturation of the 30S subunit, and at 73.4±0.1°C, corresponding to the combined 50S subunit and 70S particle [8]. Cold shocking the cells 0°C for 3 h caused a shift in the 50S/70S peak denaturation temperature to 72.1±0.5°C (Fig. 3b). The position of the 30S peak did not shift significantly. Similar results were observed with a cold shock to 5°C. Peak shoulders observed around 81°C were due to bacterial DNA [9], as with the *Clostridium* samples. The results indicate that intracellular changes in the ribosomes, such as an alteration in the association status of the 70S particles, are correlated with changes in the thermal properties of *L. monocytogenes*. The 30S and 50S subunits are more thermally labile than the associated 70S particle, so any change that causes dissociation of 70S would make the ribosome more sensitive to heat [5].

Antibiotics inhibit protein synthesis by selectively targeting bacterial 70S ribosomes while leaving eukaryotic ribosomes unaffected [4]. The effects of seven antibiotics, six active against the ribosome and one (rifampin) active against RNA polymerase, were tested on the cells to determine if the antibiotic treatment produced alterations in peak denaturation temperatures corresponding to ribosomes or their subunits. Figure 4a is the DSC curve of a control similar to that in Fig. 3a, and Fig. 4b is the curve of cells treated with kanamycin. The 50S/70S peak shifted from 73.4±0.1 to 72.1±0.7°C, a shift which was similar to the temperature reduction in cells that had been cold shocked (Fig. 3). Treatment with tetracycline removed the 30S transition that had been observed around 67°C (Fig. 4c). Thus, DSC analysis showed evidence of structural changes in the ribosomal protein. Treatment with chloramphenicol, erythromycin, puromycin, rifampin, or streptomycin produced results that were similar to those of the control.

Cells were also cold shocked from 37 to 0°C for 3 h and then thermally challenged at 60°C to determine thermal tolerance. Previous research revealed that the D_{60} value of *L. monocytogenes* is 75.6 s [13]. Kanamycin and tetracycline, which measurably altered the DSC curves of *L. monocytogenes* cells, were the antibiotics that caused reductions in thermal tolerance; chloramphenicol, erythromycin, puromycin, rifampin, and streptomycin did not alter the D_{60} values. Compared with the controls, kanamycin and tetracycline each reduced the D_{60} value by 20 s. These 26% reductions were approximately the same as those observed following cold shocks of 37 to 0°C [13] and 37 to 5°C. The antibiotic treatment data indicate that ribosomal changes have a significant impact on the thermal resistance of *L. monocytogenes*. Cold shock and certain antibiotics alter the state and modify the structure of ribosomes, as reflected by changes in the DSC curves. The results are probably due to disassociation of the 30S subunits, which are more thermally labile and more effectively denatured by heat.

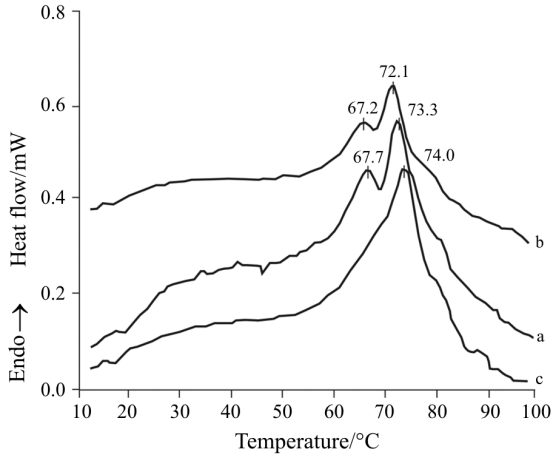

Fig. 4 DSC of *L. monocytogenes* cells treated with antibiotic; a – control; b – kanamycin-treated cells; c – tetracycline-treated cells

Conclusions

DSC is a valuable tool for examining the thermal properties of foodborne bacteria, in particular the damage to ribosomal proteins due to heat, cold, or antibiotic treatment. Protein synthesis in *C. perfringens* and *L. monocytogenes* ribosomes is more efficiently destroyed during heating when conformational changes and disassociation of the 30S subunits are induced by temperature shocks, as evidenced by DSC scans.

Acknowledgements

Mention of brand or firm name does not imply endorsement by USDA over others of a similar nature not mentioned.

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