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# Hydrostatic Pressure Effects on *Escherichia coli*: Site of Inhibition of Protein Synthesis

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Utilizing whole-cell preparations of *Escherichia coli*, it appears that 670 atm inhibits protein synthesis during elongation, while not affecting aminoacyl transfer ribonucleic acid formation, polysomal integrity, or amino acid permeability.

It has been shown that the translational phase of protein synthesis in *Escherichia coli* is totally inhibited at 670 atm of pressure in whole-cell preparations (1, 7-9, 12, 14). It has not been shown at which step or steps this inhibitory effect occurs. Although Arnold and Albright (2) have reported pressure inhibition of cell-free aminoacyl transfer ribonucleic acid (tRNA) binding to ribosomes and a reduced stability of the messenger ribonucleic acid (mRNA)-ribosome complex, it has been pointed out that results of experiments on whole-cell and those on cell-free extracts may not be comparable (2, 9, 11, 15). The data on whole-cell preparations presented here suggest that the inhibitory effect of pressure is on the process of translocation, possibly in conjunction with the tRNA binding or termination steps.

Cellular permeability to amino acids while under pressure was investigated first. Log-phase cultures of *E. coli* Hfr C were subjected to 670 atm of pressure, and then $^{14}$C-amino acids were added while the culture was under pressure (6). Control cultures were treated identically but were kept at atmospheric pressure. Five minutes after the application of pressure, both pressure and control samples were treated as described in Table 1. The results (Table 1) indicate that, even though incorporation into protein in the 670-atm sample is negligible compared to that of the atmospheric control, its acid-soluble radioactivity present as $^{14}$C-amino acids, $^{14}$C-leucine, or $^{14}$C-phenylalanine is equal to or greater than the atmospheric control. Thus it appears that 670 atm does not lower the level of these amino acids present in the acid-soluble material of the cell, even though their incorporation into protein is effectively stopped.

Since amino acids are internally available, the next step at which a pressure inhibition might occur may be during the formation of aminoacyl tRNA. To investigate this possibility, commercially available $^{14}$C-leucyl tRNA was centrifuged on a 5 to 20% sucrose gradient and was found to peak at the same fraction as tRNA extracted from log-phase cultures of *E.*

TABLE 1. *Amino acid permeability at 37 C under 670 atm of hydrostatic pressure*[a]

| | Trichloroacetic acid-soluble material[b] | | Trichloroacetic acid-insoluble material[b] | |
|---|---|---|---|---|
| $^{14}$C amino acid | 670 atm | 1 atm | 670 atm | 1 atm |
| L-Leucine ..... | 162 | 85 | 350 | 4,400 |
| L-Phenylalanine | 142 | 64 | 400 | 4,668 |
| L-Amino acids | 2,110 | 2,235 | 1,726 | 24,073 |

[a] Each experiment utilized 50 ml of log-phase cells grown to 2.5 × $10^8$ cells/ml. Cells were pulsed with $^{14}$C-amino acids (0.4 μCi/ml) either at 1 atm or under 670 atm of pressure. They were then chilled, pressure was released, and each sample was washed three times with cold nutrient broth, the final wash containing negligible radioactivity. Each sample was suspended in a small amount of cold 5% trichloroacetic acid for 20 min. The precipitated material was pelleted. The acid supernatant fluid was removed and saved, and the precipitate was again extracted for 20 min with cold 5% trichloroacetic acid. After pelleting, the second acid supernatant fluid was combined with the first, and the radioactivity was assayed by using liquid scintillating methods. The precipitate was solubilized, protein was measured (10), and its radioactivity was determined.

[b] Expressed as counts per minute.

[1] This paper is taken in part from a thesis to be submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology at Rensselaer Polytechnic Institute.

*coli* that had been pulsed with $^{14}$C-leucine for 15 sec. This was used as a marker in pressure and control experiments. In the control samples, $^{14}$C-leucine was added to cultures, and then 670 atm of pressure was applied 15 sec later (PA); in the experimental sample, 670 atm was applied first and then $^{14}$C-leucine was immediately added (PB). After 5 min at 670 atm, both samples were chilled and the pressure was released. The tRNA was extracted (5) and analyzed as described (Fig. 1). The data (Fig. 1) show that $^{14}$C-leucyl tRNA can be formed while *E. coli* is under 670 atm of pressure (PB) and that the extent of tRNA charging is the same as in the control (PA). Similar results were obtained when $^{14}$C-amino acids and $^{14}$C-phenylalanine were used.

To investigate the effect of 670 atm upon polysomal integrity, log-phase cultures were either subjected to 670 atm or left at atmos-

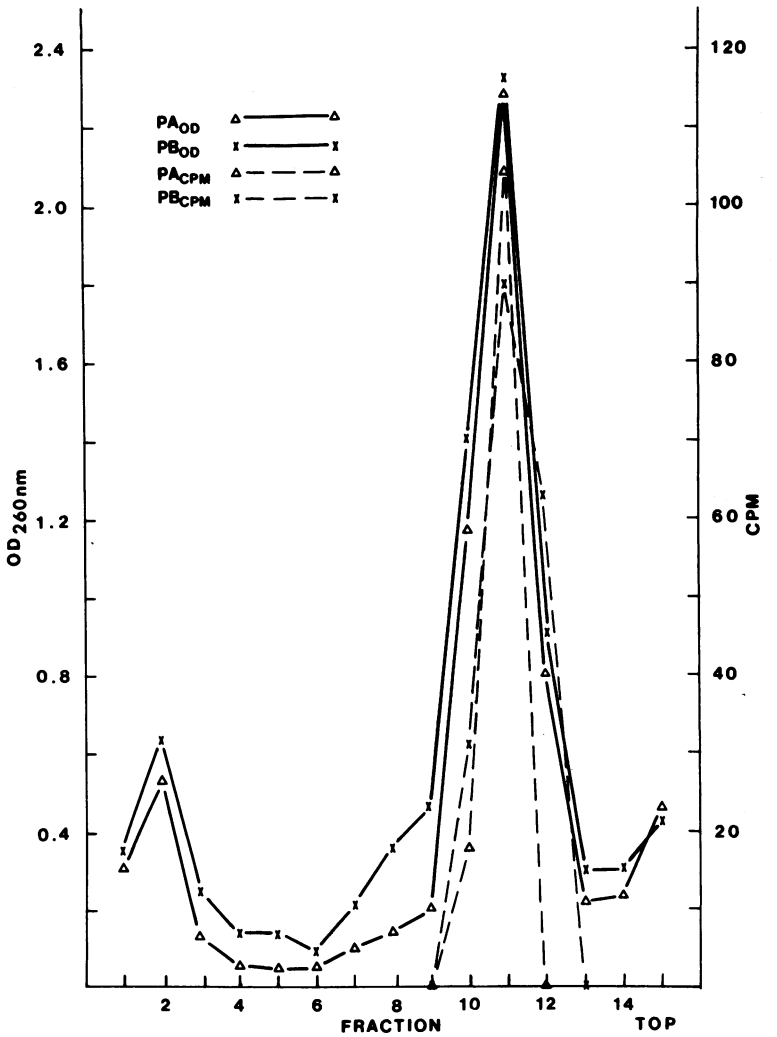


FIG. 1. *Aminoacyl tRNA formation under 670 atm at 37 C. Cultures of Escherichia coli were labeled with $^{14}$C-amino acids (0.4 μCi/ml) either before or after application of 670 atm of pressure. Pressure chambers were chilled to 1 to 2 C before pressure release, and tRNA was extracted by the method of Kjeldgaard and Kurland (5). The tRNA was centrifuged on a linear 5 to 20% sucrose gradient for 5 hr at 65,000 rev/min in a Beckman SW65 rotor. Absorbancy readings were taken before radioactivity determinations were performed in an Intertechnique liquid scintillation counter. PA is the addition of radioactivity at 15 sec before application of 670 atm of pressure. PB is the application of 670 atm of pressure followed by the addition of radioactivity. Similar results were obtained when $^{14}$C-leucine or $^{14}$C-phenylalanine was used in place of $^{14}$C-amino acids.*

pheric pressure. The cultures were then treated as described in Fig. 2. Contrary to the cell-free pressure-induced instability of the mRNA-ribosome complex reported by Arnold and Albright (2), polysomes extracted from whole cells (Fig. 2) were found to be stable under pressure. In several experiments, pressure treatment appeared somewhat to enhance polysomal preservation.

Experiments on nascent peptide formation are perhaps the most significant in relation to the delineation of a specific site for the pressure effect. In these experiments, $^{14}$C-amino acids were added to log-phase cultures, and 670 atm of pressure was applied 15 sec later (PA); in another sample, 670 atm of pressure was first applied to the culture, followed by addition of $^{14}$C-amino acids (PB). Five minutes after pressure application, both samples were chilled and treated as described in Fig. 2. The results (Fig. 2) indicate that, if cells are first allowed to form nascent peptides at atmospheric pressure, a subsequent application of 670 atm of pressure does not remove the already formed nascent proteins (PA). If, however, 670 atm of pressure is first applied and then $^{14}$C-amino acids are added, no labeling of nascent peptides is found (PB), indicating that elongation of the growing peptide chain is inhibited.

In summary, the foregoing indicate that the inhibition of protein synthesis in *E. coli* by high pressure is not found at the level of amino acid permeability, aminoacyl tRNA formation, or maintenance of polysomal integrity.

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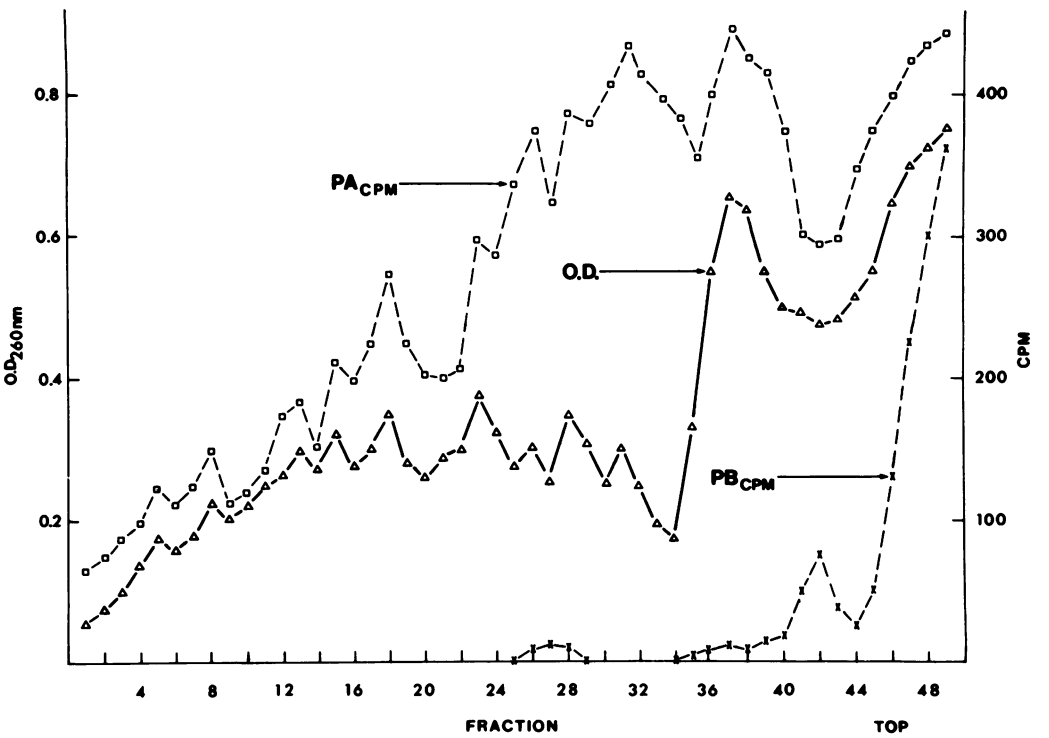


FIG. 2. *Effect of 670 atm on the integrity of polysomes and the formation of nascent peptides at 37 C. Polysomes were extracted from Escherichia coli by the method of Ron, Kohler, and Davis (13) or by using that of Godson and Sinsheimer (3, 4). Polysomes were centrifuged on linear 15 to 30% sucrose gradients for 3 hr at 25,000 rev/min in a Beckman SW25.1 rotor. Absorbancy readings were taken before determination of radioactivity in a liquid scintillation counter. O.D. is the polysomal profile obtained for cultures that are maintained at 1 atm or those subjected to 670 atm of pressure.* $PA_{cpm}$ *is the radioactivity pattern obtained when cells are either pulse-labeled for 15 sec with* $^{14}$*C-amino acids (0.4 μCi/ml) at 1 atm or are labeled with* $^{14}$*C-amino acids 15 sec before application of 670 atm.* $PB_{cpm}$ *is the radioactivity pattern obtained when cells were first subjected to 670 atm of pressure and* $^{14}$*C-amino acids were then added.*

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