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The ATPase Activity of Chaperonin GroEL Is Highly Stimulated at Elevated Temperatures

Jose A. Mendoza,[1] Terri Warren, and Pam Dulin

Department of Chemistry, California State University, San Marcos, San Marcos, California 92096-0001

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The chaperonin GroEL is a heat-shock protein that stabilizes folding intermediates by forming binary complexes. The release of bound polypeptides as active proteins requires ATP hydrolysis by GroEL. The ability of GroEL to support the folding of urea-unfolded rhodanese and to hydrolyze ATP was investigated at high temperatures. We found that the chaperonin-mediated folding of rhodanese and the ATPase activity of GroEL are temperature dependent. The GroEL ATPase activity, however, increases very strongly over the range of temperatures that is physiologically relevant for *Escherichia coli* growth. Further, GroES partially suppresses the GroEL ATPase activity in the same temperature range.

All living organisms have an increased rate of expression of specific genes encoding for the so-called heat-shock proteins (HSPs) in response, at the cellular level, to stressful conditions, e.g. heat shock (1). It is generally believed that HSPs protect cells from the toxic effects resulting from heat or other stresses. The *E.coli* chaperonins (or GroE proteins) are GroEL and GroES were shown to function as ''molecular chaperones'', which mediate the folding and correct assembly of polypeptides (2).

GroEL appears to be stable under conditions that lead to the accumulation of denatured protein (3). When *E.coli* is exposed to moderately high temperatures, the concentration of GroEL is dramatically increased. However, the ability of the GroE proteins to assist the folding of other polypeptides has not been investigated at temperatures higher than 37 °C, e.g. under heat-shock conditions.

In this study, we report that both the chaperonin-mediated folding of rhodanese and the ATPase activity of GroEL are temperature dependent. The highest rates of ATP hydrolysis by GroEL, however, were observed in the range of heat-shock temperatures.

EXPERIMENTAL PROCEDURES

GroEL and GroES were purified, as described (4,5), from lysates of *E.coli* cells bearing the multicopy plasmid pGroESL (6). Rhodanese from bovine liver was purified as described (7).

Rhodanese at 90 μg/mL was unfolded in 8 M urea for 1 h. Unfolded rhodanese was diluted to 3.6 μg/mL in a buffer containing GroEL (2.5 μM), 50 mM Tris-HCl, pH 7.8, 200 mM βME, 50 mM sodium thiosulfate, 10 mM $MgCl_2$, and 10 mM KCl at 10 °C. After incubation for 5 min, GroES (2.5 μM) and 2 mM ATP were added to the mixture and this was incubated for 90 min at the desired temperature. Then, samples were assayed for rhodanese activity (8).

GroEL (2.5 μM) was incubated in a buffer containing 50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, and 10 mM KCl at the desired temperature. After incubation for 5 min, addition of 1 mM ATP was made to initiate the hydrolysis reaction. Periodically, 120 μL-aliquots were removed and mixed with 3 mL of the 360-3 diagnostic reagent (Sigma) which forms a complex with phosphate released after ATP hydrolysis, and absorbance at 340 nm was read at 25 °C using a UV-1601 Shimadzu Spectrophotometer.

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[1] To whom correspondence should be addressed. Fax: (619) 750-4130.

Abbreviations used: ATP, adenosine triphosphate; HSP, heat shock proteins.

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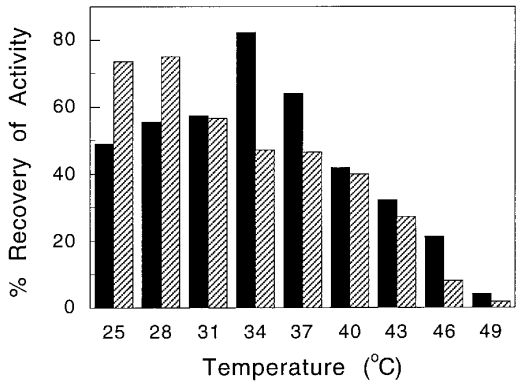

FIG. 1. Chaperonin-assisted refolding of active rhodanese after initial low-temperature formation of the GroEL-rhodanese complex and its subsequent incubation at several temperatures. The complex between GroEL and unfolded rhodanese was prepared at 10°C and supplemented with GroES and ATP. After incubation for 20 min (filled bars) or 90 min (diagonal bars), at the indicated temperature, rhodanese activity was then assayed, as described under Experimental Procedures.

RESULTS AND DISCUSSION

Previously, it was reported that GroEL remains stable at relatively high temperatures (9). At approximately 62 °C, GroEL undergoes a cooperative transition, that leads to denaturation. The unfolding transition was detected by far UV-CD. In the present investigation, the temperature-dependence of the chaperonin-assisted refolding of urea-unfolded rhodanese was studied in the 25 to 64 °C. Also, the effect of temperature on the ATPase activity of GroEL was examined. Since the ATPase activity of GroEL is significantly inhibited by the co-chaperonin GroES, we also examined this effect at various temperatures.

Fig. 1 shows the temperature-dependence of the chaperonin-assisted refolding of urea-unfolded rhodanese. The binary complex of GroEL and unfolded rhodanese was prepared by mixing the two proteins at 10 °C, as previously shown for the efficient formation of the complex (10,11). Then, MgATP and GroES were added, incubation at the indicated temperature was carried out and the refolding was followed. Thus, as shown in Fig. 1, higher refolding temperatures, resulted in a lower degree of recovery of enzyme activity.

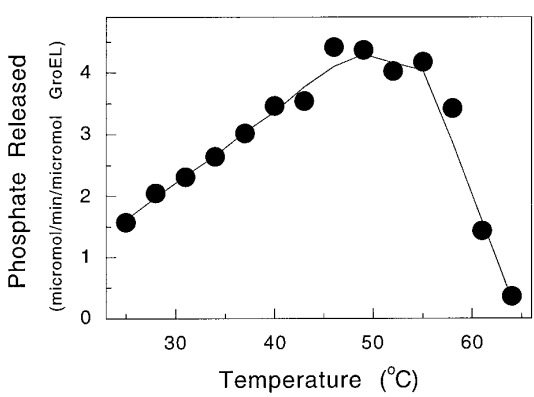

FIG. 2. ATPase activity of GroEL as a function of temperature. GroEL (2.5 μM), in the presence of $MgCl_2$ (10 mM), KCl (10 mM) and ATP (1 mM), was incubated at the indicated temperature. The phosphate released after ATP hydrolysis was determined as described under Experimental Procedures.

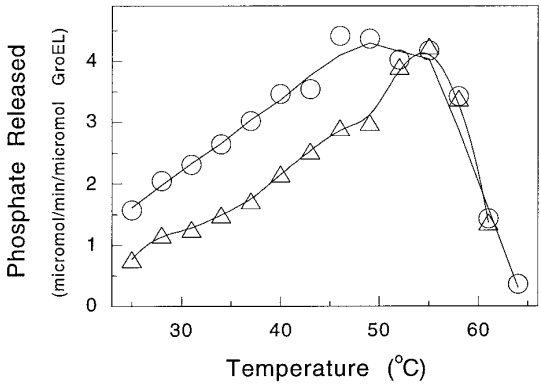

FIG. 3. Effect of GroES in the ATPase activity of GroEL at several temperatures. GroEL (2.5 μM), in the absence (open circles) or presence (open triangles) of GroES (2.5 μM) was incubated at the indicated temperature. The samples also contained $MgCl_2$ (10 mM), KCl (10 mM), and ATP (1 mM). The phosphate released after ATP hydrolysis was determined as described under Experimental Procedures. The data for GroEL in the absence of GroES correspond to that of Fig. 2.

The ability of GroEL to hydrolyze ATP was investigated at temperatures higher than 25 °C. We found that the ATPase activity of GroEL is highly temperature dependent. As shown in Fig. 2, the chaperonin's ATPase activity gradually increased in the 25 °C to 52 °C range. Above 52 °C, the ATPase activity declined sharply as a function of temperature.

The co-chaperonin GroES was previously shown to decrease by about one half the rate of ATP hydrolysis by GroEL (12-14). We investigated if this effect was temperature dependent by assaying the ATPase activity of GroEL in the presence of GroES in the 25-61 °C range. As shown in Fig. 3, GroES suppressed the hydrolysis of ATP by GroEL to a very similar extent in the temperature range of 25 °C to 49 °C. Interestingly, at 52 °C or higher temperatures, GroES did not inhibit the ATPase activity of GroEL.

Since the release of many GroEL-bound polypeptides requires ATP hydrolysis (15), a faster rate of ATP hydrolysis by GroEL, at higher temperatures, might allow the chaperonin to function more efficiently in mediating the folding of other polypeptides. A similar temperature dependence was previously reported for the ATPase activity and autophosphorylation of the heat-shock protein dnaK (16). It was proposed that dnaK functions as a molecular thermometer signaling the increase of temperature in the cell. Our results with GroEL suggest that, generally, heat-shock proteins might have an increased activity under stressful conditions, e.g. under high temperatures. This proposal is consistent with the strong inhibition of the chaperonin-assisted refolding of the enzyme rhodanese (10) and the highly reduced ATPase activity of GroEL at low temperatures (17).

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