DnaK from *Thermus thermophilus* (TDnaK) is unique because significant fractions of cellular TDnaK exist as a trigonal K J complex that consists of three copies each of TDnaK, TDnaJ, and an assembly factor T Dafa. Here, chaperone functions of the K J complex and free TDnaK plus free TDnaJ (K+J) were compared. Substrate proteins were completely denatured at 72–73 °C or 89 °C in the absence of the presence of the K J complex or K+J and were subsequently incubated at a moderate temperature of 55 °C. T GrpE and ATP were always included in the K J complex and K+J, and TClpB was supplemented at 55 °C. At 72–73 °C, both the K J complex and K+J suppressed heat aggregation of substrate proteins. During the next incubation at 55 °C, K+J, assisted by TClpB, was able to disaggregate the heat aggregates and efficiently reactivate activities of the proteins, whereas the K J complex was not; it reactivated only the soluble inactivated proteins. When substrate proteins were heated to 89 °C, both the K J complex and K+J were no longer able to prevent heat aggregation, and because of selective, irreversible denaturation of T Dafa the K J complex dissociated into K+J, which then exhibited disaggregation activity during the next incubation at 55 °C. Thus, TClpB-assisted disaggregation activity belongs only to K+J, and T Dafa is a potential thermosensor for converting the K J complex to K+J in response to heat stress.

Protein aggregation is one of the major damaging consequences of a stress situation such as heat shock. The central cellular defense against protein aggregation consists of molecular chaperones that bind unfolded proteins and prevent aggregation (1, 2). Once the aggregates are formed, however, the usual chaperones cannot handle them, and the cooperative function of HSP104 and HSP70 can dissolve aggregates and help the proteins to restore their native structures (3–7). In prokaryotes, as we have demonstrated for chaperones from a thermophilic eubacteria, *Thermus thermophilus*, heat-damaged proteins were rescued by the cooperative chaperone functions of ClpB (bacterial HSP104), DnaK (bacterial HSP70), DnaJ, and GrpE (4, 11). ClpB forms a homo-hexameric complex (580 kDa) as an active species and needs ATP hydrolysis for the function (12–17). DnaK also has ATPase activity and works with DnaJ and GrpE (termed the DnaK set) (2). Cooperation between the DnaK set and ClpB in disaggregation and reactivation of the aggregated proteins was also reported for *Escherichia coli* and mitochondrial homologues (5–7, 18).

Unlike other bacteria, however, approximately half the population of cellular DnaK of *T. thermophilus* is purified as a stable trigonal ring complex (19). This complex (330 kDa), termed K J1 hereafter, is composed of three copies each of TDnaK (69 kDa), TDnaJ (33 kDa), and T Dafa (the prefix T designates proteins of *T. thermophilus*). T Dafa is a small protein necessary to assemble TDnaK and TDnaJ into K J (20); T GrpE (22 kDa) is isolated as a homodimer (21–23). The stable K J has been reported only for DnaK and DnaJ from *T. thermophilus*, but the chaperone function of K J has been studied with a general interest because the current model for the mechanism of DnaK function assumes only transient but not stable interaction between DnaK and DnaJ (4, 11, 16, 19, 23). Related to this contention, the chaperone activity of uncomplexed TDnaK and TDnaJ (termed K+J) has been reported (14, 24), but the difference from that of K J is not clear. Here, we carefully compared chaperone activities of the K J set (K+J plus T GrpE) and those of the K+J set (K+J plus T GrpE). The results indicate that the ability of disaggregation of the previously formed aggregate in cooperation with TClpB belongs only to K+J and that K J must be dissociated into K+J to elicit this function, which indeed occurs under heat stress, in which T Dafa is heat-inactivated.

**EXPERIMENTAL PROCEDURES**

*Plasmids—* A plasmid containing genes for T Dafa(L2V) named pET-Dafa(L2V) was constructed as follows. A DNA fragment containing the sequence of the tadaA gene with an L2V mutation was prepared with PCR by using Ex-Taq DNA polymerase (Takara). The pMKJ8 plasmid (20) was used as a PCR template. The forward primer was an oligonucleotide having an NdeI site, and a 5′-end sequence of the tadaA gene with an L2V mutation was used. As the reverse primer, an oligonucleotide having a 3′-end sequence of the gene and a BamHI site was used. The amplified DNA fragment was cloned into the NdeI and BamHI sites of pET23c (Novagen), and the base sequence was confirmed. To make a pET-TDnaK(N-His10) for expression of His-tagged TDnaK, a TDnaK-expression plasmid, pMDRS (20), was digested with NdeI-BamHI, and the amplified DNA fragment was ligated into the NdeI and BamHI sites of pET23c (Novagen). The plasmids were transformed into *E. coli* DH5α, and the transformants were grown in Luria-Bertani medium containing ampicillin.

*Protein preparation—* TClpB was purified as described previously (24). ClpB was overproduced in *E. coli* BL21(DE3) cells harboring the plasmid pET-TClpB. The cell pellets were resuspended in lysis buffer (10 mM Tris, pH 8.0, 10 mM DTT, and 200 mM NaCl) and disrupted by ultrasonication. The heat-aggregated proteins were pelleted by centrifugation at 15,000 × *g* for 20 min. TClpB was affinity-purified using a Ni-NTA column. The column was washed with buffer A (20 mM Tris, pH 8.0, 200 mM NaCl, 1 mM DTT) and eluted with buffer B (20 mM Tris, pH 8.0, 200 mM NaCl, 1 mM DTT, and 0.5 M imidazole). The peak fractions were pooled and concentrated.

*Chaperone activity assay—* Heat-aggregated proteins were reactivated by the chaperone activities of TClpB and T Dafa. The aggregated proteins of DnaK, DnaJ, and GrpE (the prefix T designates proteins of *T. thermophilus*) were prepared in lysis buffer (20 mM Tris, pH 8.0, 200 mM NaCl, and 10 mM DTT) by heating at 75 °C for 1 min. The aggregated proteins were incubated in assay buffer (20 mM Tris, pH 8.0, 200 mM NaCl, and 10 mM DTT) for 10 min at 55 °C. Next, TClpB (100 nM) and T Dafa (500 nM) were added, and the mixtures were incubated for 10 min at 55 °C. The activity of the reactivated proteins was determined by using the assay buffer (20 mM Tris, pH 8.0, 200 mM NaCl, and 10 mM DTT) containing ATP. The mixtures were incubated for 10 min at 37 °C. The concentrations of the proteins were determined by the Bradford method (25) using bovine serum albumin as a standard. The activity of the reactivated proteins was expressed as a percentage of the highest activity, which was set as 100%.

*SDS-PAGE and Western blot analysis—* Heat-aggregated proteins were separated on 8% SDS-polyacrylamide gels. The gel was stained with Coomassie brilliant blue R250. Western blots were prepared as described previously (24). The blots were incubated with the anti-DnaK antibody (24) and detected by using peroxidase-conjugated anti-rabbit IgG antibody and ECL Western blotting detection reagents (25).

Received for publication, August 8, 2003, and in revised form, January 13, 2004 Published, JBC Papers in Press, January 16, 2004. DOI 10.1074/jbc.M308782200

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*HEAT STRESS CONVERTS THE FORMER TO THE LATTER, AND ONLY THE LATTER CAN DO DISAGGREGATION IN COOPERATION WITH ClpB*  

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‡ Supported in part by a research fellowship of the Japan Society for the Promotion of Science for Young Scientists.

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the fragment was ligated into NdeI-BamHI sites of the pET16b (Novagen)

Proteins—Glucose-6-phosphate dehydrogenase (G6PDH) and α-glucosidase from *Bacillus steatorrhophilus* were purchased from Unitech and Sigma, respectively. 3-α-threo-isopropylmalate dehydrogenase from *T. thermophilus* (TIPMDH) was expressed in *E. coli* and purified as described (23). K7 complex, TGrpE, and TClpB were expressed in *E. coli* and purified as described (4, 23). Additional gel filtration by G3000SWXL (Tosoh) was performed for K7 to remove trace amounts of free TDaK and TDaJ. TDaK was expressed in *E. coli* BL21(DE3) carrying pMDK6. The cells were suspended in Buffer A (25 mM Tris-HCl, pH 7.5, and 5 mM MgCl2) and disrupted by sonication. The cell extracts were heat-treated at 80 °C for 30 min and centrifuged at 100,000 × *g* for 40 min. The supernatant was applied to a Toyopearl DEAE-column (Tosoh) equilibrated with Buffer A. The column was eluted with Buffer A containing 300 mM NaCl. Fractions containing TDaK and TDaJ were pooled, and solid ammonium sulfate was added, to a concentration of 800 mM. The solution was applied to a Toyopearl butyl column (Amersham Biosciences) to exchange the buffer to 50 mM MOPS-NaOH buffer, pH 7.5, 150 mM KCl, and 5 mM MgCl2. The TDaK fractions were frozen by liquid nitrogen for storage at −80 °C until use. His-tagged TDaK was expressed in *E. coli* carrying pET-TD-DaK (Tosoh) and purified by the same procedures as TDaK. TDaJ was expressed in *E. coli* carrying pMDJ10 (20). One-half of the amount of expressed TDaJ was in the soluble fraction and was heat-stable. TDaJ was purified with the same procedures used for TDaK purification, except that the pass-through fraction of the Toyopearl DEAE-column (Tosoh) contained TDaJ and was used for further purification. To isolate the TDaA expressed in *E. coli*, we used the mutation L2V, which prevents rapid degradation in the cell (23). For simplicity, hereafter we call this mutant TDaA. TDaA was expressed as inclusion bodies in *E. coli* carrying pET-DaA(L2V). The cells were suspended in Buffer B (25 mM Tris-HCl, pH 7.5, and 1 mM EDTA), disrupted by a French press, and centrifuged at 12,000 × *g* for 20 min. The pellet was washed twice with Buffer B. From the pellet, TDaA was extracted using Buffer B containing 4 mM urea. The extract was concentrated by Ultra-free and loaded to a G3000SWXL gel filtration column equilibrated with the buffer (50 mM MOPS-NaOH, pH 7.5, 150 mM KCl, 5 mM MgCl2, and 4 mM urea). The peak fraction was frozen by liquid nitrogen and stored at −80 °C until use. Throughout this article concentrations of proteins are expressed as monomers except for K7 (expressed as a trigonal complex), TGrpE (expressed as a dimer), and TClpB (expressed as a hexamer).

Aggregation and Disaggregation of Proteins—G6PDH (53 kDa, tetramer), α-glucosidase (65 kDa, monomer), and TIPMDH (37 kDa, dimer) were used as substrate proteins. These substrate proteins (0.2 µM, expressed as a monomer) were dissolved in 250 µl of the reaction mixture (50 mM MOPS-NaOH, pH 7.5, 5 mM MgCl2, 150 mM KCl, 1 mM dithiothreitol, and 3 mM ATP) containing the indicated chaperones. When the temperature was reduced to 55 °C after heat treatment and the reactivation incubation at 55 °C for 90 min was finished, an aliquot of the solution was centrifuged at 20,000 × *g* for 10 min at 4 °C. Precipitated proteins were washed by the buffer, and proteins in the supernatant were precipitated by 10% trichloroacetic acid and loaded to SDS-PAGE (15%).

**FIG. 1. Progress of heat aggregation and effect of the K7 and K7 sets.** G6PDH (A), α-glucosidase (B), and TIPMDH (C) were heat-treated at 72, 73, and 89 °C, respectively. After incubation for the indicated times, the solutions were centrifuged, and supernatant (sup) and precipitates (ppt) were analyzed with SDS-PAGE. Right, the reactivation treatment was performed in the presence of the K7 and K7 sets, respectively. The concentrations of chaperones were 0.2 µM K7 (trigonal complex), 0.6 µM TDaK (monomer), 0.6 µM TDaJ (monomer), and 0.1 µM TGrpE (dimer). Experimental details are described under “Experimental Procedures.”

**RESULTS**

**Preventing Aggregation by K7 and K7**—The substrate proteins were heat-treated for the indicated period and subjected to centrifugation to separate soluble and aggregate fractions that were visualized by SDS-PAGE. When G6PDH and α-glucosidase, both from *B. steatorrhophilus*, were heat-treated at 72 and 73 °C, all the proteins were recovered in the precipitation after 8 and 10 min, respectively (Fig. 1, A and B). However, if the TDaK set, in the form of either K7 or K7, was included in the solution during the heat treatment, a significant fraction of the proteins remained soluble after 8 and 10 min, respectively, with the K7 set being slightly more efficient (Fig. 1, A and B, right panels). Thus, both the K7 and K7 set have a protective effect on the substrate proteins from heat aggregation. It should be added, however, that the proteins that remained soluble were not native but denatured because the enzyme activities of G6PDH and α-glucosidase were completely abolished, irrespective of the presence or absence of the K7 and K7 sets. Next, TIPMDH was examined as a representative substrate protein derived from the same bacterium from which

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**TABLE 1.** Function of Trigonal DnaK-DnaJ Complex

| Protein | G6PDH | α-Glucosidase | TIPMDH |
|---------|-------|--------------|--------|
| Time (min) | 0 | 2 | 8 |
| sup | 0 | 0 | 0 |
| ppt | 4 | 0 | 0 |
| Time (min) | 5 | 10 | 15 |
| sup | 0 | 0 | 0 |
| ppt | 4 | 0 | 0 |
| Time (min) | 10 | 30 | 100 |
| sup | 0 | 0 | 0 |
| ppt | 4 | 0 | 0 |

**Fig. 1. Progress of heat aggregation and effect of the K7 and K7 sets.**

- **A.** G6PDH (A), α-glucosidase (B), and TIPMDH (C) were heat-treated at 72, 73, and 89 °C, respectively. After incubation for the indicated times, the solutions were centrifuged, and supernatant (sup) and precipitates (ppt) were analyzed with SDS-PAGE. Right, the reactivation treatment was performed in the presence of the K7 and K7 sets, respectively. The concentrations of chaperones were 0.2 µM K7 (trigonal complex), 0.6 µM TDaK (monomer), 0.6 µM TDaJ (monomer), and 0.1 µM TGrpE (dimer). Experimental details are described under “Experimental Procedures.”

- **B.** α-Glucosidase activity was assayed at 55 °C by the absorbance at 405 nm in the assay solution (100 mM Tris-HCl, pH 8.8, 40 mM MgCl2, 1 mM NADP+, 3 mM glucose-6-phosphate) (26); α-glucosidase activity was assayed at 55 °C by the absorbance at 405 nm in the assay solution (50 mM sodium phosphate, pH 6.8, 2 mM α-threo-isopropylmalate) (26); and α-glucosidase activity was assayed at 55 °C by the absorbance at 405 nm in the assay solution (150 mM KCl, 1 mM dithiothreitol, and 3 mM ATP) containing the indicated chaperones.

- **C.** TIPMDH activity was assayed at 55 °C by the absorbance at 340 nm in the assay solution (100 mM potassium phosphate, pH 7.8, 1 mM MgCl2, 0.8 mM NAD+, 0.4 mM isopropylmalate) (25).

- **K7 Complex Formation**—Indicated chaperone components were dissolved in 250 µl of the reaction mixture (50 mM MOPS-NaOH, pH 7.5, 5 mM MgCl2, 150 mM KCl, and 1 mM dithiothreitol) and incubated as indicated. After incubation, a 200-µl aliquot was loaded to a Ni-NTA super flow column (Qiagen), equilibrated with the buffer (50 mM MOPS-NaOH, pH 7.5, 5 mM MgCl2, and 150 mM KCl), and washed with the same buffer. If needed, the column was also washed by the same buffer containing 50 mM imidazole. The proteins bound to the column through the His tag were eluted with the same buffer containing 200 mM imidazole. The proteins in each fraction were precipitated by 10% trichloroacetic acid and loaded to SDS-PAGE (15%).

- **TdaA Aggregation**—The K7 complex was dissolved at 2 µM in 25 µl of the reaction mixture (50 mM MOPS-NaOH, pH 7.5, 5 mM MgCl2, 150 mM KCl, 1 mM dithiothreitol, and 3 mM ATP) containing 0.4 mg/ml bovine serum albumin as a carrier protein to recover small amounts of aggregates. The mixture was incubated at 55 or 89 °C for 30 min, and an aliquot of the solution was centrifuged at 20,000 × *g* for 10 min at 4 °C. Precipitated and supernatant fractions were analyzed with SDS-PAGE (15%).

**RESULTS**

- **Preventing Aggregation by K7 and K7**—The substrate proteins were heat-treated for the indicated period and subjected to centrifugation to separate soluble and aggregate fractions that were visualized by SDS-PAGE. When G6PDH and α-glucosidase, both from *B. steatorrhophilus*, were heat-treated at 72 and 73 °C, all the proteins were recovered in the precipitation after 8 and 10 min, respectively (Fig. 1, A and B). However, if the TDaK set, in the form of either K7 or K7, was included in the solution during the heat treatment, a significant fraction of the proteins remained soluble after 8 and 10 min, respectively, with the K7 set being slightly more efficient (Fig. 1, A and B, right panels). Thus, both the K7 and K7 set have a protective effect on the substrate proteins from heat aggregation. It should be added, however, that the proteins that remained soluble were not native but denatured because the enzyme activities of G6PDH and α-glucosidase were completely abolished, irrespective of the presence or absence of the K7 and K7 sets. Next, TIPMDH was examined as a representative substrate protein derived from the same bacterium from which...
The KJ set was included from the beginning of heat treatment. The concentrations of chaperones were 0.6 μM and precipitated (ppt) of the solutions were centrifuged, and whole proteins in the supernatant (sup) fractions were analyzed by SDS-PAGE. Only incubations were continued for another 90 min. At the time of temperature shift, the bands of substrate proteins are shown. In the case of the supernatant fraction of B. stearothermophilus and complete aggregation required a 30-min incubation at 89 °C, an upper limit temperature of T. thermophilus growth (Fig. 1C). In contrast to G6PDH and α-glucosidase, both the K+J and KJ sets were apparently unable to prevent aggregation of TIPMDH at 89 °C (Fig. 1C, right panel). Hereafter, conditions of heat treatment were fixed to 72 °C for 8 min for G6PDH, 73 °C for 10 min for α-glucosidase, and 89 °C for 30 min for TIPMDH.

**TCPlB-assisted Disaggregation by K+J and KJ**—The ability of TCPlB-assisted disaggregation was compared between the K+J and KJ sets. The ability was assessed from the comparison of the amount of aggregates before and after a 90-min incubation at 55 °C. In the experiments shown in Fig. 2, columns 1 and 2, all the proteins (G6PDH, α-glucosidase, and TIPMDH) were aggregated during heat treatment in the absence of chaperones. These aggregates did not spontaneously become soluble during subsequent incubation at 55 °C (not shown). When TCPlB plus the K+J set was added at the time of the temperature shift to 55 °C, a large fraction of the aggregates (>50%) was disaggregated during subsequent incubation at 55 °C (Fig. 2, column 1). By contrast, if TCPlB plus the KJ set, instead of the K+J set, was added at the time of the temperature shift, only a small amount of aggregates (<15%) was disaggregated and transferred into the soluble fraction (Fig. 2, column 2). These results show the clear difference between the K+J and KJ sets; the former has TCPlB-assisted disaggregation activity of the previously formed aggregates, but the latter has only a trivial amount of such activity.

As described, when G6PDH and α-glucosidase were heat-treated in the presence of the K+J or KJ set, a significant amount of the proteins remained soluble. The subsequent incubation at 55 °C with supplemented TCPlB resulted in marginal (K+J set) or no further (KJ set) decrease in the amount of aggregates (see the band densities of precipitates, Fig. 2, columns 3 and 4). For TIPMDH, all the proteins were aggregated by heat treatment in the presence of the K+J or the KJ set, but, in either case, a significant fraction was disaggregated during the subsequent incubation at 55 °C (Fig. 2, columns 3 and 4). This result for the K+J set is consistent because the K+J set has activity of TCPlB-assisted disaggregation, but the result for the KJ set is enigmatic because the KJ set does not have such activity, as described above; this is because of the dissociation of KJ into K+J at 89 °C, as described later.

**Reactivation of Heat-treated Proteins by K+J and KJ**—Before the 55 °C incubation, the proteins showed no enzyme activities (<5%) even when a significant amount of proteins existed in the soluble fractions. However, after an incubation at 55 °C, a significant amount of enzyme activities of G6PDH, α-glucosidase, and TIPMDH was reactivated (Fig. 3, A–C, columns 1, 3, and 4), except when KJ was added after heat treatment (Fig. 3, A–C, column 2). A comparison with the results in Fig. 2 shows that the yields of reactivation are roughly parallel to the amount of protein in the soluble fractions after a 90-min incubation at 55 °C. It appears that during the 55 °C incubation, the K+J and KJ sets reactivated the pre-existing but inactivated soluble proteins with the aid of TCPlB. In addition, the K+J set reactivated newly solubilized proteins by the action of disaggregation, giving a slightly better yield of reactivation than the KJ set. The good recovery of TIPMDH by the KJ set shown in Fig. 3C, column 4, is the
result of the dissociation of KJ into K+J at 89 °C as described later.

TDafA Inhibits λClpB-assisted Disaggregation Activity of K+J Set—The effect of isolated TDafA on the λClpB-assisted dissociation/reactivation of G6PDH by the K+J set was tested. G6PDH was heat-treated at 72 °C for 8 min in the absence of a chaperone and subsequently incubated at 55 °C with λClpB, K+J set, and various concentrations of TDafA.

The results showed that the reactivation yield of the heat-aggregated G6PDH decreased as concentration of TDafA in the solution increased (Fig. 4). When the amount of TDafA reached 1:1 molar stoichiometry to TDnaK, the reactivation was nearly completely inhibited. In addition, as G6PDH was heat-treated with the K+J set and TDafA (equimolar to TDnaK and TDnaJ), 50–60% of G6PDH activities were recovered by subsequent incubation with λClpB (not shown). This reactivation yield was almost the same as when G6PDH was heat-treated with the KJ set, and less than that was obtained for K+J (~80%). These results suggest that TDafA acts on TDnaK and TDnaJ to form the K+J complex, resulting in a loss of disaggregation activity of previously formed aggregates.

Dissociation of KJ into K+J at 89 °C—To test whether TDafA really induced the assembly of TDnaK and TDnaJ, we prepared TDnaK that had a His10 tag at the N terminus. The chaperone activity of the TDnaKhis set was confirmed to be intact (data not shown). The formation of KJ was detected by a Ni-NTA column. In the absence of TDafA, only TDnaKhis was adsorbed to the column, and all of the TDnaJ passed alone through the column (Fig. 5A, lane 1). With the addition of TDafA, by contrast, most TDnaJ was retained on the column and was eluted together with TDnaKhis and TDafA by the wash with imidazole (Fig. 5A, lane 2). It was confirmed that KJ was stable during the procedures of experiments in Fig. 2, column 4 (Fig. 5A, lane 3), and therefore interaction with denatured protein did not induce decay of KJ. By using the reconstituted KJ, the heat stability of this complex was examined. KJ was exposed to the indicated temperatures for 30 min and applied to a Ni-NTA column (Fig. 5B). From 25 to 85 °C, a majority of TDnaJ was retained on the column and was eluted together with TDnaKhis and TDafA by the wash with imidazole. At 89 °C, however, ~70% of KJ decayed in 30 min, producing uncomplexed, individual components. It appears that activity of TDafA as an assembly factor was destroyed at 89 °C. Indeed, when KJ complex was heated to 89 °C for 30 min and centrifuged, TDafA was recovered in the precipitated fraction, whereas most TDnaK and TDnaJ remained in the supernatant fraction (Fig. 5C). To know if the products of decayed KJ have chaperone, especially disaggregation, activity, we substituted KJ in the experiment shown in Fig. 3, column 2, for the KJ that had been already heat-treated at 89 °C for 30 min and measured the reactivated enzyme activities of the substrate proteins after a 90-min incubation at 55 °C (Fig. 6). The results were very different from those of unheated KJ in that heated KJ could dis aggregate and reactivate the aggregated proteins as efficiently as K+J. Moreover,
Fig. 7. Models for functions of the DnaK chaperone of T. thermophiles. A, chaperone functions of KJ and K+J. Both the KJ and K+J sets can prevent formation of aggregation and reactivate soluble denatured proteins in the presence of TCPb. TCPb-assisted disaggregation activity, however, belongs only to the K+J set. GrpE and ATP are required for all functions but are not shown in the illustration. B. TCPb is a potential thermosensor. In response to heat shock, TCPb is irreversibly denatured, and KJ dissociates to K+J, which starts disaggregation of the heat aggregates.

As reported previously (4), the KJ set from T. thermophiles can suppress heat aggregation of substrate proteins and help reanimate heat-inactivated proteins in cooperation with TCPb. However, it is now clear that only soluble fractions of the denatured proteins can be reanimated by the KJ set and that efficient disaggregation of the previously formed aggregates in cooperation with TCPb is possible only by the K+J set (Fig. 7A). The mechanism underlying this difference is yet unknown, but it is likely that TCPb-assisted disaggregation activities are mediated through the transient and sequential action of TDnA and TDnAa that cannot be carried out by KJ. Another possibility is that KJ cannot bind to the aggregate because KJ and isolated TDnA have different peptide-binding repertoires (19, 20).

Because the K+J set can carry out all aspects of chaperone function, one might wonder why T. thermophiles cells possess one-half the cellular fraction of TDnA as KJ. In the case of E. coli, a counterpart of neither TCPb nor the KJ complex has been found; DnAa and DnAa exist individually in the cell. We speculate that KJ in T. thermophiles cells, with an optimum growth temperature of approximately 75 °C, participates in reactivation of the soluble denatured proteins and suppression of occasional aggregation in cooperation with other chaperones under normal growth conditions. However, when exposed to a stress temperature above 85 °C, TCPb is denatured, and KJ dissociates to K+J to counteract increasing aggregates (Fig. 7B). If this really is the case, TCPb is a kind of thermosensor that strengthens the cellular ability to defend against heat shock. For this purpose, TCPb may be made as a heat-labile sticky protein. In fact, isolated TDnA tend to stick to each other even at room temperature, and 4 M urea is needed to prevent aggregation.

Acknowledgments—We thank T. Murakami and J. Suzuki for technical assistance of DNA sequencing, Dr. F. Motojima for providing us with purified TIPMDH, and Drs. K. Motohashi and Y. Kato-Yamada for the helpful discussions.

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Trigonal DnaK-DnaJ Complex Versus Free DnaK and DnaJ: HEAT STRESS CONVERTS THE FORMER TO THE LATTER, AND ONLY THE LATTER CAN DO DISAGGREGATION IN COOPERATION WITH ClpB
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J. Biol. Chem. 2004, 279:15723-15727.
doi: 10.1074/jbc.M308782200 originally published online January 16, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M308782200

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