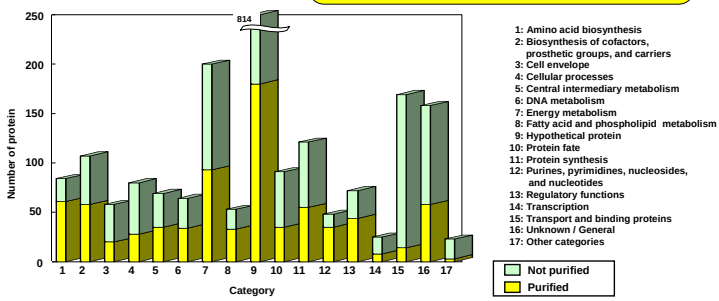
Abstract

To facilitate structural and functional analyses of *Thermus thermophilus* proteins, it is necessary to overproduce various proteins easily and efficiently. We utilized heat treatment as an effective purification step for *T. thermophilus* proteins overproduced in *E. coli*. After heat treatment, the target proteins were purified at room temperature by combination of hydrophobic interaction, ion exchange, hydroxyapatite and gel filtration column chromatography. If the expression level of a protein was insufficient, N-terminal His-tag was attached to the protein. The His-tag generally improved expression level, and the proteins overproduced were easily purified by heat treatment and chelating column chromatography. These methods allow us to prepare *T. thermophilus* proteins efficiently. We have purified 801 proteins out of 2,236 ORFs of *Thermus thermophilus* HB8 until now.

Current status

Total ORF number 2236

Total protein purified 801



Tag-free protein — Purification procedure

Cell suspension

Lysis buffer: 20 mM Tris-HCl, 50 mM NaCl, 5 mM β-mercaptoethanol, pH 8.0

Sonication

OUT PUT 8, DUTY 50 and 1 min × 10

Heat treatment

70℃, 10 min in water bath

Ultracentrifugation

Separate heat treated cell lysate to supernatant (HS) and pellet (HP).
40,000 rpm, 1 hour, 4℃

Ammonium sulfate scouting

Check an occurrence of precipitation and determine a salt concentration condition for hydrophobic column scouting in the range of 1.05 M to 1.5 M ammonium sulfate.

Hydrophobic column scouting

RESOURCE ISO, RESOURCE PHE

Figure 1-1
Analytical HPLC system (Shimadzu)

First step purification

- **When hydrophobic column can be used**
  - → **RESOURCE ISO, PHE column chromatography**
- **When hydrophobic column can not used**
  - → **SuperQ-TOYOPEARL 650 column chromatography**
  - → **Ammonium sulfate precipitation**

Figure 1-2
Preparative HPLC system (Shimadzu)

The following purification steps

- **Ion-exchange column chromatography**
  RESOURCE Q, Mono Q
  RESOURCE S, Mono S
- **Hydroxyapatite column chromatography**
  Bio-scale CHT
- **Affinity column chromatography**
  HiTrap Heparin, HiTrap Blue
- **Hydrophobic column chromatography**
  RESOURCE ETH, RESOURCE ISO, RESOURCE PHE
  HiTrap Butyl, HiTrap Octyl

Figure 1-3
AKTA explorer 10S (Amersham Biosciences)

Final step purification

- **Gel filtration**
  Superdex 75 pg, Superdex 200 pg, Sephacryl-S200

Figure 1-4
Gel filtration system (Shimadzu)

Protein concentration determination

Measurement of UV absorption at 280 nm

His-tagged protein — Purification procedure

Cell suspension

Lysis buffer:20 mM Tris-HCl, 0.5 M NaCl, 5 mM β–mercaptoethanol, pH 8.0

Sonication

OUT PUT 6.5, DUTY 50 and 1 min × 10

Heat treatment

70℃, 10 min in water bath

Centrifugation

Separate heat treated cell lysate to HS and HP.
15,000 rpm, 30 min, 4℃

HiTrap Chelating HP (5 ml) (affinity column)

(If necessary)
HiPrep 26/10 Desalting (desalting column)

RESOURCE Q (6 ml) (anion exchange column)

HiLoad 16/60 Superdex 200 pg (gel filtration column)

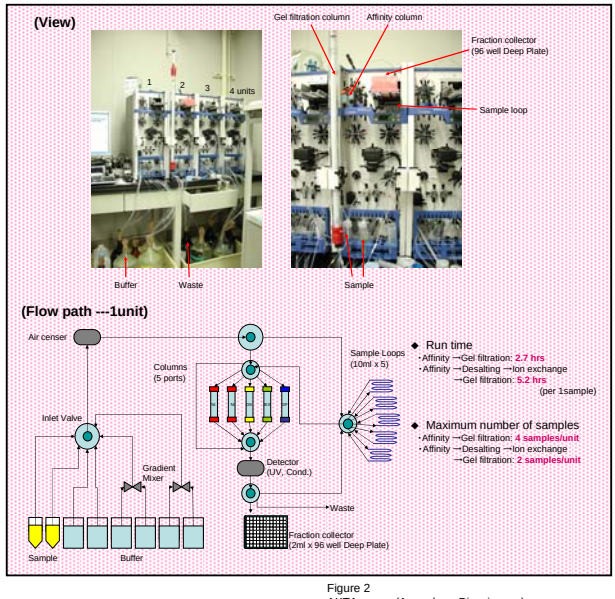


Figure 2
AKTAxpress (Amersham Biosciences)

Protein concentration determination

Measurement of UV absorption at 280 nm

Conclusion

- Proteins of *Thermus thermophilus* HB8 are efficiently and rapidly purified by heat treatment and combination of hydrophobic interaction column chromatography, ion exchange column chromatography, hydroxyapatite column chromatography, and size exclusion column chromatography.
- So far, 801 proteins were already purified in this project.
- His-tag attachment was useful for improving protein production and purification efficiency.

Members