HAYASHIBARA
NAGASE Group

TREHALOSE SG JP, USP-NF, Ph. Eur., CP

For Culture Media Use

What is TREHALOSE SG ?

- TREHALOSE SG is a dihydrous, crystalline and non-reducing disaccharide consisting of two glucose molecules linked by an α,α-1,1 bond.
- TREHALOSE SG is white crystalline powder.
- TREHALOSE SG is very soluble in water and very heat stable.
- TREHALOSE SG is an injectable grade of pharmaceuticals and is monographed as being low endotoxin.

Dihydrous crystalline trehalose

Evaluation of the suppression of protein aggregation in CHO cell culture ①

Materials & Methods (1)

1. Protein solution of a bispecific antibody (bispecific single-chained diabody with Fc, scDb-Fc, 0.8 mg/mL) was prepared with or without addition of trehalose (200 mM) in culture medium.
2. Circular dichroism (CD) spectroscopy of the scDb-Fc protein contained in the supernatant was measured.

Materials & Methods (2)

1. The scDb-Fc (0.8 mg/mL) solution prepared in the same manner as described in M & M (1) was heat-treated at 60℃ for 5 minutes.
2. The precipitate was removed by centrifugation.
3. The soluble fraction of scDb-Fc protein in the supernatant was measured.

Fig.1 CD spectrum analysis

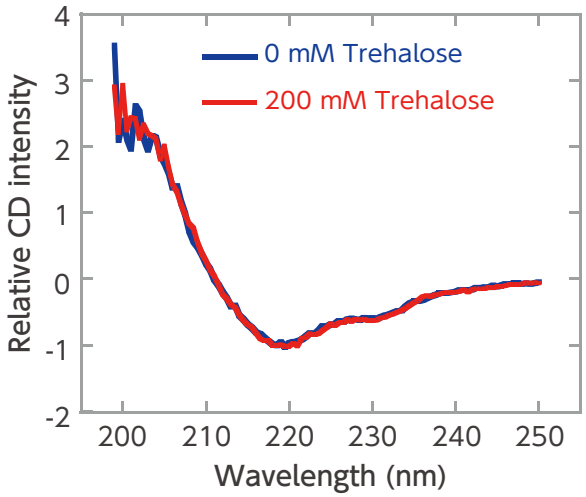
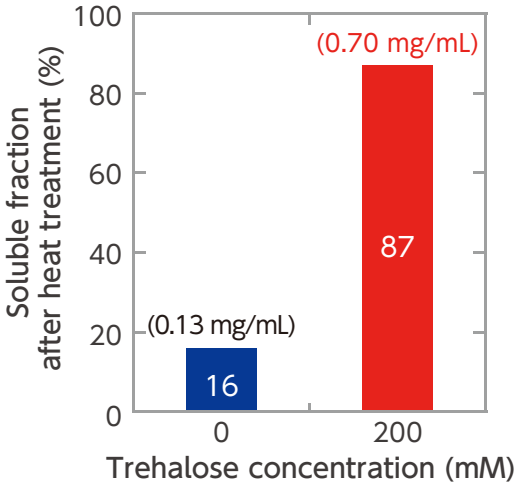


Fig.2 Effect on heat denaturation



Results

▲ Addition of trehalose (200 mM) had no effects on the structure of scDb-Fc protein (Fig. 1).
▲ Protein aggregation caused by heat denaturation was greatly reduced by trehalose (Fig. 2).

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Evaluation of the suppression of protein aggregation in CHO cell culture ②

Materials & Methods

1. The CHO Top-H cell line producing a bispecific antibody (single-chained diabody with Fc: scDb-Fc) was grown in cell culture media containing 150 mM trehalose. The cells were then cultured in an animal cell culture bioreactor (1 L scale, medium capacity 0.7 L) with or without addition of 150 mM trehalose.
2. After purifying the scDb-Fc protein from the culture supernatant by protein A affinity chromatography, the secondary structures (monomer, dimer and large aggregates), and their cohesiveness were evaluated by circular dichroism (CD)/fluorescence spectroscopy and gel filtration column chromatography, respectively.

Results

- ▲ A decrease in the ratio of large aggregates was observed by adding trehalose to the culture medium as compared with the culture medium without trehalose. On the other hand, the ratio of the monomeric scDb-Fc protein, which is an indication of no aggregation, increased while no effect on the dimer was observed (Fig. 3).
- ▲ The secondary structure of the dimer was similar to that of the monomer with antibody-like β-strand structure (Fig. 4).
- ▲ The large aggregates had both a non-native β-strand structure and a misfolded structure in which a hydrophobic region is largely exposed (Fig. 5).

Trehalose concentration	Large Aggregates	Dimer	Monomer
0 mM	17.5%	10.3%	72.2%
150 mM	5.9% ↓	8.7%	85.4% ↑

Fig. 3 Gel filtration column chromatogram

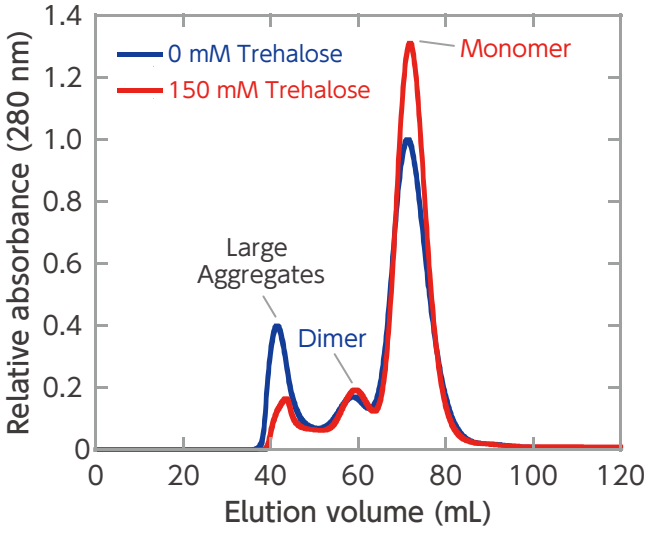


Fig. 4 CD spectrum analysis

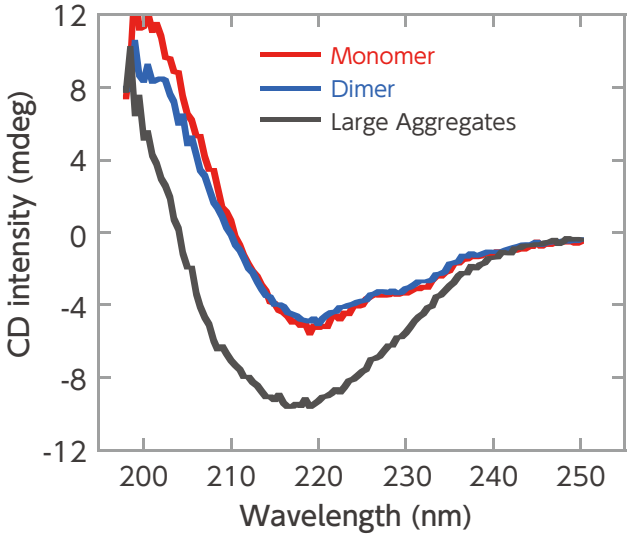
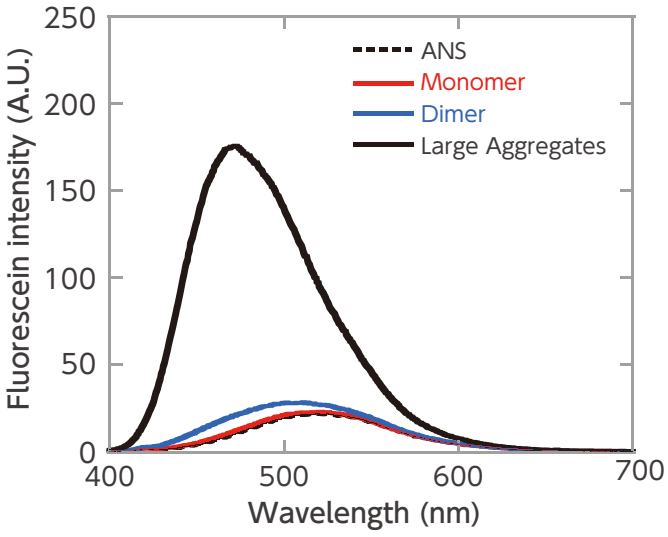


Fig. 5 Fluorescence spectrum analysis



ANS: 8-anilino-1-naphthalenesulfonic acid

Conclusion

- It was possible to culture the antibody-producing CHO cell line in the presence of TREHALOSE SG.
- TREHALOSE SG can suppress antibody aggregation, especially the formation of high-order aggregates, during the cell culture process.
- Use of TREHALOSE SG appears to increase efficiency of functional antibody production.

Reference

● Suppression of Antibody Aggregation in CHO Cell Culture by Trehalose Addition
Masayoshi Onitsuka and Takeshi Omasa: Institute of Technology and Science, The University of Tokushima. 16th Trehalose Symposium (2012)