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Hyperthermophilic TmHU: Features and Applications

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HU proteins are a group of small, homo- or heterodimeric proteins which are abundant in all prokaryotes. Each subunit of the protein is about 10 kDa in size (approximately 90 residues). They act as bacterial analoga of histone proteins, i. e. they bind into the minor groove of double-stranded DNA, thereby bending it; this leads to effective DNA condensation. DNA binding is usually non-sequence-specific. TmHU is the HU protein from the marine hyperthermophilic eubacterium *Thermotoga maritima*. It is a homodimer in solution, consisting of a helical stem region and two extended arms which are formed by antiparallel β-strands. The expression of the protein in recombinant *E. coli* is simple and straightforward; the protein is not toxic for *E. coli* and yields are usually 20 to 40 mg of purified protein per L medium. The purification is simplified by heat precipitation of all host proteins. TmHU does not contain Trp or Tyr residues and is therefore spectroscopically transparent in the UV region between 250 and 300 nm.

The protein has a high calculated pI (10.4) due to a very high number of basic amino acids; 30 % of its residues are Arg or Lys. The protein binds to dsDNA with a K_D of 73 nM, as determined by surface plasmon resonance measurements (BIACORE). The DNA binding is highly cooperative; the size of the binding site was determined to be 9 to 10 base pairs. Due to its hyperthermophilic origin, the melting point T_M is high (96 °C), well above the maximum growth temperature of *Thermotoga maritima*. Refolding of the thermally denatured protein is reversible with a yield of 88 %. An interesting feature of the protein is its protection of dsDNA from melting; we observed an increase in T_M of a synthetic dsDNA fragment of up to 47 °C when preincubated with TmHU. The protein also protects DNA from enzymatic digestion with a DNase, making it an attractive protecting factor for nucleic acids.

Due to its DNA binding and condensating properties, the protein was used as a reagent for the transfection of plasmids into eukaryotic cells. The optimal transfection protocol includes a heat precipitation step, incubating TmHU and DNA at elevated temperatures; during this process a dense precipitate is formed which is highly active with respect to cell transfections. Using this protocol, we achieved a 50 to 1,000-fold increase in transfection efficiency compared to other methods, e. g. DEAE-dextran mediated transfection. Both transient and stable transfections were possible. Confluent cells and loosely attached cells were also transfected; no limitations with respect to the cell line used was observed so far, and the addition of serum to the transfection assay which is often inhibiting does not show any negative effect on TmHU-based transfection. Addition of chloroquine does not increase yields further, suggesting that endosomal release of the particles taken up is not limiting. We could not detect any toxic effect for the target cells so far, even at very high TmHU concentrations in the medium.

When added to a standard transfection assay using lipofection (before the addition of the lipid), a synergistic increase of the transfection efficiency is observed; the increase in lipofection

efficiency is usually 5-fold to more than 30-fold, depending on the type of lipid and on the cell type used in the assay. When used *in vivo* (injection into mouse muscle) under optimal conditions, a 2- to 10-fold higher transfection efficiency (compared to similar protocols using naked DNA) is observed. This effect may be due to the high transfection efficiency of the protein, but in part also due to the DNase protection effect. The protein contains (accidentally) two active NLS (*nuclear localization signals*) per subunit in its sequence; these may be responsible for the high transfection efficiency.

Apart from the DNA transduction described before, the protein works also as a shuttle for peptides and proteins. Both eGFP and a toxic peptide fused to the protein are transported efficiently into eukaryotic cells. Taking these data together, it is demonstrated that the hyperthermophilic TmHU protein from *Thermotoga maritima* has interesting features for cell biological applications and for therapeutical usage as an efficient gene transfer system, for vaccination, or for the transport of therapeutic peptides and proteins into cells.

References

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