FG winds
Issue 9
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Isolation of drug target protein (4)
Biotin-Streptavidin Binding assay【Biotinylated MTX】

Introduction

Streptavidin is a protein that binds to biotin with a very strong affinity, and biotin is used as a low-molecular-weight compound that can be easily and effectively introduced into compounds, proteins, nucleic acids, etc. as a label. For this reason, immobilization of biotinylated ligands on streptavidin beads and purification experiments of target substances using them are being conducted in a wide range of research fields.
There are two approaches to experiments with streptavidin beads, “The direct method” and “The indirect method”. The method of reacting the ligand previously bound to the beads with the target is called the direct method, and the method of reacting the ligand with the target and then binding the ligand-target complex to the beads is called the indirect method. Use these methods properly according to the purpose of the experiment.
In this study, chemical pull-down of DHFR by MTX (methotrexate) was performed using biotinylated MTX and streptavidin beads. In addition to comparing performance with other companies' beads, it also shows the importance of the length of the spacer arm that connects MTX and biotin. This is because streptavidin can cause steric hindrance, which can interfere with the binding of ligands to target proteins.

Result

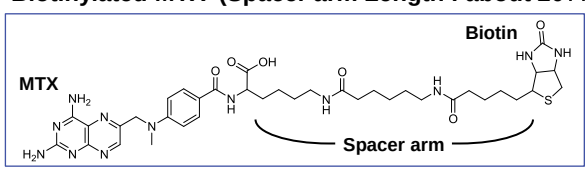
Biotinylated MTX with different spacer arm lengths were immobilized on streptavidin beads, and DHFR was purified from HeLa cell lysate. As a control, the same operation is performed for the MTX amino group derivative (without biotin) immobilized on NHS beads.

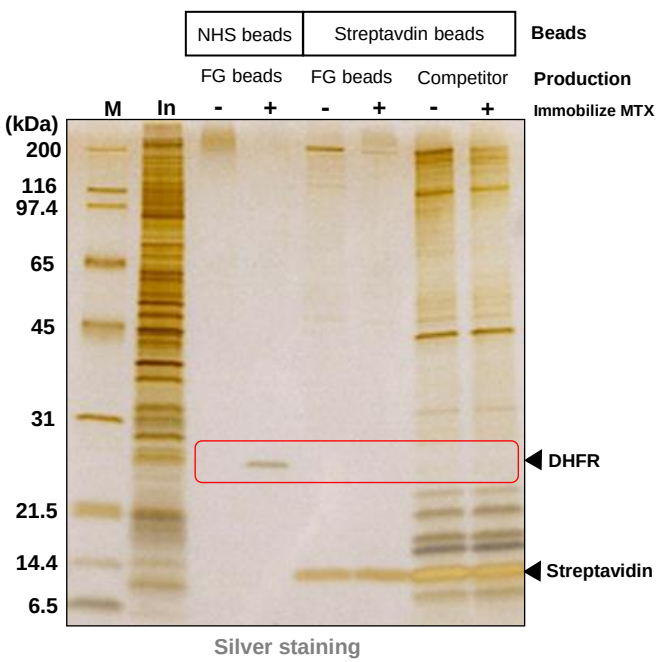
The following points can be confirmed from the two in the figure below.

・FG beads have less non-specific adsorption than other companies' beads.

・DHFR could not be purified with a spacer arm of about 20 Å, but could be purified with a spacer arm of about 30 Å. From this, it was confirmed that the length of the spacer arm should be set to about 30 Å or more when biotinlating the low molecular weight compound.

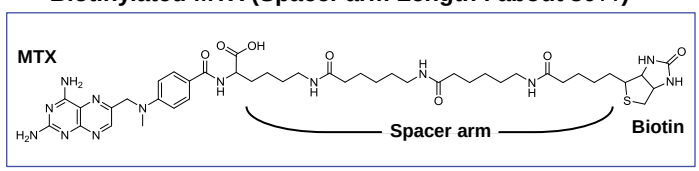
Biotinylated MTX (Spacer arm Length : about 20 Å)

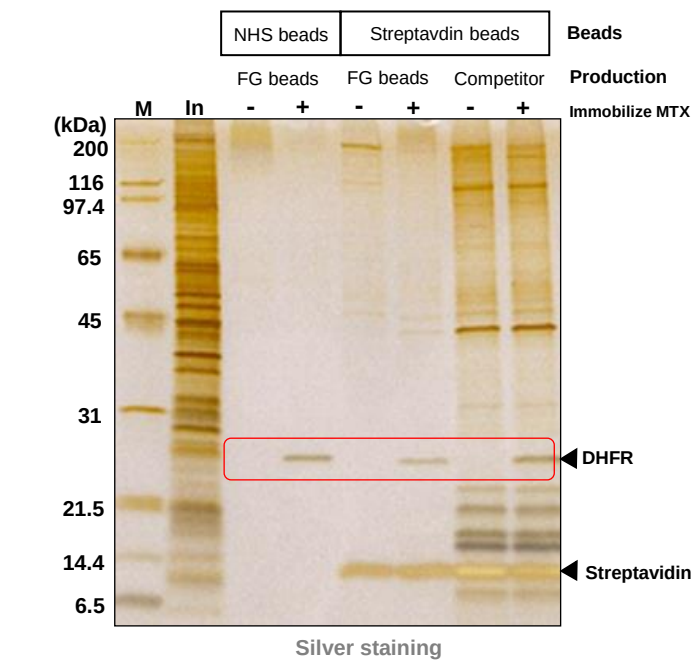




Silver staining

Biotinylated MTX (Spacer arm Length : about 30 Å)





Silver staining

Materials and method

■Materials

1. Streptavidine beads
2. Biotinylated MTX
3. PBS(-)(137mM NaCl, 2.7mM KCl, 8.1mM $Na_2HPO_4 \cdot 12H_2O$, 1.5mM KH_2PO_4)
4. Wash buffer (10mM HEPES-NaOH(pH7.9), 50mM KCl, 0.2mM EDTA, 10% glycerol)
5. HeLa cell extracts (cytosolic fraction) — 3mg/ml
6. 100mM KCl buffer (20mM HEPES-NaOH(pH7.9), 100mM KCl, 1mM $MgCl_2$, 0.2mM $CaCl_2$, 0.2mM EDTA, 10% glycerol, 0.1% NP-40, 1mM DTT, 0.2mM PMSF)
7. SDS sample buffer (62.5mM Tris-HCl(pH6.8), 0.005% BPB, 2% SDS, 10% glycerol, 5% 2-mercapto ethanol)

■FG beads® information

Product name	Streptavidin beads
Product number	TAS8848N1170
Storage temperature	2-8ºC
Storage buffer	10mM HEPES(pH7.9), 50mM KCl, 1mM EDTA, 10%glycerol
Size of beads	190nm ± 20 nm
Binding capacity (for 1mg of beads)	Free biotion >1200pmol
	Biotinylated Antibody >13ug
	Biotinilated ssDNA (50mer) >13ug
	Biotinylated BSA >3ug

■ Method 1 (Immobilize biotinylated MTX)

1. Immobilize biotinylated MTX on beads
Transfer 0.5mg beads to a tube, and wash beads with PBS(-) 3times at 4ºC.
Add 0.1mM biotinylated MTX diluted in 200ul PBS(-) to beads, and 200ul PBS(-) to another beads (Control beads).
Mix for 2hour at 4ºC.

2. Wash
Wash biotinylated MTX immobilized beads and Control beads with Wash buffer 3 times at 4ºC, and storage with 200ul Wash buffer.

■ Method 2 (Affinity Purification)

1. Beads equilibration
Wash 0.5mg of each MTX immobilized beads with 100mM KCl buffer 3 times at 4ºC.

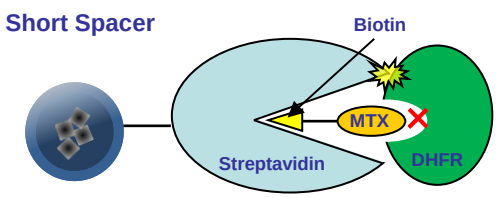
2. Reaction
Add 200ul HeLa cell extract and resuspend beads.
Incubate with rotation for 2hour at 4ºC.

3. Wash
Separate magnetically and remove supernatant .
Wash beads with 100mM KCl buffer 3 times at 4ºC.

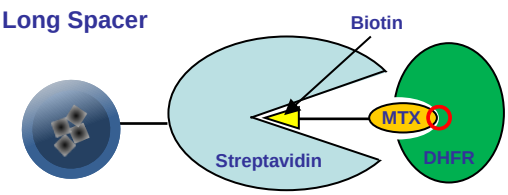
4. Elution
Add 40ul of SDS sample buffer and resusupend beads.
Boil for 5min at 98ºC, and remove beads.

5. Analyze the samples by SDS-PAGE and silver stainning.

■ Influence of Spacer Lengh on Steric hindrance



MTX has no accessibility for DHFR



MTX has accessibility for DHFR