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In vitro comparison of complementary interactions between synthetic ligands and tissue plasminogen activator (t-PA) by means of high performance monolithic disk chromatography

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1 Introduction

t-PA enables efficient dissolution of blood clots by converting plasminogen in its active form, plasmin, dissolving the major component of blood clots, fibrin. t-PA and plasminogen possess a high affinity binding site for fibrin, but also some synthetic polymers can provide the stimulating effects of plasminogen activation. HPMDC is a very fast, efficient and suitable tool for the isolation of biological active compounds.

Affinity interactions of different oligo/polymer forms of linear and branched lysine derivatives were compared to natural affinity counterparts to t-PA (mAb, plasminogen, fibrinogen) to make a practical choice of affinity systems to be used in down stream processing of recombinant t-PA.

2 Immobilisation of protein ligands and solid phase peptide synthesis

Solid phase peptide synthesis

· solid phase peptide synthesis by Fmoc strategy was accomplished inside the cartridge (housing plus syringe), deprotection of side chains outside of cartridge due to chemical irresistance towards 90 % TFA.

Immobilisation of protein ligands

· wash disk with ethanol, ethanol-water (1:1) and water.
· immerse disk in 0.1 M sodium carbonate buffer (pH 9.3) for 2 hours.
· transfer disk to 1 ml 5.0 mg/ml protein solution in the same buffer. let reaction proceed for 16 hours at 34 °C without stirring.
· wash with initial sodium phosphate buffer.
· wash with PBS solution containing 0.02 % sodium azide for storage at 4 °C.

3 Analysis

Synthetic peptides were analyzed by RP-HLPC and size exclusion chromatography of acidic hydrolyzed samples. The amount of protein ligands coupled to the matrix was monitored by Lowry assay. Affinity characteristics were calculated from frontal analysis. Model solutions of t-PA from 0.01 to 0.5 mg/ml were used. ELISA was carried out to determine t-PA concentration of the samples. Puritiy was observed by SDS-PAGE with silver staining. Standard mixtures of proteins were passed through the sorbents. In all cases non-specific interaction did not exceed 2 % from applied protein sample.

4 Results

Ligand	$q_{immobil}$ [µmolx10/m]²	q_{adsorb} [µmolx10/m]²	$q_{immobil}/q_{adsorp}$	K_{diss} [µmol/L]
mAb	0.20	0.10	2.00	0.2
Pmg	0.45	0.05	9.00	0.9
Fbg	0.05	1.30	0.04	14.0
K_{15}A (den.)	9.20	0.30	27.70	4.9
K_{12}	13.10	0.30	43.00	4.5
K_8	15.90	0.40	38.80	2.7
K_4	16.60	0.30	58.80	1.0
K_8GPRP	14.30	0.10	142.90	5.4
K_4GPRP	14.30	0.10	158.90	4.9
GPRP	15.90	0.10	175.90	1.8

5 Results

Synthetic ligands	t-PA µg	Yield %
K_{15}A	2.5	71
K_{12}	1.3	37
K_4	1.0	29
K_8GPRP	1.4	40
K_4GPRP	1.2	34
GPRP	3.0	86

Crude CHO cell supernatant was used to evaluate the purification efficiency of t-PA from a biological sample. The disk was loaded with sample in PBS buffer (pH 7.0). Specific bound t-PA was eluated with 0.01 M HCl after washing with 2 M NaCl. Total amount of t-PA was 3.5 µg.

mAb monoclonal antibodies
Pmg plasminogen
Fbg fibrinogen
HPMDC high performance monolithic disc chromatography
K_{15}A (den.) dendrimer peptide
K_n, K_8GPRP linear peptides
peptides in single letter code

6 Discussion

The amount of immobilized ligands is a function of molecular size of ligands. Highest capacity has been found for small ligands due to higher diffusion coefficient and less sterical hindrances inside the pores. While homo- and heteropeptides show no significant differences, capacity decreases for dendrimeric K_{15}A and proteins.

Adsorption capacity and values of K_{diss} show strong interaction between t-PA and mAbs. A specific complex with t-PA and every second mAb is formed. Structure safety and biological function of proteins is preserved on the surface.

About 30 to 60 peptides bind to one t-PA molecule due to the size difference between t-PA-peptide complex and single peptide. A transition from linear to branched forms of lysine cause no significant changes in $q_{ads.}$ Comparing dissociation constants it can be noticed that polymer forms of lysine show less intensive coupling, while K_{12} and dendrimeric ligand take intermediate position between polymer and short peptides. The data demonstrate that affinity properties of GPRP are unaffected by introduced lysine residues or increasing its length.

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