Cell penetrating peptides. How do they get through membranes?

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In 1988 it was discovered, that a natural polycationic protein, the trans-acting activator of transcription (TAT) of the human immunodeficiency virus (HIV-1), passed very efficiently through cell membranes of cultured mammalian cells. Covalently binding the TAT protein (or a specific peptide sequence of it) to proteins or fluorescent markers allowed these molecules also to traverse the cell membrane. Following this discovery, additional polycationic peptides of natural (vp22 and AntP) and synthetic origin (transportan) have been identified which also facilitate cellular uptake of attached cargos such as genes, proteins, or even nanoparticles. These peptide sequences were named alternatively cell-penetrating peptides (CPP), protein transduction domains (PTD), or Trojan horse peptides. Their common characteristic feature is a large content of basic amino acids, in particular arginin, leading to highly charged molecules. A fairly large range of these peptides is commercially available now but in spite of their potential use for gene transfer, drug delivery, and intracellular imaging their mode of action is still mysterious.

A recent theory for the translocation suggests the formation of micellar structures within the lipid part of the cell membrane. We have hence studied the binding of the protein transduction domain (PTD) of human (HIV-1) TAT peptide (amino acid residues 47-57, electric charge z_p =+8) to membranes containing various amounts of negatively charged lipid (POPG). Monolayer expansion measurements demonstrate that TAT-PTD insertion between lipids is possible only for loosely packed monolayer films. For densely packed monolayers (surface pressure > 29 mN/m) and for lipid bilayers, no insertion is possible. Under the latter conditions the peptide remains superficially adsorbed at the outer surface of the membrane. Membrane adsorption was then quantified with isothermal titration calorimetry (ITC). The reaction enthalpy is $\Delta H^0 \approx$ -1.5 kcal/mol peptide and is almost temperature-independent with $\Delta C^0_p \approx 0$ kcal/(mol K), indicating equal contributions of polar and hydrophobic interactions to the reaction heat capacity. The binding mechanism can be described by an electrostatic attraction/chemical adsorption model. The overall binding constant is $K_{app} \approx 10^3$-10^4 M^{-1} and the electrostatic attraction energy, calculated with the Gouy-Chapman theory, accounts for ≈ 80% of the binding energy. In contrast, the chemical adsorption constant, describing the partitioning of the peptide between the lipid water interface and the membrane surface, is small and is $K_p \approx$ 1-10 M^{-1}. Deuterium and phosphorus-31 nuclear magnetic resonance demonstrate that the lipid bilayer remains intact upon TAT-PTD binding. The NMR data provide no evidence for nonbilayer structures and also not for domain formation. The integrity of the membrane bilayer is further supported by the absence of dye efflux from single-walled lipid vesicles. A peptide translocation mediated by the lipid components of the membrane can therefore be excluded [1].

A more realistic translocation pathway is based on the binding of the protein transduction domain (PTD) to extracellular glycosaminoglycans. We have studied the interaction of TAT-PTD with three different glycosaminoglycans with high sensitivity ITC and provide the first quantitative thermodynamic description of these equilibria. The polysulfonated glycosaminoglycans were found to exhibit multiple identical binding sites for TAT-PTD with only small differences between the three species as far as the thermodynamic parameters are concerned. Heparan sulfate (HS, molecular weight, 14.2 ± 2 kDa) has 6.3 ± 1.0 independent binding sites for TAT-PTD which are characterized by a binding constant $K_0 = (6.0 \pm 0.6) \times 10^5$ M^{-1} and a reaction enthalpy $\Delta H^0_{pep} = -4.6 \pm 1.0$ kcal/mol at 28^0C. The binding affinity, ΔG^0_{pep}, is

determined to equal extent by enthalpic and entropic contributions. The HS-TAT-PTD complex formation entails a positive heat capacity change of ΔC^0_p = +135 cal/mol peptide, which is characteristic of a charge neutralization reaction. This is in contrast to hydrophobic binding reactions which display a large negative heat capacity change. The stoichiometry of 6–7 TAT-PTD molecules per HS corresponds to electric charge neutralization. Light scattering data demonstrate a maximum scattering intensity at this stoichiometric ratio, suggesting cross-linking and/or aggregation of HS-TAT PTD complexes. Two other glycosaminoglycans, namely heparin and chondroitin sulfate B, were also studied with ITC. The thermodynamic parameters are K_0 = (6.0 ± 0.8) x 10^5 M^{-1} and ΔH^0_{pep} = -5.1 ± 0.7 kcal/mol for heparin and K_0 = (2.5 ± 0.5) x 10^5 M^{-1} and ΔH^0_{pep} = -3.2 6± 0.4 kcal/mol for chondroitin sulfate B at 28^0C. The close thermodynamic similarity of the three binding molecules also implies a close structural relationship. The ubiquitous occurrence of glycosaminoglycans on the cell surface together with their tight and rapid interaction with the TAT protein transduction domain makes complex formation a strong candidate as the primary step of protein translocation [2].

We have measured the transport of CPPs across the membrane of live cells with confocal microscopy. It has been argued that the observed transport is an artifact caused by chemical fixation of the cells, a common preparation method for microscopic observation. We have synthesized a fluorescent derivative of the HIV-1 TAT protein transduction domain (Fg-$CPP^{TAT(PTD)}$) and have observed its uptake into non-fixated living fibroblasts with time-lapse confocal microscopy, thus eliminating the need for fixation. We find that Fg-$CPP^{TAT(PTD)}$ enters the cytoplasm and nucleus of non-fixated fibroblasts within seconds arguing against the suggested artifact of cell-fixation. Using differential interference contrast microscopy, we furthermore detect dense aggregates on the cell surface. Several observations suggest that these aggregates consist of Fg-$CPP^{TAT(PTD)}$ bound to membrane-associated heparan sulfate (HS). The aggregates grow in parallel with Fg-$CPP^{TAT(PTD)}$ uptake and are detected only on fibroblasts showing Fg-$CPP^{TAT(PTD)}$ uptake. The aggregation of Fg-$CPP^{TAT(PTD)}$ on the membrane surface bears close resemblance to earlier reports of "capping" of cell surface molecules combined with a polarized endocytotic flow. Enzymatic removal of extracellular HS reduced both Fg-$CPP^{TAT(PTD)}$ uptake and aggregate formation demonstrating that HS is involved in the uptake mechanism[3].

We have investigated the effect of non-labeled $CPP^{TAT(PTD)}$ on the metabolism of intact fibroblasts by measuring the extracellular acidification rate (ECAR) with a cytosensor microphysiometer. Short exposure (<2.5 min) of the cells to CPP concentrations of 500 μM caused a 22% reductionof the ECAR which was however reversible upon superfusing the cells with buffer only. At repeated exposure, recovery to baseline values was incomplete suggesting that the peptide is toxic in long-term applications [3].

References

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