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Adaptive translocation: the role of hydrogen bonding and membrane potential in the uptake of guanidinium-rich transporters into cells

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Abstract

A mechanistic hypothesis is presented for how water-soluble guanidinium-rich transporters attached to small cargoes (MW ca. <3000) can migrate across the non-polar lipid membrane of a cell and enter the cytosol. Positively charged and water-soluble, arginine oligomers can associate with negatively charged, bidentate hydrogen bond acceptor groups of endogenous membrane constituents, leading to the formation of membrane-soluble ion pair complexes. The resultant less polar, ion pair complexes partition into the lipid bilayer and migrate in a direction, and with a rate, influenced by the membrane potential. The complex dissociates on the inner leaf of the membrane and the transporter conjugate enters the cytosol. This mechanism could also be involved in the translocation of guanidinium-rich molecules that are endocytosed due to their size or the conditions of the assay, across the endosomal membrane.

Keywords: Arginine-rich transporters; Membrane potential; Ion pairs; Drug delivery; Protein translocation; Endocytosis; HIV tat; Antennapedia; FGF

Contents

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1. Introduction

Biological membranes have evolved in part to prevent xenobiotics from passively entering cells [1]. Numerous organisms have developed proteins, many of which are transcription factors that breach these biological barriers through a variety of mechanisms [2]. The protein HIV tat, for example, when used in vitro, rapidly enters the cytosol (and nucleus) of a wide spectrum of cells after endocytosis [3]. However, the nine amino acid peptide required for the uptake of HIV tat, residues 49–57 (RKKRRQRRR), appears itself to utilize an additional mechanism as evident from its uptake even at 4 °C, by a route differentiated from the intact protein [4]. We have found that guanidinium-rich oligomers enter suspension cells more effectively than the tat nonamer [5] often without the production of observable endocytotic vesicles [6,7]. We describe herein studies on the cellular uptake mechanism of guanidinium-rich transporters conjugated to small molecules (MW ca. <3000).

Pertinent to the formulation of a mechanism, our previous studies demonstrated that the guanidinium head groups of tat 49–57 are critical for its uptake into cells. Replacement of any of the arginine residues with alanine diminished uptake. Conversely, replacement of all non-arginine residues in the tat nonamer with arginines provided transporters that exhibit superior rates of uptake. Charge itself is necessary, but not sufficient, as is evident from the comparatively poor uptake of lysine nonamers [5,6]. The number of arginines is also important, with optimal uptake for oligomers of 7–15 residues [6,8]. Backbone chirality is not critical for uptake. Even the position of attachment and length of the side chains can be altered as shown with guanidinium-rich peptoids that exhibit highly efficient uptake. Changes in the backbone composition and in the side chain spacing also can increase uptake [5,9–12]. Even highly branched guanidinium-rich oligosaccharides and dendrimers are efficient transporters [7,13–15]. In contrast to receptor-mediated uptake, an increase in conformational flexibility generally favors uptake.

2. Adaptive partitioning: the conversion of water-soluble to membrane-soluble conjugates through ion pairing of guanidinium ions and fatty acids

Several mechanisms could accommodate the above structure function relationships for guanidinium-rich transporters and some could operate concurrently. A receptor-mediated process is inconsistent with the broad range of structural modifications that promote uptake and especially the observation that more flexible systems work better. Conventional passive diffusion across the non-polar interior of the plasma membrane is difficult to reconcile with the polarity of the arginine oligomers and the dependency of uptake on the number of charges. In contrast to passive diffusion in which a migrating conjugate maintains its polarity, the polar, positively charged guanidinium oligomers could adaptively diffuse into the non-polar membrane by recruiting negatively charged cell surface constituents to transiently produce a less polar, ion pair complex. Indeed the polarity and bioavailability of many proteins and peptides can be changed by the intentional pre-formation of a non-covalent complex with anionic agents such as SDS [16]. To test whether a highly water-soluble guanidinium-rich oligomer could be rendered lipid soluble through ion pair formation, a fluoresceinated arginine octamer (Fl-aca-D-Arg_8-$CONH_2$) was added to a bilayer of octanol and water. Not surprisingly, the highly polar charged system partitioned almost exclusively (>95%) into the water layer (Fig. 1). When, however, a surrogate for a membrane bound fatty acid salt, namely, sodium laurate, was added to this mixture, the transporter partitioned completely (99%) into the octanol layer [17]. The relative partitioning was quantified by separation of the layers and analysis of the dissolved agents.