Translocation of a β-Peptide Across Cell Membranes
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Biological membranes are critical for life because the hydrophobic barrier between cytoplasm and external medium allows cells to regulate entry of water-soluble molecules from the environment. This barrier effect, however, drastically limits the delivery of polar molecules (peptides, nucleic acids, drugs) to intracellular targets. Over the past decade, several short peptides have been found to cross biological membranes rapidly.1 For example, HIV Tat and related DNA-binding proteins were shown in the late 1980s to move spontaneously into nuclei from the extracellular milieu,2 and more recent work has demonstrated that this translocation activity depends on relatively short segments within such proteins.3 The Tat 47–57 translocation sequence is among the shortest examples.4 Hydrophobic segments from signal peptides5 and arginine oligomers6 also move across cell membranes, as do peptoid (i.e., N-alkyl-glycine) oligomers bearing multiple guanidinium-tipped side chains.7 Translocating peptides can deliver diverse cargos, including proteins,8 peptide nucleic acids,9 and drugs,10 to intracellular targets. Here we show that a β-amino acid analogue of Tat 47–57 (1) translocates across human cell membranes with efficiency comparable to Tat 47–57 itself (2).

β-Amino acid oligomers (“β-peptides”) have been studied extensively in recent years.11 They can adopt well-defined protein-like secondary structures, and several β-peptides with interesting biological activities have been reported.12 We were motivated to undertake the present study because β-peptides are resistant to degradation by proteases13 and because our ability to control β-peptide secondary structure might ultimately be useful for elucidating the mechanism of translocation.

β-Peptide 1 is constructed from β-substituted β-amino acid residues (“β-residues”), which are available enantiospecifically from the corresponding α-amino acid residues via methodology developed by Seebach et al.14 as modified by Müller et al.15,16 Both the Tat fragment 2 and β-peptide analogue 1 bear N-terminal fluorescein labels, allowing detection of cell membrane transit via confocal fluorescence microscopy.

Figure 1 shows representative results with HeLa cells for 1 and 2.17 Neither molecule is observed more than faintly within the cells when incubated with them at a concentration of 0.05 μM, but at 0.10 μM both molecules can be unambiguously detected inside the cells. The intracellular fluorescence intensity rises when the concentration of 1 or 2 is increased to 0.5 μM. (The background fluorescence in these images was unaffected by additional washing of the cells prior to fixation.) Similar results were obtained when fixation was omitted.

We conducted control experiments to determine the effect of length on 1. In truncation studies of Tat 48–60, Vivés et al. showed that Arg-55, Arg-56, and Arg-57 were critical for translocation; in contrast, the presence or absence of Pro-58, Pro-59, and Gln-60 had little effect on translocation.34 Therefore, we examined truncated β-peptide 3, which lacks the three C-terminal β3-homoarginine residues of 1, and the α-peptide analogue 4. No cell penetration could be detected after treatment with up to 1 μM 3 or 4 (data in Supporting Information), a 10-fold higher concentration than is required for detectable cell penetration by full-length β-peptide 1 and its α-analogue 2. In addition, free fluorescein did not enter the cells under these conditions. These results show that a minimum β-peptide length and charge is required for movement across cell membranes; comparable conclusions have been drawn from studies of arginine oligomers6 and peptoid analogues.7

![Figure 1](image-url)
To identify the major intracellular destination of these molecules, cells were co-stained with propidium iodide, which binds specifically to nucleic acids. The co-staining results with Tat fragment 47–57 of HeLa cells were co-stained with propidium iodide, which binds specifically to nucleic acids. The overlay images show that peptides 1 and 2 co-localize with propidium iodide to the nucleus; a result confirmed by bright-field microscopy (not shown); the bright spots within each nucleus represent nucleoli. The microscope settings were identical for each peptide and dose. Bar: 10 μm.

β-peptide 1 represents a platform that can be used to explore the effects of conformational constraints on translocation activity.

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Supporting Information Available: Discussion of the synthesis and purification of β-peptide 1, confocal microscopy images of cells incubated with truncated peptides 3 and 4, and circular dichroism spectra of compound 5 in water and methanol (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References
(16) A discussion of the synthesis and purification of 1 is included in the Supporting Information.
(17) HeLa cells were obtained from the American Type Culture Collection (Manassas, VA). HeLa cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 μg/mL), with antibiotics added at 22° C until the end of incubation. In addition, cells were then washed three times with PBS at 4° C until the end of the fixation procedure. Fixed cells were then incubated with 0.2% paraformaldehyde (4% w/v) in PBS for 5 min at room temperature. For experiments at 4° C, the protocol was the same except that all incubations were performed at 4° C until the end of the fixation procedure. Fixed cell monolayers were permeabilized with Triton X-100 (0.1% w/v) for 10 min, and washed twice with PBS (pH 7.3). Cells were stained with 5 μM fluorescein-labeled peptides (as above) in PBS for 5 min at room temperature. Cells were then washed three times with PBS and mounted onto glass microscope slides using mounting medium for fluorescence. The distribution of the fluorescence was examined on a Zeiss Axiosvert 100TV confocal microscope equipped with a Krypton/argon laser and 63x oil immersion objective lens (Leica). Images were captured with BioRad Laser Sharp MRC1024 and Adobe Photoshop v4.0.1 software.