Cell-Penetrating Peptides


The many futures for cell-penetrating peptides: how soon is now?

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Abstract

Studies of CPPs (cell-penetrating peptides), sequences that are also commonly designated as protein transduction domains, now extend to a second decade of exciting and far-reaching discoveries. CPPs are proven vehicles for the intracellular delivery of macromolecules that include oligonucleotides, peptides and proteins, low-molecular-mass drugs, nanoparticles and liposomes. The biochemical properties of different classes of CPP, including various sequences derived from the HIV-1 Tat (transactivator of transcription) [e.g. Tat-(48–60), GRKKRRQRRRPPQ], and the homeodomain of the Drosophila homeoprotein Antennapedia (residues 43–58, commonly named penetratin, RQIKIWFQNRRMKWKK), also provide novel insights into the fundamental mechanisms of translocation across biological membranes. Thus the efficacy of CPP-mediated cargo delivery continues to provide valuable tools for biomedical research and, as witnessed in 2007, candidate and emerging therapeutics. Thus it is anticipated that the further refinement of CPP technologies will provide drug-delivery vectors, cellular imaging tools, nanoparticulate devices and molecular therapeutics that will have a positive impact on the healthcare arena. The intention of this article is to provide both a succinct overview of current developments and applications of CPP technologies, and to illustrate key developments that the concerted efforts of the many researchers contributing to the Biochemical Society’s Focused Meeting in Telford predict for the future. The accompanying papers in this issue of Biochemical Society Transactions provide additional details and appropriate references. Hopefully, the important and eagerly anticipated biomedical and clinical developments within the CPP field will occur sooner rather than later.

CPP (cell-penetrating peptide) design and mechanisms of membrane translocation

The identification and early applications of Tat (transactivator of transcription)-derived sequences [1] and penetratin [2] as archetypical CPPs has since been consolidated by the identification of many additional CPP families. Thus, in addition to the many predominantly polycationic peptide sequences [Tat, penetratin and R8 (octa-arginine), etc.], other classes of monomeric CPP include amphipathic peptides (e.g. transportan, GWTLNSAGYLLGKINLKALAALAKKIL [3]) and sequences containing multiple histidine or proline residues (see Pujals et al. [4] on pp. 794–796). Additionally, CPPs may be entirely synthetic or fragments of peptides and proteins such as human calcitonin and cadherin. It is also possible to predict, using QSAR (quantitative structure–activity relationship) analysis, polycationic cryptomic CPPs hidden within the primary sequences of proteins [5]. Such an approach will expand the repertoire of known CPP vectors and also offers great promise for the identification of rhegnylogic CPPs that are themselves biologically active. Other more unusual CPPs include pentapeptides derived from the Bax-binding domain of Ku70 (see Gomez et al. [6] on pp. 797–801) that can rescue cells from apoptosis. Various chimeraic peptides capable of membrane translocation have also been developed, and these may provide improved pharmacodynamic and pharmacokinetic parameters compared with monomeric sequences. Thus it is possible to include...
likely that, at low micromolar concentrations, CPPs such as CPP import requires endocytosis [8]. Thus it would seem that, at low micromolar concentrations, CPPs such as Tat and R₄ can both bind initial components of proteoglycans on the cell surface (see Abes et al. [9] on pp. 775–779 and Futaki et al. [10] on pp. 784–787). Subsequently to this electrostatic interaction, energy-dependent endocytosis leads to the internalization of the CPP into subcellular endosomal compartments. These events may be regulated by the activation of small G-proteins such as Rho-A and Rac-1 that induce a re-organization of the F-actin (filamentous actin) cytoskeleton [10]. Endocytosis is used here in a broad sense and may include a combination of macropinocytosis and other endocytic events dependent upon clathrin and lipid rafts. Indeed, the re-organization of lipid rafts to the cell surface may lead to increased CPP uptake during inflammation. Thus different contributions of various endocytotic mechanisms may account for observed differences in translocation efficacies that are cell-type- and/or sequence-dependent (see Poon and Gariépy [11] on pp. 788–793). The molar fraction of CPP or CPP-delivered cargo that escapes the endosomal compartment remains a matter of debate, but, clearly, this must happen to allow a bioactive cargo to reach and modulate intracellular targets located in the cytoplasm, nucleus or other cellular compartment. The employment of multiple histidine residues, sensitive to lysosomal acidification, or unsaturated lipids as components of chimaeric CPPs may facilitate the release of translocated materials effectively trapped in endosomes. Higher concentrations of polycationic CPPs probably induce other modes of cellular entry, leading to a more generalized cytoplasmic distribution of CPP. It is uncertain whether other CPP families also utilize energy-dependent endocytosis as a predominant mode of cellular penetration. Indeed, studies with various inhibitors of discrete endocytotic pathways are often misleading. Although debate is ongoing, robust functional assays with measurable biological responses should at least establish the degree of endosomal entrapment/release and provide a strategy to overcome this possible limitation. An assay system that can accurately determine endosomal leakiness on a cellular level would greatly facilitate such studies.

### CPP-mediated delivery of oligonucleotides

CPPs have been used to affect the intracellular delivery of many classes of bioactive cargo. However, there appears to be an increasing interest in the application of CPPs for the delivery of oligonucleotides (see Abes et al. [9], Järver et al. [12] on pp. 770–774 and Moschos et al. [13] on pp. 807–810). Oligonucleotides translocated by CPPs include charge neutral PNA (peptide nucleic acid) and PMOs (phosphorodi- amidate morpholino oligomers), and double-stranded siRNA (short interference RNA). The delivery of uncharged oligonucleotides, PNA and PMO, can be achieved using a variety of tandem constructs utilizing single CPPs such as Tat and R₄-penetratin (see Moschos et al. [13] and Abes et al. [9]) or more sophisticated biohuttle carriers (see Pipkorn et al. [14] on pp. 829–832). Arginine-rich CPPs including (R-Ahx-R)₄ (6-amino hexanoic acid-spaced oligoarginine) (see Moulton et al. [15] on pp. 826–828) and R₄-penetratin are particularly useful for PNA or PMO delivery although it is uncertain whether a reducible disulfide bond, connecting CPP and oligonucleotide, is a necessary or advantageous component of such conjugates. However, the CPP-mediated delivery of siRNA is a more challenging problem, since multiple anionic charges of this biomolecule inhibit the translocation efficiency of single CPP sequences. Progress towards the effective delivery of siRNA has been made using non-covalent CPP–siRNA complexes that markedly improve cellular import. Thus it is possible to stabilize cell-permeable nanoparticles by combining siRNA with chimaeric CPPs at appropriate ratios. Other strategies employ CPPs to translocate cationic liposomes to which charged oligonucleotides are complexed.

### Biomedical and therapeutic applications

There is now convincing evidence that CPPs have therapeutic potential, and, indeed, diverse biomedical applications of CPP technologies have dramatically advanced in recent years. This section summarizes some of these advances, but is, necessarily, only a biased fraction of the many developments happening on a worldwide scale.

Improved delivery of oligonucleotides (see above) offers enormous potential for therapeutic intervention in diseased cells where aberrant protein expression is a causative phenomenon. Potential strategies for gene-expression control include splice correction, steric block and RNA interference. It is noteworthy that the Tat peptide has been widely employed in such studies and can be used to deliver therapeutic siRNA when joined to a dsRNA (double-stranded RNA)-binding domain as a fusion protein. Moreover, data presented on behalf of Steven F. Dowdy (Howard Hughes Medical Institute, University of California) elegantly demonstrated the progress being made using RNA interference for in vivo applications. siRNA targeting of a splice variant of the EGF (epidermal growth factor) receptor (EGFRvIII) and additional targets can be used to treat glioblastoma-bearing mice. This mode of delivery, using multiple Tat domains, provides the dual function of masking the anionic charge of siRNA, while facilitating membrane translocation. Other possibilities for effective intracellular delivery include the
application of self-assembling peptide dendrimers (legomers) and short nucleic acid sequences (aptamers [11]).

Tat has also been employed in the construction of nano-carriers that enable the specific delivery of drugs to acidified pathological sites such as tumours and infarcts (see Torchilin [16] on pp. 816–820). The use of Tat to deliver BCL6 peptide inhibitors modulates B-cell phenotype and is being developed as a potential anti-lymphoma therapeutic (see Melnick [17] on pp. 802–806). Tat also enables the intracellular delivery of modular antigen-translocating molecules that may be useful for the treatment of allergies and the production of preventative vaccines (see Rhyner et al. [18] on pp. 833–834). It is significant that KAI Pharmaceuticals (see Chen and Harrison [19] on pp. 821–825) has also utilized Tat in the design of isoform-specific peptide modulators of protein kinase C. Such constructs, currently in clinical trials, have utility for acute myocardial infarction and cerebral ischaemia. Using a peptide phage display library to screen for novel protein transduction peptides, some have been discovered that are able to facilitate internalization of large protein complexes into cells, which can be useful for the treatment of conditions such as inflammatory bowel disease, diabetes and muscular dystrophy (see Tilstra et al. [20] on pp. 811–815).

It is without doubt that from their first serendipitous discovery to the present day, CPPs have opened many doors to a wealth of biomedical and therapeutic opportunities. An abundance of drug-discovery data and now a growing mass of in vivo applications are witness to the utility of CPPs as biomedical tools and molecular therapeutics. Considering the current inefficiency of traditional drug-discovery programmes, it is now clearly imperative that we advance CPP technologies further into drug development and the clinical setting.

References


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