

PEPTIDE-ENHANCED CATIONIC LIPID TRANSFECTIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Ser. No. 08/658,130, filed Jun. 4, 1996, U.S. Pat. No. 5,736,392 which in turn is a continuation-in-part of U.S. application Ser. No. 08/477,354, filed Jun. 7, 1995 (now abandoned), both of which are incorporated by reference in their entirety herein.

FIELD OF THE INVENTION

Compositions containing peptides, optionally conjugated to nucleic acid-binding groups, to lipids or to dendrimers, and transfection agents, including cationic lipids and dendrimer polymers, useful for transfecting eukaryotic cells are disclosed. Also disclosed are methods of transfecting eukaryotic cells employing such compositions.

BACKGROUND OF THE INVENTION

Lipid aggregates such as liposomes can function to facilitate introduction of macromolecules, such as DNA, RNA, and proteins, into living cells. Lipid aggregates comprising cationic lipid components can be effective for delivery and introduction of large anionic molecules, such as nucleic acids, into certain types of cells. See Felgner, P. L. and Ringold, G. M. (1989) *Nature* 337:387-388 and Felgner, P. L. et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:7413. Since the membranes of most cells have a net negative charge, anionic molecules, particularly those of high molecular weight, are not readily taken up by cells. Cationic lipids aggregate to and bind polyanions, such as nucleic acids, tending to neutralize the negative charge. The effectiveness of cationic lipids in transfection of nucleic acids into cells is thought to result from an enhanced affinity of cationic lipid-nucleic acid aggregates for cells, as well as the function of the lipophilic components in membrane fusion.

Dendrimers are a new type of synthetic polymers with regular, dendric branching with radial symmetry composed of an initiator core, interior layers (or generations) of repeating units, radially attached to the core and an exterior surface of terminal functional groups. See: D. A. Tomalia and H. D. Durst (1993) in E. Weber (ed.) *Topics in Current Chemistry*, Vol. 165: *Supramolecular Chemistry I-Directed Synthesis and Molecular Recognition*, Springer-Verlag, Berlin, pp.193-313. The size, shape and surface charge density of the dendrimer is controlled by choice of core, repeating unit, number of generations and terminal functional group. See: U.S. Pat. Nos. 5,527,524; 5,338,532; 4,694,064; 4,568,737; 4,507,466; and PCT patent applications; WO8801179; WO8801178; WO9524221; and WO9502397. "STAR-BURST" (Trademark, Dendritech, Inc.) or dense star polyamidoamine dendrimers have been reported to mediate efficient transfection of DNA into mammalian cells (J. F. Kukowska-Latolla et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:4897-4902 and A. Bielinska et al. (1996) *Nucleic Acids Res.* 24(11):2176-2182). PCT patent application WO9524221 relates to bioactive or targeted dendrimer conjugates; PCT patent applications WO9319768 and WO9502397 relate to polynucleotide delivery systems comprising dendrimers.

Transfection agents, including cationic lipids and dendrimers, are not universally effective for transfection of all cell types. Effectiveness of transfection of different cells

depends on the particular transfection agent composition and the type of lipid aggregate or dendrimer-complex formed. In general, polycationic lipids are more efficient than monocationic lipids in transfecting eukaryotic cells. Behr, J-P. et al. (1989) *Proc. Natl. Acad. Sci.* 86:6982-6986, Hawley-Nelson, P., et al. (1993) *FOCUS* 15:73 and U.S. Pat. No. 5,334,761 (Gebeyehu et al.). Behr et al. and EPO published application 304 111 (1990), for example, describe improved transfection using carboxyspermine-containing cationic lipids including 5-carboxyspermylglycine dioctadecyl-amide (DOGS) and dipalmitoylphosphatidylethanolamine 5-carboxyspermylamide (DPPES). Despite their relative effectiveness, however, successful transfection of eukaryotic cell cultures using polycationic lipid reagents often requires high dosages of nucleic acid (approximately 10^7 DNA molecules per cell). For transfection, the optimal charge ratio of DNA/dendrimer was found to be between 1:5 and 1:50 and G5 (generation 5)-G10 dendrimers were reported capable of mediating transfection. Transfection efficiency of a given dendrimer varied with cell type, as has been observed with cationic lipid-mediated transfection (J. F. Kukowska-Latolla et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:4897-4902).

Many biological materials are taken up by cells via receptor-mediated endocytosis. See: Pastan and Willingham (1981) *Science* 214:504-509. This mechanism involves binding of a ligand to a cell-surface receptor, clustering of ligand-bound receptors, and formation of coated pits followed by internalization of the ligands into endosomes. Both enveloped viruses, like influenza virus and alphaviruses, and non-enveloped viruses, like Adenovirus, infect cells via endocytotic mechanisms. See: Pastan, I. et al. (1986) in *Virus Attachment and Entry into Cells*, (Crowell, R. L. and Lonberg-Holm, K., eds.) *Am. Soc. Microbiology*, Washington, p. 141-146; Kielian, M. and Helenius, A. (1986) "Entry of Alphaviruses" in *The Togaviridae and Flaviviridae*, (Schlesinger, S. and Schlesinger, M. J., eds.) Plenum Press, New York p.91-119; FitzGerald, D. J. P. et al. (1983) *Cell* 32:607-617. Enhancement of dendrimer-mediated transfection of some cells by chloroquine (a lysosomotropic agent) suggests that endocytosis is involved in at least some dendrimer-mediated transfections.

The introduction of foreign DNA sequences into eukaryotic cells mediated by viral infection is generally orders of magnitude more efficient than transfection with cationic lipid or dendrimer transfection agents. Viral infection of all the cells in a culture requires fewer than 10 virus particles per cell. Although the detailed mechanism of fusion is not fully understood and varies among viruses, viral fusion typically involves specific fusogenic agents, such as viral proteins, viral spike glycoproteins and peptides of viral spike glycoproteins. Vesicular stomatitis virus (VSV) fusion, for example, is thought to involve interaction between the VSV glycoprotein (G protein) and membrane lipids (Schlegel, R. et al. (1983) *Cell* 32:639-646). The VSV G protein reportedly binds preferentially to saturable receptors such as acidic phospholipid phosphatidylserine (Schlegel, R. and M. Wade (1985) *J. Virol.* 53(1):319-323). Fusion of influenza virus involves hemagglutinin HA-2 N-terminal fusogenic peptides. See Kamata, H. et al. (1994) *Nucl. Acids Res.* 22(3):536-537.

Cell binding can also be enhanced, accelerated or made selective with peptides that bind cell receptors. For example, the penton-base protein of the Adenovirus coat contains the peptide motif RGD (Arg-Gly-Asp) which mediates binding to integrins and viral internalization via receptor-mediated endocytosis (Wickham, T. J. et al. (1995) *Gene Therapy* 2:750-756).