

CATIONIC OLIGOPEPTIDES HAVING MICROBICIDAL ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of application Ser. No. 705,455, filed Feb. 25, 1985, now abandoned, which is a continuation-in-part of application Ser. No. 609,410, filed May 11, 1984, now U.S. Pat. No. 4,659,692, issued Apr. 21, 1987, which is a continuation-in-part of application Ser. No. 443,087, filed Nov. 19, 1982, now U.S. Pat. No. 4,543,252, issued Sept. 24, 1985, which is a continuation-in-part of application Ser. No. 341,394, filed Jan. 21, 1982, now abandoned, which disclosure are incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

There is a continued interest in the development of novel microbicides which do not have adverse effects on the host, but have high potency against an undesirable pathogen or other microorganism. One area of interest in locating compounds which are microbically active are naturally occurring compounds which a host uses in its natural defense.

The function of macrophages and granulocytes, e.g., neutrophils, in host defense is well documented. There are numerous reports describing the cellular antimicrobial efficiency. See, for example, Hocking and Golde, *N. Engl. J. Med.* (1979) 301:580-587, who describe the role of rabbit alveolar macrophages, and Lehrer, *J. Clin. Invest.* (1972) 51:2566-2572 and Mandell, *Infect. Immun.* (1974) 9:337-341, who describe the antimicrobial activity of human neutrophils and human polymorphonuclear neutrophils.

Studies of the effect of granule extracts on microorganisms may be found in Rest et al., *Infect. Immun.* (1978) 19:131-137; Rest et al., *ibid* (1977) 16:145-151 and Modrzakowski et al., *ibid* (1979) 23:589-591. See also, Modrzakowski and Spitznagel, *ibid* (1979) 25:597-602, and Shafer et al., *ibid* (1984) 43:834-838, who report cationic proteins of greater than 30 kDal isolated from granules having bactericidal activity. As macrophages and granulocytes are thought to arise from a common stem cell and as these cells contain certain constituents in common (e.g. lysozyme), macrophages and neutrophils appear to be a potential source of compounds having microbicidal activity.

In order for naturally occurring compositions to be useful, there are many criteria. If one is to synthesize an oligopeptide, it is desirable that the number of amino acid units be relatively small. While hybrid DNA technology offers an opportunity to make large proteins, where a microbicidal protein is of interest, the toxic effect of the product on the host evidently creates difficulties in its economic synthesis. Secondly, the microbicidal compound should be independently active and not require the presence of a number of other materials for its activity. Where only a mixture of materials have the microbicidal activity, formulation problems can become severe. Thirdly, one is concerned with the environment in which the naturally occurring compound is active, that is, whether the naturally occurring compound can be formulated in such a way as to be acceptable to the host to be protected from the invading microorganism. Furthermore, by determining the amino acid composition and specific sequence, the pep-

tide may be varied to enhance such properties as stability to degradation, biocidal activity, spectrum activity, and the like. Compounds having biocidal activity against a wide variety of microorganism are particularly important where there is no adverse effect on the host.

2. Description of the Prior Art

Zeya and Spitznagel, *J. Bacteriology* (1966) 91:755-762 describe cationic proteins of polymorphonuclear leukocyte lysosomes from guinea pigs. Zeya and Spitznagel, *Science* (1966) 154:1049-1051, describe the antimicrobial specificity of leukocyte lysosomal cationic proteins. Zeya and Spitznagel, *J. of Exp. Med.* (1968) 127:927-941, describe arginine-rich proteins of polymorphonuclear leukocyte lysosomes from rabbits. Patterson-Delafield et al., *Infection and Immunity* (1980) 30:180-192, report the isolation in impure form of microbicidal cationic proteins from rabbit alveolar macrophages. Patterson-Delafield et al., *Infection and Immunity* (1981) 31:723-731 (published Jan. 23, 1981) reports amino acid composition and functional attributes of impure microbicidal cationic proteins from rabbit alveolar macrophages. See also the references cited therein. Selsted et al., *J. of Biol. Chem.* (1983) 258:14485-14489, describe MCP-1 and MCP-2. See also, Lehrer et al., *Infection and Immunity* (1983) 42:10-14.

SUMMARY OF THE INVENTION

Novel antimicrobial oligopeptides are provided, having sequences comparable to cationic oligopeptides found in leukocytes. Particularly, the antimicrobial compounds have high cysteine and basic amino acid content, particularly arginine and share a common framework structure over a wide range of mammalian species.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 illustrates the effect of the peptides of the present invention on Vero cells infected with HSV-1.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Microbicidal cationic proteins are provided having high cysteine and arginine content and consisting of cationic proteins derived from macrophages and granulocytes, fragments containing antimicrobial activity, and modified oligopeptides, having one or more amino acids substituted by a different amino acid from that found in the macrophages and granulocytes. The naturally occurring oligopeptides are characterized by having six cysteines and from two to four arginines, which are substantially conserved. The cysteines and arginines are dispersed throughout the oligopeptide, so that the cysteines provide for the opportunity for extensive crosslinking, intramolecularly and intermolecularly, covalently and non-covalently, and the arginines provide for positive charges throughout the molecule at a wide range of pHs, so as to be highly cationic. The subject compounds may be readily attached to a wide variety of other compounds, both poly(amino acids) and non-proteinaceous compounds, through one or more sulfur linkages or peptide linkages.

While the cysteine framework structure is preserved, as well as the excess of basic amino acids, (particularly arginine) over acidic amino acids, there appears to be substantial differences as to the intervening oligopeptides, depending on the source.