

tional equivalents of the proteins or fragments thereof of the present invention can have 10 or fewer conservative amino acid changes, more preferably seven or fewer conservative amino acid changes, and most preferably five or fewer conservative amino acid changes. The encoding nucleotide sequence will thus have corresponding base substitutions, permitting it to encode biologically functional equivalent forms of the proteins or fragments of the present invention.

[0057] It is understood that certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Because it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence and, of course, its underlying DNA coding sequence and, nevertheless, obtain a protein with like or superior properties. It is thus contemplated by the inventors that various changes may be made in the amino acid sequences of the proteins or fragments of the present invention, or corresponding DNA sequences that encode said polypeptides, without appreciable loss of their biological utility or activity. It is understood that codons capable of coding for such amino acid changes are known in the art.

[0058] In making such changes, the hydrophobic index of amino acids may be considered. The importance of the hydrophobic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte and Doolittle, *J. Mol. Biol.* 157, 105-132 (1982)). It is accepted that the relative hydrophobic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

[0059] Each amino acid has been assigned a hydrophobic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, *J. Mol. Biol.* 157, 105-132 (1982)); these are isoleucine (+4.5), valine (+4.2), leucine (+3.8), phenylalanine (+2.8), cysteine/cystine (+2.5), methionine (+1.9), alanine (+1.8), glycine (-0.4), threonine (-0.7), serine (-0.8), tryptophan (-0.9), tyrosine (-1.3), proline (-1.6), histidine (-3.2), glutamate (-3.5), glutamine (-3.5), aspartate (-3.5), asparagine (-3.5), lysine (-3.9), and arginine (-4.5).

[0060] In making such changes, the substitution of amino acids whose hydrophobic indices are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

[0061] It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Pat. No. 4,554,101, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

[0062] As detailed in U.S. Pat. No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0), lysine (+3.0), aspartate (+3.0 \pm 1), glutamate (+3.0 \pm 1), serine (+0.3), asparagine (+0.2), glutamine (+0.2), glycine (0), threonine (-0.4), proline (-0.5 \pm 1), alanine (-0.5), histidine (-0.5), cysteine (-1.0), methionine (-1.3), valine (-1.5), leucine (-1.8), isoleucine (-1.8), tyrosine (-2.3), phenylalanine (-2.5), and tryptophan (-3.4).

[0063] In making such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

[0064] In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention differ in nucleotide sequence from those encoding a *D. v. virgifera* protein or fragment thereof set forth in SEQ ID NO: 1 through SEQ ID NO: 9112 or fragment thereof due to the fact that one or more codons encoding an amino acid has been substituted for a codon that encodes a nonessential substitution of the amino acid originally encoded.

[0065] Isolation and identification of nucleic acids of the present invention are described in detail in Examples. All or a substantial portion of the nucleic acids of the present invention may be used to isolate cDNAs and nucleic acids encoding *D. v. virgifera* protein homologues or fragments thereof from the same or other species.

[0066] A "substantial portion" of a nucleotide sequence comprises enough of the sequence to afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. In general, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., in situ hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

[0067] Isolation of nucleic acids encoding protein homologues using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

[0068] For example, structural nucleic acids encoding a *D. v. virgifera* protein or fragment thereof, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the nucleic acids of the present invention as DNA hybridization probes to screen cDNA or genomic libraries from any desired species employing methodology well known to those skilled in the art. Methods for forming such libraries are well known in the art. Specific oligonucleotide probes based upon the nucleic acids of the present invention can be designed and synthesized by methods known in the art. Moreover, the entire sequences of the nucleic acids can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available in vitro transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic DNAs under conditions of appropriate stringency.