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Peptidology: short amino acid modules in cell biology and immunology

Minireview Article

G. Lucchese[1], **A. Stufano**[1], **B. Trost**[2], **A. Kusalik**[2], and **D. Kanduc**[1]

[1] Department of Biochemistry and Molecular Biology "Ernesto Quagliariello", University of Bari, Bari, Italy
[2] Department of Computer Science, University of Saskatchewan, Saskatoon, Canada

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Summary. Short amino acid motifs, either linear sequences or discontinuous amino acid groupings, can interact with specific protein domains, so exerting a central role in cell adhesion, signal transduction, hormone activity, regulation of transcript expression, enzyme activity, and antigen-antibody interaction. Here, we analyze the literature for such critical short amino acid motifs to determine the minimal peptide length involved in biologically important interactions. We report the pentapeptide unit as a common minimal amino acid sequence critically involved in peptide-protein interaction and immune recognition. The present survey may have implications in defining the dimensional module for peptide-based therapeutical approaches such as the development of novel antibiotics, enzyme inhibitors/activators, mimetic agonists/antagonists of neuropeptides, thrombolitic agents, specific anti-viral agents, etc. In such a therapeutical context, it is of considerable interest that low molecular weight peptides can easily cross biological barriers, are less susceptible to protease attacks, and can be administered at high concentrations. In addition, small peptides are a rational target for strategies aimed at antigen-specific immunotherapeutical intervention. As an example, specific short peptide fragments might be used to elicit antibodies capable of reacting with the full-length proteins containing the peptide fragment's amino acid sequence, so abolishing the risk of cross-reactivity.

Keywords: Regulatory peptides – Epitopic peptides – Minimal peptide length – Pentapeptide unit

Short peptide modules in cell biology

Peptide-protein interactions are central to cell biology. Browsing through various scientific fields, from enzymology to toxicology, from receptor binding to immunology, there is widely documented evidence that important biological functions are carried out by short peptides able to specifically interact with defined protein domains.

The canonical example is represented by the enzymatic active site. Enzymes are proteinaceous molecules with molecular weight ranging from 10^4 to 10^5 kDa. Nonetheless, only a few amino acids form the core of the enzymatic activity or the "active site", the region in the enzyme where the substrate is converted into the product. E.g., chymotrypsin is a 25 kDa serine protease which hydrolyzes cleavage of polypeptide chains on the carbonyl side of aromatic amino acids such as phenylalanine and tyrosine. The active site of the enzyme is formed by 3 polar residues (H_{57}–D_{102}–S_{195}) only, i.e. the so-called catalytic triad. Figure 1 illustrates the chymotrypsin molecule and its active site.

In addition to minimalist catalytic motifs, a number of short peptides have been reported to exert fundamental roles in biological processes. To list only a few:

- the pentapeptide FTVCL, which corresponds to residues 33–37 of human C-reactive protein, mediates cell attachment in vitro (Mullenix et al., 1994), whereas the pentapeptide YIGSR from a cell-binding domain of the B1 chain of laminin prevents cell attachment to laminin (Olson et al., 1991);
- peroxisomal targeting signal receptor interacts with cargoes and import machinery components through conserved WXXXF/Y pentamer motifs (Otera et al., 2002);
- the high-affinity phosphorylated YLPQTV peptide inhibits Stat3 dimerization and DNA binding (Coleman et al., 2005);
- the 6-mer SEQIKA peptide affects the *in vitro* expression of the high-risk human papillomavirus type 16 E7 oncoprotein by increasing E7 mRNA stability and, at the same time, inhibiting transcript translation (Kanduc, 2002);

- immunosuppressory activity, comparable to that of cyclosporine, is shown by a pentapeptide fragment, corresponding to the ubiquitin$_{52–56}$DGRTL sequence (Szewczuk et al., 2004);
- cyclosporin itself is a cyclic, undecamer peptide that was originally isolated because it binds to and inhibits the immunophilin cyclophilin (Schreiber, 1991);
- substance P is an undecapeptide that appears involved in the regulation of pain, asthma, psoriasis, inflammatory bowel disease, emesis, migraine, schizophrenia, depression and anxiety (O'Connor et al., 2004; Datar et al., 2004);
- ketolide resistance is conferred by short peptides, the highest level of resistance being associated with a pentapeptide (MRFFV) (Tripathi et al., 1998);
- the macrolide antibiotic erythromycin binds at the entrance of the nascent peptide exit tunnel of the large ribosomal subunit and blocks synthesis of peptides longer than between six and eight amino acids. Expression of a short open reading frame in 23 S rRNA encoding five amino acids confers resistance to erythromycin by a mechanism that depends strongly on both the sequence and the length of the peptide (Lovmar et al., 2006);
- pepstatin A, an inhibitor of aspartyl proteases, is a pentapeptide (Mothes et al., 1994);
- a cyclic pentapeptide derived from the second EGF-like domain of Factor VII is an inhibitor of tissue factor dependent coagulation and thrombus formation (Orning et al., 2002).

As regards hormones and hormone-receptor interactions, again it is evident that powerful hormonal actions start from short peptide motifs. Thyrotropin-releasing hormone is a tripeptide hormone that stimulates the release of thyroid-stimulating hormone and prolactin by the anterior pituitary (Boler et al., 1969); pentagastrin corresponds to the 5-mer AWMDF (Surewicz and Epand, 1985); the pentapeptide EGSLQ, corresponding to the C-terminal five residues of human proinsulin C-peptide, mimics several of the effects of the full-length peptide: displaces cell membrane-bound C-peptide, elicits transient increase in intracellular Ca(2+) concentration and stimulates MAP kinase signalling pathways and Na(+), K(+)-ATPase (Johansson et al., 2002). On the whole, the examples are numberless and add to the well known fundamental actions exerted by oligopeptides in biology and physiology, from vasopressin and oxytocin to insulin and somatostatin, from cholecystokinin and gastrin to leptin, prolactin, growth hormone, et cetera, et cetera.

Likewise, hormone-receptor recognition is based on short modules: α-melanocyte-stimulating hormone possesses two motifs, a pentapeptide (EHFRW) and a tetrapeptide (GKPV), capable of independently triggering the hormone receptor (Eberle and Schwyzer, 1976). To cite one more, the esapeptide WLDIIW is an antagonist of endothelin and, at the same time, exerts a gonadotropin releasing hormone agonistic activity (i.e., induces luteinizing hormone release from rat pituitary) (Yahalom et al., 2000). The 6-mer WLDIIW sequence also illustrates that the same small peptide module may have important multiple effects.

We conclude that short amino acid sequences have critical roles at all levels of biological complexity and are central elements of life.

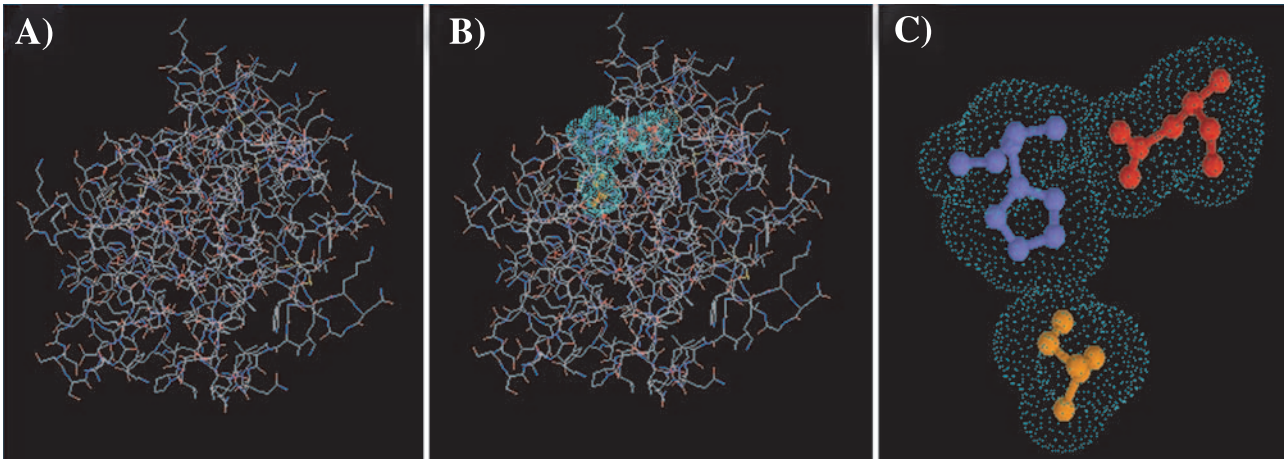

Fig. 1. Chymotrypsin enzyme and molecular resolution of its active site. **A** Chymotrypsin molecule; **B** Chymotrypsin molecule with the catalytic triad or active site; **C** The 3 polar amino acid residues (H_{57}-D_{102}-S_{195}) forming the catalytic triad. Images are from website: www-biol.paisley.ac.uk, by kind authorization of Dr. Marco Cardosi

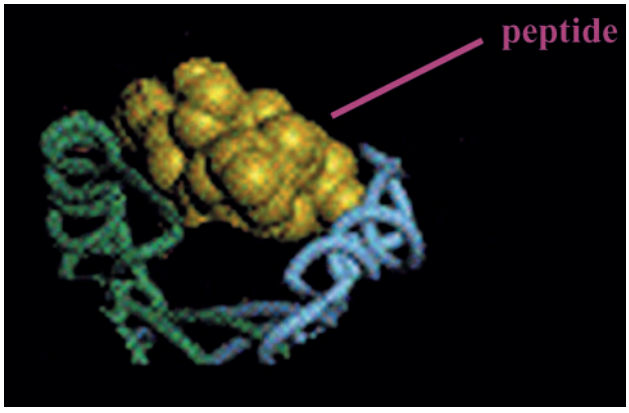

Fig. 2. The protein antigen-antibody immunorecognition process occurs in a context defined by short amino acid motifs. The interaction between the antibody hypervariable regions (in green and blue), and the peptide amino acid residues (in yellow) is shown

Short peptide modules in immunology

Based on the above-listed examples and considerations, it is no wonder that the antigen-antibody immunorecognition process occurs in a context defined by short amino acid motifs (Fig. 2).

These have been defined as oligomers in consecutive steps using various experimental procedures. Epitope scanning strategy has allowed location of the area of the sequence in which immunologically relevant antigenic sequences are to be found (Vanderlugt et al., 2000; Thrasyvoulides et al., 2001; Kaliyaperumal et al., 2002). Then, using experimental approaches such as NMR immunoanalysis and site-directed mutagenesis, precise amino acid epitope definition at a resolution of a single amino acid can be reached (Geysen et al., 1984; Dummer et al., 2004).

Currently, the epitopic motifs of a number of antigenic proteins have been exactly detailed at the molecular level. Again, a recurring module is represented by pentameric peptides, as reported in the myriad of examples that follow:

- the major part of the epitope structure of metallothionein to the MT189-14-7 monoclonal antibody is located within the NH_2-terminal acetylated pentapeptide, Ac-MDPNC (Kikuchi et al., 1990);
- the pentapeptide PDTRP and the glycopentapeptide PDT(O-alpha-D-GalNAc)RP are ligands of the monoclonal antibody SM3, raised against mucin 1 (MUC-1) characteristically present on breast cancer cells (Moller et al., 2002);
- a pentapeptide unit (aa_{31-35}DPAFR) is the hepatitis B preS1 epitope (Borisova et al., 1999; Lachmann et al., 1999);
- the EYAV (aa338–341) and LILNR (aa453–457) oligopeptides from bovine serum albumin, the major beef allergen, bind to patient IgE antibodies and are found to be the cores of the IgE-binding epitopes (Tanabe et al., 2002);
- the anti-Fya blood group antigen recognizes epitope aa_{38-43}DGDYGA (Wasniowska et al., 2004);
- the EDP208 pilus contains a major determinant in the N-terminal dodecapeptide, with the antigenic region being represented by the N-terminal pentapeptide, TDLLA (Worobec et al., 1985);
- pentapeptide consensus sequences (phage display QXPW/FP; pepscan QQPFP) are the gliadin epitope recognized by the monoclonal antibody R5 (Osman et al., 2001);
- MAB383 and MAB664 are directed against the extracellular region of kinase domain receptor (KDR) of vascular endothelial growth factor (VEGF), which is the main human receptor responsible for the angiogenic activity of VEGF. Two different sets of five residues from KDR (namely: I_{256}, D_{257}, E_{261}, L_{313}, and T_{315} and Y_{262}, P_{263}, S_{264}, S_{265}, and K_{266}), are critical for binding to MAB383 and MAB664, respectively (Lu et al., 2000);
- polyclonal rabbit Ab 12484 raised against enzymatically active full-length human transaldolase recognizes an antigenic determinant corresponding to linear pentapeptide epitope (residues 27–31) (Esposito et al., 1999);
- mAb-994, raised against the mucin-2 glycoprotein secreted by the epithelial cells of human colon, recognizes malignant human colon tissues as well as pentapeptides with the TX1TX2T motif present in mucin-2 (Windberg et al., 2004);
- mAbs raised against the myelin proteolipid protein recognize the proteolipid protein carboxyl-terminal pentapeptide (aa272–276), and the terminal phenylalanine residue is found particularly important (Yamamura et al., 1991);
- human IgE pentapeptide DSDPR and some of its related peptides have the capacity to inhibit the binding of immunoglobulin E to the mast cells of the skin (Godzhaev and Agaeva, 2000);
- a pentapeptide, PIWTR, seems to function as a dominant epitope common to all eukaryotic HSP90 molecular chaperones (Kishimoto et al., 2005).

These studies identified short immunodominant motifs and, in addition, defined the minimal peptide size required for antibody binding. The important conclusion is that B

cell epitopes are usually comprised of 5 aa or less in contiguity. Defining such a minimal epitopic length unit is preliminary to the definition of the regulated interactions between antigenic peptide motifs and antibodies and may have implications for peptide-based immunotherapeutical approaches. These short fragments are frequently represented in the primary sequence of a protein. The antibodies elicited by these peptides are directed against specific regions of the protein of interest and so have a predetermined specificity. Such antibodies are useful tools for studying, identifying and purifying proteins and maybe used in therapeutical approaches too, in neutralization of harmful autoantibodies or as reagents for passive vaccination and targeted immunotherapy of cancer diseases. As a final consideration, when antibodies against specific peptide sequences have to be induced, the shorter the sequence, the less is the risk of side cross-reactivity (Kanduc, 2006).

Concluding remarks

Peptidology, the science of peptides, is the new sub-proteomic multifaceted approach able to analyze the molecular aspects of protein expression, modification, interactions, inhibition, signalling, organization and function at physiological and pathological levels. While a proteome constitutes the protein informational database of an organism, it is the peptides that actually serve as the functional basic units of cellular processes. Therefore, thorough analysis at the amino acid level of critical peptide motifs may give clear-cut insights into (de)regulated pathways and networks involved in the physiology or pathogenesis of disease, and may lead to identification of finely defined amino acid sequences applicable to the detection, diagnosis, prognosis and treatment of specific diseases.

References

Boler J, Enzmann F, Folkers K, Bowers CY, Schally AV (1969) The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline amide. Biochem Biophys Res Commun 37: 705–710

Borisova G, Borschukova O, Skrastina D, Dislers A, Ose V, Pumpens P, Grens E (1999) Behavior of a short preS1 epitope on the surface of hepatitis B core particles. Biol Chem 380: 315–324

Coleman DR 4th, Ren Z, Mandal PK, Cameron AG, Dyer GA, Muranjan S, Campbell M, Chen X, McMurray JS (2005) Investigation of the binding determinants of phosphopeptides targeted to the SRC homology 2 domain of the signal transducer and activator of transcription 3. Development of a high-affinity peptide inhibitor. J Med Chem 48: 6661–6670

Datar P, Srivastava S, Coutinho E, Govil G (2004) Substance P: structure, function, and therapeutics. Curr Top Med Chem 4: 75–103

Dummer R, Mittelman A, Fanizzi FP, Lucchese G, Willers J, Kanduc D (2004) Non-self discrimination as a driving concept in the identification of an immunodominant HMWMAA epitopic peptide sequence by autoantibodies from melanoma cancer patients. Int J Cancer 111: 720–726

Eberle A, Schwyzer R (1976) Hormone-receptor interactions. The message sequence of alpha-melanotropin: demonstration of two active sites. Clin Endocrinol [Suppl] 5: 41S–48S

Esposito M, Venkatesh V, Otvos L, Weng Z, Vajda S, Banki K, Perl A (1999) Human transaldolase and cross-reactive viral epitopes identified by autoantibodies of multiple sclerosis patients. J Immunol 163: 4027–4032

Geysen HM, Meloen RH, Barteling SJ (1984) Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. Proc Natl Acad Sci USA 81: 3998–4002

Godzhaev NM, Agaeva GA (2000) Conformational features of a pentapeptide as an element of the active center of human immunoglobulin E. Biofizika 45: 581–585

Johansson J, Ekberg K, Shafqat J, Henriksson M, Chibalin A, Wahren J, Jornvall H (2002) Molecular effects of proinsulin C-peptide. Biochem Biophys Res Commun 295: 1035–1040

Kaliyaperumal A, Michaels MA, Datta SK (2002) Naturally processed chromatin peptides reveal a major autoepitope that primes pathogenic T and B cells of lupus. J Immunol 168: 2530–2537

Kanduc D (2002) Translational regulation of human papillomavirus type 16 E7 mRNA by the peptide SEQIKA, shared by rabbit alpha(1)-globin and human cytokeratin 7. J Virol 76: 7040–7048

Kanduc D (2006) Defining peptide sequences: from antigenicity to immunogenicity through redundancy. Curr Pharmacogen 4: 33–37

Kikuchi Y, Irie M, Ikebuchi H, Sawada J, Terao T, Nakayama S, Iguchi S, Okada Y (1990) Antigenic determinants on rat metallothionein: fine epitope mapping for a murine monoclonal antibody and rabbit polyclonal antisera. J Biochem (Tokyo) 107: 650–654

Kishimoto J, Fukuma Y, Mizuno A, Nemoto TK (2005) Identification of the pentapeptide constituting a dominant epitope common to all eukaryotic heat shock proteins 90 molecular chaperones. Cell Stress Chaperones 10: 296–311

Lachmann S, Meisel H, Muselmann C, Koletzki D, Gelderblom HR, Borisova G, Kruger DH, Pumpens P, Ulrich R (1999) Characterization of potential insertion sites in the core antigen of hepatitis B virus by the use of a short-sized model epitope. Intervirology 42: 51–56

Lovmar M, Nilsson K, Vimberg V, Tenson T, Nervall M, Ehrenberg M (2006) The molecular mechanism of peptide-mediated erythromycin resistance. J Biol Chem 281: 6742–6750

Lu D, Kussie P, Pytowski B, Persaud K, Bohlen P, Witte L, Zhu Z (2000) Identification of the residues in the extracellular region of KDR important for interaction with vascular endothelial growth factor and neutralizing anti-KDR antibodies. J Biol Chem 275: 14321–14330

Moller H, Serttas N, Paulsen H, Burchell JM, Taylor-Papadimitriou J, Meyer B (2002) NMR-based determination of the binding epitope and conformational analysis of MUC-1 glycopeptides and peptides bound to the breast cancer-selective monoclonal antibody SM3. Eur J Biochem 269: 1444–1455

Mothes E, Shoeman RL, Traub P (1994) Pepstatin A: polymerization of an oligopeptide. Micron 25: 189–217

Mullenix MC, Kaumaya PT, Mortensen RF (1994) Cell attachment peptide of C-reactive protein: critical amino acids and minimum length. J Cell Biochem 54: 343–353

O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F (2004) The role of substance P in inflammatory disease. J Cell Physiol 201: 167–180

Olson AD, Pysher T, Bienkowski RS (1991) Organization of intestinal epithelial cells into multicellular structures requires laminin and functional actin microfilaments. Exp Cell Res 192: 543–549

Orning L, Fischer PM, Hu CK, Agner E, Engebretsen M, Husbyn M, Petersen LB, Orvim U, Llinas M, Sakariassen KS (2002) A cyclic pentapeptide derived from the second EGF-like domain of Factor VII is an inhibitor of tissue factor dependent coagulation and thrombus formation. Thromb Haemost 87: 13–21

Osman AA, Uhlig HH, Valdes I, Amin M, Mendez E, Mothes T (2001) A monoclonal antibody that recognizes a potential coeliac-toxic repetitive pentapeptide epitope in gliadins. Eur J Gastroenterol Hepatol 13: 1189–1193

Otera H, Setoguchi K, Hamasaki M, Kumashiro T, Shimizu N, Fujiki Y (2002) Peroxisomal targeting signal receptor Pex5p interacts with cargoes and import machinery components in a spatiotemporally differentiated manner: conserved Pex5p WXXXF/Y motifs are critical for matrix protein import. Mol Cell Biol 22: 1639–1655

Schreiber SL (1991) Chemistry and biology of the immunophilins and their immunosuppressive ligands. Science 251: 283–287

Surewicz WK, Epand RM (1985) Role of peptide structure in lipid-peptide interactions: high-sensitivity differential scanning calorimetry and electron spin resonance studies of the structural properties of dimyristoylphosphatidylcholine membranes interacting with pentagastrin-related pentapeptides. Biochemistry 24: 3135–3144

Szewczuk Z, Stefanowicz P, Wilczynski A, Staszewska A, Siemion IZ, Zimecki M, Wieczorek Z (2004) Immunosuppressory activity of ubiquitin fragments containing retro-RGD sequence. Biopolymers 74: 352–362

Tanabe S, Kobayashi Y, Takahata Y, Morimatsu F, Shibata R, Nishimura T (2002) Some human B and T cell epitopes of bovine serum albumin, the major beef allergen. Biochem Biophys Res Commun 293: 1348–1353

Thrasyvoulides A, Sakarellos-Daitsiotis M, Philippou G, Souvatzoglou A, Sakarellos C, Lymberi P (2001) B-cell autoepitopes on the acetylcholinesterase-homologous region of human thyroglobulin: association with Graves' disease and thyroid eye disease. Eur J Endocrinol 145: 119–127

Tripathi S, Kloss PS, Mankin AS (1998) Ketolide resistance conferred by short peptides. J Biol Chem 273: 20073–20077

Vanderlugt CL, Neville KL, Nikcevich KM, Eagar TN, Bluestone JA, Miller SD (2000) Pathologic role and temporal appearance of newly emerging autoepitopes in relapsing experimental autoimmune encephalomyelitis. J Immunol 164: 670–678

Wasniowska K, Lisowska E, Halverson GR, Chaudhuri A, Reid ME (2004) The Fya, Fy6 and Fy3 epitopes of the Duffy blood group system recognized by new monoclonal antibodies: identification of a linear Fy3 epitope. Br J Haematol 124: 118–122

Windberg E, Uray K, Illyes E, Skribanek Z, Price MR, Sebestyen F, Hudecz F (2004) Heteroclitic recognition of combinatorial TX1TX2T peptide mixtures by mucin-2 protein specific monoclonal antibody. J Pept Sci 10: 56–65

Worobec EA, Paranchych W, Parker JM, Taneja AK, Hodges RS (1985) Antigen-antibody interaction. The immunodominant region of EDP208 pili. J Biol Chem 260: 938–943

Yahalom D, Koch Y, Ben-Aroya N, Fridkin M (2000) Hexapeptide and cyclic pentapeptide endothelin antagonists directly activate pituitary gonadotropin-releasing hormone receptors. Mol Pharmacol 57: 718–724

Yamamura T, Konola JT, Wekerle H, Lees MB (1991) Monoclonal antibodies against myelin proteolipid protein: identification and characterization of two major determinants. J Neurochem 57: 1671–1680

Authors' address: Darja Kanduc, Department of Biochemistry and Molecular Biology "Ernesto Quagliariello", University of Bari, Via Orabona 4, 70125 Bari, Italy,
Fax: +39 080 544 3317, E-mail: d.kanduc@biologia.uniba.it