Review

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The use of post-translationally modified peptides for detection of biomarkers of immune-mediated diseases‡

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Biomarkers are decision-making tools at the basis of clinical diagnostics and essential for guiding therapeutic treatments. In this context, autoimmune diseases represent a class of disorders that need early diagnosis and steady monitoring. These diseases are usually associated with humoral or cell-mediated immune reactions against one or more of the body's own constituents. Autoantibodies fluctuating in biological fluids can be used as disease biomarkers and they can be, thus, detected by diagnostic immunoassays using native autoantigens. However, it is now accepted that post-translational modifications may affect the immunogenicity of self-protein antigens, triggering an autoimmune response and creating neo-antigens. In this case, post-translationally modified peptides represent a more valuable tool with respect to isolated or recombinant proteins. In fact, synthetic peptides can be specifically modified to mimic neo-antigens and to selectively detect autoantibodies as disease biomarkers. A 'chemical reverse approach' to select synthetic peptides, bearing specific post-translational modifications, able to fishing out autoantibodies from patients' biological fluids, can be successfully applied for the development of specific *in vitro* **diagnostic/prognostic assays of autoimmune diseases. Herein, we report the successful application of this approach to the identification of biomarkers in different autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis. Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.**

Keywords: autoimmune diseases; peptide antigenic probes; post-translational modifications; autoantibodies; neo-antigens

A video of this lecture is available at http://www.eurpepsoc.com/view/0/30epsawards.html

Background

Among the many outputs of the 'omics' revolution, the characterization of a great number of new biomarkers is having a relevant position. Biomarkers are 'decision-making tools' particularly important in the drug development process and, most importantly, in diagnostics [1,2]. In fact, although the term biomarker originally refers to analytes in biological fluids, nowadays any measurement that predicts a person's disease state or response to therapy (with putative diagnostic and/or prognostic value) can be called a biomarker. Moreover, focus in biomarker research is shifting from methods that can analyze one marker at a time to the so-called profiling methods that allow the simultaneous measurement of a broad range of markers.

Biomarkers are the foundation of evidence-based medicine helping clinicians to decide who should be treated, how and with what. It is thus imperative that biomarker development should be accelerated along with therapeutics. Consequently, the interest in any kind of biomarker is growing exponentially, boosted by hopes of immediate biomedical applications of the HUman GenOme (HUGO) Project.

An increasing number of individuals throughout the world is affected by autoimmune diseases, a large and diverse group of disorders that are categorized by tissue injury or pathology. Although the incidence and prevalence of individual autoimmune diseases are not high, the population burdens of the disease are large and underestimated. Thus, reliable diagnostic/prognostic tools are necessary for an early diagnosis and for monitoring disease activity. In some cases, there are therapies that can change the outcome and improve the quality of life, but an early diagnosis is absolutely necessary to halt disease progression. On the other hand, these therapies are expensive and not devoid of potentially serious side effects. The evaluation of their efficacy by means of specific prognostic assays has become a crucial issue.

In general, these diseases are associated with humoral or cell-mediated immune reactions against one or more of the body's own constituents. The immune response usually involves activation of both T and B cells, the latter producing antibodies that can be detected in sera of patients and can be used to guide the clinical management of certain diseases. Involvement of autoantibodies in disease progression is widely accepted, yet the mechanisms of their generation and of the initiating events of the clinical disease are not yet completely identified. While the T-cell response has been widely studied, the critical role of B cells has been reevaluated. In fact, B cells and autoantibodies are active participants in triggering the autoimmune response and are undoubtedly effective disease biomarkers.

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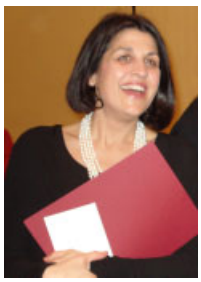
Biography



Anna Maria Papini was born in 1959 in Florence, Italy. In 1990, she obtained her PhD in chemical sciences developing a multidisciplinary project based on peptide sciences following an international research pathway (University of Sherbrooke; Max Planck Institute of Biochemistry, Martinsried; University of Florence). Since 1995, she is leading the Peptide Chemistry Laboratory at the University of Florence with the research addressing both improvements and developments of synthetic methodologies and the design of peptide neo-antigens for the discovery of specific autoantibodies as biomarkers of autoimmune diseases. With her newly developed 'chemical reverse approach', she succeeded in using particular post-translationally modified synthetic peptides to detect and monitor immune biomarkers as diagnostic, prognostic and theragnostic tools. These scientific achievements have been recognized in 2008 by the peptide research community with the 1st D. Theodoropoulos Award and the Zervas Lecture Award. The expertise gained in basic research led her in 2003 to found EspiKem as the first spin-off of the University of Firenze for the synthesis of post-translationally modified peptide, peptidomimetic and protein antigens, and in 2006 to establish Toscana Biomarkers as an R&D Biotech company for the development of innovative immunoassays based on synthetic neo-antigens to detect biomarkers in autoimmune diseases. This scientific & technology transfer activity was honoured by multiple awards among which in 2008 by the Frost & Sullivan Excellence in Research Award in the European autoimmune disease diagnostics market. Most recently, she has been appointed by the University of Cergy-Pontoise to organise a European Centre of Excellence of Molecular Detection for Life Sciences in the context of a cooperation between Région Val d'Oise and Regione Toscana.

Antibodies and Autoimmune Diseases

In 1906, Wassermann, looking for syphilis markers, introduced a routine diagnostics assay that later was realized detecting anti-cardiolipin autoantibodies [3]. Since then, autoantibodies have been used as disease biomarkers for the development of diagnostic assays [4]. Many examples of the involvement of autoantibodies in the pathogenesis of infectious and non-infectious diseases are reported. Nowadays, the clinical value of autoantibodies-based tests is more and more recognized and accepted. However, the practical value of autoantibodies-based tests strongly depends on the disease sensitivity and specificity of the used antigenic source. In fact, the precise specificity of the antibody and particularly the recognition of specific epitopes in the test conditions are assuming increasing importance.

While the practical value of autoantibodies has been realized in some clinical conditions, it remains underutilized in the majority of diseases. In fact, sera from patients suffering from autoimmune disorders often contain multiple types of autoantibodies. Some autoantibodies can be exclusive of a disease and thus used as biomarkers for diagnosis; others fluctuate with disease exacerbations or remissions and are extremely valuable in the follow up of patients [5]. Therefore, serial evaluations of autoantibodies from the quantitative and qualitative point of view are of utmost importance in the management of autoimmune disorders, in particular in development and clinical evaluation of personalized therapeutic treatments (biomarker with theragnostic value).

Antibodies may reflect the presence, nature and intensity of the immune response. Since in autoimmune diseases, the immune response is itself part of the disease process, it is possible to use autoantibodies as markers of disease activity. For example, the increase of titer of anti-double-stranded (ds) DNA antibodies in sera of systemic lupus erythematosus patients precedes nephritic flares.

In some autoimmune diseases, autoantibodies can be present before appearance of the first clinical symptoms, thus, enabling to predict both the likelihood of clinical diseases and the rate of progression of the diseases. In type I diabetes and in thyroiditis, autoantibodies may be detected in peripheral blood years before the destruction of hormone-secreting cells leading to "*ouvert*" clinical symptoms. Anti-citrullinated peptides antibodies, which are characteristic of sera of rheumatoid arthritis patients, may be detected in healthy subjects (blood donors) years before they develop the disease.

Autoimmune diseases, which affect at least 5% of the population, might be prevented by avoiding those environmental factors triggering the disease (primary prevention) or by use of specific therapy that modulates the destructive process before the onset of clinical symptoms (secondary prevention). Thus, the detection of a particular autoantibody in otherwise healthy individuals might suggest an increased risk of future autoimmune disease. Such an approach could be especially promising for diseases with a long pre-clinical period, a feature of many organ-specific autoimmune diseases, thus leading to disease prediction and consequently allowing early diagnosis and possibly strategies for disease prevention.

Synthetic Post-Translationally Modified Peptides for the Discovery of New Biomarkers of Autoimmune Diseases: a 'Chemical Reverse Approach'

In this scenario, identification of autoantibodies, as disease biomarkers, can be achieved with simple biological assays using native antigens. However, growing evidences indicate that post-translational modifications (i.e. acetylation, lipidation, citrullination, glycosylation, etc.), either native or aberrant, may play a fundamental role for specific autoantibody recognition in autoimmune diseases [6]. These observations account, at least in part, for the limited success met until now in the discovery of biomarkers for autoimmune diseases using proteomic analysis and/or protein microarrays. As alternative, a 'chemical reverse approach' based on the use of patients' sera to screen focused libraries of synthetic modified peptides can lead to the effective identification of specific probes able to characterize highly specific autoantibodies as disease biomarkers of autoimmune disorders [7].

This approach is defined 'reverse' because the screening of the antigenic probes is guided by autoantibodies circulating in biological fluids of autoimmune-disease patients. The autoantibody recognition drives the selection and the optimization of

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the 'chemical' structure of focused peptide libraries. Thus, autoantibodies in patients' sera allow the identification of synthetic post-translationally modified peptides mimicking newly generated antigenic epitopes (neo-antigens) possibly at the origin of the disease. Peptides identified by this approach, that recognize selectively and specifically autoantibodies in patients sera, can be used as antigenic probes in immunoenzymatic assays to detect disease biomarkers.

In details, as the autoantibody can be directed to the protein domain exposing a post-translationally modified residue, the chemical reverse approach should be focused on (a) identification of the side-chain modification involved in the pathogenetic mechanism of a specific autoimmune disease by screening peptide libraries based on post-translational diversity; (b) structure–activity relationship studies for the selection of the best peptide sequence fitting with the antibody antigen-binding site by screening of parallel peptide libraries aimed at the characterization of the role of amino acids and conformation.

This screening of libraries of synthetically modified peptides can lead to the identification of specific neo-antigens able to characterize highly specific autoantibodies as disease biomarkers. An interesting example in this context can be found in the field of systemic lupus erythematosus, a disease characterized by the production of autoantibodies directed against a wide spectrum of nuclear, cytoplasmic and cell membrane autoantigens [8]. Anti-dsDNA, anti-ribosomal P protein and anti-Sm-protein antibodies are found only sporadically in other connective tissue disorders and thus have been included in the serological criteria for diagnosing this disease. The anti-Sm antibodies are detectable in 5% to 30% of patients and are directed against the proteins that constitute the common core of small nuclear ribonucleoprotein particles particularly of the B/B′, D1 and D3 protein type. The SmD1 protein contains several immunoreactive regions, although the dominant epitope was detected in the *C*-terminal portion of the molecule [9–13]. Even if the synthetic peptides used in these studies differ in length, they all include the stretch 97–114 consisting of nine Gly-Arg dipeptide repeats. These peptides when used in enzyme linked immunosorbent assays (ELISAs) allowed the detection of antibodies in a similar percentage of systemic lupus erythematosus sera (25–36%). The *C*-terminal region of SmD1 has a high sequence homology with SmD3 [14]. Thus, antibodies specific for one of the Sm proteins also cross-react with other Sm components. It was subsequently shown that Sm B/B′, D1 and D3 are post-translationally modified at the arginine residues by methylation of their guanidine function [15]. Other nuclear proteins containing Gly-Arg repeats are asymmetrically dimethylated, while Sm and myelin basic protein are the only known human proteins that bear symmetrically dimethylated arginines [16]. The Gly-Arg repeats 97–114 of SmD1, synthesized with dimethylated arginine residues, were specifically recognized by most systemic lupus erythematosus sera containing anti-Sm antibodies [16]. In a recent report, the effect of this arginine methylation on the antigenicity of Sm peptides was analyzed into more details. It was observed that one particular peptide containing a single symmetrically dimethylated arginine residue in a specific position represents a more sensitive and reliable antigen for the detection of a subclass of anti-Sm autoantibodies [17]. This example may represent a proof-of-concept of the use of post-translationally modified peptides for the characterization of specific biomarkers for autoimmune diseases.

Citrullinated Peptides Recognize Highly Specific Autoantibodies, Biomarkers of Rheumatoid Arthritis

Rheumatoid arthritis is up to now the most common autoimmune disease in humans, and is characterized by the inflammation of synovial membranes that can lead to the destruction of synovial joints. Diagnosis of this disease has long been based on determination of IgM anti-rheumatoid factor. However, these antibodies frequently occur in many inflammatory diseases as well as generally even in healthy elderly individuals compromising the specificity of such a diagnostics. Moreover, technical difficulties in measuring anti-perinuclear-factor and anti-keratine antibodies as highly specific rheumatoid arthritis biomarkers never allowed their routine use. The discovery of a critical role of deimination of filaggrin in triggering the autoantibody response [18,19] allowed to set up new methods for the serological diagnosis of rheumatoid arthritis [20].

Deimination is a post-translational modification that involves the conversion of arginine into citrulline residues by the Ca-dependent enzyme peptidylarginine deiminase. The conversion of the guanidino to an ureido group decreases (i) the net positive charge of the protein, thus potentially affecting its three-dimensional structure, (ii) the ability to interact with other proteins and (iii) the susceptibility to proteolytic digestion. Filaggrin and myelin basic protein are the best known examples of physiologically deiminated proteins, but several proteins can be deiminated if the enzyme is released extracellularly (because of cell death) or is activated intracellularly by calcium influx (cell activation, apoptosis, etc).

A further step in the set up of diagnostic assays for rheumatoid arthritis was achieved by the identification of deiminated sequences of filaggrin that are recognized by a high percentage of patients sera [21,22]. A more sensitive assay was obtained modifying the peptide structure to optimally expose the citrulline moiety in cyclic peptide structures that allowed detection of antibodies in up to 70% patients [23,24]. This cyclic peptide assay has been industrially developed, yielding a family of products that is indeed the brighter example of a diagnostic assay based on modified peptides and a true blockbuster in its sector.

Anti-filaggrin antibodies react with several proteins expressed in synovial tissue and mainly with deiminated fibrinogen [25]. On the other hand, the ''Sa-antigen'' that is specifically recognized by antibodies present in 50% patients [26] has been identified as deiminated vimentin [27]. Thus, disease-specific antibodies that recognize different deiminated proteins, represent a family of antibodies of overlapping specificities and can be collectively named 'anti-citrullinated peptide/protein antibodies' [28]. These antibodies have been shown to correlate well with disease severity, evaluated on the basis of radiological damage and functional impairment. Moreover, these antibodies are present already in the early phases of rheumatoid arthritis when the differential diagnosis from other chronic arthritis or post-infectious self-limited forms may not be easy just by clinical aspects.

An example of citrullinated peptide/protein antigens is the peptide corresponding to the 35–58 sequence of the EBNA I protein, encoded by the Epstein-Barr virus. When this peptide is synthesized as tetrameric multiple antigen peptide with the arginine residues replaced by citrulline, it is specifically recognized by sera of rheumatoid arthritis patients [29,30].

Table 1. Glycosylated peptide sequences as synthetic antigens

Name (no.)	Peptide sequence
[Asn^{31}(Glc)]hMOG-(30–50) (**1**)	KN(Glc)ATGMEVGWYRPPFSRVVHL
[Asn^{84}(Glc)]hMBP-(83–99) (**2**)	EN(Glc)PVVHFFKNIVTPRTP
CSF114(Glc) (**3**)	TPRVERN(Glc)GHSVFLAPYGWMVK
[Ser^{7}(Glc)]CSF114 (**4**)	TPRVERS(Glc)GHSVFLAPYGWMVK
MBH36(Glc) (**5**)	RGKYTYN(Glc)GITYEGR
[Thr^{9}]CSF114-(Glc) (**6**)	TPRVERN(Glc)GTSVFLAPYGWMVK
Ac-[c(Dap^{5},Asp^{10})]-CSF114(Glc) (**7**)	Ac-TPRV-c[Dap-RN(Glc)GHD]VFLA-PYGWMVK
Scramble CSF114(Glc) (**8**)	LAKVSYN(Glc)FRMETRVGWHPVGP
[Pro^{7},Asn(Glc)^{8},-Thr^{10}]CSF114 (**9**)	TPRVERPN(Glc)HTVFLAPYGWMVK
[Gly^{7},Asn(Glc)^{8},-Thr^{10}]CSF114 (**10**)	TPRVERGN(Glc)HTVFLAPYGWMVK

CSF114(Glc), the First Synthetic Probe Able to Detect Autoantibodies in Multiple Sclerosis Patients' Sera as Disease Biomarkers

Multiple sclerosis is an inflammatory demyelinating disease of the central nervous system. The pathogenesis of this disease has not been yet elucidated, but an autoimmune mechanism against myelin antigens is thought to contribute to its immunopathological mechanisms, even if the target antigens responsible for inflammation and demyelination remain elusive. Different self-proteins have been investigated as potential targets for T or B cells in multiple sclerosis. The most extensively studied putative self-antigens are components of normal myelin of the central nervous system (myelin basic protein, proteolipid lipoprotein, myelin oligodendrocyte glycoprotein, etc.), or of their post-translational modified forms. It is now well established that some post-translational modifications can generate new self-antigens or even mask antigens normally recognized by the immune system in physiological conditions. In particular, aberrant glycosylations are known to affect in various manner immune responses and to exert marked effects on immune tolerance [31]. In line with these observations, in a previous work, we established the ability of the glucosylated analogue of the immunodominant epitope of myelin oligodendrocyte glycoprotein (MOG), [Asn^{31}(Glc)]hMOG(30–50) (**1**) (Table 1), to detect autoantibodies in multiple sclerosis patients, but not in healthy controls, by solid-phase ELISA, while the unglucosylated analogue hMOG(30–50) was inactive [32]. We subsequently showed that both the glucosylated and non-modified hMOG(30–50) adopted similar solution conformations in the environment used for NMR analysis (water/hexafluoroacetone (HFA) mixture) [33]. We concluded, therefore, that the ability of [Asn^{31}(N-β-Glc)]hMOG(30–50) (**1**) to detect autoantibodies in multiple sclerosis sera was linked to characteristics other than conformation and that the specific autoantibody binding site on the MOG glycopeptide **1** was related to the N-linked glucose moiety.

Therefore, a series of peptide and glycopeptides were synthesized, unrelated to the MOG sequence, to characterize the autoantibody recognition in sera of multiple sclerosis patients (Table 1). One was [Asn^{84}(Glc)]hMBP(83–99)] (**2**), a glucosylated partial sequence of an immunodominant epitope of myelin basic protein not naturally glycosylated. The second was a completely unrelated sequence, termed CSF114(Glc) (**3**). A structure-based design yielded CSF114(Glc) (**3**) as an artificial sequence characterized by a β-turn structure, with a conformational propensity to optimally expose the sugar moiety (glucose) [34,35]. CSF114(Glc) (**3**) allows the identification of a population of multiple sclerosis patients in which the autoantibody titer correlates well with disease progression [36,37]. This result, together with the observation that the *N*-glucosylated asparagine alone was able to bind multiple sclerosis autoantibodies in a solution-phase competitive ELISA experiment, allowed us to determine the minimal epitope as the Asn(Glc) moiety.

Our interest was to further investigate the role of glycosyl moiety in autoantibody recognition in multiple sclerosis sera using different glycomimetic derivatives of the CSF114 peptide sequence. For this purpose, the CSF114-type sequence was modified by the introduction of a series of natural carbohydrates [36] and of alkaloid-type sugar mimics [38] (Figure 1).

By testing these CSF114-analogues, characterized by glycoamino acids diversity (Figure 1), we were able to definitively determine that the minimal epitope requires an Asn(Glc) moiety. In fact, glycopeptides lacking Asn(Glc) displayed negligible inhibitory activity in competitive ELISA and also failed to detect IgG autoantibodies in solid-phase ELISA. Moreover, screening of differently glycosylated peptides confirmed the importance of Asn(Glc), a chemical residue not present in eukaryotic *N*-oligosaccharide chains. Therefore, *N*-glucosylation has been characterized as one of the possible post-translational modifications associated with the autoantibody-mediated demyelination in multiple sclerosis.

The small series of glycopeptides based on structural diversity (compounds **4–8** in Table 1) allowed to determine the importance of the type I′ β-turn structure [39] for optimal exposure of the minimal epitope (*N*-linked glucose). Glycopeptides **4–7**, as well as CSF114(Glc) (**3**), all possessing the β-hairpin motif, revealed increased IgM and/or IgG titers by solid-phase ELISA [36]. Moreover, these glycopeptides, containing the minimal epitope Asn(Glc), displayed affinity for multiple sclerosis autoantibodies comparable to CSF114(Glc) (**3**) in a competitive ELISA. Differently, glycopeptide **8**, an unstructured peptide scrambled in its amino acid sequence relative to CSF114(Glc) (**3**), did not recognize autoantibodies, as it is unable to expose the Asn(Glc) moiety for recognition by the antibodies under the solid-phase conditions of the assay (Figure 2). Structural studies by circular dichroism, 1D- and 2D-^{1}H NMR combined with molecular dynamics simulations demonstrated that CSF114(Glc) (**3**) possesses a β-hairpin structure with the minimal epitope Asn(Glc) at position $i+1$ of a type I′ β-turn, nicely exposed to antibody recognition.

With the aim of optimizing the glycopeptides/antibody interactions, we analyzed the glucosylated peptides **9** and **10** based on different turn structures around the antigenic Asn(Glc) residue. Results confirmed the role of conformation in recognition and binding of the synthetic antigenic peptide to multiple sclerosis autoantibodies. In particular, [Pro^{7},Asn(Glc)^{8},Thr^{10}]CSF114 (**9**) shows a type I β-turn motif around residues 6–9 while [Gly^{7},Asn(Glc)^{8},Thr^{10}]CSF114 (**10**) displays a type II′ β-turn motif around the same residues (Figure 3) [40]. In both cases, the residue Asn(Glc) is placed at position $i+2$ of the β-turn and the designed peptides showed high affinity to antibody recognition, with [Pro^{7},Asn(Glc)^{8},Thr^{10}]CSF114 (**9**) being one log unit more active than its precursor CSF114(Glc) (**3**) (IC_{50} = 11.8 nM *vs.* 137 nM). According to our re-

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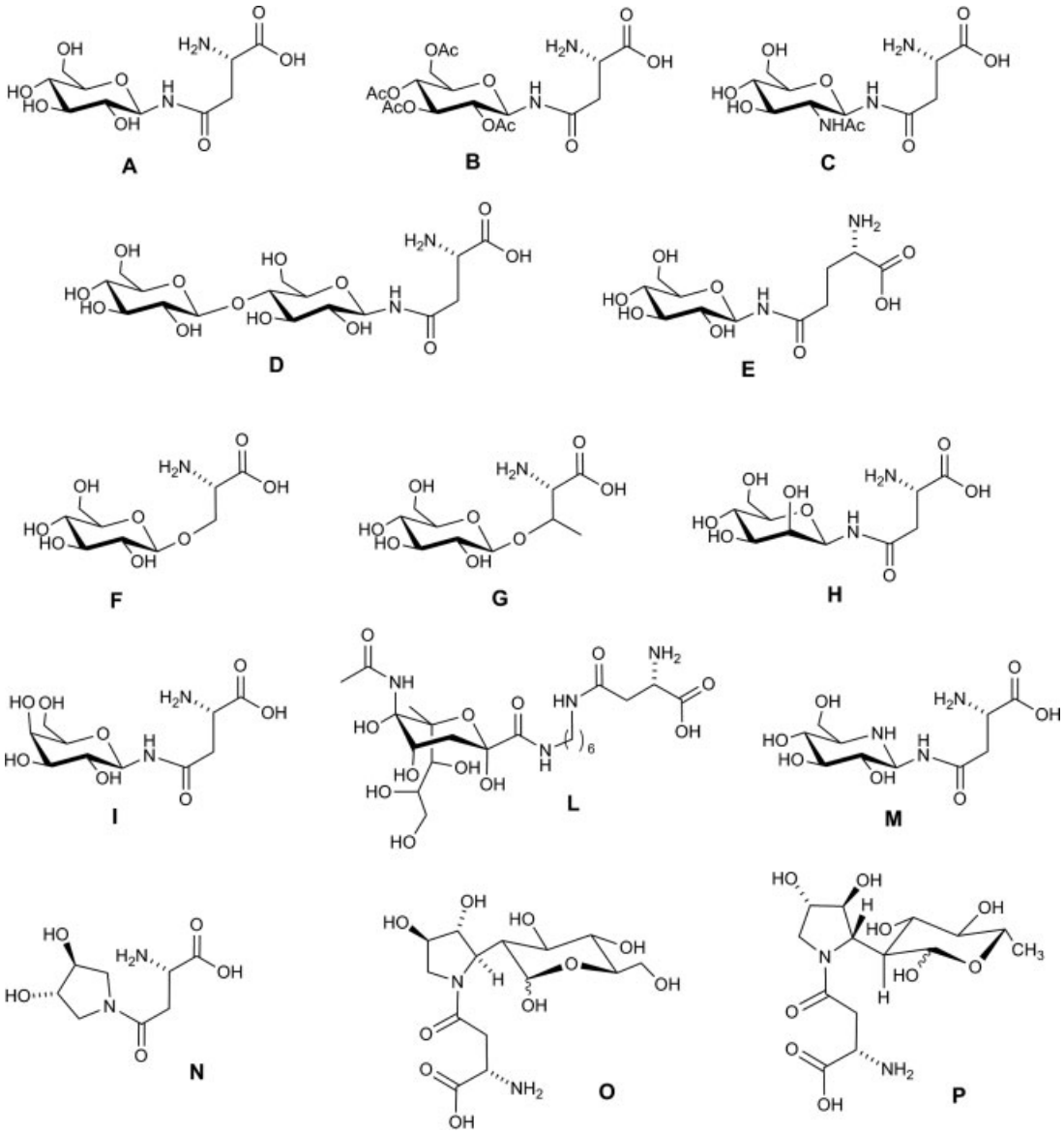

Figure 1. Chart of the glycosylated amino acids introduced in the focused library of CSF114-analogues at position 7: Asn(Glc) (A), Asn(GlcOAc4) (B), Asn(GlcNAc) (C), Asn(Glc4βGlc) (D), Gln(Glc) (E), Ser(Glc) (F), Thr(Glc) (G), Asn(Man) (H), Asn(Gal) (I), Asn(Neu5Ac-NHhexaNH$_2$) (L), Asn(DNJ) (M), Asn(DHPyr) (N), Asn(DHPyr-2-deoxyGlc) (O), Asn(DHPyr-2-deoxyRha) (P).

sults, multiple sclerosis autoantibodies recognize glycopeptides structures in the order type I > type II′ > type I′.

At the clinical level, CSF114(Glc) (**3**) detected autoantibodies in multiple sclerosis sera by a simple solid-phase ELISA. We followed for up to 6 months a consistent group of untreated multiple sclerosis patients ($n = 40$) with a clearly defined disease, by using the ELISA assay with CSF114(Glc) (**3**) as antigen. The IgM and IgG antibody titers revealed with CSF114(Glc) (**3**) and recorded at regular intervals, paralleled the occurrence of magnetic resonance imaging lesions and disease progression in approximately 40% of the patients' population (16/40). These results showed the potent value of IgM as well as IgG antibody titers in detecting disease progression. In this context, quantification of the IgG content has been found to have a high prognostic value [36].

Moreover, we analyzed the autoantibodies in terms of IgM and IgG class as well as IgG subclasses with CSF114(Glc) (**3**) as antigen in the sera of 186 multiple sclerosis patients, 166 blood donors, 25 patients affected by meningitis/encephalitis, 41 affected by systemic lupus erythematosus and 49 affected by rheumatoid arthritis. The IgM antibody level as revealed by CSF114(Glc) (**3**) was significantly increased in multiple sclerosis patients *versus* blood donors ($p < 0.001$). The IgG response was restricted to the subclass IgG2. The IgM antibodies were found in 30% of relapsing/remitting multiple sclerosis patients and, at lower levels, in subjects affected by meningitis/encephalitis. No IgM or IgG antibodies to CSF114(Glc) were detected in systemic lupus erythematosus and rheumatoid arthritis patients' sera [37].

To limit operator-dependent procedures, which are the common disadvantages of ELISA, we modified the chemical structure of CSF114(Glc) to develop an electrochemical assay. In this perspective, we have recently reported the set up of an electrochemical probe for the detection of specific autoantibodies in multiple sclerosis patients' sera [41]. We introduced

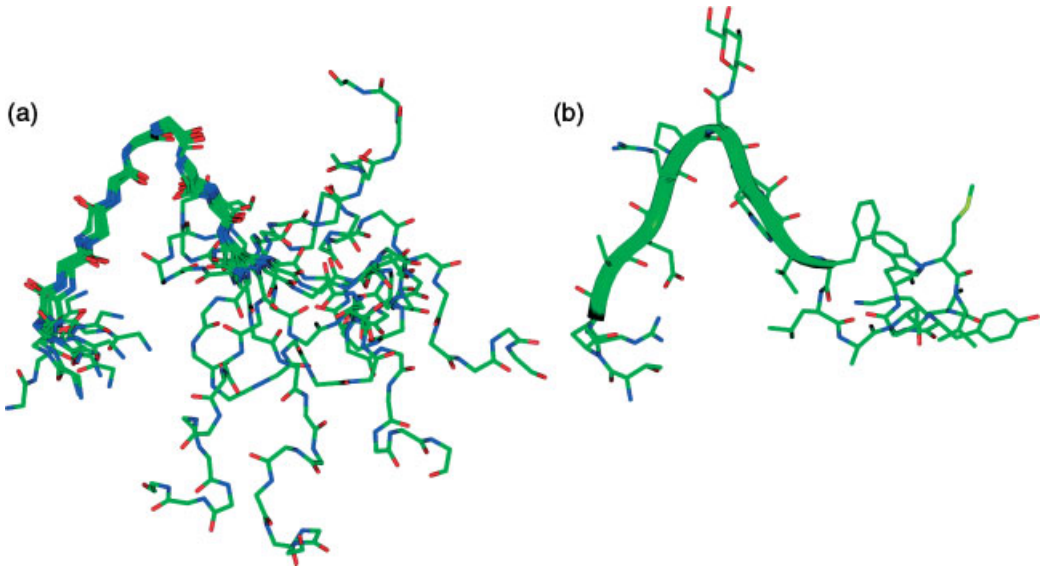

Figure 2. (a) Superposition of the ten lowest-energy conformers of [Pro^{7},Asn(Glc)^{8},Thr^{10}]CSF114 (**9**). (b) Lowest-energy conformer of [Pro^{7},Asn(Glc)^{8},Thr^{10}]CSF114 (**9**) [40]. Reprinted with permission from Ref. 40. Copyright 2008 American Chemical Society.

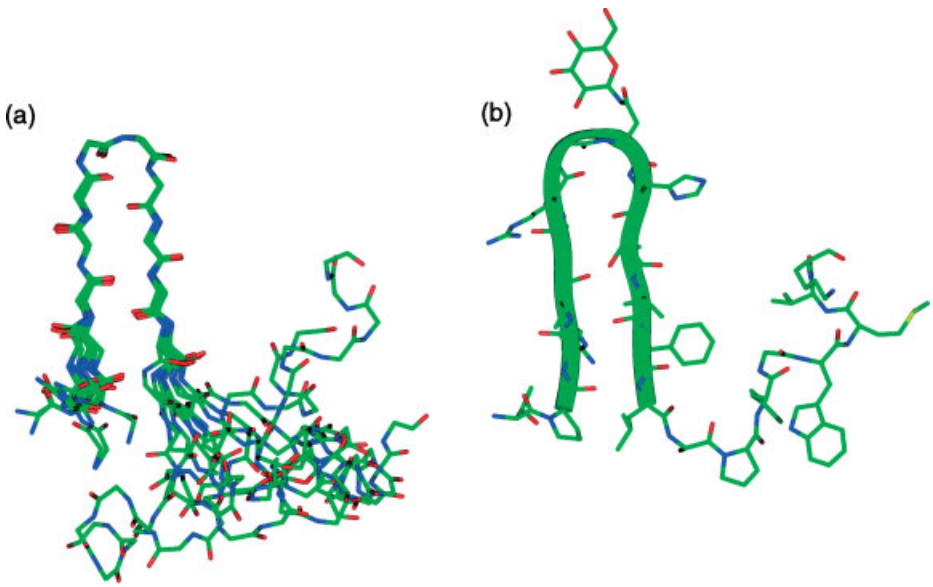

Figure 3. (a) Superposition of the ten lowest-energy conformers of [Gly^{7},Asn(Glc)^{8},Thr^{10}]CSF114 (**10**). (b) Lowest-energy conformer of [Gly^{7},Asn(Glc)^{8},Thr^{10}]CSF114 (**10**) [40]. Reprinted with permission from Ref. 40. Copyright 2008 American Chemical Society.

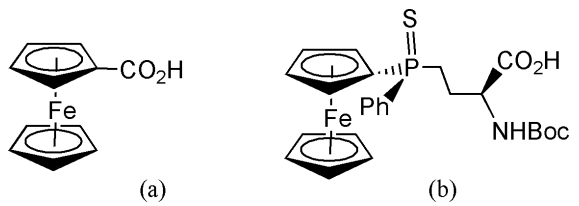

Figure 4. Structure of ferrocenyl derivatives introduced in CSF114(Glc) for the development of an electrochemical assay to detect autoantibodies in multiple sclerosis [41].

at the *N*-terminus of CSF114(Glc) a ferrocenyl carboxylic acid (Fc) or a ferrocenyl-thio-phosphine derivative of 1-aminobutyric acid [4-FcPhP(S)Abu; Figure 4]. The electrochemical properties of ferrocene, coupled to thio-phosphine ability to build simple monolayers on gold surfaces, allowed peptide anchoring on the detection electrode. The glucosylated ferrocenyl peptide 4-FcPhP(S)Abu-CSF114(Glc) and the unglucosylated 4-FcPhP(S)Abu-CSF114 were adsorbed on gold surface to form a self-assembled monolayer via the sulfur atom. The electrochemical response of the ferrocenyl group was measured by cyclic voltammetry. Only the *N*-glucosylated peptide was able to recognize isolated anti-CSF114(Glc) autoantibodies. The detection of the antigen–antibody interaction was also realized in solution using the peptide Fc-CSF114(Glc) and a gold electrode properly modified to avoid unspecific reactions. Also in this case, it was possible to detect anti-CSF114(Glc) antibodies, however further studies are necessary to investigate the use of different protective monolayers.

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We demonstrated that autoantibodies could be detected by monitoring electrochemically the interaction with Fc-CSF114(Glc) and 4-FcPhP(S)Abu-CSF114(Glc) with a sensitivity comparable to the ELISA method. In this novel electrochemical method, the modified peptide acts both as an antigen and as an electrochemical probe to read out antigen/antibody interaction.

Perspectives

Peptides represent unique chemical tools because of the amino acid diversity and of the different type of synthetic modifications, which can affect the chemical structure, the spatial conformation and the biological activity. Thus, peptides can be used as synthetic antigenic sources to mimic the native antigens especially when post-translational modifications are supposed to trigger the autoimmune response. In fact, as it was demonstrated, synthetic peptides bearing post-translational modifications can be used to understand the molecular mechanisms at the basis of most autoimmune diseases. The development of synthetic peptides interacting with high affinity with autoantibodies, biomarkers of autoimmune diseases, can guide the set up of diagnostic/prognostic peptide-based immunoassays. In this perspective, peptide sciences can contribute not only to the comprehension of the molecular mechanisms of autoimmune diseases but also to the development of diagnostic/prognostic immunoassays particularly useful to guide therapeutic treatments. Moreover, as we have recently demonstrated, peptides can be used to set up new technologies based on the electrochemical properties of suitable chemical groups added to the amino acid sequence. This new research field is of great interest to improve the specificity and the sensitivity of the antigen/antibody recognition and to set up fast, simple, reliable and low-cost diagnostic assays.

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