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A Diepitopic Sequential Oligopeptide Carrier (SOC_n) as Mimic of the Sm Autoantigen: Synthesis, Conformation and Biological Assays

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Abstract: Anti-Sm (Sm: U1-U6 RNA-protein complex) antibodies are usually considered highly specific for systemic lupus erythematosus (SLE), while anti-U1RNP (U1RNP: U1RNA-protein complex) are thought of as diagnostic criteria for the mixed connective tissue disease (MCTD). However, both antibody specificities coexist in SLE and MCTD, in varying percentages. Although the anti-Sm/anti-U1RNP immunological cross-reactivity has been initially attributed to a common motif, PPXY(Z)PP (where X, Y, Z are various amino acids), found in the Sm, U1-A and U1-C autoantigens, it appears that the conformational features of the Sm epitopes also play an important role in the immunoreactivity. The PPGMRPP and PPGIRGP main epitopes of the Sm antigen were coupled in duplicate to the tetrameric Ac-(Lys-Aib-Gly)$_4$-OH, SOC_4, carrier to form the [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC_4 construct as a mimic of the native Sm. It was found that: (i) the 3_{10} helical structure of SOC_4 allows the epitopes to adopt an exposed orientation, similar to their free forms, that facilitates their recognition from the anti-Sm antibodies, and (ii) the U1-RNP cross-reactivity is minimized.

Keywords: carrier of the Sm epitopes; diepitopic sequential oligopeptide carrier; NMR study of SOC_n; sequential oligopeptide carriers $(SOC)_n$; Sm autoantigen; Sm epitopes grafted to SOC_n; Sm mimic

INTRODUCTION

Antibodies directed against components of the spliceosome complex are common in several autoimmune disorders, particularly systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD) [1]. These autoantibodies are classified into two major groups: (i) the anti-Sm (Sm: U1-U6RNA-protein complex) that mainly recognize four polypeptides (B, B′, D and N) in complex with U1-U6RNAs, and (ii) the anti-U1RNP (U1RNP: U1RNA-protein complex) that bind the polypeptides 70KD, A and C in complex with U1RNA. Although anti-Sm antibodies are usually considered highly specific for SLE, and anti-U1RNP are thought of as diagnostic criteria for MCTD, both antibody specificities coexist in SLE and MCTD in varying percentages [2,3].

The three major epitopes of Sm contain the PPGMRPP sequence, and the fourth, the sequence PPGIRGP. Proteins U1-A and U1-C of the U1RNP complex have in common the motif PPXY(Z)PP, where X, Y and Z are various amino acids. The sequence conservation between U1-A, U1-C and Sm suggests that this proline-rich motif is an important functional domain, and predicts an immunological cross-reactivity [4–6]. However, substitution and deletion studies of PPGMRPP indicated that it is not only the proline content which is important for immunoreactivity, but rather their position within the

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sequence, related directly to the conformational features of the epitope [6]. The significance of the conformation adopted by PPGMRPP in relation to the immunoreactivity of this epitope was also demonstrated by our research group [7].

In this regard, differentiations in the way that PPPGMRPP and PPPGIRGP are recognized by anti-Sm monoclonal antibodies were attributed to differences in their conformations. Substitution of Ile in PPGIRGP by Met results to the epitope analogue PPGMRGP, which is very similar to the main Sm epitope PPGMRPP. However, the PPGMRGP analogue does not show anti-Sm reactivity [8]. This observation indicates the importance of the PPGIRGP epitope, which does not exist in U1RNP, and for this reason, was used in our construct for mimicking the Sm antigen and increasing the anti-Sm specificity of the conjugate. Interestingly, epitope mapping of U1-A and U1-C revealed the sequences PPGMIPP (166-172) and PPGAMPP (181-187) for U1-A, as well as APGMRPP (119-127) and APAMIPP (49-55) for U1-C, but not the PPGIRGP found only in the Sm antigen [9].

A new sequential oligopeptide carrier (SOC_n) of antigenic/immunogenic peptides, formed by the repetitive Lys-Aib-Gly triplet, was successfully developed in our laboratory, and applied as antigenic substrate in immunoassays and for the generation of specific immune responses. A detailed conformational analysis of Ac-(Lys-Aib-Gly)$_4$-OH, SOC_4, which has shown the best biological applications, using ^{1}H-NMR spectroscopy and molecular modeling indicated that it adopts a distorted 3_{10}-helix. Although the Lys-N$^\varepsilon$H$_2$ side-chains in SOC_4 are expected to be directed towards the same site of the 3_{10}-helix, the distance between lysines and the length of their side-chains seems to facilitate an appropriate orientation of the conjugated antigens, which does not allow their interactions [10–13].

The PPGMRPP and PPGIRGP main epitopes of the Sm antigen were coupled in duplicate to the tetrameric Ac-(Lys-Aib-Gly)$_4$-OH, SOC_4, carrier to form the [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC_4 construct (Figure 1), as a mimic of the native Sm antigen. It is expected that the SOC_n carrier will allow the epitopes to assume an exposed orientation that will facilitate their recognition by the anti-Sm antibodies, and that the U1-RNP cross-reactivity will be minimized.

We report here on the synthesis, conformational characterization by ^{1}H-NMR technique and biological assays of [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC_4. As

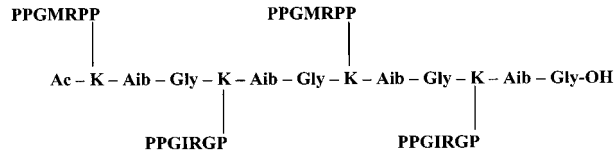

Figure 1 Schematic representation of [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC_4.

reference compounds, for both conformational and biological assays, we employed PPGMRPP-NH_2 and PPGIRGP-NH_2, which resemble the PPGMRPP and PPGIRGP epitopes when coupled to the Lys-N$^\varepsilon$H$_2$ groups of SOC_4. Synthesis, ^{1}HNMR study and biological assays of PPGMRPP-NH_2 and (PPGMRPP)$_5$-SOC_5 used in this report are given in reference [14].

MATERIALS AND METHODS

Solvents were purchased from Labscan Ltd (Ireland), while trifluoroacetic acid (TFA), diisopropylethylamine (DIEA) and piperidine were purchased from Merck Schuchardt (Germany), and were used without further purification. 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), 1-hydroxy-benzotriazole (HOBt), *tert*-butyloxycarbonyl (Boc)-, 9-fluorenylmethyloxycarbonyl (Fmoc)- and allyloxycarbonyl (Alloc)-protected amino acids were purchased from Bachem Biochemica GmbH (Germany) and Neosystem Laboratoire (France). Boc-Gly-OCH$_2$Pam resin (Pam: phenylacetamidomethyl) was from Calbiochem-Novabiochem GmbH (Germany), and 4-methylbenzhydrylamine resin was purchased from Neosystem Laboratoire (France). Dialysis tubes, which are cellulose membranes benzoylated in order to reduce pore size with a molecular mass cut-off of $\sim$1500, were obtained from Sigma (Deisenhofen, Germany). DMSO-d_6 was purchased from EURISO-TOP (Gif-sur-Yvette, France). High-performance liquid chromatography (HPLC) grade acetonitrile (CH_3CN) was purchased from E. Merck Darmstadt (Germany).

Synthesis of PPGIRGP-NH_2

The synthesis of PPGIRGP-NH_2 was carried out manually by a stepwise solid-phase procedure [14–16] using the 4-methyl-benzhydrylamine resin. Arginine was introduced as Boc-L-Arg(Tos)-OH, (Tos, toluene-4-sulfonyl), derivative. Couplings were performed using a 3/2.9/3/3/1 molar ratio in amino acid/TBTU/HOBt/DIEA/resin. Deprotection and coupling reactions were monitored either by Kaiser

ninhydrin or 2,4,6-trinitrobenzenesulfonic acid (TNBSA) tests. The PPGIRGP-NH_2 peptide was cleaved from the resin with anhydrous HF in the presence of phenol and anisole as scavengers. The crude material (yield 80%) was subjected to semi preparative HPLC using a C_{18} 25 cm × 100 mm, 5u, Hypercil reverse phase column with solvent A, H_2O/0.1% TFA and B, CH_3CN/0.1% TFA. Programmed gradient elution (4 mL/min) was applied (90/10-70/30), elution time 30 min. The yield of the purified product was 60%. Amino acid analysis of the purified PPGIRGP-NH_2 gave Pro/Gly/Ile/Arg (2.8/1.9/1.0/1.2); expected (3/2/1/1). The analytical HPLC confirms the purity of the peptide.

Synthesis of ((PPGMRPP)$_2$, (PPGIRGP)$_2$)-SOC_4

The synthesis of the sequential oligopeptide carrier was carried out by solid-phase peptide synthesis (SPPS) [15–17] using the Boc-Gly-OCH_2-Pam resin. Aminoisobutyric acid was introduced as Boc-Aib-OH and lysine either as Boc-L-Lys(Fmoc)-OH or as Boc-L-Lys(Alloc)-OH (after its conversion to the free carboxylate form from the corresponding DCHA salt), so as to shape an alternate ABAB motif, where A and B represent the PPGMRPP and PPGIRGP epitopes covalently attached to the Lys-$N^{\varepsilon}H_2$ groups (Figure 2). The three N-protecting groups (Boc-, Fmoc- and Alloc-) constitute an orthogonal system,

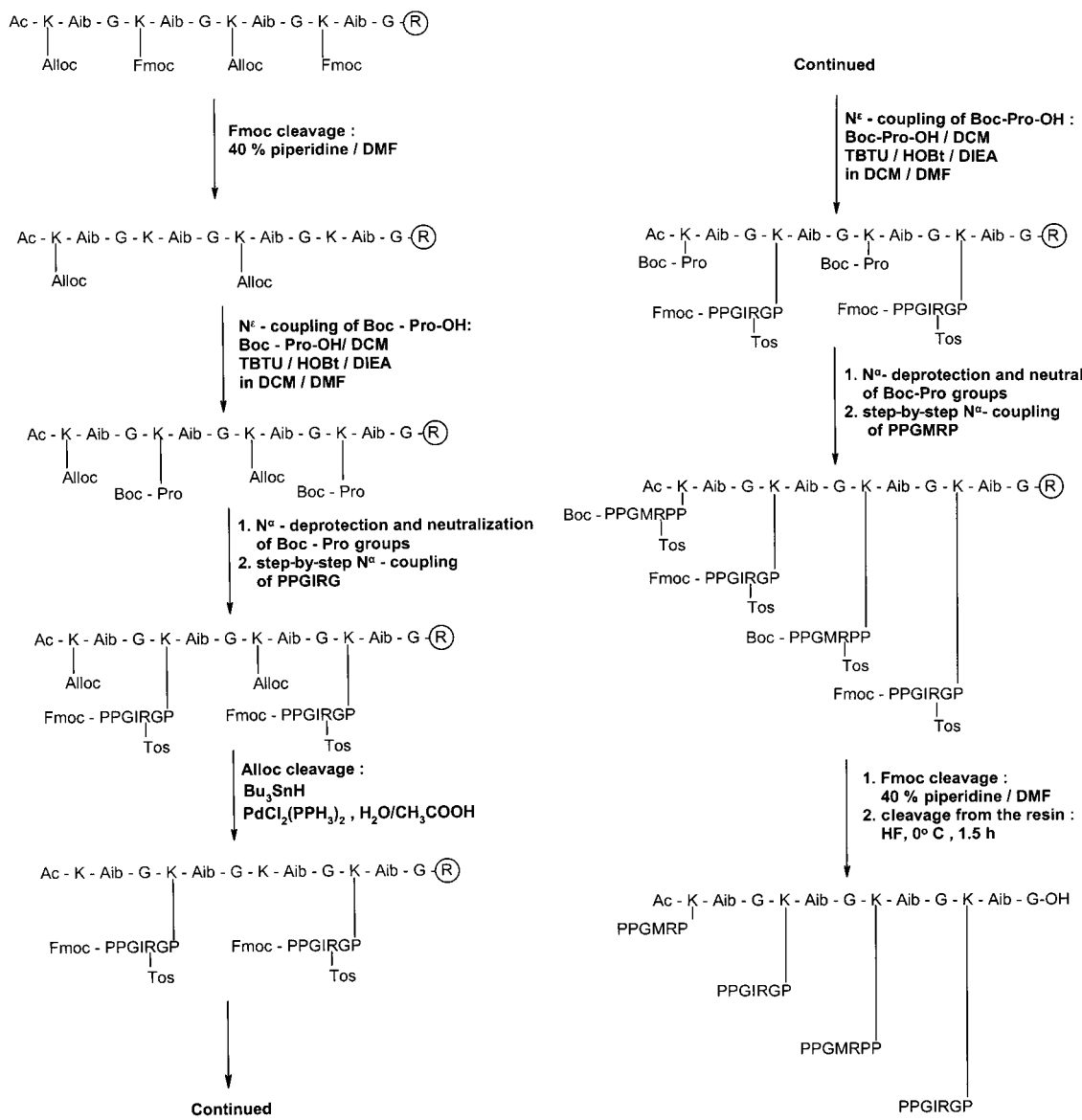

Figure 2 Steps in the synthesis of [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC_4.

which allows the synthesis of the sequential oligopeptide carrier Ac-(Lys-Aib-Gly)$_4$-, SOC$_4$-, with two functionalities.

Couplings were performed using a 3/2.9/3/3/1 molar ratio in amino acid/TBTU/HOBt/DIEA/resin, and were monitored either by Kaiser ninhydrin or 2,4,6-trinitrobenzenesulfonic acid (TNBSA) tests and repeated if necessary. After formation of the desired length of the oligopeptide carrier, the *N*-terminal Boc group was cleaved, and Lys was N$^{\alpha}$-acetylated with Ac$_2$O in pyridine, using a 30:1 ratio of Ac$_2$O/-NH$_2$ groups. Initially, the heptapeptide PPGIRGP was covalently attached, from the *C*-terminus, by SPPS to the first and third Lys-N$^{\varepsilon}$H$_2$ groups of SOC$_4$, which were available after removal of the Fmoc groups. After completion of the step-by-step synthesis of the PPGIRGP epitope, the Alloc groups were removed under hydrostannolytic cleavage [18,19], followed by synthesis of the PPGMRPP epitopes by SPPS.

Arginine was introduced as Boc-L-Arg (Tos)-OH, while methionine was used without side protection as Boc-L-Met-OH. Dimethylsulfide was added to each cycle of Boc-removal, after coupling of Boc-L-Met-OH, to avoid oxidation of the Met side-chain. Finally, the peptide was cleaved from the resin support by HF in the presence of *p*-cresol, *p*-thiocresol and dimethylsulfide as scavengers. HF was evaporated under vacuum, the resin was washed with diethyl ether and the peptide was extracted with 2 M aqueous acetic acid. The crude peptide (yield 55%) was dialysed against water, using dialysis tubes with molecular mass cut-off *ca* 1500 (80% yield after dialysis). Maximum purity was achieved using semi-preparative HPLC using a C$_{18}$ 25 cm $\times$ 100 mm, 5u, Hypercil reverse phase column with solvent A, H$_2$O/0.1% TFA and B, CH$_3$CN/0.1% TFA. Programmed gradient elution (4.4 mL/min) was applied (85/15–50/50), elution time 30 min. The purified [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC$_4$ (yield 40%) gave satisfactory amino acid analysis: Gly 10.4 (10), Arg 3.4 (4), Pro 14 (14), Met 1.4 (2), Ile 2.1 (2), Lys 3.8 (4). The ES mass spectrum of [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC$_4$, as well as the corresponding LC, is shown in Figure 3. The calculated $[M+H]^+$ is 3957.79, and that found is 3957.00 $\pm$ 1.38. The positive-ion spectrum shows the $[M+3H]^{+3}$ ion at 1320.66, the $(M+4H)^{+4}$ ion at 990.09 and the $(M+5H)^{+5}$ at 792.81.

^{1}H-NMR Experiments

^{1}HNMR experiments of PPGMRPP-NH$_2$ are given in details in Reference [14]. The NMR samples of [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC$_4$ and PPGIRGP-NH$_2$ were prepared by dissolving the solid material in

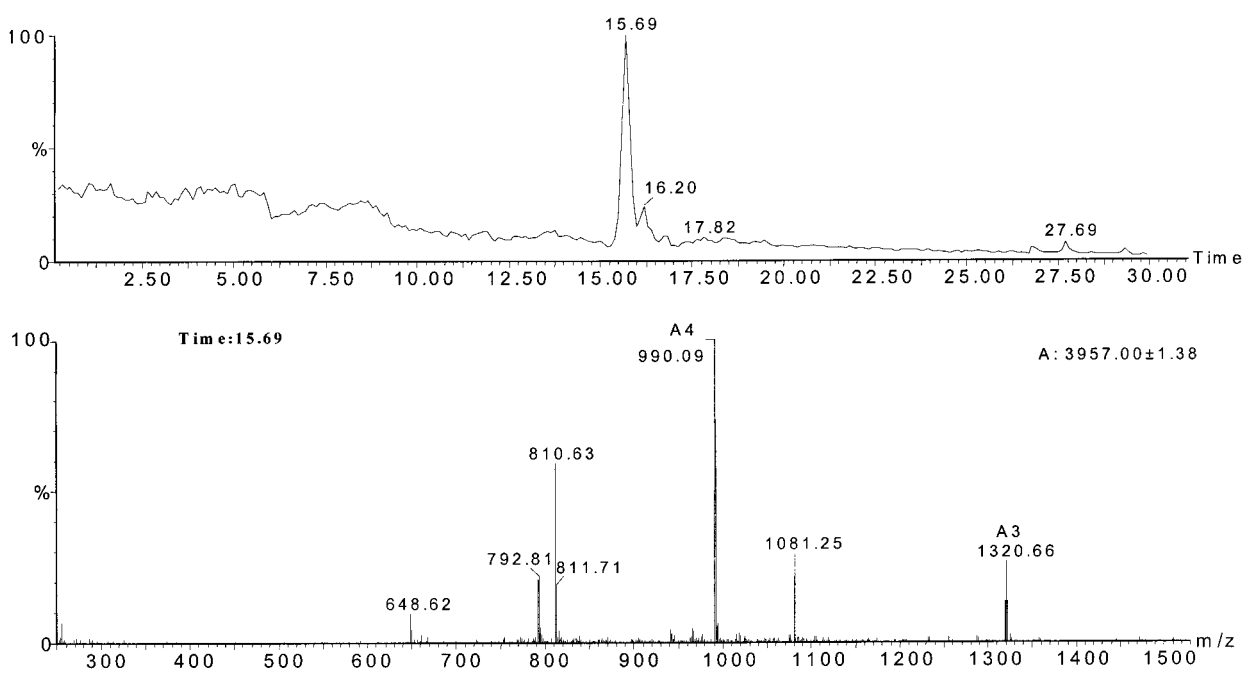

Figure 3 LCMS analysis of the purified [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC$_4$.

H_2O, and adjusting the pH to the desired value with NaOH or HCl. The aqueous solutions were lyophilized, and weighed amounts were dissolved in DMSO-d_6 at concentrations of *ca* 5.5×10^{-3} M for PPGIRGP-NH_2 and *ca* 3×10^{-3} M for [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC_4. At these concentrations, intermolecular associations were not observed, as was proven by dilution experiments. The NMR spectra were recorded on a Bruker AMX 400 spectrometer at 300 K, using the standard COSY, TOCSY and NOESY microprograms. The NOESY spectra were recorded using two mixing times (180 and 350 ms), and the TOCSY spectra one mixing time (100 ms). The spectral width in F_1 and F_2 was 5000 Hz.

Biological Experiments

ELISA assays of PPGMRPP-NH_2 and (PPGMRPP)$_5$-SOC_5 are given in detail in Reference [14]. The PPGIRGP-NH_2 and [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC_4 peptides were used as antigenic substrates in ELISA assays according to the following protocol. The antigens were coated (10 μg/mL in carbonate buffer pH 9.6, 50 μg/well) to 96-well ELISA plates (NUNC, DENMARK) by overnight incubation at 4°C. The non-specific binding sites were blocked with 10% bovine serum albumin in PBS (BS 10%) for 2 h at room temperature. Sera diluted in 10% BS (1/100) were added 50 μg/well for 1 h at room temperature. After washing with PBS-Tween 20 (0.05%) (five times) alkaline phosphatase conjugated anti-human IgG was added (1/2500 in 10% BS) and incubated for 30 min at room temperature. Finally, the plates were washed (five times) with PBS-Tween, and substrate solution was added to each well. The absorbance was read at 405 nm. RNA immunoprecipitation was applied as reference method for the anti-Sm and anti-U1RNP positive sera [20]. The number of tested sera was 17 with anti-Sm/U1RNP, and 15 with anti-U1RNP specificity. The ELISA experiment was performed in duplicate.

RESULTS AND DISCUSSION

Conformational Studies

^{1}H-NMR study of PPGMRPP-NH_2 is given in detail in Reference [14]. Almost all proton resonances (NH,

Table 1 Chemical Shifts δ (ppm) and Temperature Coefficient Values $\Delta\delta/\Delta T$ (10^{-3} ppm K^{-1}), in Parentheses, for PPGIRGP-NH_2 in DMSO-d_6 (5.5×10^{-3} M) at 300 K, Referenced to Me_4Si (pH 5)

	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	Others
Conformer A						
Pro[1]		4.34	2.19	1.84	3.09/3.41	
Pro[2]		4.39	2.11/2.36	1.86	3.48/3.63	
Gly[3]	8.19 (−4.2)	3.69/3.76				
Ile[4]	7.70 (−5.2)	4.24	1.88	1.07/1.41	0.83	
Arg[5]	8.16 (−5.7)	4.30	1.71	1.55/1.71	3.11	$N^{\varepsilon}H$:7.57 $N^{\eta}H_2$:7.11
Gly[6]	7.92 (−5.3)	3.85/3.98				
Pro[7]		4.18	2.03/2.29	1.98/2.03	3.44/3.55	
$CONH_2$ (*trans*)	7.27 (−4.9)					
$CONH_2$ (*cis*)	6.93 (−6.2)					
Conformer B						
Pro[1]		3.96	2.36	1.85	3.11/3.18	
Pro[2]		4.47	2.02/2.27	2.02	3.43/3.50	
Gly[3]	8.49 (−3.6)	3.80				
Ile[4]	7.91 (−5.3)	4.23	1.73	1.07/1.41	0.82	
Arg[5]	8.13 (−5.6)	4.34	1.71/1.94	1.71	3.11	$N^{\varepsilon}H$:7.63 $N^{\eta}H_2$[a]
Gly[6]	7.88 (−5.6)	3.59/3.86				
Pro[7]		4.34	2.19	1.88	3.41	
$CONH_2$ (*trans*)	7.18 (−5.8)					
$CONH_2$ (*cis*)	6.53 (−8.6)					

[a] Not identified.

Table 2 Chemical Shifts δ (ppm) of the Peptide Epitopes of [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC$_4$ in DMSO-d_6 (3×10^{-3} M) at 300 K, Referenced to Me$_4$Si (pH 5)

	NH	C$^{\alpha}$H	C$^{\beta}$H	C$^{\gamma}$H	C$^{\delta}$H
PPGMRPP					
Pro^1		4.34	2.32	1.82/1.86	3.08/3.18
Pro^2		4.38	2.13	1.87	3.49/3.65
Gly^3	8.28	3.71			
Met^4	7.88	4.39		2.41	
Arg^5	8.13	4.45	1.71	1.53	3.07
Pro^6		4.56	2.15	1.86	3.51/3.70
Pro^7		4.21	2.02		3.54/3.63
PPGIRGP					
Pro^1		4.34	2.32	1.82/1.86	3.08/3.18
Pro^2		4.38	2.13/2.41	1.87	3.49/3.65
Gly^3	8.23	3.73			
Ile^4	7.71	4.24	1.89	1.07/1.39	0.81/0.83
Arg^5	8.16	4.33	1.88		3.08
Gly^6	7.96	3.86/3.99			
Pro^7		4.24	2.04	2.04	3.46/3.55

C$^{\alpha}$H and side-chain aliphatic protons) of PPGIRGP-NH$_2$ and [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC$_4$ were assigned by the combined use of COSY, TOCSY and ROESY experiments (Tables 1 and 2).

PPGIRGP-NH$_2$. The presence of two sets of NH peaks of varying intensities in the 1D ^{1}H-NMR spectrum, as well as the presence of exchange cross connectivities in the NH region of the ROESY spectrum, argue in favour of multiple conformers, possibly owing to the *cis–trans* isomerism of the X-Pro peptide bonds (Pro^2 and Pro^7). Based on the combined use of the COSY, TOCSY and ROESY experiments, it was possible to assign all of the proton resonances of the two conformers (A and B) (Figure 4), and estimate the percentage of each one by integration of the peak areas in the NH region ($\sim$ 75% of A and $\sim$ 25% of B).

Considering the chemical shift values of RN$^{\varepsilon}$H and RN$^{\eta}$H$_2$ (7.57 and 7.11 ppm, respectively) of conformer A, and the fact that the protons of each methylene group of Arg side-chain are magnetically equivalent [7,21–23], we conclude that the Arg side-chain is not implicated in the structure stabilization of PPGIRGP-NH$_2$. Also, the high absolute

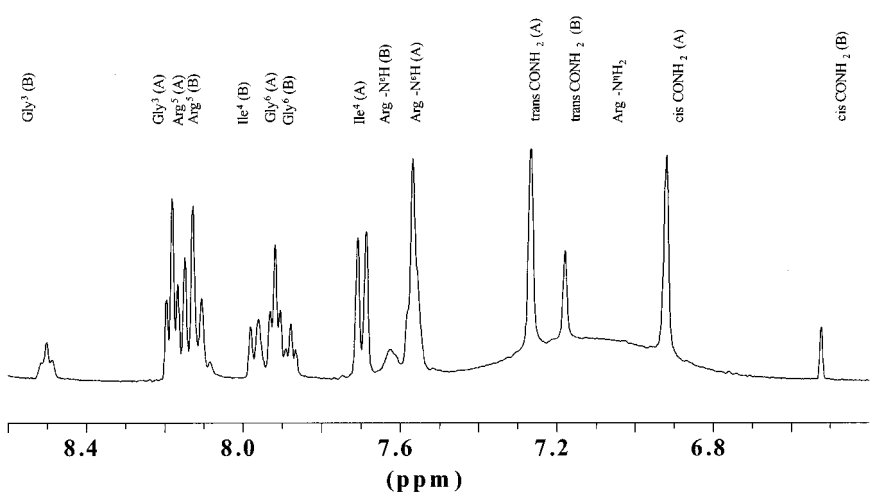

Figure 4 The NH region of the 400 MHz ^{1}H-NMR spectrum of PPGIRGP-NH$_2$. Conformers (A) and (B).

temperature coefficient values of all of the NH protons indicate that they are exposed to the solvent and do not participate in intramolecular hydrogen bonds (Table 1).

Medium and intense NOE connectivities [$d_{\alpha N}(i, i)$, $d_{\alpha N}(i, i+1)$ and $d_{NN}(i, i+1)$] detected in the central part of PPGIRGP-NH_2 could be attributed to a rigid conformation. However, the high coupling constant values $^3J_{\alpha N}$ of Arg and Ile (8.0 and 8.7 Hz, respectively), as well as the absence of intramolecular hydrogen bonds (high absolute temperature coefficient values of NH protons) support an extended conformation, which is common for both conformers (A and B) [24,25]. On the other hand, the intense NOE cross peaks P^1-$C^{\alpha}H/P^2$-$C^{\delta}H$ and G^6-$C^{\alpha}H/P^7$-$C^{\delta}H$ indicate the *trans* forms of these peptide bonds for conformer A, while the intense NOE connectivities P^1-$C^{\alpha}H/P^2$-$C^{\alpha}H$ and G^6-$C^{\alpha}H/P^7$-$C^{\alpha}H$ suggest the *cis* forms for conformer B.

((PPGMRPP)$_2$, (PPGIRGP)$_2$)-SOC$_4$. Identification of almost all of the proton resonances of [$(PPGMRPP)_2$, $(PPGIRGP)_2$]-SOC_4 was accomplished by comparative combination of the ^{1}H-NMR spectra of the free PPGMRPP-NH_2 (analysed in Reference [14]) and PPGIRGP-NH_2, with the spectrum of the SOC_4 conjugate (Table 2). The very small variations between the chemical shifts of PPGMRPP-NH_2 and PPGIRGP-NH_2 free and bound to the SOC_4 carrier indicate a conservative structure of the epitopes in the two states (Table 3). The close similarity of the NH and $C^{\alpha}H$ chemical shifts of the free and bound epitopes and the common NOE pattern suggest that the epitopes display the same extended structure in both states. Interestingly, we were also able to identify the majority of the proton resonances of the carrier, SOC_4, bearing both epitopes, as shown in Figure 4. The reported findings (Table 3, Figure 5) provide convincing evidence that the epitopes grafted to SOC_4 do not interact with the carrier and preserve their original structure.

One may note the close similarity of the NH chemical shift differences of the SOC_4 carrier in its free form, and coupled to the PPGMRPP and PPGIRGP epitopes (Figure 6). It appears very probable that the carrier would retain its helical conformation, previously identified [10,11], even after anchoring of the epitopes. Thus, it was confirmed our initial concept of designing a carrier with predetermined structural motif (reviewed in References [26,27]), in order to achieve a favourable spatial arrangement of the conjugated peptides and to obtain potent artificial antigens with potential molecular recognition properties.

Biological Assays

A comparison of sera reactivities with anti-Sm/U1RNP and anti-U1RNP specificities in anti-[$(PPGMRPP)_2$, $(PPGIRGP)_2$]-SOC_4, anti-PPGIRGP-NH_2, anti-PPGMRPP-NH_2 (reported in Reference [14]) and anti-$(PPGMRPP)_5$-SOC_5 (reported in Reference [14]) ELISA experiments is shown in Table 4.

Although [$(PPGMRPP)_2$, $(PPGIRGP)_2$]-SOC_4 and $(PPGMRPP)_5$-SOC_5 (14) are almost equally recognized by anti-Sm/U1RNP positive sera, the diepitopic conjugate is poorly recognized by anti-U1RNP positive sera. This finding is attributed to the presence of the PPGIRGP epitope on SOC_4. On the other hand, PPGIRGP-NH_2 is less specific for anti-U1RNP compared with the SOC_4 conjugate. Both conjugates SOC_4 and SOC_5 are better recognized by

Table 3 NH and $C^{\alpha}H$ Proton Chemical Shift Differences $\Delta\delta$ (ppm) of PPGMRPP-NH_2 and PPGIRGP-NH_2 Free and Bound to SOC_4

δ[[$(PPGMRPP)_2$,$(PPGIRGP)_2$]-SOC_4]-δ(PPGMRPP-NH_2)			δ[[$(PPGMRPP)_2$,$(PPGIRGP)_2$]-SOC_4]-δ(PPGIRGP-NH_2)		
	$\Delta\delta$NH	$\Delta\delta C^{\alpha}H$		$\Delta\delta$NH	$\Delta\delta C^{\alpha}H$
Pro^1		−0.06	Pro^1		0.00
Pro^2		0.01	Pro^2		−0.01
Gly^3	0.04		Gly^3	0.04	
Met^4	0.01	−0.01	Ile^4	0.01	0.00
Arg^5	0.0	0.0	Arg^5	0.00	0.03
Pro^6		−0.01	Gly^6	0.04	0.01
Pro^7		0.01	Pro^7		0.06

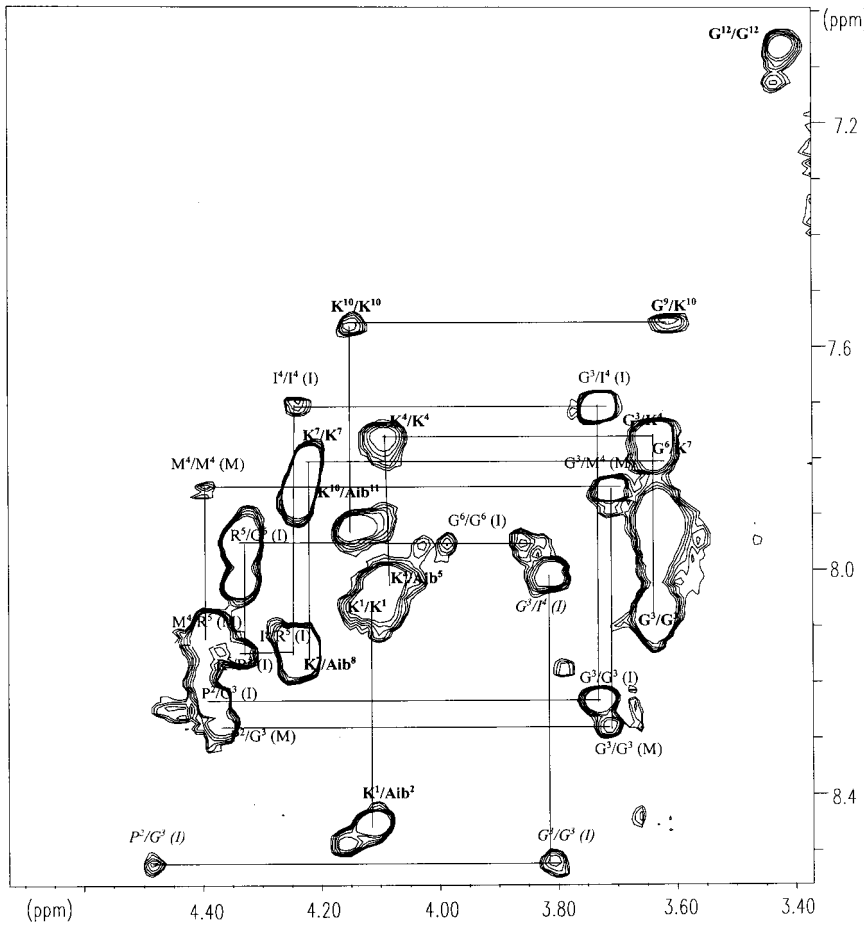

Figure 5 The $C^{\alpha}H_i/NH_{i+1}$ region of the 400 MHz NOESY spectrum of [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC$_4$. The sequential assignment of PPGMRPP is given by (M), PPGIRGP (conformer A) by (I), and PPGIRGP (conformer B) by italics (I). The sequential assignment of SOC$_4$ are given in bold.

anti-Sm/U1RNP positive sera compared with PPGIRGP-NH$_2$ and PPGMRPP-NH$_2$, respectively, possibly owing to the exposed orientation of the epitopes when they are grafted to the carrier. It is concluded that the combined use of anti-(PPGMRPP)$_2$, (PPGIRGP)$_2$-SOC$_4$ and anti-(PPGMRPP)$_5$-SOC$_5$ ELISA experiments can be applied to differentiate anti-U1RNP positive sera.

CONCLUSIONS

The PPGMRPP and PPGIRGP sequences, main epitopes of the Sm autoantigen, were grafted to the SOC$_4$ carrier with the aim at constructing a mimic of this autoantigen with minimum anti-U1RNP cross-reactivity, owing to common U1RNP proline rich motifs.

Conformational analysis of [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC$_4$ pointed out that both epitopes retain the structural characteristics found in their free forms, and indicated that they do not interact to each other, being thus disposable to molecular recognitions. Moreover, the carrier SOC$_4$ preserves its helical conformation, even after anchoring of the epitopes, which probably induces an exposed orientation of the epitopes so that they are better recognized by the autoantibodies, compared with their monomeric forms.

Biological studies indicated that although [(PPGMRPP)$_2$, (PPGIRGP)$_2$]-SOC$_4$ and (PPGMRPP)$_5$-SOC$_5$ are almost equally recognized by anti-Sm/U1RNP positive sera, the diepitopic conjugate is poorly recognized by anti-U1RNPs.

It is concluded that coupling of the main epitopes of Sm to the SOC$_4$ carrier results in a mimic of Sm which, combined to the helical structure of the

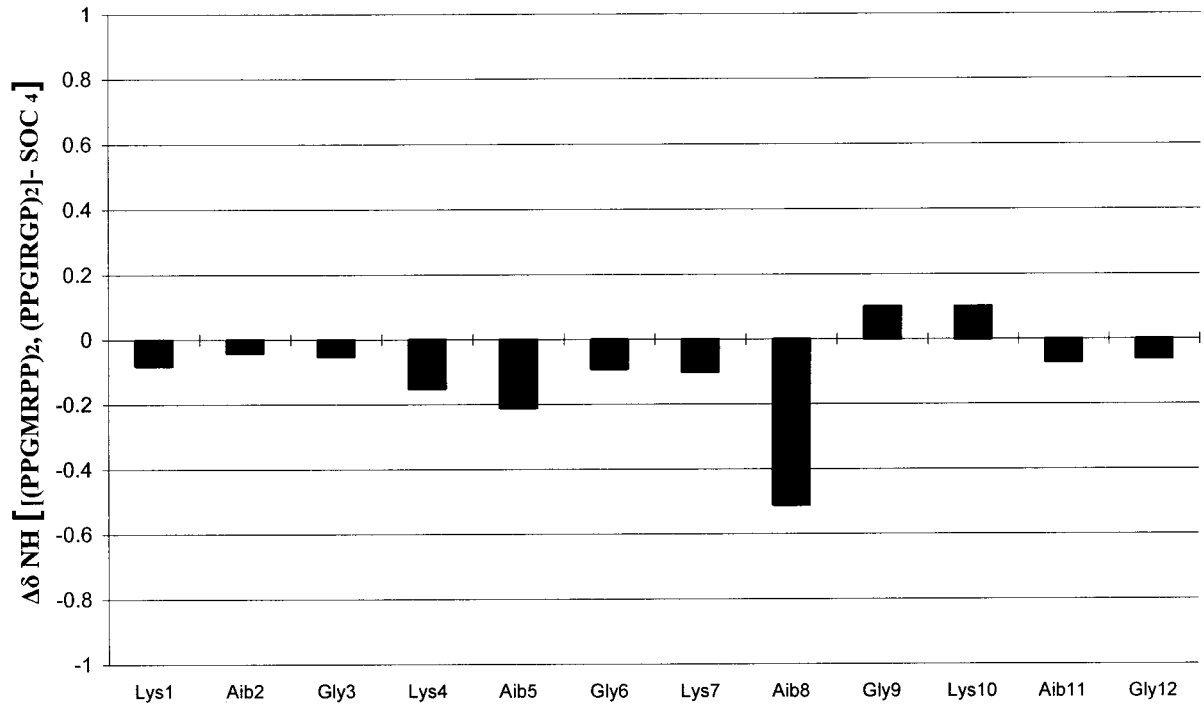

Figure 6 NH chemical shift differences of the carrier SOC_4 in its free form and bearing the epitopes PPGMRPP and PPGIRGP.

Table 4 Reactivity of Anti-Sm and anti-U1RNP Positive Sera to [$(PPGMRPP)_2$ $(PPGIRGP)_2$]-SOC_4, PPGIRGP-NH_2, PPGMRPP-NH_2 and $(PPGMRPP)_5$-SOC_5 in ELISA Assays

Antigenic substrate	Anti-Sm/U1RNP(+) $n = 17$	Anti-U1RNP(+) anti-Sm(−) $n = 15$	Normals $n = 20$
[$(PPGMRPP)_2$, $(PPGIRGP)_2$]-SOC_4	15/17	2/15	1/20
PPGIRGP-NH_2	10/17	5/15	1/20
PPGMRPP-NH_2[a]	10/17	9/15	1/20
$(PPGMRPP)_5$-SOC_5[a]	16/17	9/15	1/20

[a] Reference [14].

carrier, allows the epitopes to retain their initial conformation, being also well exposed and disposable to molecular recognitions. To this end, the combined use of anti-[$(PPGMRPP)_2$, $(PPGIRGP)_2$]-SOC_4 and anti-$(PPGMRPP)_5$-SOC_5 ELISA assays can be applied to discriminate anti-U1RNPs, which are considered as MCTD markers, from anti-Sm, which are considered as SLE markers.

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