Journal of Peptide Science
J. Peptide Sci. **11**: 254–257 (2005)
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.660

JOURNAL OF PEPTIDE SCIENCE

Sequential oligopeptide carriers: from thermal polymerization to the construction of reconstituted antigenic and immunogenic conjugates‡

CONSTANTINOS SAKARELLOS,* DIMITRIOS KRIKORIAN and MARIA SAKARELLOS-DAITSIOTIS

Department of Chemistry, Section of Organic Chemistry and Biochemistry, University of Ioannina, 45110 Ioannina, Greece

Received 10 January 2005; Accepted 19 January 2005

Keywords: antigenic conjugates; arginine rich polypeptides; histone models; immunogenic conjugates; synthetic carriers; thermal polymerization

The development of 'Sequential Oligopeptide Carriers', SOC_n, for anchoring antigenic determinants of various functionalities constitutes one of the main subjects on which our research has been focused over the last years. The starting point of this research dates back to early 1970s at the time of our postdoctoral stay in Murray Goodman's laboratory in San Diego.

PREPARATION OF SEQUENTIAL POLYPEPTIDES USING MATRIX–CONTROLLED THERMAL POLYMERIZATION

This was our first contact with the field of sequential polypeptides. This method was initially applied by Goodman and coworkers to the synthesis of polydepsipeptides [1] and later it was extended by Goodman, Gilon and two of us to the synthesis of the sequential polypeptides poly(Val-Val-Ala), poly(Gly-Val-Ala) and poly(Val-Ala-Gly), which were obtained in excellent yield and high molecular weight [2,3]. Preliminary attempts at the bulk polymerization of the 'monomer' peptide *C*-terminal pentachlorophenyl (Pcp) active esters were unsuccessful, even at high temperature, because of trapping of the liberated pentachlorophenol in the reaction mass. Deposition of $F_3CCOOH^{\bullet}$H-Val-Val-Ala-OPcp, $F_3CCOOH^{\bullet}$H-Gly-Val-Ala-OPcp and $F_3CCOOH^{\bullet}$H-Val-Ala-Gly-OPcp on an inert matrix, micron-size celite, in a ratio of peptide : celite of 2 : 3 and polymerization at 125 °C, well below the melting points of the monomers, under high vacuum (0.025 mm), resulted in the sublimation of the liberated pentachlorophenol and in a rapid polymerization. After 24 h the extent of polymerization, monitored by quantitative IR absorption spectroscopy, was calculated to be 90%. The yield and the degrees of polymerization were higher than those obtained by solution polymerization.

Comparison of the extent of racemization in the poly(Gly-Val-Ala) and poly(Val-Ala-Gly) constitutes a general approach for assessing optical purity during the synthesis of sequential polypeptides since both yield the same polymer, but only the *C*-terminal Ala may racemize in the former case.

SEQUENTIAL POLYPEPTIDES AS MODELS OF ARG-RICH HISTONES

After our return from San Diego to Greece the study of sequential polypeptides as models of Arg-rich histones was our second effort in this field. The use of simplified model polypeptides proved to be quite reasonable since histones are conformationally flexible molecules and their 3D-structure in solution is strongly dependent upon the microenvironment. Polypeptides $(Arg\text{-}Xxx\text{-}Gly)_n$, where Xxx represents the amino acid residues Ala, Val, Leu, Ile, Nle and Nva, were prepared by solution polymerization of the corresponding tripeptide active esters, and their structure and stereospecific interactions with DNA were studied by circular dichroism in various microenvironments [4–10]. The extent of helical, β-structure and β-turn conformations of the polymers was analysed in relation to the bulkiness, length and C_β-branching. Thus, when Xxx stands for Val or Nva, the degree of helical structure, in hexafluoroisopropyl alcohol–water mixtures, increases in the order Val → Nva (45% and 80% α-helix, respectively), which is consistent with the hypothesis that the lack of the β-branched side chain of the Xxx residue in the repeating trimer (referring to the same alkyl group-C_3H_7) favours the helical structure. The percentages of structural ordering for poly(Arg-Ile-Gly), poly(Arg-Nle-Gly) and poly(Arg-Leu-Gly) (10%,25% and 55%, respectively) showed that, contrary to β-branching, C_γ branching of Leu does not constitute a further conformational constraint to the α-helix. It was concluded that these sequential Arg-containing polypeptides are plausible models for histone fractions f_3 and f_2a_1.

* Correspondence to: Constantinos Sakarellos, Department of Chemistry, Section of Organic Chemistry and Biochemistry, University of Ioannina, 45110 Ioannina, Greece; e-mail: ksakarel@cc.uoi.gr

‡ Selected paper part of a special issue dedicated to the memory of Murray Goodman.

The technique of continuous-flow salt gradient linear dialysis in aqueous solutions was applied to mixtures of DNA with polypeptides at various ratios. It was found that the latter induce a decrease of the characteristic DNA CD band at 275 nm, depending on the nature of the Xxx residue, which evidences the onset of the 10.2 B-DNA (10.2 base pairs/turn instead of 10.4) conformation. A large negative DNA CD band at 275 nm in 2,2,2-trifluoroethanol/water (40 : 60 v/v) mixtures containing 0.35 to 0.5 M NaCl was attributed to the formation of the condensed structure of Ψ-DNA, invariably found *in vivo*. A drastic decrease of this band was observed in the presence of the polypeptides. It was concluded that the synthetic Arg-rich histone polypeptide models slightly unwind B-DNA and reduce the formation of Ψ-DNA aggregates. Therefore, they could be used in modulating DNA function.

With the aim of further contributing to the field of sequential polypeptides, the synthetic oligopeptide carriers of antigenic and immunogenic epitopes $(Lys\text{-}Aib\text{-}Gly)_n$, SOC_n, have been developed in our laboratory in order to formulate potent diagnostics in solid-phase immunoassays and effective immunogens as vaccine candidates. The conceptual and experimental framework of this research is presented below.

CONCEPT AND DESIGN OF SEQUENTIAL OLIGOPEPTIDE CARRIERS (SOC_n)

The conventional approach to obtain potent antigens or immunogens is to conjugate B and T cell epitopes (antigenic determinants) to a protein carrier, for example bovine serum albumin. To avoid several drawbacks of ambiguous composition, the alteration of the biologically 'active' conformation of the coupled epitopes and the generation of non-specific antibodies originated from the protein carrier, a number of artificial carriers have appeared in the literature the past decades [11].

With the aim at optimizing epitope presentation and helping in the reconstitution of an antigenic or immunogenic protein, a new class of synthetic oligopeptide carriers, SOC_n, was modelled and successfully applied in our laboratory [reviewed in references 12–14]. The SOC_n carrier, formed by the repetitive motif Lys-Aib-Gly, incorporates Lys for anchoring B/T cell epitopes, Aib for inducing a helical structure to the carrier backbone and Gly for its small stereochemical volume (Figure 1).

One of the major guidelines in designing the SOC_n carrier was to build up constructions with predetermined 3D-structure so that the attached epitopes would be characterized by a defined spatial orientation. In fact, detailed conformational analysis by ^1H NMR experiments and molecular modelling [15] provided evidence for the occurrence of a distorted 3_{10}-helix with a somewhat curved axis and a RMS deviation between every

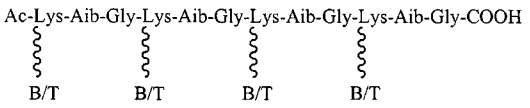

Figure 1 Schematic representation of the tetrameric sequential oligopeptide carrier, SOC_4, formed by the repeating tripeptide unit Lys-Aib-Gly. B and/or T cell epitopes are anchored to the Lys-$N^{\varepsilon}H_2$ groups.

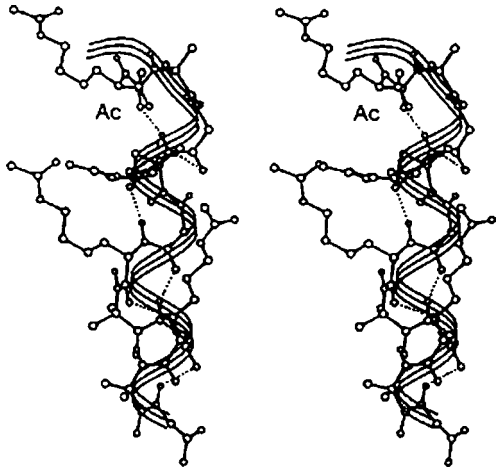

Figure 2 Minimized average structure of the sequential oligopeptide carrier (SOC_4) from the *in vacuo*-restrained MD simulation during the last 20 ps. Comparison with the ribbon representing a canonical 3_{10}-helix of the SOC_4 backbone [15]. (Copyright 1996, with permission from Elsevier Science).

atom pair of a canonical 3_{10}-helix and the SOC_n of less than 1.4 Å (Figure 2). Studies of the SOC_n-conjugates by ^1H NMR revealed that the carrier retains its helical conformation even after anchoring the B/T cell epitopes on the Lys-$N^{\varepsilon}H_2$ groups. It was also demonstrated that the coupled epitopes preserve their initial 3D-structural characteristics and reactivity [16,17].

SOC_n-CONJUGATES AS ANTIGENIC SUBSTRATES IN IMMUNOASSAYS

Covalent attachment of B/T cell epitopes, in multiple copies, to the Lys-$N^{\varepsilon}H_2$ groups of SOC_n resulted in the production of potent antigenic conjugates with enhanced antibody recognition and consequently in the development of sensitive, highly reproducible and reliable immunoassays [18,19]. A few selected examples are presented in the following.

The PPGMRPP epitope appears to be the dominant epitope of the Sm autoantigen against which the majority of autoantibodies in patients with systemic lupus erythematosus (SLE) is directed. This epitope was coupled to SOC_5. The $(PPGMRPP)_5$-SOC_5 construct, tested against sera with different autoantibody specificities, exhibited, in ELISA tests, 98% sensitivity and 68% specificity for anti-Sm and 82% and 86% for

anti-Sm/U1RNP antibodies, respectively. Taking into consideration that in ELISA experiments, using the Sm/U1RNP purified complex, the sensitivity in detecting anti-Sm/U1RNP was 74%, we concluded that the $(PPGMRPP)_5$-SOC_5 conjugate offers a clear advantage and is a better alternative as an antigenic substrate in ELISA assays [20].

B and T cell epitopes encoded by complementary nucleic acids are capable of inducing the generation of anti-idiotypic and anti-clonotypic antibodies that could interact with autoantibodies and autoreactive T cells, respectively. In this regard, such interactions could be an ideal treatment of autoimmune diseases since they could 'neutralize' the harmful autoreactive cells, resulting in the regulation or suppression of autoimmune responses [21]. The complementary epitope HOOC-PRTFSFYLYLALFRH-NH_2 (cpep349–364) of the major B cell/minor T cell epitope NH_2-GSGKGKVQFQKKKTKF-COOH (349–364) of the La/SSB autoantigen against which is mainly directed the humoral autoimmune response in patients with Sjogren's syndrome (SS) and SLE was coupled to the SOC_4 carrier in two copies and the resulting Ac-[$(Ac)_2$, $(cpep349–364)_2$]-SOC_4 conjugate was studied. It was found that this conjugate is able to unmask the anti-La/SSB response in anti-La/SSB negative sera from patients with SS and SLE. In particular, heating at 55 °C and treatment with the complementary epitopes of the anti-La/SSB negative sera resulted in the recovery of the anti-La/SSB reactivity and thus in the establishment of an improved diagnostic tool in SLE and SS. It is worth noting that the anti-La/SSB negative sera represent a stable serological subset among patients with SS and SLE [22].

SOC_n-CONJUGATES FOR INDUCING SPECIFIC ANTIBODIES

Immunized animals with SOC_n-conjugates produced high titres of antibodies recognizing the immunogenic peptides. Depending on the epitope anchored to SOC_n, it was identified either as an immune spreading covering various peptide sequences on the protein, as well as the intact protein, or as a limited expansion of the B-cell repertoire [23]. Selected examples are presented below.

Animals immunized with $(PPGMRPP)_5$-SOC_5 developed high titres of anti-$(PPGMRPP)_5$-SOC_5 antibodies as determined in ELISA assays [24]. Precipitating anti-Sm and anti-U1RNP antibodies were detected in animal sera by RNA precipitation and Western blot on HeLa total cellular and nuclear extract. However, antibodies recognizing the native forms of Sm or U1RNP antigens did not appear in the sera of the immunized animals. It was concluded that immunizations with $(PPGMRPP)_5$-SOC_5 result in a rather site-specific response to the epitope PPGMRPP than in the expansion of the B-cell repertoire to other parts of the antigen or even to the entire antigen, in agreement with other reports in the literature [25]. It is of interest that the immune response induced by $(PPGMRPP)_5$-SOC_5 is associated with immune-mediated kidney injury, suggesting that these antibodies are crucial in inducing renal disease.

Antibodies in patients with SS are mainly directed against four distinct B cell epitopes located at positions 145–164, 289–308, 301–318 and 349–364 of the La/SSB autoantigen. Immunization of rabbits with each one of these epitopes, coupled to the SOC_4 in four copies, $(La/SSB\ epitope)_4$-SOC_4, resulted in the production of antibodies directed: (a) to the particular immunizing epitope, (b) to other epitopes of La/SSB, and (c) to the rLa/SSB autoantigen. These findings argue in favour of an immune spreading. Moreover, T cell responses against the epitopes of La/SSB were detected in spleen cells from the immunized animals, indicating that B cell epitopes function as T cell epitopes in this experimental animal [26].

The study of an adjuvant conjugated SOC_n carrier, incorporating a fragment of IL-1β (interleukin-1β), is currently in progress in our laboratory for the development of human vaccine candidates.

REFERENCES

1. Nissen D, Gilon C, Goodman M. Polydepsipeptides. 4. Synthesis of the alternating polydepsipeptides poly(Ala-Lac) and poly(Val-Lac). *Makromol. Chem. Suppl.* 1975; **1**: 23–53.
2. Sakarellos-Daitsiotis M, Gilon C, Sakarellos C, Goodman M. A new method for the preparation of sequential polypeptides using matrix-controlled thermal polymerization. *J. Am. Chem. Soc.* 1976; **98**: 7105–7107.
3. Sakarellos-Daitsiotis M, Gilon C, Sakarellos C, Goodman M. Sequential polypeptides by matrix-controlled thermal polymerization. Synthesis and racemization studies. *Biopolymers* 1977; **16**: 2507–2520.
4. Panou-Pomonis E, Sakarellos C, Sakarellos-Daitsiotis M. Sequential polypeptides containing arginine as histone models: synthesis and conformational studies. *Biopolymers* 1986; **25**: 655–675.
5. Tsikaris V, Sakarellos-Daitsiotis M, Sakarellos C, Marraud M. CD studies of synthetic polypeptides as histone models: poly(L-Arg-L-Ile-Gly), poly(L-Arg-L-Nva-Gly), and poly(L-Arg-L-Nle-Gly). *Biopolymers* 1988; **27**: 213–229.
6. Panou-Pomonis E, Sakarellos C, Sakarellos-Daitsiotis M. Circular dichroism studies on chromatin models. Interactions between DNA and sequential polypeptides containing arginine. *Eur. J. Biochem.* 1986; **161**: 185–190.
7. Tsikaris V, Sakarellos-Daitsiotis M, Panou-Pomonis E, Sakarellos C, Marraud M. Circular dichroism studies on sequential polypeptides (Arg-X-Gly) when X = L-Ala, Val, Leu, Ile, Nva, Nle in amphiphilic environments. *Int. J. Pept. Protein Res.* 1989; **33**: 195–201.
8. Tsikaris V, Panou-Pomonis E, Sakarellos C, Sakarellos-Daitsiotis M, Marraud M. Changes in the c.d. spectra of calf thymus DNA induced by sequential polypeptides in aqueous solutions. Part I. *Int. J. Biol. Macromol.* 1991; **13**: 349–354.

9. Panou-Pomonis E, Tsikaris V, Sakarellos C, Sakarellos-Daitsiotis M, Marraud M. Changes in the c.d. spectra of calf thymus DNA induced by sequential polypeptides in trifluoroethanol solutions. Part II. *Int. J. Biol. Macromol.* 1991; **13**: 355–368.
10. Sakarellos-Daitsiotis M. Nucleohistone complexes. In *Polymeric Materials Encyclopedia*, Salamone JC (ed.). CRC Press: Boca Raton, FL, 1996; **6**: 4662–4672.
11. Tam JP. Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. *Proc. Natl Acad. Sci. USA* 1988; **85**: 5409–5413.
12. Veprek P, Jezek J. Peptide and glycopeptide dendrimers. Part I. *J. Pept. Sci.* 1999; **5**: 5–23.
13. Veprek P, Jezek J. Peptide and glycopeptide dendrimers. Part II. *J. Pept. Sci.* 1999; **5**: 203–220.
14. Tam JP. Macropeptide structures. Synthesis of peptide dendrimers and protein mimetics. In *Houben-Weyl, Methods of Organic Chemistry. Synthesis of Peptides and Peptidomimetics*, Goodman M, Felix A, Moroder L, Toniolo C (eds). Thieme: Stuttgart, 2003; **E22d**: 129–168.
15. Tsikaris V, Sakarellos C, Sakarellos-Daitsiotis M, Orlewski P, Marraud M, Cung MT, Vatzaki E, Tzartos S. Construction and application of a new class of sequential oligopeptide carriers (SOC_n) for multiple anchoring of antigenic peptides. Application to the acetylcholine receptor (AChR) main immunogenic region. *Int. J. Biol. Macromol.* 1996; **19**: 195–205.
16. Alexopoulos Ch, Tsikaris V, Rizou C, Panou-Pomonis E, Sakarellos-Daitsiotis M, Sakarellos C, Vlachoyiannopoulos P, Moutsopoulos HM. A diepitopic sequential oligopeptide carrier (SOC_n) as mimic of the Sm autoantigen: synthesis, conformation and biological assays. *J. Pept. Sci.* 2001; **7**: 105–114.
17. Keramisanou D, Tsikaris V, Sakarellos-Daitsiotis M, Sakarellos C, Mikhailova AA, Strelkov LA. Multiple anchoring of myelopeptides on sequential oligopeptide carriers (SOC_n): synthesis, conformation and studies in human leukemia cells. *J. Pept. Res.* 2002; **60**: 178–185.
18. Sakarellos-Daitsiotis M, Tsikaris V, Vlachoyiannopoulos PG, Tzioufas AG, Moutsopoulos HM, Sakarellos C. Peptide carriers. A helicoid-type sequential oligopeptide carrier (SOC_n) for multiple anchoring of antigenic/immunogenic peptides. *Methods* 1999; **19**: 133–141.
19. Sakarellos-Daitsiotis M, Alexopoulos Ch, Sakarellos C. Sequential oligopeptide carriers, SOC_n, as scaffolds for the reconstitution of antigenic proteins: applications in solid phase immunoassays. *J. Pharm. Biomed. Anal.* 2004; **34**: 761–769.
20. Petrovas CJ, Vlachoyiannopoulos PG, Tzioufas AG, Alexopoulos Ch, Tsikaris V, Sakarellos-Daitsiotis M, Sakarellos C, Moutsopoulos HM. A major Sm epitope anchored to sequential oligopeptide carriers is a suitable antigenic substrate to detect anti-Sm antibodies. *J. Immunol. Methods* 1998; **220**: 59–68.
21. Sakarellos-Daitsiotis M, Cung MT, Sakarellos C, El Hilali Z, Kosmopoulou A, Voitharou C. Complementary peptide epitopes and anti-idiotypic antibodies in autoimmunity. *Protein Pept. Lett.* 2004; **11**: 367–375.
22. Routsias JG, Touloupi E, Dotsika E, Moulia A, Tsikaris V, Sakarellos C, Sakarellos-Daitsiotis M, Moutsopoulos HM, Tzioufas AG. Unmasking the anti-La/SSB response in sera from patients with Sjogren's syndrome by specific blocking of anti-idiotypic antibodies to La/SSB antigenic determinants. *Mol. Med.* 2002; **8**: 293–305.
23. Sakarellos-Daitsiotis M, Tsikaris V, Sakarellos C, Vlachoyiannopoulos PG, Tzioufas AG, Moutsopoulos HM. A new helicoid-type sequential oligopeptide carrier (SOC_n) for developing potent antigens and immunogens. *Vaccine* 1999; **18**: 302–310.
24. Vlachoyiannopoulos PG, Petrovas C, Tzioufas AG, Alexopoulos Ch, Tsikaris V, Guialis A, Nakopoulou L, Sakarellos-Daitsiotis M, Sakarellos C, Davaris P, Moutsopoulos HM. No evidence of epitope spreading after immunization with the major Sm epitope P-P-G-M-R-P-P anchored to sequential oligopeptide carriers (SOCs). *J. Autoimmun.* 2000; **14**: 53–61.
25. Mason LJ, Timothy LM, Isenberg DA, Kalsi JK. Immunization with a peptide of Sm B/B′ results in limited epitope spreading but not autoimmune disease. *J. Immunol.* 1999; **162**: 5099–5105.
26. Yiannaki E, Vlachoyiannopoulos PG, Manoussakis MN, Sakarellos C, Sakarellos-Daitsiotis M, Moutsopoulos HM, Tzioufas AG. Study of antibody and T-cell responses in rabbits immunized with synthetic human B-cell epitope analogues of La (SSB) autoantigen. *Clin. Exp. Immunol.* 2000; **121**: 551–556.