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Synthesis and evaluation of cell-attachment activity of RGDS-related molecules

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Arg-Gly-Asp-Ser (RGDS) related molecules were synthesized for the purpose of improving the cell attachment activities of RGDS oligopeptide, and their cell-attachment activities were assayed. Moreover, theoretical conformation analysis was carried out for Ac-Arg-Gly-Asp-Ser-NHMe H-Arg-Gly-Asp-Ser-OH and Ac-(Arg-Gly-Asp-Ser)$_8$-NHMe, using ECEPP and conformational energy minimization procedure. The cell attachment property of L-929 cell was more specifically inhibited by adding (RGDS)$_n$ than adding RGDS oligopeptide. Activity of cell attachment depends on the molecular weight of poly(RGDS) and RGDS$_n$-MAP. Results of conformational analysis suggested that side chains locate in the suitable position for playing the importance role as the ligand for the cell surface receptor.

1. INTRODUCTION

Recent progress in the structure and bioactivity elucidation of such extracellular, structural proteins as fibronectin, vitronectin, and collagen has provided information on the cell attachment sites of these proteins. Tetrapeptides Arg-Gly-Asp-Xaa (Xaa=Ser, Val, Ala and Thr) were suggested to be the cellular recognition determinants of fibronectin, vitronectin, and collagen, respectively[1,2]. In this work, Arg-Gly-Asp-Ser (RGDS) related molecules were synthesized for the purpose of improving the cell attachment activities of RGDS oligopeptide[3,4], and their cell-attachment activities were assayed by cell-inhibition method and cell-attachment method. Moreover, theoretical conformation analysis was carried out for Ac-Arg-Gly-Asp-Ser-NHMe H-Arg-Gly-Asp-Ser-OH and Ac-(Arg-Gly-Asp-Ser)$_8$-NHMe, using ECEPP[5] and conformational energy minimization procedure.

2. EXPERIMENTS

2.1 Oligopeptide synthesis

Scheme of liquid-phase RGDS synthesis is shown in Fig. 1.

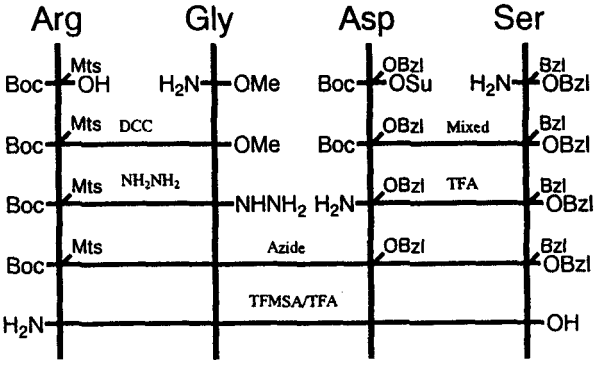


Fig. 1 Scheme of RGDS synthesis by liquid-phase procedure.

$(RGDS)_4$ and $(RGDS)_8$ were prepared by combination of solid and liquid phase procedures. The same method as mentioned above was used for the synthesis of $(RGDS)_8K_4K_2KG$ ($RGDS_8$-MAP)

(Fig. 2) and $(RGDS)_4K_2KG$ ($RGDS_4$-MAP), which were conjugated RGDS at α and ε amino group of Lys residue. Poly(RGDS) was synthesized by polymerization procedure with diphenylphosphoryl azide(DPPA)[6, 7].

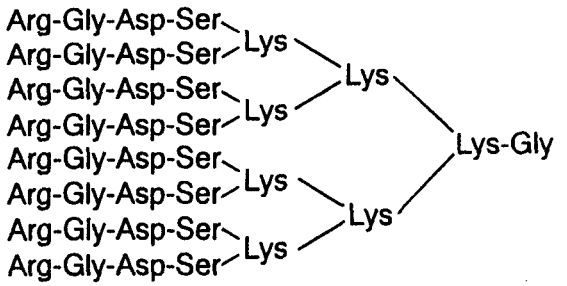


Fig. 2 Structure of $(RGDS)_8K_4K_2KG$ ($RGDS_8$-MAP).

2.2 Conformational Analysis

Conformational energies of Ac-Arg-Gly-Asp-Ser-NHMe, H-Arg-Gly-Asp-Ser-OH and ac-$(Arg\text{-}Gly\text{-}Asp\text{-}Ser)_n$-NHMe were optimized with the energy functions ECEPP (Empirical Conformational Energy Program for Peptides) and Powell algorithm. During minimization, all ϕ, ψ, and all χ values for Arg, Gly and Asp residues were allowed to vary except that $\chi^{6.2}$ of Arg was fixed at 180° or -180 ° ,and also that all ω were fixed at 180° .

3. RESULTS AND DISCUSSION

3.1 Oligopeptides Synthesis and Cell-Attachment Activity Test

The number-averaged molecular weight of poly(RGDS) was estimated to be approximately n=15~18 by GPC measurement. These peptides were characterized by amino-acid analysis and elemental analysis. Fig. 3 shows the percentage of cell attachment onto fibronectin coated dish from the suspensions of L-929 fibroblast cell including $(RGDS)_n$ molecules plotted against incubation time (2.0×10^{-4}mmol•RGDS/*ml*). The cell attachment property of L-929 cell to fibronectin coated substrate was more specifically inhibited by adding $(RGDS)_n$ than adding RGDS oligopeptide. The percentage of cell attachment from the suspensions of L-929 cell including $RGDS_n$-MAP molecules plotted against incubation time in Fig. 4. Activity of cell attachment depends on the molecular weight of $(RGDS)_n$ and $RGDS_n$-MAP.

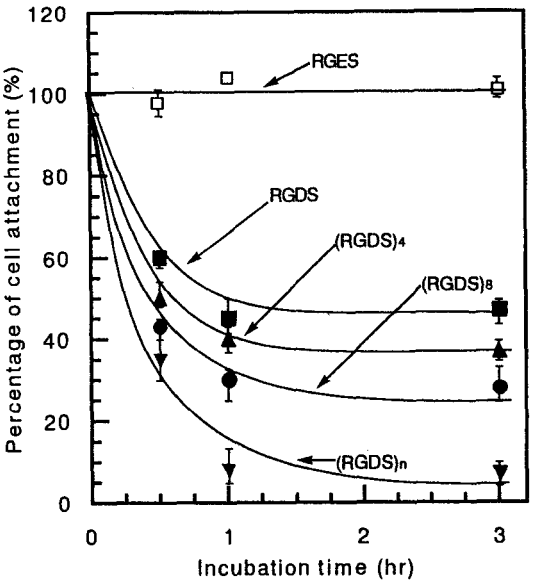


Fig. 3 Inhibition effect of $(RGDS)_n$ molecules for attachment of L-929 cells on the fibronectin coated dish.

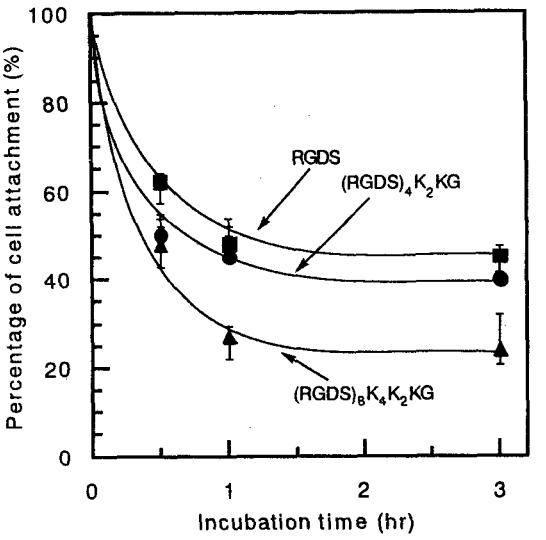


Fig. 4 Inhibition effect of $RGDS_n$-MAP molecules for attachment of L-929 cells on the fibronectin coated dish.

These results suggested that chain conformations of RGDS and poly(RGDS) would play an important role for exhibiting the cell attachment activities. Thus, we checked the chain conformations of RGDS and poly(RGDS), using molecular mechanics and optimization procedure.

3.2 Conformational Analysis

A total of 894 energy minima of Ac-Arg-Gly-Asp-Ser-NHMe was obtained in $\Delta E < 5.0$ kcal•mol^{-1}. The tetrapeptide was represented by an ensemble composed of many stable conformations. The lowest-energy conformation of Ac-Arg-Gly-Asp-Ser-NHMe is shown in Fig. 5. The backbone conformation of AAAA is an α-helical conformation. According to the calculation, Ac-Arg-Gly-Asp-Ser-NHMe is stabilized by three kinds of hydrogen bondings: i.e., (Ac-)CO•••HN(Ser), (Ac-)CO•••HO(Asp), and (Arg)CO•••HN(-NHMe) and takes non-bend structure. The guanidino residue of Arg locates at the opposite side of the carbonyl group of Asp, and the distance between hydrogen atom of -NH_2 group and hydrogen atom of -COOH group is ca. 13.1 Å.

Thus, 894 energy minima of Ac-Arg-Gly-Asp-Ser-NHMe in $\Delta E < 5.0$ kcal•mol^{-1} were used for the starting conformations of H-Arg-Gly-Asp-Ser-OH. A stable conformation of H-Arg-Gly-Asp-Ser-OH whose inter-proton distance corresponding to the results of NOE measurement in H_2O is shown in Fig. 6. H-Arg-Gly-Asp-Ser-OH is stabilized by three kinds of hydrogen bondings: i.e., (Arg)NH••• OC(Ser), (Arg)CO•••HN(Asp), and (Asp)C$^{\delta}$O••• HO(Ser) and takes type II bend structure[8] at Arg-Gly portion. The hydrogen bond (Asp)C$^{\delta}$O••• HO(Ser) is very important to support the side-chain conformation of Asp. In such chain conformation, the guanidino group of Arg residue locates at the same side as the carbonyl group of Asp side-chain, the side chain of Asp is supported by Ser with hydrogen binding.

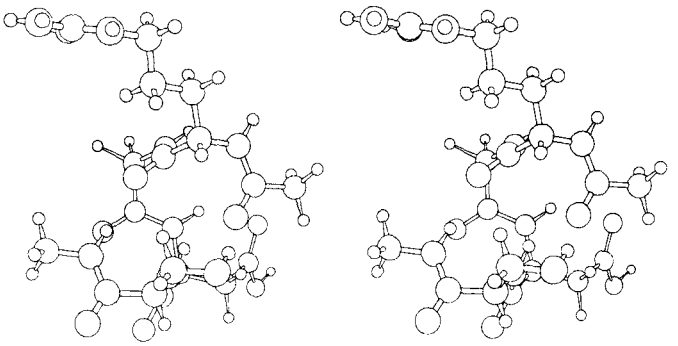


Fig. 5 The lowest-energy conformation of Ac-Arg-Gly-Asp-Ser-NHMe.

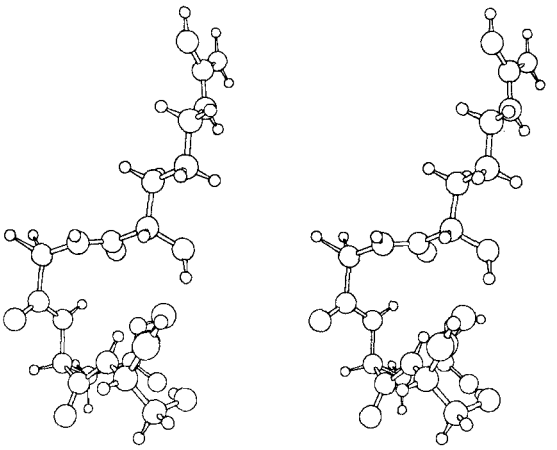


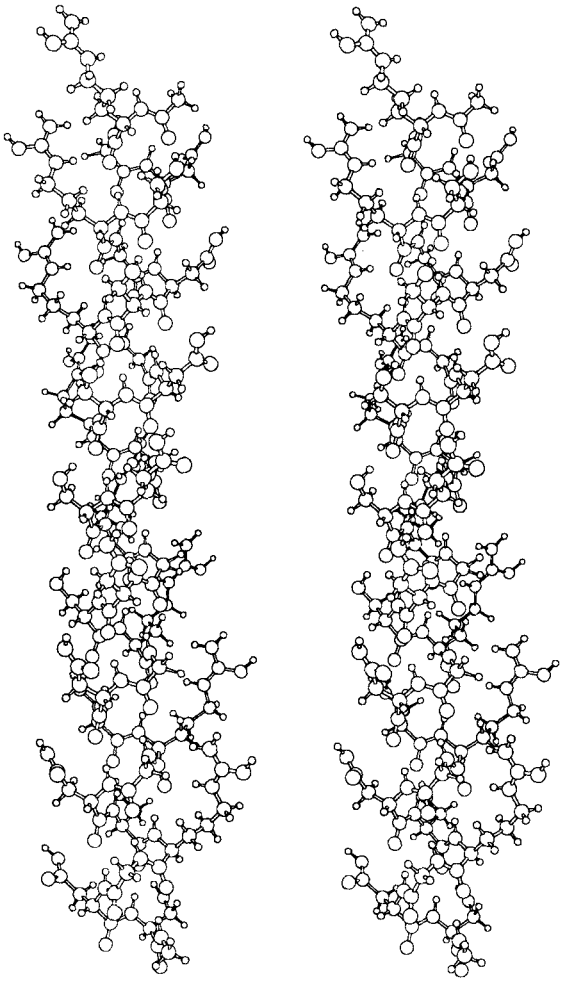
Fig. 6 The chain conformation of H-Arg-Gly-Asp-Ser-OH in H_2O.

Minimum-energy conformations of Ac-Arg-Gly-Asp-Ser-NHMe under the condition $\Delta E < 5.0$ kcal•mol^{-1} were used as the starting conformations of the peptide repeating units of Arg-Gly-Asp-Ser. And then, all minimum-energy conformations of Ac-(Arg-Gly-Asp-Ser)$_n$-NHMe (n=2,4) were used as the starting conformations for the minimization of conformational energy of Ac-(Arg-Gly-Asp-Ser)$_8$-NHMe.

A total of 114 low-energy minima of Ac-(Arg-Gly-Asp-Ser)$_8$-NHMe in $\Delta E_{res} \leq 1.0$ kcal•mol^{-1} was obtained as shown in Fig. 7, the lowest-energy conformation is a right-handed α-helical

conformation having six types of hydrogen-bonds, $(Arg_i)CO\cdots HN(Arg_{i+4})$, $(Arg_i)CO\cdots HN^{\varepsilon}(Arg_{i+4})$ $(Gly_i)CO\cdots HN(Gly_{i+4})$, $(Asp_i)CO\cdots HN(Asp_{i+4})$, $(Ser_i)CO\cdots HN(Ser_{i+4})$, and $(Ser_i)CO\cdots HO(Ser_{i+4})$. All side-chain groups of Arg, Asp and Ser are exposed to the outside of helix. These results suggested that side chains locate in the suitable position for playing the importance role as the ligand for the cell surface receptor.

A)



B)

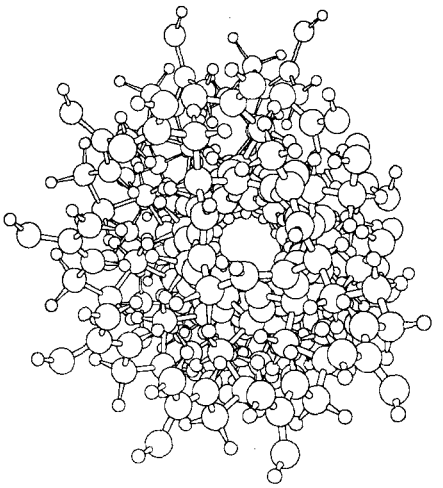


Fig. 7 The lowest-energy conformation of poly-(Arg-Gly-Asp-Ser). A) Side-view, B) Top-view.

4. REFERENCE

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