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Three-dimensional solution structure of EM703 with potent promoting activity of monocyte-to-macrophage differentiation

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Abstract—EM703, which is an erythromycin derivative synthesized by our group, has a potent promoting activity of monocyte-to-macrophage differentiation in vitro. Its activity is approximately 300 times higher than that of erythromycin A (EM-A). In this study, we determined three-dimensional (3D) solution structures of EM703 and EM-A, and compared them using a superposition method, in order to investigate the 3D structure–activity relationship. We found a distinct difference between the 3D structures of these molecules, which might be an important factor in their divergent activities.

Erythromycin A (EM-A; Fig. 1A) is a macrolide antibiotic isolated from cultures of *Streptomyces erythreus* that has been widely used to treat a variety of infectious diseases. In 1987, it was reported that EM-A was efficacious against diffuse panbronchiolitis, which is an incurable chronic inflammatory airway disease.[1,2] This finding suggested that EM-A might possess anti-inflammatory or immunomodulatory activity. During the past decade, it was shown that EM-A has a wide range of anti-inflammatory and immunomodulatory effects. For example, EM-A was found to have inhibitory activity against the inflammatory functions of neutrophils[3] and to promote differentiation of the human monocytic cell line THP-1 to macrophages.[4,5] EM-A is thought to interact with a receptor that is related to anti-inflammatory or immunomodulatory activity. Recently, we chemically modified EM-A in order to obtain derivatives with stronger promoting activities of monocyte-to-macrophage differentiation.[6] One of these derivatives, EM703 (Fig. 1B), exhibited an activity approximately 300 times higher than that of EM-A. The chemical structure of EM703 differs from that of EM-A in the C1 and C6–C15 regions. These structural differences are considered to be important for the potent activity of EM703, because all other derivatives with the same macrolide ring structure showed stronger activities than EM-A.[6] In this study, we determined the three-dimensional (3D) solution structures of EM-A and EM703, and compared them using a superposition method. This approach was intended to reveal any contrasting 3D structural features of the two macrolides that might be important for their different activities.

We employed SYBYL6.91 (Tripos, St Louis, MO, USA) to prepare initial structures for EM-A and EM703. Conformational analysis of the two macrolides was then performed using the Conformational Analyzer with Molecular Dynamics And Sampling (CAMDAS) 2.1 program[7] developed in our laboratory. CAMDAS generates the energetically accessible conformers of a target molecule by performing an MD calculation and sampling conformers along the trajectory. The procedure for the CAMDAS calculation was similar to that described previously.[8] In total, 33 (35) dihedral angles were used to cluster similar conformations for EM-A

Keywords: Erythromycin A; Erythromycin derivative; Immunomodulatory activity; NMR; Molecular dynamics; Three-dimensional solution structure; Alignment; QSAR.

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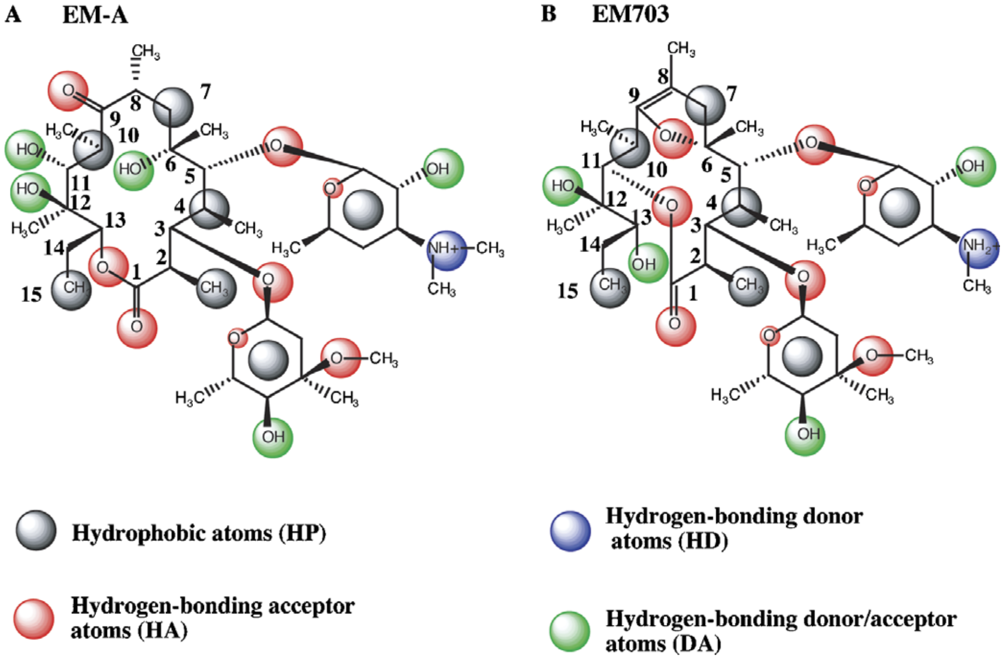

Figure 1. Chemical structures and functional atoms of EM-A (A) and EM703 (B). The circles represent the property radii of the functional atoms: large circles = 1 Å; small circles = 0.5 Å.

(EM703). From the CAMDAS calculation, 8683 (8723) different conformers were obtained for EM-A (EM703).

Next, nuclear magnetic resonance (NMR) experiments were performed to extract structural information on the two macrolides.[9] Dihedral constraints were obtained by applying the Karplus equation to vicinal proton–proton scalar coupling constants,[10] which were obtained from a high-resolution one-dimensional proton spectrum. Two-dimensional transverse rotating-frame Overhauser-enhancement spectroscopy (TROESY) was also performed in order to obtain proton–proton distance constraints.[11,12] In total, 81 (82) constraints—comprising 67 (70) distance and 14 (12) dihedral constraints—were obtained for EM-A (EM703). We then extracted conformers from the ensemble generated by the CAMDAS calculation, which satisfied the distance and dihedral constraints. As a result, three (four) conformers were obtained as solution structures of EM-A (EM703). Figures 2A and B show stereopairs of the best-fit superpositions of the heavy atoms for the solution structures of EM-A and EM703, respectively. The average pairwise root mean square deviation between the three (four) individual structures was 0.56 ± 0.11 (0.55 ± 0.13) Å for all heavy atoms of EM-A (EM703). These small values indicate that the 3D structures of the macrolides were well defined.

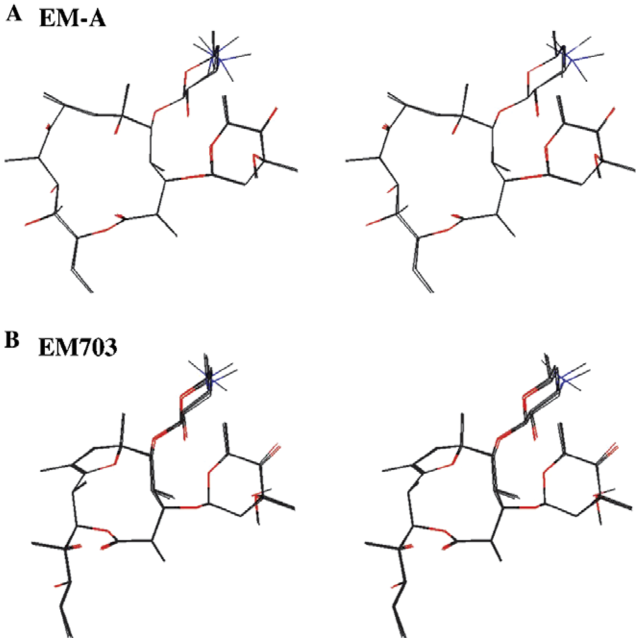

Figure 2. The stereopairs of superpositions of the 3D solution structures of EM-A (A) and EM703 (B). The superpositions correspond to the best-fit of the heavy atoms.

Finally, alignment of the 3D structures of EM-A and EM703 was carried out using the molecular-overlay program SUPERPOSE,[13] which was recently developed in our laboratory. This program is based on the premise that compounds binding to the same site of a receptor possess common 3D structural features. The program superposes two molecules based on their respective physicochemical properties. It deals with pseudo-molecules, consisting of functional atoms, instead of real molecules. The functional atoms are divided into four types: hydrophobic atoms (HPs), hydrogen-bonding donors (HDs), hydrogen-bonding acceptors (HAs) and hydrogen-bonding donors/acceptors (DAs). The functional atoms of EM-A and EM703 were defined as shown in Figures 1A and B, respectively, according to the criteria described in our previously published paper.[13] The procedure for the SUPERPOSE calculation was the same as that described previously.[8] When atomic groups with the same properties are overlapped, points are added to the score, whereas points are subtracted from the score if atomic groups with different properties are overlapped. The superposition between the two molecules with the highest score is adopted. Figure 3 shows the resulting alignment between the 3D structures of EM703 and EM-A. The functional atoms defined on the C2–C5 region of the macrolide rings, and on the two sugar rings, overlapped relatively closely (Figs. 3B and C). This was expected, because the two macrolides have almost identical conformations in these regions. The functional atoms defined on the C1 and C6–C15 regions of the macrolide rings were also found to overlap well (Figs. 3B and C). This overlap was better than expected, as the conformation of the C1 and C6–C15 regions of EM703, which are considered important to its high activity, seemed to differ from those of EM-A (Fig. 3A). Figure 4 shows the alignment of the C1 and C6–C15 regions in detail. The HP 1, HP 2, HP 3, HA 1, HA 2, HA 3, DA 1 and DA 2 atoms of EM703 overlapped with the HP I, HP II, HP III, DA III, HA III, HA II, DA I and DA II atoms of EM-A, respectively (Fig. 4C). The overall common relative spatial dispositions of these functional atoms might contribute to the interaction with a receptor. However, there is one clear difference in the spatial dispositions of the functional atoms: the HA I atom in EM-A does not overlap with any HA or DA atoms of EM703 (Fig. 4C) and is in close spatial proximity to HP 2 of EM703. The presence of the HA I atom might be one reason for the lower activity of EM-A. The hydrophobic region around the HP 2 atom of EM703 might be important for its potent activity because, for instance, it might bind to the hydrophobic pocket of a receptor. This finding could aid in the rational design of macrolides with even greater potent immunomodulatory activities.

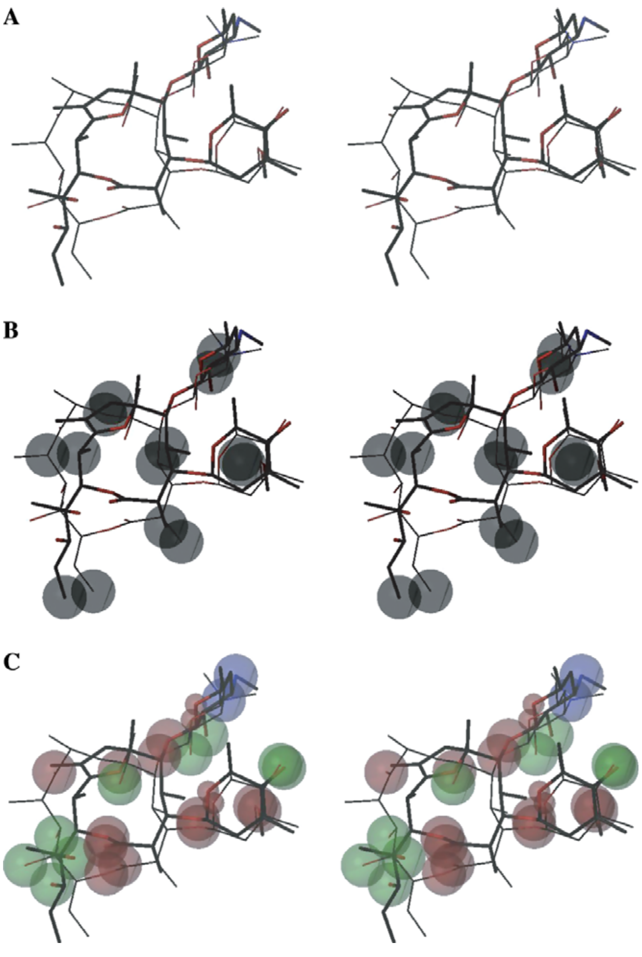

Figure 3. The stereopair of aligned 3D structures of EM-A (thin line) and EM703 (thick line) (A). The stereopair of aligned HP atoms (B). The stereopair of aligned HA (red), HD (blue) and DA (green) atoms (C).

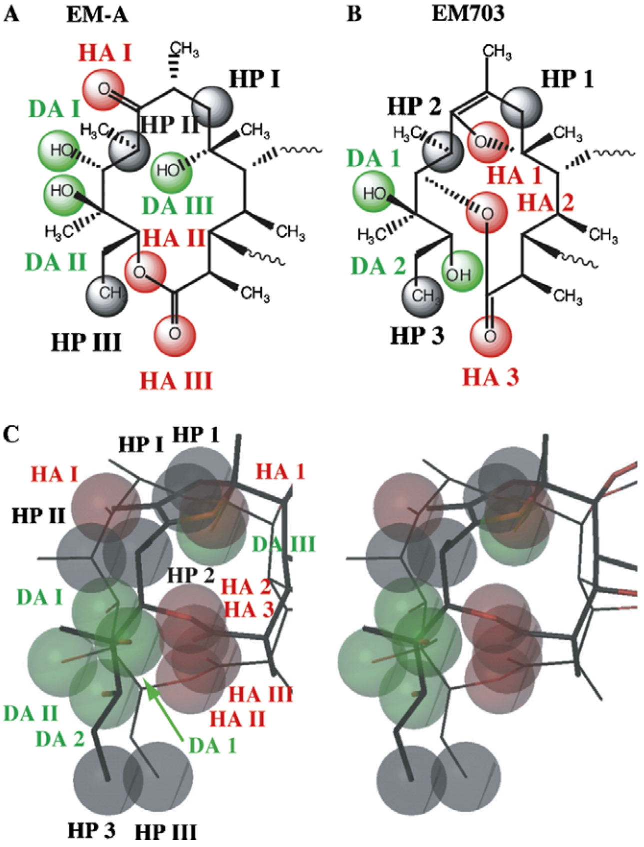

Figure 4. Numbering of functional atoms of EM-A (A) and EM703 (B) in the C1 and C6–C15 regions. Enlargement of the stereopair of aligned C1 and C6–C15 regions (C).

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.079.

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