Three-Dimensional Structure–Activity Relationship Analysis between Motilin and Motilide Using Conformational Analysis and a Novel Molecular Superposing Method

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Motilin, an erythromycin derivative, has been shown to equal activity to that of motilin as an agonist at the motilin receptor. However, there is little information on the three-dimensional (3D) structure–activity relationship between these two molecules, largely because they have quite different structures. In this study, we applied a rational computational procedure consisting of conformational analysis and a novel superposing method to investigate the 3D structure–activity relationship between motilin and motilide. We propose common 3D structural features between these molecules, which may be important for their similar activity.

Key words motilide; motilin; agonist; pharmacophore; molecular dynamics; alignment

The contractions of gastrointestinal smooth muscles are stimulated by motilin, a linear peptide hormone with 22 amino acids residues.1–5) Recently, a receptor for motilin has been identified in the human gastrointestinal system.6) It has also been found that erythromycin A (EM-A) and its derivatives work as agonists at the motilin receptor site.7) Therefore these agonists were termed “motilide(s),” meaning a motilin-like macrolide.8) From the perspective of structure–activity relationships, it is interesting that both motilin and motilide act as agonists of the motilin receptor, given that they have quite different chemical structures. They must possess some common three-dimensional (3D) structural features relative to their biological activity. To date, several studies on synthetic fragments of motilin have appeared.9–11) It has been reported that four hydrophobic residues, Phe1, Val2, Ile4, and Tyr7, are most essential for its activity.9–11) Recently, the 3D structure of motilin in SDS micelles, i.e., in a membrane-like environment, was determined using NMR spectroscopy with high resolution.12) This structure can be considered the best potential candidate for the active, i.e., receptor-bound, conformation. In this structure, Phe1 and Val2, near the N-terminus, are in almost extended conformation. Phe1, Val2, and Ile4 then form a large hydrophobic region, and Tyr7 forms another small one. The spatial disposition of these two hydrophobic regions is assumed to be essential for the activity of motilin. The entire hydrophobic region of motilin, residues 1–9, is at nearly a right angle against the axis of the clearly observable helical part in the center of the molecule. Chemical modification studies of motilide have been reported.13,14) However, little information is available in the current literature on the structure-activity relationships between motilin and motilide.

In this study, we performed the following computational calculations to investigate the 3D structure–activity relationships between motilin and motilide. First, conformational analysis was performed to obtain a number of energetically stable conformations of EM536, one of the motilides. Because the activity of EM536 is as potent as that of motilin,7) EM536 is thought to have hydrophobic regions corresponding to the Phe1, Val2, Ile4, and Tyr7 of motilin. As our point of departure, we employed the CAChe package (CAChe Scientific, Oxford Molecular Group Inc., Beaverton, OR, U.S.A.) to prepare structure files for EM536, based on the X-ray analysis of EM523, which has some cyclic structures as EM536 (personal communication). Then the Conformational Analyzer with Molecular Dynamics And Sampling (CAMDAS) program,15) developed in our laboratory, was employed. This program generates the energetically stable conformations of a target molecule by performing the high-temperature molecular dynamics (MD) calculation and sampling conformations along the MD trajectory. CAMDAS then clusters similar conformations based upon values of dihedral angles defined before calculation. A total of 7 dihedral angles used to cluster similar conformations is indicated by arrows in Fig. 1A. The dihedral angles involved in a 14-membered ring and two sugar rings were constrained in MD calculations to retain their cyclic structures that were derived from X-ray analysis. The constraint energy term was quadratic, and the force constants were 100 kcal mol⁻¹ rad⁻². We finally obtained a total of 714 distinct conformations for EM536 using CAMDAS.

Next, the alignment between the NMR structure of motilin and a total of 714 distinct conformations for EM536 were carried out using the novel molecular overlay program “SUPERPOSE,”16) assuming that the NMR structure in SDS micelles of motilin is its active conformation. SUPERPOSE, recently developed in our laboratory, enables us to obtain reliable alignment between molecules for 3D quantitative structure–activity relationship analysis (3D-QSAR). The SUPERPOSE program superposes two molecules based on their respective physicochemical properties by treating a pseudo-molecule consisting of functional atoms instead of a real molecule. The properties of the functional atoms are divided into four types, comprising hydrophobic atoms (HP), hydrogen-bonding donors (HD), hydrogen-bonding acceptors (HA), and hydrogen-bonding donors/acceptors (DA), each of which is represented as a sphere or a few spheres with a given radius depending on the chemical properties. In order to perform the superposition of two molecules systematically, first the center of mass of each molecule is translated to the origin of coordinates, and then the circumscribed rectangular boxes are calculated. The molecule with a large box volume is fixed, and then the center of mass for the molecule with a small box volume is translated and rotated. The range of translation is the maximum distance that the small box can translate inside the large box. The translational increment is 1 Å and the center of mass is translated on the body-centered cubic lattice points in the circumscribed rectangular box of large volume. The rotation is performed on each of the lattice points using three Eulerian angles. The ranges of the three Eulerian angles are 0≤φ,ψ≤350°, and 0≤θ≤180° and the

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A: EM536

B: motilin

Fig. 1. Chemical Structures and Functional Atoms of EMAS636 (A) and Motilin (B)

The large circles represent 1 Å and the small circles represent 0.5 Å with respect to the property radii of the functional atoms. The dihedral angles used to cluster similar conformations are indicated by round arrows (A).

Fig. 2. Stereopairs of the Resulting Alignment between the NMR Structure in SDS Micelles of Motilin (Green) and the Selected Conformation of EM536 (Yellow)

Phel, Val2, Ile4, and Tyr7 of motilin are displayed in red. The four HP atoms located on the propargyl group, the amino sugar ring, C13, and C15 in EM536 are represented by white spheres.

rotational increment is 10°.

Compared with other superposition techniques such as DISCO,17) SUPERPOSE has the advantage that there is no need to estimate in advance a corresponding functional group for the superposition between two molecules. Therefore we can easily obtain alignment between two molecules, even though they have quite different chemical structures as in the case of motilin and EM536. According to the criteria in our previously published paper, the functional atoms for EM536 and motilin can be defined as shown in Figs. 1A and B, respectively. The functional atoms of motilin are defined only in the region of Phel-Arg12, because the fragment of Phel-Arg12 has been shown to have activity almost identical to that of the wild form.8) After calculations using SUPERPOSE, we obtained a total of 714 different alignments between motilin and EM536. At that point, we needed to determine the best alignment among these 714. As described earlier, Phel1, Val2, Ile4, and Tyr7 are the most essential for the activity of motilin. Therefore we can use this information to determine the best alignment between motilin and EM536, i.e., we have selected the alignment where all four HP atoms corresponding to Phel1, Val2, Ile4, and Tyr7 of motilin are superposed on the HP atoms of EM536. We found only one alignment satisfying this constraint.

The selected conformation for EM536 has a difference in energy value of about 9 kcal mol⁻¹ from the most stable conformation obtained from the conformational analysis. Figure 2 shows the resulting alignment between motilin and EM536. In this alignment, the four HP atoms located on the propargyl group, the amino sugar ring, C13, and C15 in EM536 are superposed on those corresponding to Phel1, Val2, Ile4, and Tyr7 of motilin, respectively (Fig. 2). Studies on mutants of motilin have shown the importance of an aromatic side chain in position 1 for activity.10) Studies on the activity of motilides have also shown that a propargyl or an allyl substituent on the quaternary ammonium group is important for high activity.7) These experimental results seem to indicate the importance of the π electron for activity in each region.
Therefore the correspondence between Phel of motilin and the propargyl group of EM536 could be considered reasonable. In addition, the activity of motilin mutant F1A, in which Phel is replaced by an alanine residue, was reduced below 1% of that of the wild form, and the replacement of the propargyl group with a methyl one in EM536 also caused a reduction in potency of more than two log units. This coincidence suggests a correspondence between the Phel of motilin and the propargyl group of EM536. The HP atom located in the 6,9-hemiacetal region of EM536 has been considered to be a pharmacophore corresponding to the Ile4 of motilin. This is consistent with experimental results indicating that the 6,9-hemiacetal region may be important for the activity of EM536. Based on the findings described above, we have concluded that the resulting alignment in this study is reliable. Therefore the selected conformation for EM536 could be considered to be its active conformation. In addition, the resulting alignment may suggest other pharmacophores for both motilin and EM536, which may be important for their activity as agonists of the motilin receptor (Fig. 3). For example, the ethyl group at C13 in EM536 is assumed to be important for its activity, because the HP atom for this group is presumed to correspond to the one for the Tyr7 of motilin. Because motilin and EM536 exhibit several common relative spatial dispositions with respect to HA, HD, and DA atoms (Fig. 3A), the property of being a hydrogen-bonding acceptor or donor may contribute to the ability to bind with the motilin receptor. To obtain more information on the interaction mode between motilides and the motilin receptor, a 3D-QSAR study is currently under way in our laboratory.

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References and Notes