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Conformation of receptor-associated PGI_2: An investigation by molecular modeling

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SUMMARY

To elucidate the conformation of receptor-associated prostacyclin (PGI_2), we first performed structure–activity correlation analysis of over 200 PGI_2 analogues and derived from this analysis several crucial features pertaining to structural requirements for PGI_2 activity [Ah-lim Tsai and Kenneth K. Wu, Eicosanoids, 2 (1989) 131-143]. These structural features proved to be useful guidelines for selecting 'model molecules' for further investigations by molecular mechanics. By properly selecting four analogues with either rigid or uniquely oriented α-side chain structure for geometric fitting, we succeeded in maximally minimizing the degree of freedom of the carboxylate terminus of PGI_2. We were able to define the spatial relationship among the four critical functional groups, i.e., C1-COOH, C6a-O, C11-OH and C15-OH. More information is needed, however, to define the geometry of the ω-side chain, particularly for the moiety beyond C15. Nevertheless, results from structure–activity correlation analysis and molecular modeling provide useful information regarding the conformation of receptor-associated PGI_2, which assumes an 'elongated' conformation instead of the traditional 'hairpin' structure.

INTRODUCTION

Prostacyclin (PGI_2), 6,9α-oxido-11α,15α-dihydroxyprosta-(*Z*)5,(*E*)13-dienoic acid (Compound I in Fig. 1), is an arachidonate metabolite possessing diversified biological activities [1]. It inhibits platelet aggregation and induces vasodilation [2]. These biological activities appear to be mediated through its interaction with a specific receptor coupled to stimulation of adenylate cyclase [3]. Although the PGI_2 receptor has been identified on human platelets [4,5] and the receptor protein has recently been solubilized [6], its structure has not been elucidated nor have its physicochemical properties been characterized. The exact molecular mechanism by which PGI_2 interacts with its target receptor is hence unclear. Recent studies have shown that the structure of a receptor can be deduced from its binding ligands by quantitative structure–activity relationship (QSAR) analysis

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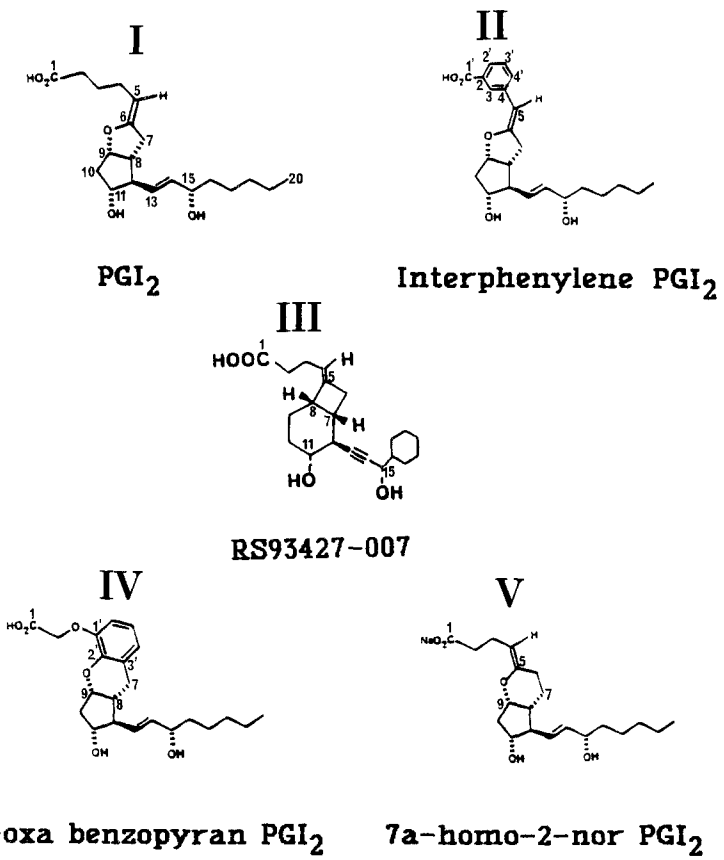


Fig. 1. Chemical structures of PGI_2 and the four selected rigid analogues used for molecular modeling.

[7,8]. In this report, we used a similar approach to study the active structure of PGI_2 by molecular modeling. Because of its potential clinical value, great efforts have been directed toward organic synthesis of PGI_2 analogues for improving chemical and metabolic stability. More than 200 analogues have been synthesized in the last decade or so [9–12]. The relative potency of these analogues against platelet aggregation has also been unambiguously documented [9–13]. Availability of this information enabled us to perform correlation analysis of the structural variations of PGI_2 with their corresponding anti-platelet activity [14]. To minimize the environmental influence on the molecule in the modeling process, we selected from the voluminous analogues several active compounds with rigid overall conformation. Spatial distances among the critical functional groups were evaluated. Our study provided a quantitative description of the conformation of the receptor-associated PGI_2 which appears to assume an elongated conformation and not the traditional 'hairpin' structure. We intend to use this study as an initial investigation of the structure–activity relationship.

EXPERIMENTAL DESIGN AND METHODS

To perform structure–activity analysis, we applied the following strategies. First, we conducted

a thorough literature search to collect information of all synthesized PGI_2 analogues published in the last 12 years. Second, we catalogued these compounds according to the types of chemical modifications. Third, we constructed a correlation table between the structural modification and the corresponding biological activity, i.e., activity of inhibition of platelet aggregation. The structural requirements were then derived from this correlation table [14]. Fourth, we selected as reference compounds several analogues which have a most rigid conformation but retain a biological activity approximately equivalent to PGI_2. We then performed force-field analysis on these analogues to obtain their stable conformers. Fifth, we carried out geometric fitting between stable PGI_2 conformers and the stable conformers of the reference compounds to define the most probable conformation of PGI_2 when it is associated with the platelet receptor. From this process, we determined the spatial coordinates of the critical functional groups and evaluated quantitatively the steric effects of various substituents in the side chains.

The program we used for molecular modeling is ALCHEMY (Tripos Associates, Inc., St. Louis, MO) [15]. Two energy-minimization programs, ALCHEMY and MM2 (ChemCad, C-Graph software, Inc., Austin, TX), were used to cross-check the reliability of the derived stable conformations. Unless otherwise specified, the data of force-field analysis presented in this paper were obtained by ALCHEMY minimization. Each PGI_2 analogue was constructed step by step from individual atoms or fragments and by partial editing of the 'yardstick' PGI_2 conformer. Each fragment generated during the building process as well as the final complete analogue was energy minimized, and the whole molecule was then fitted to the compound having special geometrical significance.

RESULTS

Structure–activity correlation studies

Several salient features concerning the general structural requirements for PGI_2 activity were derived from the correlation studies (see Ref. 14 for details). They are summarized as follows (see Fig. 1, PGI_2, for these structure features).

(1) The C1-COOH group and C11-OH and C15-OH groups are essential for platelet activity. Modifications that change the relative positions of these three groups tend to decrease the biological activity.

(2) The bicyclo[3,3,0]octane ring structure and the C5 and C13 double bonds are molecular designs to maintain its unique geometry for receptor binding.

(3) The C6a-O group, although not vital to the binding geometry, is important for the biological potency. Therefore, electron-withdrawing groups present adjacent to C6a or substitution of the C6a-O with a less electronegative atom will result in a decrease of the biological activity.

(4) Any additional hydrogen-bond donor present between C1 and C15 usually results in loss of activity, possibly due to mispairing of hydrogen bonding.

Selection of chemically rigid and biologically active PGI_2 analogues

Four analogues possessing a less flexible α-side arm were chosen: Interphenylene PGI_2, [(5*Z*,13*E*,9α,11α,15*S*)-2,3,4-trinor-1,5-inter-*m*-phenylene-6,9-epoxy-11,15-dihydroxy]-prosta-5, 13-dienoic acid (Compound II); RS93427-007, la-nor-6,9α-9a-homo-11α,15α-dihydroxy-15-cyclohexyl-ω-pentanor-prosta-(*Z*) 5-en-13-ynoic acid (Compound III); 3-oxa benzopyran PGI_2,

3-oxa-4,5,6-trinor-3,7-inter-*m*-phenylene-5,9α-oxido-11α,15α-dihydroxy-(*E*)13-enoic acid (Compound IV); and 7a-homo-2-nor PGI_2 (5,9α-oxido-11α,15α-dihydroxy-prosta(*Z*)4,*E*-(13)-dienoic acid (Compound V); (Fig. 1). Compound II has the most rigid α-chain structure. The only freedom of the α-chain is essentially the rotation around the single bond between C4 and C5. Despite relatively low biological potency (Table 1), the obligatory meta-configuration of the carboxylphenylate as the biologically active form rendered the compound useful for molecular modeling. The other three analogues, although less rigid than Compound II, have one less methylene unit in the α-side arm and therefore have a lesser degree of freedom than PGI_2. Furthermore, each of these three rigid analogues exhibits biological potency similar to PGI_2 (Table 1). With the exception of Compound III, these rigid analogues have identical lower-ring structure and an equivalent C6a oxygen as the parent PGI_2. Compound III, on the other hand, does not have this vinyl ether oxygen, and its bicyclooctane ring structure is 2-4-0 instead of 3-3-0 (Compounds I and II) or 4-3-0 bicyclononane (Compounds IV and V). It has a triple bond at C13 which appears to compensate for the decrease in activity due to the absence of C6a oxygen [14]. We decided to use Compound III as the first reference because it carries a ring structure very different from PGI_2, and an attachment point of the α-chain to the ring structure. These features of Compound III would tend to restrict the geometric fitting of the C11-OH and C15-OH to the corresponding functional groups of other analogues.

Model building and geometric fitting

(1) Model construction of the ring structure. We started with building the PGI_2 bicyclooctane ring because of its simplicity. It is known that these two 5-membered rings are present as a *cis*-configuration [16]. The upper ring adjacent to the α-side arm has only one stable conformation due to the presence of the C5 double bond. The lower 5-membered ring, however, has two stable conformers with the C11 located above or below the plane defined by the other four carbon atoms (Fig. 2A). The potential-energy difference between these two stable conformers is small, i.e., 0.43

TABLE I
SUMMARY OF THE MOLECULAR MODELING OF PGI_2 AND FOUR ANALOGUES WITH RIGID STRUCTURE

Compound	Potential energy[a] (kcal/mol)	Interatomic distance (Å)				Activity[b] index
		C1	C6a	C11-O	C15-O	
(I) PGI_2	−1.6	0	0	0	0	1
(II) Interphenylene PGI_2	−0.8	0.631	0.350	0.142	0.251[c]	0.01
(15-*n*-pentyl)		0.910	0.126	0.091	0.071	
(III) RS-93427-007	20.7	0.120	–	0.355	0.316	0.25
(IV) 3-oxa benzopyran PGI_2	−0.3	0.126	0.135	0.087	0	1
(V) 7a-homo-2-nor PGI_2	−2.7	0.122	0.119	0.100	0.081	1

[a]Figures were obtained by ALCHEMY analysis; these values can only be compared on a relative basis, as a different set of values were obtained by MM2 analysis.

[b]Activity on inhibition of human platelet aggregation. The activity of PGI_2 is regarded as 1.

[c]Results obtained by fitting of C6a, C11-O and C15-O only.

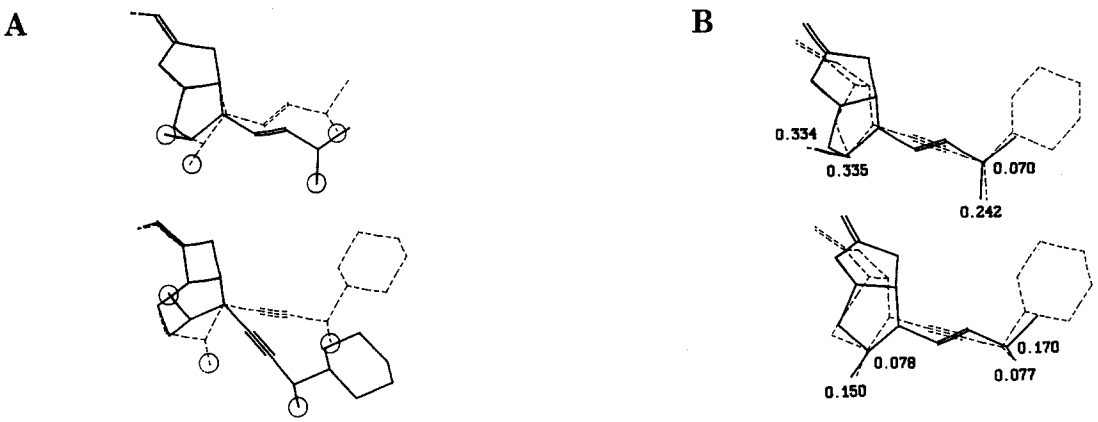


Fig. 2. The stable conformers of the ring structure with the associated C11 and C15 hydroxyl groups. (A) The two stable conformer pairs of Compounds I and III. Only the fragments containing the bicylooctane ring and the C11, C15 hydroxyl groups are shown. Included in each pair are the stable conformers which resulted from the movement of the C11-O bond to its two extreme positions. The C11 and C15 hydroxyl groups are highlighted by a circle. (B) Two matched pairs of stable conformers between Compounds I and III. Conformations of these two matching pairs are almost identical to those shown in (A). Fitting was conducted on C11, C11-O, C15 and C15-O, and the interatomic distances of corresponding atoms are indicated in Å.

kcal/mol. The upper 4-membered ring of III is also present as a single conformation, while the lower cyclohexyl ring has several potential stable conformations. The stable bicyclooctane conformers generated by fusing cyclohexane (chair or boat form) with cyclobutane converged to four major configurations by ALCHEMY or MM2. All these conformers exhibit a 'twisted' cyclohexane in their bicyclooctane ring structure due to high angle-bending energy exerted by the top 4-membered ring. The steric energy of each conformer is not much different, indicating an easy conversion among these conformers through pseudorotation*. We analyzed two conformers with the lowest energy generated by pseudorotation of the 6-membered ring as shown in Fig. 2A. Both conformers contained a twist-form cyclohexyl ring and interconversion between them was achieved by simply yanking the C11-O bond back and forth. This movement resulted in a dramatic difference in the spatial relationship between the C11 and C15 hydroxyl groups (from ca. 5 Å to ca. 7 Å). These two conformers had a similar geometry in the ring structure as the two stable bicyclooctane conformers of PGI_2. Geometric fitting of the C11 and C15 hydroxyl groups of this pair of stable Compound III conformers to that of PGI_2 was performed. The two matching pairs are shown in Fig. 2B with the interatomic distances of C11, C15, C11-O and C15-O affixed. These two matched pairs have ring conformations very similar to those shown in Fig. 2A. The bottom pair with the C11-O pointing forward appears to have a better geometric fitting at these two critical functional groups. In fact, the ring conformation of the bottom pair coincided with the X-ray data of Compound III and was proposed originally as the conformation for Compound III analogues [17]. However, geometric fitting between I and V provides solid evidence indicating a different ring conformation as shown in Fig. 2B.

*Theoretically, pseudorotation of the twisted cyclohexyl group of Compound III generates more than 4 local minima because of small energy differences. Advanced molecular mechanics tools will be necessary to obtain these additional intermediate conformers.

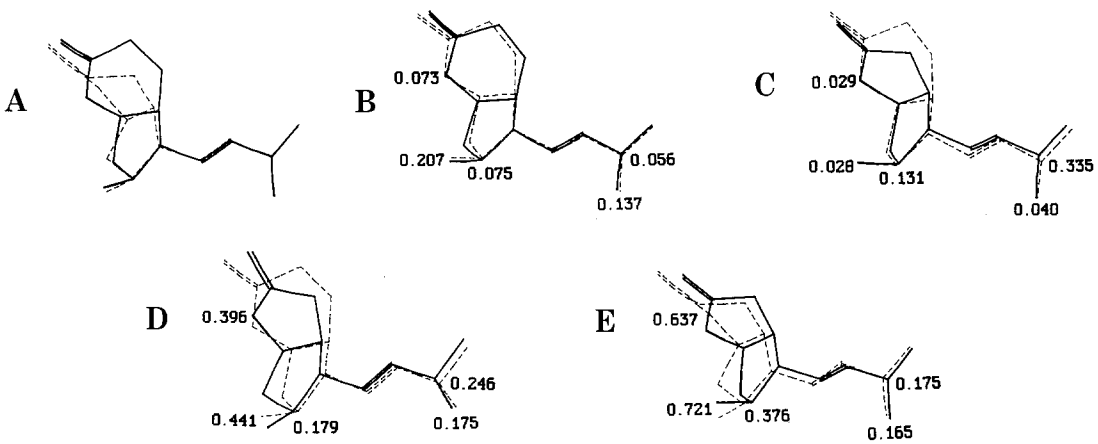


Fig. 3. Stable conformers of Compound V ring structure with attached C11 and C15 hydroxyl groups and its geometric fitting with the PGI_2 fragment. (A) Two most stable conformers obtained by ALCHEMY and MM2 minimization. Only moieties containing the ring structure and two hydroxyl functional groups were constructed. These two conformers were aligned for C11, C11-O, C15 and C15-O. (B) Geometric fitting of two conformers generated from an initial cyclopentane unit with the fifth methylene carbon (corresponding to C11 carbon on PGI_2) being above or below the plane defined by the other four methylene carbons. Fitting of these two conformers was carried out for C6a, C11-O and C15-O. (C – E) Geometric fittings between two stable conformers of Compound V and the two stable conformers of PGI_2 as shown in Fig. 2A. Fittings were also performed for C6a, C11-O and C15-O. The interatomic distances are indicated in Å.

Compound V contains a characteristic top 6-membered ring. Energy minimization of the bicyclononane and the attached exocyclic double bond yielded only two stable conformations. Presence of the exocyclic double bond renders the top 6-membered ring very rigid, which yielded only two possible stable conformers as a result of pseudorotation (Fig. 3A). Moreover, the torsional factor exerted by the top ring forced the lower 5-membered ring to be close to a planar conformation and severely limited the orientation of the C11-OH. In fact, we constructed the ring structure of V using both conformers of cyclopentane as the initial building fragments and ended up with an almost identical lower ring structure (Fig. 3B). When geometric fitting was performed between the two PGI_2 conformers shown in Fig. 2B and the two stable conformers of V for the three critical groups C6a-O, C11-O and C15-O, only one pair showed good fitting for all three groups (Fig. 3C). The other two pairs (Figs. 3D and E) exhibited poor fitting not only for the C6a-O, but also for the C11-O. Therefore, we considered the top pair of Fig. 2B to be the correct receptor-associated conformation.

To support this proposition, model fitting of the C11 and C15 hydroxyl groups was further performed between Compounds III and V. Only one of the conformer pairs could be closely superimposed (Fig. 4B). The C11-O and the ω-side chain of this superimposable Compound III were antiparallel to each other. The selective fitting between these 2 analogues was a result of the relative rigid spatial orientation of C11 and C15 hydroxyl (numbering according to I) defined by the ring structure and the C13 triple bond (or double bond). Geometric fitting was also conducted between the stable bicyclononane conformer of V and the bicyclooctane conformer of III for the two critical hydroxyl groups. As shown in Fig. 4A, stable conformers other than those two given in Fig. 4B always showed poor fitting despite rotational adjustment around the triple bond of III (or double bond of V). Furthermore, as depicted in Fig. 4B, the C15-O bond must point downward

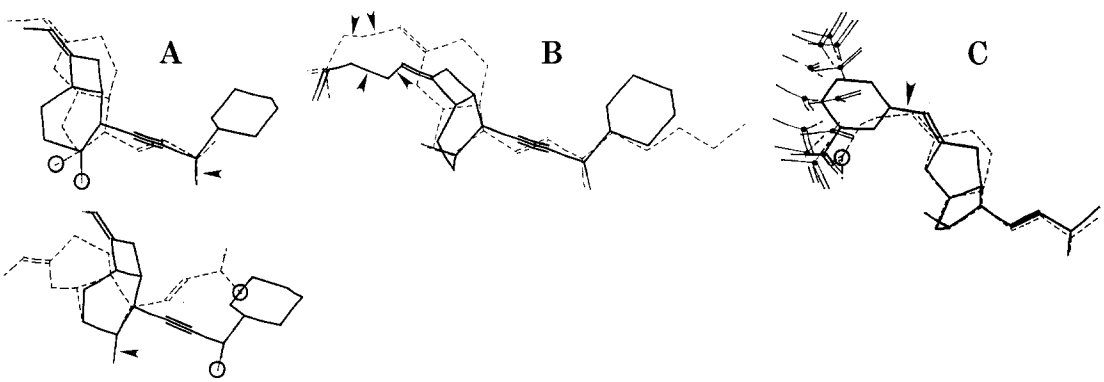


Fig. 4. Fitting between Compounds III and V (A and B) and determination of the location of C1 (B and C). (A) Demonstration of the mismatch between Compound V and one of the two stable conformers of Compound III. Two moieties subjected to geometric fitting were first aligned by either C11-O or C15-O bonds (indicated by solid arrows), and the rest of the molecule was rotated around each specific bond to achieve the shortest interatomic distances between corresponding C11 or C15 oxygen atoms (highlighted by an open circle). (B) Demonstration of a match of Compound V and one of the two stable conformers of Compound III. Fitting is also shown for the C1 atom. This was done by torsional motions of the α-side chain around four single bonds (indicated by solid arrows) and successively adjusted to other rigid analogues, i.e., Compounds IV and II. (C) Final adjustment of the C1 coordinates by Compound II. The latter analogue was first aligned with Compound V for their C6a, C11-O and C15-O atoms. The trajectory of the C1 carboxyl of Compound II (in 30° increments) was generated as a consequence of rotating the *m*-carboxyphenyl moiety around the only rotatable bond (indicated by solid arrow). Adjustment of the C1 coordinates obtained from the process described in Fig. 4B was reached by minimizing the interatomic distances among all C1 atoms of each rigid analogue.

instead of upward in order to fit with other rigid analogues. The unique twist conformation of the cyclohexyl ring not only helps to minimize the overall molecular potential energy but also provides a favored α-chain orientation by raising the upper 4-membered ring and bringing the carboxyl terminus closer to the corresponding carboxyl of V. The spatial relationship between C6a-O and the two hydroxyl groups was further confirmed by using a third rigid analogue, Compound IV. When model building and energy minimization was performed on this analogue, we found that this compound displayed great similarity to V. As shown in Fig. 5D, both analogues carried identical lower-ring conformation and very similar upper-ring geometry. The presence of a benzene ring in IV slightly deformed the middle 6-membered ring structure. Since both IV and V possess similar anti-platelet activity as PGI_2, we believe that the spatial relationship defined by these two analogues and III is valid.

Location of C1 in the active conformation of PGI_2. Fitting of the C1 atom would seem to have numerous possibilities due to the flexibility of the C1-C4 side arm in both III and V. However, since the C4-C5 double bond assumed a specific configuration (*Z*-configuration in V and *E*-configuration in III), the possible spatial coordinates of the C3 atom after energy minimization were significantly reduced. Therefore, the freedom of motion of the α-side arm for both analogues was limited to rotation around the C3-C4 and the C2-C3 single bonds. As the exocyclic double bonds at C4 in both compounds were physically well separated (Fig. 4B), docking of the C1 atoms became more selective. Further adjustment of the α-chain orientation was done by rotating the C1′-O and O-C2 bonds of IV in the same docking procedure. After these two cycles of geometric fitting, the location of the C1 atom in the active form of PGI_2 was substantially defined.

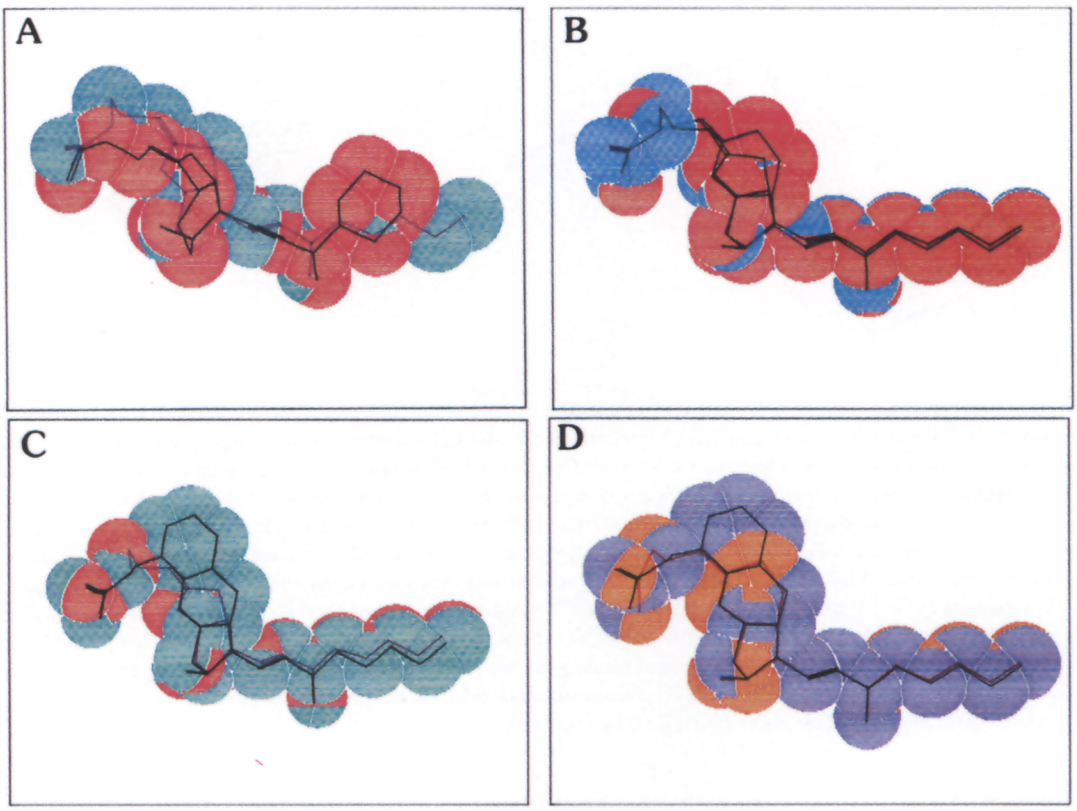


Fig. 5. Geometric fittings among PGI_2 and its rigid analogues. (A–C) Model fittings between Compounds I and III, I and V, and I and IV, respectively. (D) Fitting between Compounds IV and V. Fittings were conducted on the C1, C11-O, C15-O and C6a where applicable. Space-filling representation is used to show the general shape of each molecule.

The final fitting of the C1 carboxylate was based on the use of the compound with the most rigid α-side arm, i.e., Compound II as the reference compound. We first generated the trajectory of its C1 atom by rotating the *m*-carboxyphenyl moiety around the C4-C5 single bond (Fig. 4C). We then varied the two changeable torsional angles, C5-C4-C3-C2 and C4-C3-C2-C1 of III and 7a-homo-2-nor PGI_2, and C2′-C1-O-C2 or C1′-O-C2-C1 of IV. After energy minimization, we were able to superimpose the C1 of III with those of IV and V and also to keep it very close to the C1 of II. We found the geometric fitting of the carboxylate to be very rigid due to extremely limited freedom in locating common spatial coordinates of the C1 atoms of these four analogues.

We then oriented the α-chain of PGI_2 so that the C1 carbon coincided with the predetermined coordinates. This fitting procedure only allowed us to locate the spatial coordinates of the C1 of receptor-bound PGI_2. The coordinates of C2 and C3 remained ambiguous.

Conformation of ω-chain of active PGI_2. The conformation of the ω-chain could not be as well characterized as the α-chain. The information derived from the correlation study and the model fitting indicated that the C13 double bond could be replaced by a triple bond without jeopardizing the activity. The C15 hydroxyl group is pointing downward as shown in Fig. 2B. Published data in the literature show that the structure of the ω-side chain beyond C15 could be highly variable

A

Parameters for receptor-bound PGI_2:

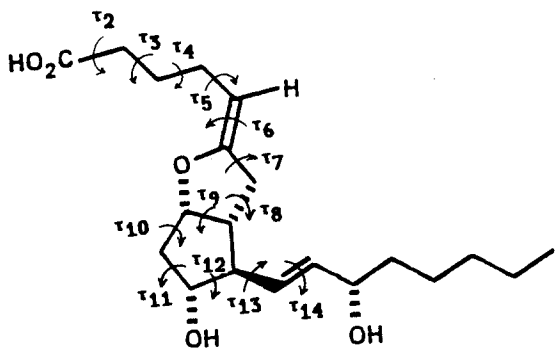
Distances: (Å)

	C6a	C11-0	C15-0
C1	3.65	5.98	11.19
C6a	-	3.23	7.68
C11-0	-	-	6.04

Dimension: (Å)

C1-C20: 16.2
C_1OH-$C_{20}H$: 18.1

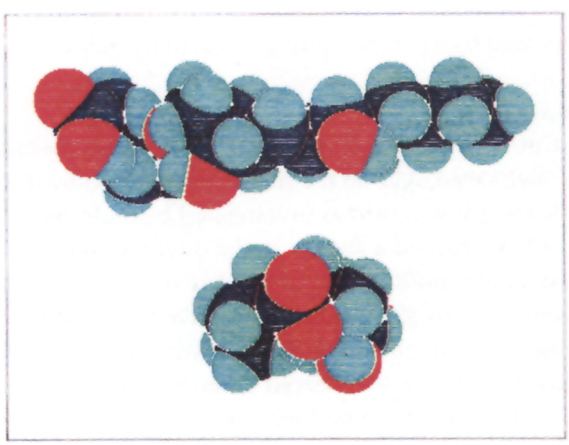
C7-C11: 3.7
C7βH-C11αH: 5.5



Torsional Angles (°)

τ1	58.6	τ8	133.3
τ2	-83.8	τ9	-24.6
τ3	-80.8	τ10	23.4
τ4	84.9	τ11	104.7
τ5	176.3	τ12	144.7
τ6	-175.5	τ13	-178.9
τ7	-13.3	τ14	81.5

B



C

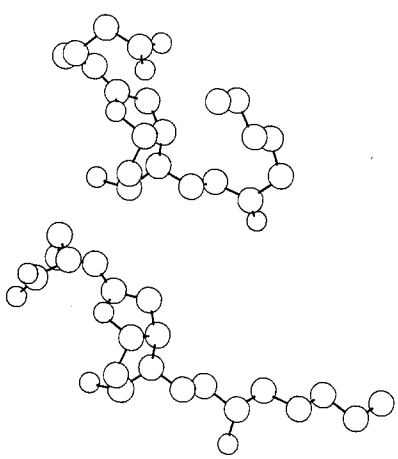


Fig. 6. Characteristics of the receptor-associated PGI_2 molecule. (A) Parameters of receptor-associated PGI_2. Torsional angles τ_{14}: (C15-O)-C15-C14-C13, τ_{13}: C15-C14-C13-C12, τ_{12}: C14-C13-C12-C11, τ_{11}: C13-C12-C11-C10, τ_{10}: C12-C11-C10-C9, τ_9: C11-C10-C9-C8, τ_8: C10-C9-C8-C7, τ_7: C9-C8-C7-C6, τ_6: C8-C7-C6-C5, τ_5: C7-C6-C5-C4, τ_4: C6-C5-C4-C3, τ_3: C5-C4-C3-C2, τ_2: C4-C3-C2-C1, τ_1: C3-C2-C1-(C4-OH) (B) The upper model shows the elongated geometry of this ligand, represented as space-filling model; the lower model illustrates a 90° rotation of the molecule with the C1 carboxylate facing the reader to show the relative dimension of the molecule. (C) One of the stable conformers of PGI_2 which exhibits the shortest distance between the termini of two side arms.

without losing significant biological potency [9,10]. The number of carbon units after C15 could be varied from 3 to 7 and could be substituted with cyclic alkane or aromatic fragments. Correlation of chemical modifications after C15 is not clear-cut. Literature data sometimes showed conflicting results. For example, 15-cyclohexyl-ω-pentanor PGI_2 exhibited a biological potency only 15% of the 15-n-pentyl counterpart [18], while both 15-cyclopentyl-ω-pentanor carbacyclin and 15-cyclohexyl-ω-pentanor interphenylene PGI_2 displayed significantly improved potency over their 15-n-pentyl derivatives [19,20]. Comparison of the molecular model of the 15-cyclohexyl-ω-pentanor PGI_2 with the fully-extended 15-n-pentyl PGI_2 revealed a fairly close match between the C16-C18 in the n-pentyl moiety and the first three carbon atoms in the chair-form of the cyclohexyl moiety. The increase in the rigidity at the tail by introducing a cyclohexyl group might lead to an increase in the biological potency. This ordering effect can also be applied to interpret the activity changes that resulted from other modifications at the tail portion of the ω-side chain [21,22]. At this writing, we did not have sufficient data to unequivocally determine the configuration of C18-C20 in PGI_2.

Based on these modeling features, we summarized the key parameters for the receptor-associated PGI_2 assuming a fully-extended ω-side chain after C15 (Fig. 6A). The spatial relationship of the four critical functional groups in PGI_2, C1-COOH, C6a-O, C11-OH and C15-OH, are clearly defined. The values for torsional angles were given for the structure up to C15-OH because of lack of structural information beyond C15. The three torsional angles, τ_2, τ_3, τ_4, could assume many different values due to the flexibility of the α-chain.

Fittings of each of the three rigid analogues with the PGI_2 molecule as well as fitting of Compound IV to V are shown as spatial drawings in Fig. 5. The α-side chains of III and IV were greatly stretched, while the α-arm of PGI_2 had to assume a loop structure from C2 to C5 and was not fully extended. As shown in Table 1, Compound III assumed the highest potential energy due to the strong angle bending of the ring structure. Compound II exhibited the worst fitting of the C1 atom with the parent PGI_2, which was accompanied by a corresponding low activity index.

Characterization of the steric effect of PGI_2 analogues

Although the conformation of the ω-side arm was less defined than the α-side arms, the steric effect delineated from our correlation studies might shed light on the specific role played by the ω-side arm in the receptor binding. Therefore, this factor was further investigated by molecular mechanics. Addition of a methyl group to C15 of PGI_2 caused a change in the orientation of the ω-chain, a shift of the C15 hydroxyl oxygen in its stable conformation towards the *R*-configuration by 0.6 Å, and a concomitant change in τ_{14} from 81.5° to 52.3°. Similar negative influence on the biological activity was observed for 16,16-dimethyl PGI_2. In this case, the hydroxyl oxygen was shifted by 0.4 Å. On the other hand, 17,17-dimethyl PGI_2 was observed to have insignificant spatial perturbation of the 15-hydroxyl group. Comparison of the biological activity of these analogues shows a trend of a local steric effect on the 15-OH. The global steric effect was demonstrated by 15-adamantyl-ω-pentanor interphenylene PGI_2 and iloprost (6,9α-carba-11α,15α-dihydroxy-16-methyl-prosta-(*E*)5,(*E*)13-dien-18-ynoic acid). Neither the presence of a bulky adamantan group nor the addition of a methyl group on C16 and a C18 triple bond, as in iloprost, caused any direct interference of the spatial orientation of 15-OH. We recently found that the two 16-methyl stereoisomers of iloprost exhibited quite different platelet activity, with the 16*S* isomer being about 20-fold more active than the 16*R* isomer (Table 2, [23]). This difference was a result of a

TABLE 2
PARAMETERS OF THE NINE STABLE ROTAMERS OF THE 16*R* AND *S* STEREOISOMERS OF ILOPROST

Rotamer number	Steric energy (kcal/mol)		τ_1[a]		τ_2[b]	
	R	*S*	*R*	*S*	*R*	*S*
1	−5.54	−5.64	176.4	171.6	−177.6	−177.6
2	−5.56	−5.75	60.9	64.0	168.2	−174.6
3	−5.76	−5.74	−65.1	−59.2	166.0	−173.6
4	−5.87	−5.77	178.6	168.8	71.8	60.9
5	−6.88	−6.70	−69.8	−64.1	73.6	62.8
6	−6.10	−5.81	61.0	64.3	68.5	62.5
7	−6.00	−6.10	−62.4	−54.4	−66.1	−65.6
8	−7.16	−7.40	66.9	71.5	−67.7	−76.0
9	−5.76	−5.87	180.0	171.8	−61.1	−61.2

[a]Torsional angle of C14-C15-C16-C17.
[b]Torsional angle of C15-C16-C17-C18.

slow association of 16*R* isomer with its receptor [23]. We postulated that in the 16*S* isomer the presence of 16*S*-methyl and the stiff C17-C20 tail might result in a rigid and 'correct' molecular conformation for binding; the 16*R* isomer, on the contrary, assumed a rigid and unfavored conformation for accessing the receptor site [23]. Molecular mechanics analysis was conducted to determine the validity of this proposal. As indicated in Table 2, conformer pair 8 is the most stable species among the 9 pairs of stable rotamers generated by rotation around C15-C16 and C16-C17 single bonds. The geometry of the ring structure relative to the ω-chain in conformer 8 is very similar to our previous data obtained by another energy minimization program, PCMODEL [23]. The spanning from the end of the C_{17}-C_{20} rigid tail to the C_{16} methyl carbon is much larger for the *R* isomer than the *S* isomer (6.3 Å vs. 4.9 Å) (Fig. 7). Such an orientation at the tail end is likely to hinder the association of the ligand with its receptor. Despite the lack of any direct perturbation of the spatial orientation of C15-OH, the addition of the C15-adamantyl group might exert a similar steric effect as the *R* isomer of iloprost. The 'cage-like' structure of the adamantan group exhibited steric hindrance on all three coordinate axes and could not be alleviated by rotation around the C15-C16 single bond. Such a bulky substituent present at the ω-side arm therefore causes a significant loss of biological activity.

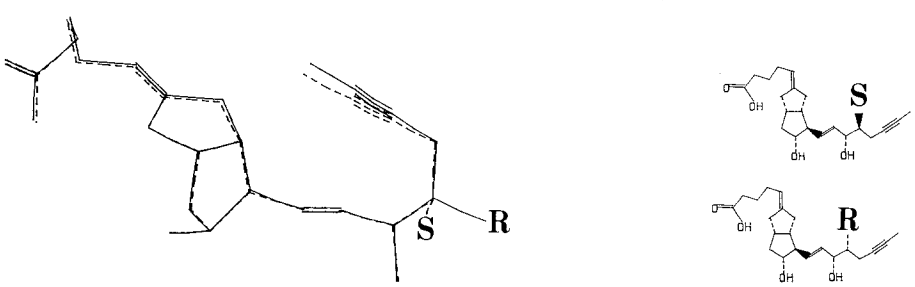


Fig. 7. Spatial representations of the *R* and *S* pair of the number 8 stable rotamer of iloprost. The configuration of the 16-methyl group is indicated by '*S*' and '*R*' in both isomers.

DISCUSSION

Evaluation of the structure–activity relationship by the molecular-mechanics approach is based on the presumption that biological potency is directly proportional to its binding affinity. This presumption is theoretically and practically valid [12, 14]. The molecular mechanics study proves useful for gaining insight into the conformation of receptor-bound PGI_2. In this report, we used two energy minimization programs to obtain the stable conformers of several selected rigid analogues and their fragments. We found that the more rigid a molecule, the better convergence of its energy calculation. Of the five compounds investigated (Fig. 1), the three rigid ones, i.e., II, III, and IV, yielded essentially identical conformations by ALCHEMY and MM2 minimization, while the two less rigid compounds, i.e., I and V, appeared to be too flexible for these two minimizations to converge. This difference in the outcome of minimization, however, has only minor influence on the results of our geometric fitting.

Our molecular modeling studies provide important information about the relative spatial coordinates of the four critical functional groups of the active form of the PGI_2 molecule. More efforts, however, are needed to obtain a definitive quantitative description of the overall conformation. Several potential approaches exist. First, attachment of diversified substituents at various positions of the PGI_2 molecule could be valuable for defining the 'local' and 'global' steric effects of these substituents on receptor binding and useful in measuring the dimension of receptor size. Second, systematic modification of the α-chain length should be useful in determining the minimum and maximum length requirements for activity. For instance, 1-nor PGI_2 could be synthesized and tested for its activity. This is important because given the finding that the α-side arm of the PGI_2 molecule is curved in the bound state, the α-side arm of the PGI_2 α-dinor would be too short to reach the receptor. By synthesizing analogues with an additional carbon at the carboxyl end of α-dinor PGI_2, the spatial requirement for receptor-binding might be satisfied. Third, synthesis of additional new analogues with rigid and correct spatial relationships among C1, C11-O and C15-O and evaluation of their biological activity will be useful to support the propositions made in this work.

In this study, we only calculated the minimum steric energy of molecules in vacuum without considering such surrounding factors as solvent interaction. The possibility that the ligand conformation in the binding process could be changed by the receptor protein should not be overlooked. The α-side arm, which is considered to be the lead group in entering the receptor, shows a flexible structure and is subject to environmental influence. However, when PGI_2 is associated with the receptor, it must assume the unique conformation as depicted in Figs. 6A and B in order to have biological activity. Since this conformation is well-defined through force-field analysis and model fitting using four active analogues possessing a specific α-side arm, we believe the result is much less sensitive to external factors. We are currently seeking advanced tools available in mainframe computer systems to perform further studies. The possible conformation changes caused by solvent molecules, such as water, will be evaluated in depth.

The elongated shape of PGI_2 is unique among the prostaglandin molecules. It differs from the prostaglandins A, E and F series, which have been shown to assume a 'hairpin' shape as evidenced by their crystal structures [24], and by the preparation of biologically active macrolide formed by covalently bridging the α- and ω-side arms [25]. The bicyclooctane ring structure and the C5-C13 double bonds of PGI_2 severely limit the possible proximity of the two termini of the upper and

lower side arms. The closest distance attainable by reorienting the flexible ends of the two side arms (C1-C4 and C15-C20) could never be less than 3 Å, as shown in Fig. 6C. This conformation, which is closest to a hairpin structure, actually has a lower energy of formation (−5.1 vs. −1.6 kcal/mol in Table 1). Although this conformation has higher strain in angle bending, folding of the two side arms apparently gains significant van der Waals interactions and leads to an overall lower steric energy. The geometry of PGI_2 is also different from the 'L shape' of the PGB_1 molecule [26] and the 'scorpion shape' of thromboxane [27]. The extended molecular geometry of PGI_2 (Fig. 6B) is different from all other prostanoids and should be taken into consideration when performing structure–activity studies. The crystal structure of PGI_2 analogues, e.g. iloprost [28] and 15-cyclohexyl-ω-pentanor-inter-phenylene PGI_2 (CG4305) [29], but not PGI_2, have been determined. Comparison of the models based on the X-ray data with those obtained by molecular mechanics as reported here reveals that the orientation of the α- and ω-side chains, and particularly the orientation of the C11-OH, are different between these two methods (X-ray data indicated an orientation as shown in Fig. 2B, bottom, and our modeling approach suggested an orientation as shown in Fig. 2B, top). Extrapolation of the crystal structure or the solid/solution conformation of the ligand itself determined by NMR [30] to obtain the receptor-associated configuration might be misleading. A definite solution to this dilemma would have to involve the analysis of the data derived from the co-crystal of purified receptor and the bound ligand.

We conclude from these studies that the receptor-associated PGI_2 assumes an elongated conformation and not a traditional 'hairpin' structure. We further propose that C1 carboxylate of PGI_2 is likely to be the leading probe for entering the receptor and the platelet receptor might have a cleft or channel-like structure rather than an open domain. This is supported by several pieces of evidence: (a) a free C1 carboxyl is essential for activity; (b) the length and orientation of the α-side arm do not show much variability; (c) the ω-side chain can be quite variable beyond C15; (d) the tail portion of the ω-side arm elicits the steric effect; and (e) the molecular geometry is elongated. We propose that the size of the entrance of the receptor could be gauged by using PGI_2 analogues containing different bulky substituents at its ω-side arm. Once PGI_2 has entered the receptor channel, a sequential binding of the four critical functional groups (or even three groups excluding C6a-O) to their specific target amino acids could generate conformational change(s) of the receptor leading to a signal transduction through G protein(s) to adenylate cyclase.

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