# Solution conformation and electrostatic potential distribution of prostaglandins and thromboxanes and their relation to specificity

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## 1. INTRODUCTION

A number of physico-chemical studies directed towards understanding of the molecular mechanism of action of prostaglandins (PGs) and thromboxanes (TXs) have been reported [1–8]. However, the root cause of their differential activity is still unclear, because several factors [9] are involved in this phenomenon. A systematic study of conformational flexibility (in vacuo) and molecular electrostatic potential distribution on different PG analogs was therefore undertaken [10–15]. Good correlation was observed between theoretically calculated parameters and contractile activity due to PGs [14–16].

These molecules are seldom present in vacuo. A knowledge of their conformations in an electrolytic environment with counter ion distribution is the next step towards understanding their specificity. Here we report a conformational study on $PGE_2$, $PGB_1$ and $TXB_2$ with the incorporation of solvent and counter ion field. Static environmental and atmospheric effects due to diffused counter ion screening have been considered. These 3 analogs were chosen because of known differences in their structures [3,7,8] and activity [9]. We also present results on molecular electrostatic potential distribution on $TXB_2$. Similar results on $PGE_2$ and $PGB_1$ were reported earlier [11,13]. The relation of these differences to biological activity of these molecules is shown here.

## 2. METHODS

Starting data, viz. bond lengths, bond angles and fixed dihedral angles, were taken from X-ray crystallographic studies on these molecules [3,7,8]. Conformational energy was calculated using atom-atom potentials consisting of non-bonded (attractive and repulsive), torsional, electrostatic and polarization contributions [17]. Electrostatic and polarization terms were corrected for both the static environmental effect by multiplying by a factor $1/\epsilon$, where $\epsilon$ is the dielectric permeability, and the diffuse counter ion distribution by introducing a Debye-Hückel screening factor $1/\exp(K \cdot r_{ij})$ where $K$ is the Debye length, dependent on the concentration and charge of the ions [18], and $r_{ij}$ is the distance between the 2 charged atoms. These charges were evaluated by the CNDO/II [19] method. Other parameters were the same as in our earlier publications [11,12].

Rotations were allowed around $C_7$-$C_8$ ($\psi$), $C_{12}$-$C_{13}$ ($\theta$), $C_{14}$-$C_{15}$ ($\phi$), $C_{15}$-$C_{16}$ ($\alpha$) bonds in PGs and $C_4$-$C_5$ ($\theta$), $C_7$-$C_8$ ($\phi$), $C_6$-$C_7$ ($\psi$) bonds in $TXB_2$ (see fig.1 for nomenclature). Isoenergy contours $\psi\theta$ ($\psi\phi$ in the case of $TXB_2$) were drawn for fixed $\phi$ and $\theta$ values, respectively. The latter were varied

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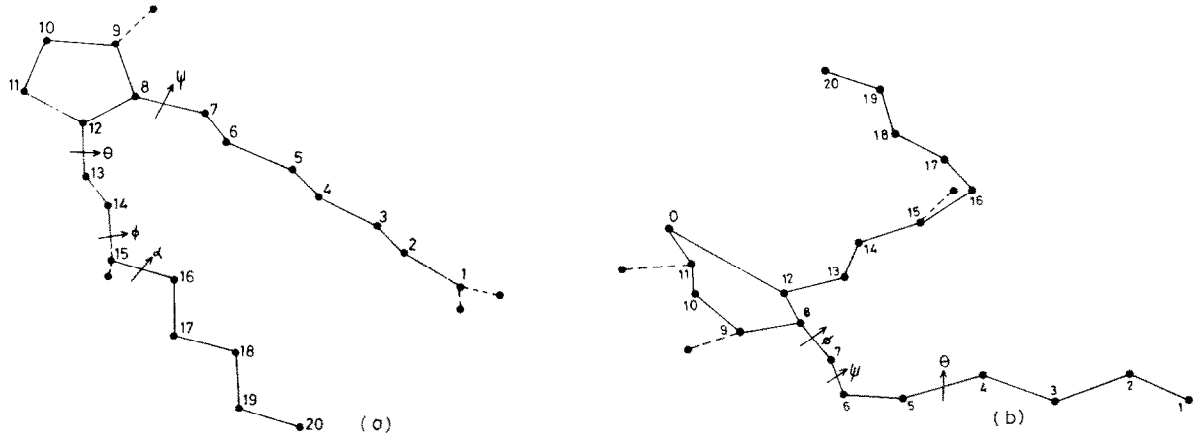


Fig.1. Nomenclature for rotational angles in (a) $PGB_1$ and (b) $TXB_2$.

in 30° steps. A $\psi\alpha$ map in $PGB_1$ was constructed using crystallographic values of $\phi$ and $\theta$, as well as minimum energy values obtained earlier. These rotations were chosen because of the relatively greater flexibility around these bonds, as well as known differences in their values in single crystals [3,7,8]. The conformation maps were drawn for 3 different values at dielectric permeability ($\epsilon$ = 3, 8 and 20) and 4 different cation concentrations ($\mu$ = 0.001, 0.01, 0.1 and 1 M). Only monovalent cations were considered.

Molecular electrostatic potential mapping in three dimensions was done on the basis of the CNDO/II (usual ZDO) density matrix and approximation 1 in [20].

Table 1

Summary of the conformations obtained at $\mu$ = 0.1 M using dielectric constant ($\epsilon$) = 3.0

| | $PGE_2$ | | | | $PGB_1$ | | | | | $TXB_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\phi$ | $\psi$ | $\theta$ | $\Delta E$ | $\phi$ | $\psi$ | $\theta$ | $\alpha$ | $\Delta E$ | $\psi$ | $\phi$ | $\theta$ | $\Delta E$ |
| Cryst. | 247.8 | 300.4 | 114.1 | 0.89 | 123.3 | 268.1 | 5.0 | 166.7 | 3.733 | 92.0 | 44.0 | −142.0 | 0.98 |
| | 240 | 300 | 120 | 0.0 | 270 | 120 | 120 | C | 0.0 | 80 | 40 | 260 | 0.0 |
| | 240 | 60 | 160 | 2.72 | 270 | 260 | 100 | C | 1.751 | 220 | 40 | 260 | 0.002 |
| | 240 | 140 | 160 | 2.95 | 270 | 240 | 240 | C | 1.914 | 80 | 40 | 220 | 0.333 |
| | 90 | 300 | 80 | 2.50 | 270 | 100 | 260 | C | 2.226 | 200 | 40 | 220 | 0.459 |
| | 90 | 60 | 100 | 3.03 | 90 | 120 | 120 | C | 1.849 | 240 | 40 | 180 | 0.623 |
| | 90 | 60 | 160 | 3.08 | 90 | 280 | 120 | C | 2.932 | 100 | 40 | 180 | 1.186 |
| | 90 | 160 | 100 | 3.20 | 90 | 240 | 240 | C | 1.178 | 240 | 40 | 150 | 0.971 |
| | | | | | 90 | 100 | 260 | C | 1.774 | 80 | 40 | 150 | 1.048 |
| | | | | | 90 | 260 | 0 | C | 2.755 | | | | |
| | | | | | C | 100 | C | 120 | 3.539 | | | | |
| | | | | | C | 260 | C | 120 | 3.653 | | | | |
| | | | | | C | 100 | C | 300 | 3.823 | | | | |
| | | | | | C | 260 | C | 300 | 3.980 | | | | |
| | | | | | 270 | 120 | 120 | 160 | 0.561 | | | | |
| | | | | | 270 | 120 | 120 | 280 | 1.831 | | | | |

C, crystallographic value. All energies are in kcal/mol

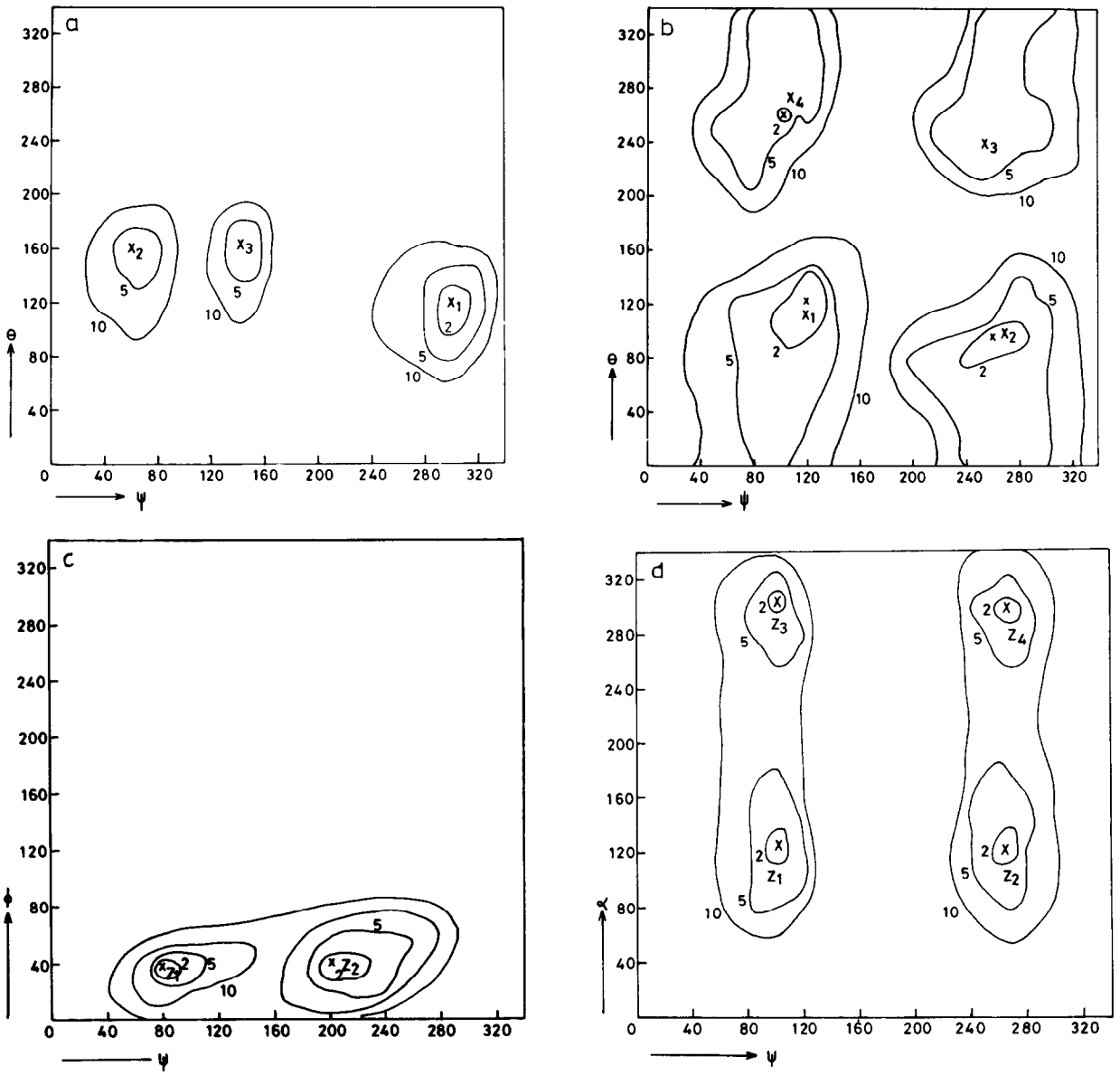


Fig.2. Conformational maps. (a) $\psi\theta$ – $PGE_2$, $\epsilon = 3.0$, $\mu = 0.001$, $\phi = 240°$; (b) $\psi\theta$ – $PGB_1$, $\epsilon = 20.0$, $\mu = 0$, $\phi = 270°$; (c) $\psi\phi$ – $TXB_2$, $\epsilon = 3.0$, $\mu = 0$, $\theta = 220°$; (d) $\psi\alpha$ – $PGB_1$, $\epsilon = 3.0$, $\mu = 0.1$.

## 3. RESULTS AND DISCUSSION

A summary of the conformational minima obtained (global as well as local) along with their relative energies at $\epsilon = 3.0$ and $\mu = 0.1$ M is presented in table 1. Our results show that the absolute minimum in the case of $PGE_2$ is close to the crystallographic conformation of this molecule [3]. It has an energy (at $\epsilon = 3.0$ and $\mu = 0.1$ M) lower than that of the crystallographic conformation by 0.89 kcal/mol. The position of the energy minima remains the same on varying either the dielectric constant of the medium or the salt concentration. In $TXB_2$ the absolute minimum is also in the same

region as the crystallographic conformation [8] (table 1), having 2 non-aligned chains (fig.1b). The conformation of this molecule is also invariant under different salt concentrations. The theoretical conformation proposed by us for $PGB_1$ is significantly different from the L-shaped conformation based on crystallographic studies by Detita et al. [7]. The 'hairpin' orientation of the 2 chains observed by us is similar to that of other PG analogs [15] (fig.1a). Also its conformation does not change with electrolytic environment and counter ion concentration.

A series of low-energy minima (local minima) has been observed in all the 3 cases (fig.2). Their relative order remains the same at different ionic concentrations. These results are not unexpected, considering the hydrophobic nature of the fatty acid side chains and the dominance of non-bonded interactions. The partitioning of the energy of the absolute minima at different dielectric constants and salt concentrations is presented in table 2. The same trend was observed for local minima. Data on energy partitioning for different salt concentrations at $\epsilon = 20$ and 8 have not been presented as their net contribution to the conformation energy was very small, and the changes were similar to those for $\epsilon = 3.0$. One can see that the non-bonded term varies in the same direction as the electrostatic term but the relative weight of the electrostatic term varies with changes in counter ion current and dielectric permeability. An increase in the dielectric constant or the cation concentration leads to a decrease in the relative weight of the electrostatic term. The effect of 1.0 M concentration of monovalent ion is similar to that of doubling the dielectric constant (table 2). Changes in the ionic concentration or dielectric constant do not affect the conformation of absolute minima or flexibility of the molecule. Molecules behave as if they were in vacuo. The 'hairpin' or $\pi$-shaped conformation is maintained for PGs. Thus in this case chain alignment differences alone cannot explain their differential activity [16,21]. Quantitative and qualitative differences in the activity of PGs arise from some other physical property. By contrast, differences in the activity of PGs and TXs arise because of differences in the chain orientations. This was also noted by Fortier et al. [8].

The molecular electrostatic potential of ring fragment $TXB_2$ in the $xy$ plane for $Z = 0.13$ Å (a plane which contains the absolute minimum) is shown in fig.3. The potential distribution of $\alpha$ and $\omega$ chains was similar to that of other PG analogs. It can be seen that $E_{min}$ for $TXB_2$ has a value ($-52.07$ eV) much lower than those of $PGE_2$ ($-32.78$ eV) or $PGB_1$ ($-18.655$ eV) reported earlier [11,13]. These values show a linear relationship with the number of oxygen atoms in the rings (3,2,1 in case of $TXB_2$, $PGE_2$ and $PGB_1$), as expected since oxygen is the only electron acceptor in all 3 cases. It is possible that these differences are exaggerated here to some extent because of the use of the CNDO/II method. However, qualitatively the same trend would be expected if more sophisticated methods such as ab initio molecular orbital techniques were used. We believe that the differences in electrostatic potential of the ring play an important role in specific recognition of these hormones.

To sum up, the flexible chain structure facilitates interaction of these hormones with their receptors. Different receptors are selected by PGs and TXs depending on their chain conformations. However, a change in the nature of the binding to the receptor due to alteration of the electrostatic potential of the rings is responsible for quantitative differences in their interactions. The electrolytic environment with counter ion field can reduce specificity in the action of analogs belonging to the same series, but does not alter their overall biological effect.

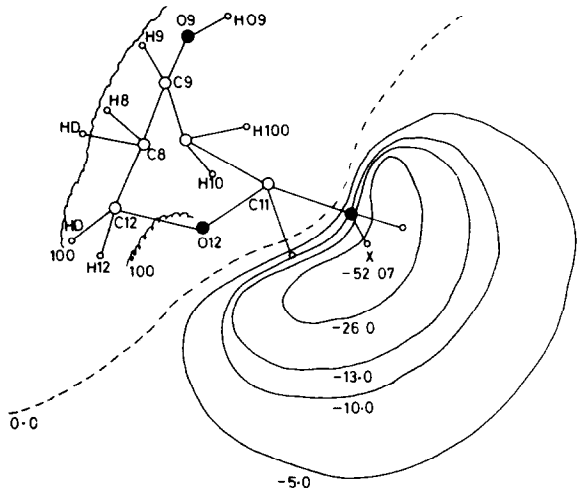


Fig.3. Isopotential map for $TXB_2$ ring fragment for $Z = 0.13$ Å.

Table 2

Comparison of non-bonded, electrostatic and total conformational energies of global minima in $PGE_2$, $PGB_1$ and $TXB_2$ for different values of dielectric constant ($\epsilon$) and reduced molarity of the solution ($\mu$) (polarization and torsional contributions not shown)

| | Angles | | | | $\epsilon$ | $\mu$ (M) | Non-bonded | Electrostatic | $E_{tot}$ (kcal/mol) |
|---|---|---|---|---|---|---|---|---|---|
| | $\phi$ | $\psi$ | $\theta$ | $\alpha$ | | | | | |
| $PGE_2$ | 240 | 300 | 120 | C | 20.0 | 0 | −8.969 | −1.486 | −10.063 |
| | | | | | 8.0 | 0 | −8.969 | −3.652 | −13.039 |
| | | | | | 3.0 | 0 | −8.969 | −9.740 | −18.210 |
| | | | | | 3.0 | 0.001 | −8.969 | −9.485 | −18.039 |
| | | | | | 3.0 | 0.01 | −8.969 | −8.957 | −17.763 |
| | | | | | 3.0 | 0.1 | −8.969 | −7.483 | −16.021 |
| | | | | | 3.0 | 1.0 | −8.969 | −4.274 | −12.989 |
| $PGB_1$ | 270 | 120 | 120 | C | 20.0 | 0 | −10.663 | −1.382 | −11.290 |
| | | | | | 8.0 | 0 | −10.663 | −3.345 | −14.683 |
| | | | | | 3.0 | 0 | −10.663 | −8.921 | −19.126 |
| | | | | | 3.0 | 0.001 | −10.663 | −8.671 | −19.035 |
| | | | | | 3.0 | 0.01 | −10.663 | −8.163 | −18.506 |
| | | | | | 3.0 | 0.1 | −10.663 | −6.745 | −17.037 |
| | | | | | 3.0 | 1.0 | −10.663 | −3.711 | −13.898 |
| $TXB_2$[a] | 40 | 80 | 260 | C | 20.0 | 0 | −1.777 | −1.193 | −3.011 |
| | | | | | 8.0 | 0 | −1.777 | −2.982 | −5.031 |
| | | | | | 3.0 | 0 | −1.777 | −7.954 | −7.999 |
| | | | | | 3.0 | 0.001 | −1.777 | −7.789 | −7.089 |
| | | | | | 3.0 | 0.01 | −1.777 | −7.036 | −6.709 |
| | | | | | 3.0 | 0.1 | −1.777 | −6.535 | −6.012 |
| | | | | | 3.0 | 1.0 | −1.777 | −3.903 | −4.011 |

[a] In $TXB_2$ the non-bonded term is apparently increased due to incorporation of some atom pairs of the ring which are second neighbours

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