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# Theoretical Studies of the Binding of Trifluoperazine Derivatives to Site (82–93) of Calmodulin: Effect of Lengthenings of the Methylene Linker Chain on the Binding Affinity

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**SUMMARY**

A theoretical study was performed of the comparative binding affinities to fragment (82–93) of calmodulin (CaM) of trifluoperazine (TFP) and three derivatives, in which the methylene chain linking the phenothiazine ring and the piperazinium group was lengthened by addition of one to three methylenes. The backbone of the oligopeptide was held in the $\alpha$-helical conformation. The computations were performed with the SIBFA procedures (sum of interactions between fragments computed *ab initio*), which use empirical formulas based on *ab initio* self-consistent field computations. The interaction energy is the sum of the intermolecular phenothiazine derivative-oligopeptide interaction energy and of the separate intramolecular energy variations of the ligand, on the one hand, and of the oligopeptide, on the other hand, upon relaxing the conformations of side chains Glu 84, Glu 87, Phe 89 and Phe 92 due to complex formation. All three derivatives were found to display a higher binding affinity than did TFP itself, an optimal affinity being found for a four- and a five-methylene linker chain. In as much as fragment (82–93) of CaM is a plausible candidate receptor site for phenothiazines, these results imply that two such compounds should be endowed with a significantly greater anti-CaM activity than TFP itself.

The activation of multifarious metabolic events hinges upon CaM (reviewed in Refs. 1 and 2). Molecules endowed with high CaM-binding affinities can interfere with the CaM-dependent processes, which encompass enzymatic activation (3, 4), microtubule assembly (5), DNA repair (6–8), cell proliferation (9), tumor cell growth (10–12), etc. An outstanding class of CaM inhibitors is provided by phenothiazine (PZ) derivatives and, more notably, TFP. Extensive structure-activity relationships are available for this class of compounds (13, 14). The most important structural modifications involve substitutions on the PZ nucleus and modification of the cationic head. To our knowledge, the effect of increasing the length of the hydrocarbon chain linking the PZ ring and the cationic head was solely examined in the chlorpromazine series. Let $n$ denote the number of methylene groups of the linker chain. A significant and gradual increase of the binding affinity was reported upon passing from $n = 2$ to $n = 4$ methylenes, chlorpromazine itself being characterized by $n = 3$ (13). The effect of further lengthenings ($n > 4$) was not investigated, and it was underlined in Refs. 12 and 14 that $n = 4$ may not necessarily represent the optimal distance required for optimal anti-CaM activity in the PZ series.

The present study will be devoted to analogs of TFP. Progressive lengthenings of the linker chain will be undertaken, starting from $n = 3$ methylenes in TFP proper, up to $n = 6$. By means of theoretical computations, we will assess the effect of such lengthenings on the binding affinity to fragment (82–93) of CaM.

The structural formulas of the four investigated PZ derivatives are given in Fig. 1. They will be denoted by *1–4*. Their chemical names are: 2-trifluoromethyl-10-[3-(piperazino)propyl]phenothiazine (*1*), 2-trifluoromethyl-10-[4-(piperazino)butyl]phenothiazine (*2*), 2-trifluoromethyl-10-[5-(piperazino)pentyl]phenothiazine (*3*), and 2-trifluoromethyl-10-[6-(piperazino)hexyl]phenothiazine (*4*). The primary sequence of fragment (82–93) is shown in Fig. 2. The choice of this fragment as a candidate receptor site was previously discussed (15), in line with an explicit proposal by Reid (16, 17). The oligopeptide backbone is held in the $\alpha$-helical conformation, consistent with the recent resolution of the X-ray structure of CaM (18) as well as with spectroscopic evidence on related troponin C (19).

A previous study was devoted to a comparison of the binding affinities to this site of four PZ derivatives: promethazine, promazine, trifluopromazine, and TFP itself. Their overall binding affinities were ranked in the same order as those of their experimentally determined affinities (15). The usefulness of the present approach should now be assessed by testing its predictive value for the design of prospective CaM antagonists.

**ABBREVIATIONS:** CaM, calmodulin; PZ, phenothiazine; TFP, trifluoperazine; SCF, self-consistent field; SIBFA, sum of interactions between fragments computed *ab initio*.

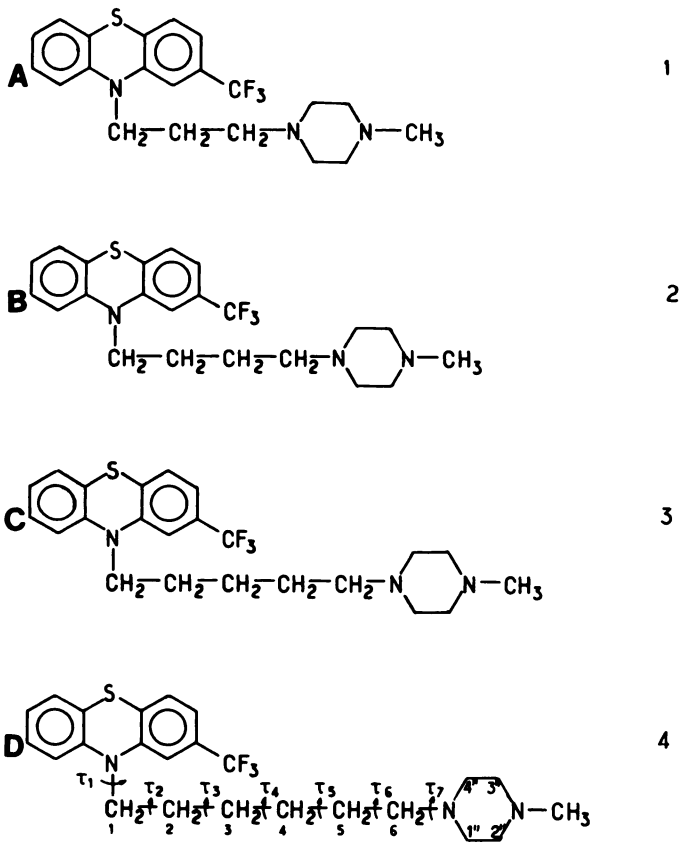

Fig. 1. The four investigated PZ derivatives: structural formulas, atom numbering, and definition of torsion angles. A. *1*, Three-methylene linker chain (TFP). B. *2*, Four-methylene linker chain. C. *3*, Five-methylene linker chain. D. *4*, Six-methylene linker chain.

## Procedure

The variations of the conformational energy change of the PZ derivative and of the rotated side chains of the oligopeptide upon complex formation are computed with the SIBFA procedure (20, 21). Within this methodology, each investigated molecule is built of elementary constitutive fragments separated by single bonds, and the variation of the intramolecular energy upon a conformational change is obtained as the variable part of the sum of the interactions between the fragments expressed as:

$$\delta E = E_{MTP} + E_{pol} + E_{rep} + E_{disp} + E_{tor} \quad (1)$$

The intermolecular PZ-oligopeptide interaction energies are computed by the SIBFA 2 procedure as the sum of five terms:

$$\Delta E = E_{MTP} + E_{pol} + E_{rep} + E_{disp} + E_{CT} \quad (2)$$

In expressions 1 and 2, $E_{MTP}$ and $E_{pol}$ denote, respectively, the electrostatic and polarization contributions, computed using a multipolar expansion of the *ab initio* SCF molecular wave functions of the fragments, and $E_{rep}$ and $E_{disp}$ are the repulsion and dispersion contributions, respectively. $E_{tor}$ is a torsional energy contribution, calibrated in Ref. 20 for elementary rotations along C—C and C—O bonds, and $E_{CT}$ is a charge-transfer contribution (see Refs. 20 and 22 for details).

Standard bond lengths and valence angles were adopted throughout (23). The internal geometry of the phenothiazine ring was taken from Ref. 24. The *ab initio* SCF computations on the constitutive fragments were performed using our usual basis set (25). In the computations of the intramolecular energy of the oligopeptide, the value of $E_{MTP}$ was simplified to that of the sole monopole-monopole component, which enabled a considerable reduction of the total computing time. The validity of this assumption was tested and justified in our previous work (15).

The search for the optimal configuration of the complex was performed by energy minimization (26) of the sum of $\delta E_{CaM}$ (oligopeptide) *plus* $\delta E_{phen}$ (phenothiazine derivative) *plus* $\Delta E$ (PZ derivative-CaM oligopeptide). The involved variables in the minimization process are the six variables defining the position of the drug with respect to the oligopeptide, the torsional angles $\tau$ of the drug, and the torsional angles of Glu 84, Glu 87, Phe 89, and Phe 92. The conformations of the remaining polar side chains, which do not participate in any direct interaction with the PZ derivatives, were previously energy-minimized (see Ref. 15 for discussion). The variation of intramolecular energy of CaM, $\delta E_{CaM}$, thus incorporates the variation of the mutual interactions of each of these four side chains with the three other mobile ones *and* all the remaining side chains, as well as with the $\alpha$-helical backbone.

For each PZ derivative, the choice of the starting configuration prior to energy minimization was assisted by molecular modeling using an interactive computer display on a Spectragraphics machine. Each starting configuration was selected on the basis of an adequate location, on the one hand, of the piperazinium moiety with respect to Glu 84 and Glu 87 and, on the other hand, of the PZ ring with respect to Phe 89, Phe 92, and Ile 85. Unfavorable short-range contacts (if any) were eliminated by performing preliminary energy minimizations using a truncated form of $\Delta E$, in which $E_{MTP}$ is approximated by its sole monopole-monopole term; this also helped to optimize the combination of both electrostatic and hydrophobic interactions for each PZ derivative prior to a more complete evaluation of $\Delta E$.

The computations were performed on the CRAY-1 computer of the Centre de Calcul Vectoriel pour la Recherche.

## Results and Discussion

**Conformational preferences of PZ derivatives prior to interaction.** Conformational energy maps as a function of the torsional angles $\tau_1$ (S—N—$C_1'$—$C_2'$) and $\tau_2$ (N—$C_1'$—$C_2'$—$C_3'$) using 20° increments were drawn. The torsional angles along the terminal paraffinic C—C bond and the following C—N bond were held at 180°. This choice is in keeping with the conclusions of studies devoted to the conformational behavior of PZs (27). The results for *1* were previously exposed in Ref. 15. For *2*, three submaps were drawn, corresponding to $\tau_3$ values of 180°, 60°, and −60°. For *3*, nine submaps were drawn, corresponding to the nine combinations of values of 180° and ±60° for $\tau_3$ and $\tau_4$, and for *4*, twenty-seven submaps were drawn, corresponding to the twenty-seven combinations of values of 180° and ±60° for $\tau_3$, $\tau_4$, and $\tau_5$.[1] Energy minimizations of the best so-derived local and global minima were then performed, by relaxing all the torsional angles (up to seven for *4*). The intrinsically preferred conformations of all four PZ derivatives are *gauche-gauche* conformations along $\tau_1$ and $\tau_2$, in which the piperazinium group is folded over the electron-rich PZ ring. The best conformations energy-wise which were thus derived are characterized by the following set of angles:

*1:* $\tau_1 = 35°$, $\tau_2 = 295°$, $\tau_3 = 200°$, $\tau_4 = 180°$

[1] Unpublished maps, available upon request.

- $Glu_{82}$-$Glu_{83}$-$Glu_{84}$-$Ile_{85}$-$Arg_{86}$-$Glu_{87}$-$Ala_{88}$-$Phe_{89}$-$Arg_{90}$-$Val_{91}$-$Phe_{92}$-$Asp_{93}$ -

Fig. 2. The sequence of CaM oligopeptide (82–93).

*2*: $\tau_1 = 315°$, $\tau_2 = 65°$, $\tau_3 = 270°$, $\tau_4 = 160°$, $\tau_5 = 175°$

*3*: $\tau_1 = 30°$, $\tau_2 = 280°$, $\tau_3 = 60°$, $\tau_4 = 180°$, $\tau_5 = 170°$, $\tau_6 = 175°$

*4*: $\tau_1 = 40°$, $\tau_2 = 280°$, $\tau_3 = 180°$, $\tau_4 = 295°$, $\tau_5 = 300°$, $\tau_6 = 180°$, $\tau_7 = 180°$

These conformations of the PZ derivatives will be taken as their energy zero levels in Table 1.

The preferred conformation of the CaM oligopeptide side chains prior to complexation was determined in our previous study (15) and will be taken as its energy zero.

**The CaM (82–93)-PZ complexes.** The complexes of *1–4* are represented in Fig. 3. For the sake of clarity, only the hydrogens belonging to the PZ derivatives are represented. The conformational changes of Glu 84 and Glu 87, which are brought closer together by the attraction of the PZ cationic end, are very similar in all four complexes, with $\chi_1$, $\chi_2$, and $\chi_3$ values amounting (respectively) to 230°, 270°, and 345° for Glu 84 and to 180°, 270°, and 340° for Glu 87 (15). For both Phe 89 and Phe 92, the conformations of the side chains are defined by values of $\chi_1$ and $\chi_2$ close to 270° and 90°, respectively. These values also correspond to the ones previously derived for the CaM oligopeptide prior to interaction and for the complexes of promethazine, promazine, trifluopromazine, and TFP itself (see Ref. 15).

The conformations of the PZ derivatives along $\tau_1$ and $\tau_2$ correspond to *gauche-trans* conformations, with the occurrence of one or more *gauche* conformations along paraffinic C—C bonds for *2*, *3*, and *4* (see below).

The stereochemistries of the four complexes at the level of their piperazinium moieties are similar. They are characterized by a short H-bond between the piperazinium proton and one O of Glu 87, whereas one H linked to the methylene carbon immediately preceding the piperazinium ring, and on which the positive charge is partially delocalized, bridges the two anionic oxygens of Glu 84. Hydrophobic interactions occur, in addition, between the piperazinium moiety and Val 91 (15). The paraffinic linker chain and the PZ ring are involved in hydrophobic interactions with Ile 85, Ala 88, Phe 89, and Phe 92. The involvement of these four residues in the binding of anti-CaM molecules was originally proposed in Ref. 16. The extent of participation of these residues in the overall hydrophobic interaction varies, however, according to the investigated PZ derivative, as will be tentatively analyzed below.

TABLE 1

**Values of the interaction energies in the optimized PZ derivative-oligopeptide complexes, and of the dihedral angles defining the conformation of the derivative in the complex**

In Tables 1 and 2, $\Delta E$ denotes the CaM-PZ derivative interaction energy and its components $E_{MTP}$ (electrostatic), $E_{pol}$ (polarization), $E_{CT}$ (charge transfer), $E_{disp}$ (dispersion), and $E_{rep}$ (repulsion). $\delta E_{CaM}$ and $\delta E_{phen}$ denote the variations of intramolecular energies of CaM and PZ derivative (respectively) upon complex formation. $E$ is the overall energy balance $\Delta E + \delta E_{CaM} + E_{phen}$, and $\delta E$ the difference of overall energy balance with respect to the most favorable value of $E$ takes as energy zero. $\tau_1$ to $\tau_7$ are the conformational angles of the PZ derivatives. Energies are in kcal/mol, angles are in degrees.

| | *1* | *2* | *3* | *4* |
|---|---|---|---|---|
| $\Delta E$ | −264.4 | −276.4 | −281.1 | −273.7 |
| $E_{MTP}$ | −236.6 | −241.9 | −241.6 | −236.6 |
| $E_{pol}$ | −22.4 | −25.4 | −26.8 | −25.2 |
| $E_{CT}$ | −16.1 | −16.8 | −16.4 | −16.5 |
| $E_{disp}$ | −33.7 | −39.3 | −49.8 | −40.6 |
| $E_{rep}$ | 44.4 | 46.9 | 53.5 | 45.2 |
| $\delta E_{CaM}$ | 28.7 | 29.0 | 29.5 | 27.8 |
| $\delta E_{phen}$ | 5.6 | 10.4 | 15.2 | 12.7 |
| $E$ | −230.3 | −237.0 | −236.4 | −233.2 |
| $\delta$ | 6.9 | 0.0 | 0.6 | 3.8 |
| $\tau_1$ | 32.9 | 318.0 | 38.2 | 311.8 |
| $\tau_2$ | 173.1 | 173.1 | 202.2 | 200.0 |
| $\tau_3$ | 167.4 | 58.7 | 264.8 | 182.0 |
| $\tau_4$ | 164.2 | 173.5 | 73.7 | 187.2 |
| $\tau_5$ | | 165.0 | 153.7 | 55.5 |
| $\tau_6$ | | | 172.1 | 162.1 |
| $\tau_7$ | | | | 167.9 |

The results of the interaction computations are reported in Table 1, which lists the intermolecular interaction energy, $\Delta E$, and its contributions; the intramolecular energy differences, $\delta E_{CaM}$ and $\delta E_{phen}$, of the oligopeptide and the PZ derivative respectively, with respect to their energies prior to interaction; the resulting energy balance $E = \Delta E - \delta E_{CaM} - \delta E_{phen}$; the difference, $\delta$, of interaction energies with respect to the best derived interaction energy value; and the torsional angles of the PZ derivatives in their respective complexes.

Table 1 shows that the most favorable values of $E$ are found for *2* and *3*, that is, derivatives with a four- or a five-methylene linker chain, which are found to display a significantly higher affinity for the CaM oligopeptide than TFP itself.

The trend in $E$ values is the result of contrasting trends imposed by $\Delta E$ and $\delta E_{phen}$ for the complexes of *1* to *4*, whereas the values of $\delta E_{CaM}$ remain comparable (to within 1.7 kcal/mol) in the four complexes. Although the value of $\Delta E$ is much larger for the complex of *3* than for that of *2*, this preference is opposed by a larger value of $\Delta E_{phen}$ in the former, resulting in overall comparable values of $E$ for *2* and *3*. Overall, lengthening of the paraffinic linker chain, upon passing from *1* to *2–4*, results in a strong increase of the CaM oligopeptide-PZ interaction energy, but *also* in an increase of the energy separation, $\delta E_{phen}$, between the intrinsically preferred folded conformation of the PZ derivative and its *gauche-trans* conformation involved in the complex. This increase of $\delta E_{phen}$ is caused by the increased stabilization of the folded conformation of derivatives *2–4*, due to a facilitated rapprochement of the piperazinium head and the phenothiazine ring, which is itself assisted by the occurrence of one or more *gauche* conformations along the paraffinic C—C bonds in the energy minima (see above).

It is to be recalled at this point (15) that, in the present computations, Asp 93 and Arg 90 side chains, which are not involved in direct interactions with the PZ derivatives, are mutually complexed through one carboxylate oxygen of Asp 93 and the imino and vicinal amino guanidinium protons of Arg 90. In the CaM protein as opposed to the simpler oligopeptide, Asp 93 is part of $Ca^{2+}$-binding loop III and is involved in an ionic interaction with $Ca^{2+}$. Its incorporation as part of the PZ receptor may thus be questioned. We have accordingly recomputed the interaction energies of the PZ derivatives with the CaM oligopeptide in two additional cases. In the first, Asp 93 was excluded from the energy computations, and in the second, both Asp 93 and Arg 90 were excluded. The interaction energies of *1–4* in the first case amount to −240.5, −252.2, −257.0, and −249.2 kcal/mol, respectively. In the second case, these interaction energies amount to −268.6, −280.8, −285.6, and −278.0 kcal/mol (respectively). It thus appears that, whereas the presence of these two residues will affect the absolute value of $\Delta E$ owing to long-range electrostatic effects, they will affect only very slightly the values of the differences between the different

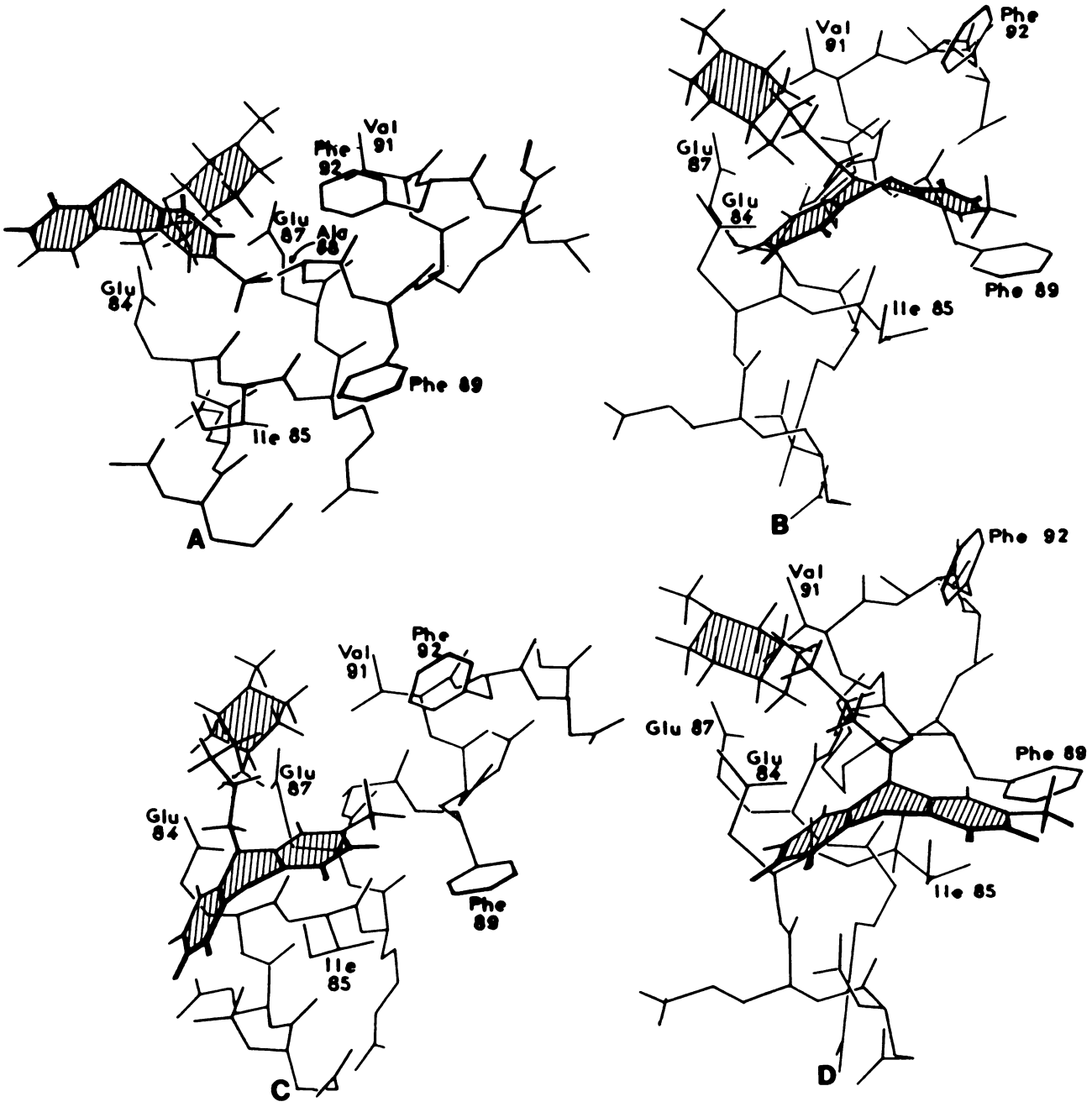


**Fig. 3.** Representation of the CaM oligopeptide complexes with *1* (A), *2* (B), *3* (C), and *4* (D). For more clarity, the PZ and piperazinium rings have been shaded.

$\Delta E$ values (and $\delta E_{CaM}$ values as well) and will not, therefore, alter the overall ordering of $E$ values.

We have attempted to delineate the role of two hydrophobic fragments of the PZ derivatives, namely, the paraffinic linker chain and the PZ ring, in complex stabilization. For that purpose we have recomputed the separate interaction energies of these two moieties with the CaM oligopeptide and with individual fragments of the oligopeptide, namely: the peptide without Glu 84 and Glu 87 side chains, the Phe 89 and Phe 92 side chains, the Ile 85 side chain, and the Ala 88 side chain. We have also computed the separate PZ-oligopeptide backbone interaction energies. The results of these computations are reported in Table 2. In the five latter decompositions, only the $E_{disp}$ and $E_{rep}$ contributions to $\Delta E$ have been reported, since these are then the predominant contributions to the corresponding $\Delta E$ values.

Table 2 shows that the contributions of the linker chain and the phenothiazine ring to the total $\Delta E$ values of Table 1 are significant, a much larger value being reached with *2–4* than with *1*. The predominant part of these energies is contributed by the two glutamates, Glu 84 and Glu 87. This appears upon comparing results (a) in Table 2 to results (b), obtained when the two glutamate side chains are excluded from the interaction. It is caused by a closer proximity, in the longer homologs *2–4*, between Glu 84 and the PZ ring as well as part of the linker chain and is facilitated by the folding of the PZ structure along

TABLE 2

**Values of the interaction energies of the paraffinic linker chain and the PZ nucleus of PZ derivatives with: (a) CaM oligopeptide; (b) oligopeptide without Glu 84 and Glu 87 side chain; (c) Phe 89 and Phe 92 side chains alone; (d) Ile 85 side chain alone; (e) Ala 88 side chain alone; and (f) oligopeptide backbone[a]**

| | | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| (a) CaM oligopeptide | $\Delta E$ | −17.6 | −30.0 | −35.3 | −30.0 |
| | $E_{MTP}$ | −6.6 | −11.7 | −11.3 | −8.4 |
| | $E_{disp}$ | −7.1 | −13.8 | −23.3 | −16.5 |
| | $E_{pol}$ | −5.2 | −8.0 | −9.7 | −9.2 |
| | $E_{CT}$ | −0.4 | −2.8 | −3.7 | −3.1 |
| | $E_{rep}$ | 1.7 | 6.3 | 12.7 | 7.2 |
| (b) CaM oligopeptide without Glu 84 and Glu 87 | $\Delta E$ | −4.3 | −7.7 | −9.9 | −8.3 |
| | $E_{disp}$ | −5.4 | −8.7 | −16.3 | −9.7 |
| | $E_{rep}$ | 1.4 | 1.8 | 5.9 | 2.6 |
| (c) Phe 89 and Phe 92 | $\Delta E$ | −1.5 | −1.2 | −1.4 | −0.3 |
| | $E_{disp}$ | −1.9 | −1.5 | −1.6 | −0.3 |
| | $E_{rep}$ | 0.5 | 0.5 | 0.2 | 0.0 |
| (d) Ile 85 | $\Delta E$ | −0.4 | −2.7 | −2.7 | −3.6 |
| | $E_{disp}$ | −0.4 | −3.6 | −6.8 | −5.2 |
| | $E_{rep}$ | 0.0 | 1.3 | 4.1 | 2.5 |
| (e) Ala 88 | $\Delta E$ | −0.9 | −1.2 | −1.3 | −0.6 |
| | $E_{disp}$ | −1.6 | −0.6 | −2.0 | −0.7 |
| | $E_{rep}$ | 0.9 | 0.1 | 0.9 | 0.0 |
| (f) Backbone | $\Delta E$ | −1.2 | −2.6 | −4.4 | −3.7 |
| | $E_{disp}$ | −1.6 | −2.7 | −6.1 | −3.5 |
| | $E_{rep}$ | 0.0 | 0.0 | 0.7 | 0.1 |

[a] Energies are in kcal/mol.

some of its paraffinic C—C bonds. In *2–4* as compared to *1*, it is translated by significantly increased values of all the energy contributions indistinctly.

The interaction energies with the hydrophobic side chains proper, but also with the sole oligopeptide backbone, are more favorable for *2–4* than for *1*. The former energies are ranked in the order: $3 > 4 > 2 > 1$, where a peak is reached for *3*, similar to the result with the total $\Delta E$ values of Table 1. The adoption by the four PZ derivatives of slightly differing orientations with respect to Phe 89, Phe 92, Ala 88, and Ile 85 (see Fig. 3) is translated by varying values of the individual contributions to overall stabilization stemming from them. Thus, *1*, *2*, and *3* are much more favorably oriented for interaction with Phe 89 and Phe 92 whereas, on account of its longer linker chain, *4* is shifted away from them. This is translated by a distinctly smaller value of the corresponding interaction energy in *4* as compared with *1–3*. In contrast, *1* is much less favorably positioned with respect to Ile 85 than *2–4*, now translated by a much less favorable interaction energy of *1* with Ile 85 side chain as compared with *2–4*.

## Conclusions

The results of the present study indicate that analogs of TFP with a four-, a five-, or a six-methylene linker chain should display a significantly larger affinity for CaM oligopeptide (82–93) than TFP itself, with a maximal affinity being reached with a four- or a five-methylene linker. The overall increases of affinity stem from a more adequate fitting of the linker chain and the PZ ring to the receptor site. This is translated by significantly more favorable values of their overall interaction energies with the residue side chains involved in the binding, and also with the backbone proper. The present finding of an enhanced affinity upon lengthening the TFP linker by one methylene group is consistent with the experimental result of Ref. 13, devoted to the related chlorpromazine series, in which it was found that a derivative with a four-methylene linker displayed a significantly enhanced anti-CaM activity compared to chlorpromazine itself, but with no longer chain derivatives then being tested.

The treatment adopted in the present study should certainly lend itself to further improvements (extension of the receptor site, inclusion of solvation effects, etc.). Despite its present limitations, and on account of the potential importance of anti-CaM molecules, the present prediction of an enhanced affinity of *2–4* for CaM oligopeptide (82–93) is certainly worthy of trials of their prospective anti-CaM activities.

The parallelism observed in our previous study (15) between theoretically computed affinity values and experimental determinations in a series of four related PZs (including TFP itself) should lend support to the validity of the model.

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