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Pharmacophoric features and Ca^{2+} ion holding capacity of verapamil

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Abstract—Ab initio Hartree–Fock calculations have been performed at the 6-31G level to study the pharmacophoric features of verapamil. Both the unprotonated and the protonated forms of verapamil have been studied. The study predicts that the drug enters the body in protonated form and is anchored to the receptor via H-bond formation involving protonated amine. Huge conformational change as well as deprotonation is required before the drug is capable of holding Ca^{2+} ions. Folded form of drug is capable of holding Ca^{2+} ion and the chiral center also seems to be involved to certain extent.

Verapamil belongs to the phenylalkylamine (PAA) group of calcium channel antagonists and has been extensively used clinically in the treatment of several cardiovascular diseases.[1] Phenylalkylamines block voltage-gated Ca^{2+} channel, by binding within the intracellular mouth of the ion conducting pore.[2] Previous studies suggest that verapamil adopts a relatively compact structure.[3] Verapamil can adopt three structural forms (or conformational shapes): extended, folded, and half folded.[4] The folded conformation is stabilized by nonbonded interactions between the two dimethoxy aryl rings situated on the opposite ends of verapamil and is suggested to be the most stable conformation of the isolated drug.

Molecular simulations for the free drug with the crystal structure of verapamil as the starting point have also been performed.[5] Solution structure of verapamil in deuterated DMSO using one-dimensional ^{1}H and ^{13}C NMR data[6] has been analyzed. The levorotatory (−) enantiomers of PAAs were found to be more potent than the dextrorotatory (+) enantiomers.[7] Three drugs from this class have been extensively studied: verapamil, D888, and gallopamil (D600) (Fig. 1). D888 contains only one meta methoxy group at the inside of the aromatic ring of the phenylethyl part, whereas verapamil contains two methoxy groups in meta and para positions. D888 blocks the channel with affinity higher than that of verapamil (~300-fold).[8] Another drug of PAA subclass is gallopamil (D600), also widely used in clinical medicine and experimental biology to block Ca^{2+} inflow into cell across the plasma membrane.[9] The conformational features of gallopamil have also been studied and gave the analysis that the neutral form of the drug is characterized by a unique conformation; whereas the protonated form exists in different conformations with great mobility of the torsional angles and of the ionized site of the molecule.[10]

Verapamil gains access to their binding sites from the cytoplasm[11] and seems to inhibit the central pore by physical occupancy. Electrophysiological data have indicated that the binding domain for PAA is located on the intracellular side of the membrane in cardiac myocyte.[12] The PAA binding pocket is composed of at least seven amino acid residues,[13] based on the alanine scanning mutagenesis of binding of the PAA derivative desmethoxyverapamil (D888). Three amino acid residues in segment IVS6—Tyr 1463, Ala 1467, and Ile 1470—are required for high affinity block by D888, because their mutations reduced the affinity 6- to 12-fold. Met 1464 is also included as a residue contributing to high affinity PAA interaction.[14] Previous studies also show that in the Ca^{2+} bound form, two verapamil molecules are arranged with a 2-fold symmetry such that the two methoxy oxygens from each molecule act as ligand to the cation.[3] Early studies indicate an inhibitory effect of Ca^{2+} on the binding of PAAs to the receptor; there is also evidence that suggests, there could be a concentration dependence of the Ca^{2+} effect.[15] Data using a

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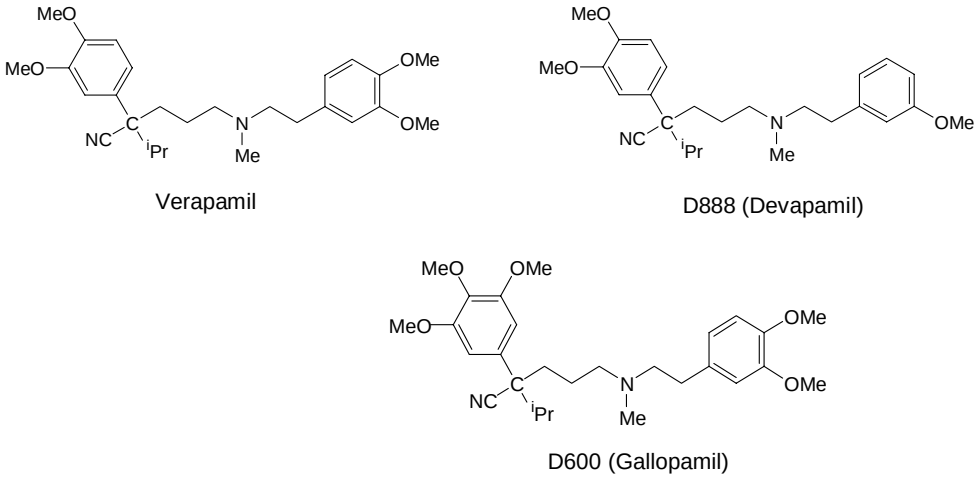

Figure 1. Phenylalkylamines.

fluorescent probe that interacts at the PAA site reveal that removal of Ca^{2+} results in a lowering of the binding affinity for these drugs.[16]

PAAs exist in the protonated form at physiological pH and their pK_a have been reported to be 9.04.[17] IC_{50} value of verapamil in μM is about 12 ± 5 (mean ± SE of four cells).[18]

The present study concentrates on conformational aspects of verapamil as well as Ca^{2+} ion holding capacity and site of Ca^{2+} ion interaction for verapamil. Ab initio HF molecular orbital calculations have been performed on verapamil using 6-31G basis set.[19,20] Complete geometry optimizations have been performed using an optimally conditioned method of Davidon and Nazareth.[21,22] Complete conformational phase space has been scanned. The starting conformations were similar to stationary points reported for gallopamil by Brasseuer and co-workers. Optimized conformations have been plotted with the help of ORTEP package. Charge environment has been studied using complete molecular electrostatic potential (MESP) contours and the same have been illustrated with the help of graphic package MOLDEN.[23] We have also performed conformational mapping, that is, the unprotonated verapamil has been superimposed onto protonated verapamil to understand the variation in the conformations. The relative capacity of the drug to hold Ca^{2+} ion has been investigated by calculating intermolecular interaction energies. The geometry of the complex has been optimized, only constraint being that the Ca^{2+} should not be covalently bound (i.e., the drug can hold the ion and also release the ion if needed, in other words use-dependent blockade[24]). The channel can never hold the ion permanently as it will result in permanent disruption of ion flow.

First of all, we have located the global minimum on the potential energy surface to be taken as the bioactive conformation in absence of information on actual bioactive conformation. The conformation corresponding to located global minima for the unprotonated and protonated forms of verapamil are shown in Figure 2A. The

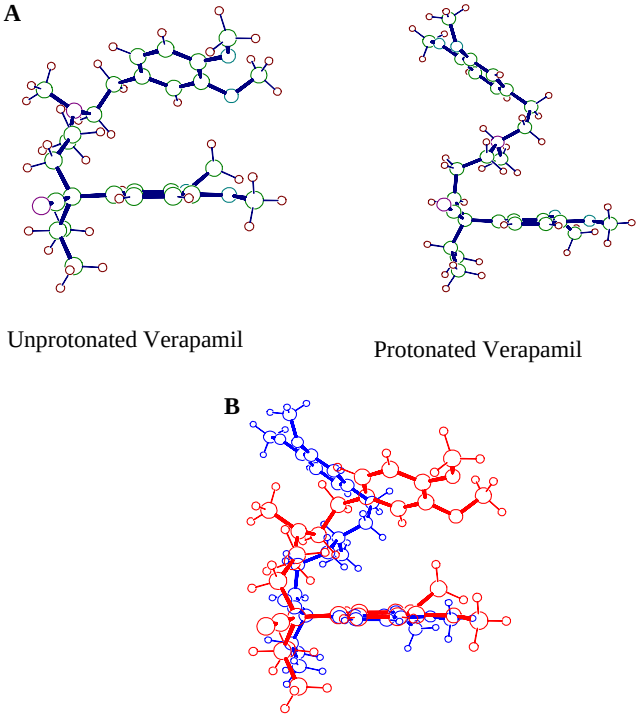

Figure 2. (A) ORTEP plots of unprotonated and protonated verapamil. (B) Unprotonated verapamil mapped onto protonated verapamil.

unprotonated form occurs in the 'sandwich' or folded conformation and the protonated in the half folded form.[†] The site of protonation is obviously the amine N. There is no other site available for protonation in PAAs. Next, we have performed the conformational mapping. It obviously indicates the huge conformational change on protonation of drug (Fig. 2B). Figure 2 shows that in both the conformations (unprotonated/

[†] Despite several tries stationary point corresponding to half folded or unfolded form could not be located on potential energy surface for unprotonated form.

folded; protonated/half folded) the aryl rings are out of plane with respect to aliphatic backbone. In the half folded form, the two aryl rings are out of plane with respect to aliphatic backbone. In the half folded form, the two aryl rings are at an angle of 71.50°. The unprotonated folded/compact form is similar to the conformations observed in the crystal; angle between the rings is predicted to be 80.85°. It is interesting to note that both the forms are '*R*' enantiomers with respect to chiral center.

Earlier studies have shown some importance of chiral center with reference to the activity of the system. Another important pharmacophoric feature is the distance between the two aryl rings, which is predicted to be 8.5 Å in the half folded, protonated form and reduced to be only 5.7 Å in the folded form on deprotonation. The calculations of complete molecular electrostatic potential maps indicate an overall negatively charged environment on PAA (Fig. 3) in unprotonated form. However, as the drug enters the body in protonated form it may be anchored to the receptor via H-bond formation involving protonated amine to a H-bond acceptor group like ionized tyrosine.

We now discuss intermolecular interaction calculations toward Ca^{2+} ion holding capacity of the drug. In the case of verapamil after several tries attractive interaction was found only in single case, where the Ca^{2+} electrostatically interacts with the methoxy groups on aryl ring close to the chiral carbon (Fig. 4). Ca^{2+} ion in its most preferable position can be seen approaching from above the aryl ring, so as to avoid steric interactions and be able to electrostatically interact with cyanide group on the chiral carbon and the two methoxy substituents on the aryl ring. There is no other site suitable for holding the Ca^{2+} ion.

To summarize, this work explains that the most favorable conformation based on gas-phase calculations for unprotonated form of verapamil is the folded structure. Protonated form is predicted to be in half folded/unfolded conformation. The pharmacophoric features extracted from optimized conformation predict that the two dimethoxy aryl groups should be disposed at an angle

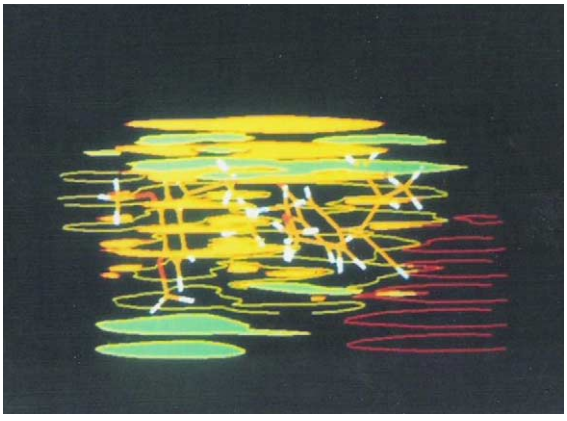

Figure 3. Molecular electrostatic potential map of verapamil.

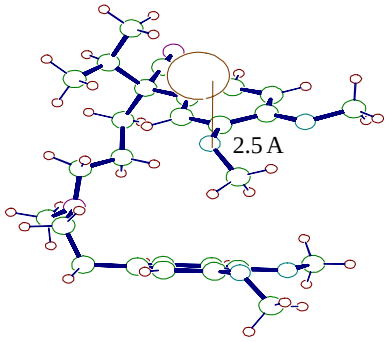

Figure 4. Intermolecular interaction energy calculation for verapamil Ca^{2+} complex.

of 80.85° in the unprotonated/folded form (only the unprotonated form is capable of holding Ca^{2+} ion and hence it is the active form). Both phenyl rings are out of plane with respect to aliphatic backbone. As the drug enters the body in protonated form it is expected to be anchored to the receptor via H-bond formation involving protonated amine of PAA. Huge conformational change is required before it can be capable of holding Ca^{2+} ion. Folded, unprotonated form is capable of holding the Ca^{2+} ion. Chiral center also seems to be involved in channel blocking in conformity with previous studies showing different potencies for different enantiomers.

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