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Docking of Acylamide and Acyl Ester in the Coordination Environment of Calcium in PLA2. Methyl formate, formamide, acetamide and the N-methylformamide and N-methylacetamide were used to simulate the steric, coordination, and hydrogen-bonding constraints for a carboxyl ester or amide between calcium and His-48 in the active site of pancreatic PLA2. *Ab initio* docking of acylamide and N-methylacylamide in the active site was carried out by a method which rigorously and systematically explores all possible independent orientations and identifies all possible positions of a docked mimic in the Eulerian space of the active site. As described below, of the 279936 possible orientations, we identified 20 sterically contact-free orientations, however only one of these satisfied the inhibition requirement.

Ab Initio Docking of Acyl Mimics in the Active Site of PLA2. Optimization of docking of an acylamide or acylester molecule in the active site of PLA2 was carried out using a systematic grid search method (1). PDB coordinates 1BP2 for bovine PLA2 and 5P2P for the inhibitor complex of a mutant of pig PLA2 were used to set up the initial binding constraints at the catalytic site. The force field parameters were from the AMBER package with a charge of 2+ assigned to Ca. The distance 'q' (Ca-O) between the calcium and the carbonyl oxygen of the amide was fixed between 2.5 to 2.6 Å. The parameters for the ligands (formamide, methyl formamide, and the ester analogs) were adopted from similar chemical environment (Table S1). The distance 'p' (δ N-His-48 to amide-NH) was maintained at hydrogen bonding distance (3.0 Å); this condition could not be satisfied with ϵ N. A set of Eulerian angles (Φ, Θ, Ψ), varied in steps of 10°, was used to explore the conformational space and to describe the rigid body motion of the mimic in the active site (2). Standard internal parameters are chosen for the generation of amide (3) in which ω can take values of 0° and 180°. The

parameter α describes the orientation of the amide with respect to His-48 and it was varied in steps of 60° . Thus 279936 ($36 \times 18 \times 36 \times 6 \times 2$) possible orientations were tested for 'contact' between formamide atoms and the PLA2 atoms. Of these only 200 are free of any steric hindrance within the overlap-limit of 1 Å. The Eulerian contact maps were generated to identify 20 distinct contact free orientations in which the carbonyl group coordinates with Ca(II) and the amide NH is H-bonded to δ N of His-48. These maps were used to identify additional constraints which lead to a single unique docking site for N-methylformamide. These orientations were also used for docking methyl formate, and for the localization of the two water molecules (W5 and W6).

Only one of these orientations (Figure S1A) distinguishes between acylamide and its N-methyl derivative. The possibility of H-bonding of the second hydrogen of acylamide with a neighboring group, other than His-48, on the protein yielded no viable interactions. Alternative possibilities were considered in terms of the interactions with water molecules. In the formamide-docked PLA2 structure the carbonyl oxygen occupies a position very close (about 2.16 Å up) to the position of W5 in the penta-coordination plane about the calcium ion in the native PLA2. A search for possible positions to include water molecules suggested that the three water (W5, W6 and W12) molecules could be accommodated in only one of the twenty orientations. This structure was further energy minimized using the AMBER program. As shown in Figure S1A, one of the water molecules is placed in the apical position above the penta-coordination plane, which is very close to the position occupied by W12 in the native structure. W5 could be accommodated within the coordination shell but somewhat shifted from its original equatorial position towards the other apical position where the backbone carbonyl of Tyr-28 is coordinated. Similarly W6 could be placed only slightly

shifted from its original position between W5 and δ N-H48. Also the additional carbonyl oxygen ligand from the substrate in the octa-coordinated complex is easily accommodated without significant distortion of the five ligand provided by the protein (Figure S1B), and its stability comes from the H-bonding network (Figure S1A). In the formamide complex the Ca...W5 oxygen distance is 2.99 Å, compared to the 2.6-2.8 Å range for all the other coordinating distances. This is perhaps due to a pull from the hydrogen bond of W5 with one of the amide hydrogens. Since such a network is not sterically possible with N-methylformamide; the methyl group does not permit simultaneous placement of W5 in the coordination shell and formation of a hydrogen-bond with His-48. This orientation of formamide is unique and it adequately accounts for the lower inhibitory potency of the N-methylformamide.

For energy minimization of the PLA2-formamide complex, the initial positions of the other two bound water molecules (W6 and W12) were obtained from the crystal structure of the native enzyme. The new position for W5, 0.76 Å away from the original crystallographic position was arrived at by conducting a grid search so as to avoid steric hindrance with the carbonyl oxygen while satisfying the coordination with calcium and maximizing its hydrogen bonding with the active site groups. The hydrogen atoms on the bound water oxygens were fixed in arbitrary orientation. The AMBER suite of programs was used to conduct a 'soft belly' minimization. The force field parameters for coordination around Ca(II) with +2.0 charge were adopted from those with similar chemical environment. For monitoring the active site interactions, docked formamide and W5, W6 and W12 were allowed to move during the minimization cycles: first 10 cycles of steepest descent followed by 90 cycles of conjugate gradient minimization with allowance to move only the water molecules. Then

minimization was repeated for the next 100 cycles (10 + 90 as before) with formamide as the only mobile group. The resultant geometry is displayed in Figure S1A.

Once parameters for the docking of formamide were established, the structure for methyl formate docked into the active site was refined by direct substitution. The CH₃ group was fixed at a torsion angle of 25° with respect to the carbonyl oxygen, i.e. without steric strains. Further refinement was carried out by energy minimization protocol as described above. However, as the water molecules showed a tendency to drift away from the calcium coordination shell, a small restraint with a force constant of 1.0 kcal/mole-Å was used on the three water molecules in the first 100 cycles, and then only on the carbonyl oxygen during the subsequent 100 cycles of minimization. The minimized structure is shown in Figure S1B. The hydrogen attached to the carbonyl group in methylformate can be replaced by an extended alkyl chain to mimic the sn-2-chain of the substrate without any unfavorable short contacts.

The catalytic environment around the carboxyl ester bond was simulated with methylformate docked in PLA2 (Figure S1B). W5 in the coordination shell and its H-bonding and distance relationships make it well positioned for a role as the catalytic water; it is polarized by coordination to Ca(II) and its deprotonation is assisted by the stabilization of W6 and His-48. In the ester structure, O1 of Asp-49 is 2.51 Å away from the proton of W5 and 2.75 Å away from its oxygen. The structures shown in Figure S1 are not only without any unfavorable steric contacts but the coordination and hydrogen bonding distances are energy minimized to the optimum range.

Electronic Structure of the Cation and Dative Bonds are Probably Not Critical. As k^*_{cat} -cofactor, 3d-cations(II) show a relatively sharp cutoff: Fe, Mn, Co and Ni support

catalysis, while the rates with Cu, Zn and Cd are lower by at least a factor of 200. We ascribe this to a difference in the coordination geometries in E^*CS complexes.

The inhibitory effect of certain cations is apparently not related to the ability of the cation to polarize the carbonyl or the water nucleophile, nor is it related to any of the features of the chemistry of the d-electrons (4). Considerations based on the rate of desolvation of cations (5-7) are relevant if the leaving tendency of the water bound to the cation is limiting, however it does not account for the difference between Co(II) and Ni(II) *versus* Cu(II) and Zn(II). To wit, water exchange rates, presumably by a dissociative mechanism, of the hexaquo ions of divalent transition metals show Co(II) and Ni(II) to be slower to substitute water ligand than Cu(II) and Zn(II) by several orders of magnitude. In as much as these dissociative rates are a direct measure or a close correlate of the "rate of desolvation" of the cations (6), they are inconsistent with the observed trend. Thus loss of water or hydroxide from the metal ion is unlikely to be involved in the transition state in the chemical step.

Table S1. Parameters for Acylamide Docked in PLA2 in Molecular Dynamics Calculations¹.

Bond length parameters:

	Force constant	Equilibrium distance
C-HC	331.	1.09
C-OS	320.	1.41

Bond Angle parameters

C-C -N	70.	116.6
C -C-HC	35.	109.5
C-C-OS	50.	109.5
HC-C-OS	35.0	109.5
C-OS-C	60.	109.5

Dihedral Parameters:

O -C -OS-C	1	0.383	0.	-3.
O -C -OS-C	1	0.2	180.	2.
HC-C -OS-C	1	0.383	0.	-3.
HC-C -OS-C	1	0.2	180.	2.

¹ All other parameters were from the Amber Package

Figure Legends

FIGURE S1. Calculated distances for the energy minimized coordination environment of calcium in the active site of PLA2 docked with [A] formamide or [B] methylformate. Note that the H-bond distances are for -O...H-.

References (to Supplementary Material section)

1. Seshadri, K., Vishweshwara, S., and Jain M. K. (1994) Proc. Indian Acad Sci. **106**, 1177-1189.
2. Goldstein, H. (1980). Classical Mechanics, p. 143. Addison-Wesley, Reading MA.
3. Ramachandran, D. N., and Sasisekharan, V. (1968). Adv. Protein Chem. **23**, 283.
4. Bock, C. W., Katz, A. K., and Glusker, J. P. (1995). J. Amer. Chem. Soc., **117**, 3754.
5. Burgess, J. (1978) Metal Ions in Solution Halsted Press, Wiley, New York.
6. Akesson, R., Petterson, L. G. M., Sandstrom, M., and Wahlgreen, U. (1994a). J. Amer. Chem. Soc. **116**, 869.
7. Akesson, R., Petterson, L. G. M., Sandstrom, M., and Wahlgreen (1994b). J. Am. Chem. Soc. **116**, 8705.

