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Unveiling the Binding Dynamics of two organic compounds with Human Serum Albumin: Integrating Computational, Spectroscopic, and Preliminary SCXRD Insights

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INTRODUCTION & AIM

- Albumin, a highly abundant, negatively charged protein at blood pH, is synthesized in the liver, has a molecular weight of 66 kDa, and exists in plasma at 30–45 g/L in healthy individuals **[1]**.
- Structurally, albumin has a cardioid shape with a single polypeptide chain divided into three domains, each containing α-helices stabilized by 17 disulfide bonds, which maintain its stability and flexibility.
- Propylene carbonate (PC) is a low-toxicity, polar, aprotic organic solvent widely utilized in applications such as adhesives, CO_2 removal, paint strippers, and cosmetics **[2]**.
- Decyl glucoside (DG), a plant-derived, eco-friendly surfactant, is widely used in personal care and household cleaning products due to its mild cleansing properties and ingredients **[3]**.
- The aim of this study is to investigate the interactions between human serum albumin (HSA) and the compounds propylene carbonate and decyl glucoside, providing molecular insights that could inform biological impacts and future industrial applications.

METHODS

Computational Methods

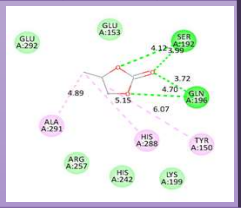
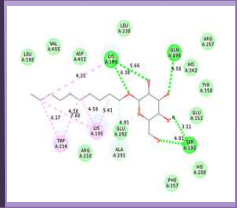
- **Docking**: The high-resolution HSA model (PDB ID: 2BX8) was modified in *PyMOL* to remove ligands and chain B, preparing it for docking of PC and DG ligands using *AutoDock* (v4.2.6) with the Lamarckian genetic algorithm. Grid dimensions were adjusted to cover key active site residues, and binding affinity was assessed via estimated free energy of binding.
- **Molecular Dynamics Simulations (MDs)**: MD simulations for HSA/PC and HSA/DG complexes were performed in *Amber 22* using the ff14SB and GAFF force fields. Systems were solvated in a TIP3P water box, equilibrated, and run under NPT conditions, with analysis of RMSD, RMSF, and binding energy conducted via CPTRAJ, *VMD*, and MM-GBSA methods.

Experimental Methods

- **Defatting Protocol**: The defatting of HSA involved pH adjustments and centrifugation to remove charcoal impurities, followed by pH readjustment to 7.0. The protein was then concentrated and buffer-exchanged using a 10K MWCO concentrator to ensure sample purity **[4]**.
- **UV-Vis Spectroscopy**: UV-Vis absorbance of HSA with and without PC and DG was measured on a Q6000 spectrophotometer (Quawell), covering 240–340 nm. This analysis allowed for assessment of structural changes in HSA upon ligand binding.
- **Crystallization Trials**: Crystallization of HSA alone and in complex with PC was attempted via the sitting-drop vapor-diffusion method. Rhombohedral crystals of HSA formed at 291 K within three weeks.

RESULTS & DISCUSSION

Table 1. The molecular docking results

Docking modes	Compound name					
	Propylene carbonate			Decyl glucoside		
	FEB (kcal/mol)	Distance from best mode		FEB (kcal/mol)	Distance from best mode	
		rmsd l.b.	rmsd u.b.		rmsd l.b.	rmsd u.b.
1	-4.5	0.000	0.000	-6.6	0.000	0.000
2	-4.4	17.261	17.819	-6.4	1.404	2.429
3	-4.3	18.260	18.807	-6.0	11.908	14.242
4	-4.2	19.609	20.161	-5.8	20.077	22.537
5	-4.2	19.659	20.707	-5.7	11.813	14.004
6	-4.1	17.913	18.474	-5.7	18.527	20.805
7	-4.1	20.195	21.345	-5.6	10.695	12.671
8	-4.1	19.624	20.731	-5.5	9.677	11.735
9	-4.1	37.570	37.945	-5.5	20.368	22.653
Protein – Ligand Interactions depicted by *Discovery studio*						

Computational results:

- **Docking:** The DG molecule exhibited lower FEB values than PC, indicating stronger binding to HSA, while RMSD variations reflect the smaller size of PC compared to DG (**Table 1**).
- **MDs:** The dynamic behavior of the HSA/PC complex is characterized by a slight repositioning occurred at 1.7 ns over the initial 2 ns, while the HSA/DG complex remains relatively stable **(Fig. 1a and b)**. RMSD and RMSF plots confirm the structural stability and MM/GBSA analysis demonstrate that van der Waals forces predominantly govern the binding interactions between PC and DG ligands and the HSA protein (**Table 2 & Fig. 1c and d**).

Experimental results:

- **UV analysis:** The absorption spectra confirm both HSA/PC and HSA/DG complex formation, with increased UV intensity suggesting interactions affecting HSA's peptide helices (**Fig. 2a**).
- **Crystallization:** HSA protein crystals formed in three weeks, exhibiting a rhombohedral shape and suitable size (**Fig. 2b**) for X-ray data collection, which verified their proteinaceous nature.

Table 2. MM/GBSA analysis for the two protein complexes

	HSA/PC complex	HSA/DG complex
ΔE_{vdW}	-15.76 ± 1.64	-44.04 ± 3.04
ΔE_{ele}	-0.02 ± 4.44	-32.65 ± 11.23
ΔE_{MM}	-15.78 ± 4.60	-76.70 ± 10.43
ΔG_{GB}	7.77 ± 3.96	48.84 ± 9.98
$\Delta G_{nonpolar}$	-2.34 ± 0.13	-6.69 ± 0.27
$\Delta G_{solvation}$	5.43 ± 3.90	42.15 ± 9.90
ΔH	-10.35 ± 1.71	-34.55 ± 3.65

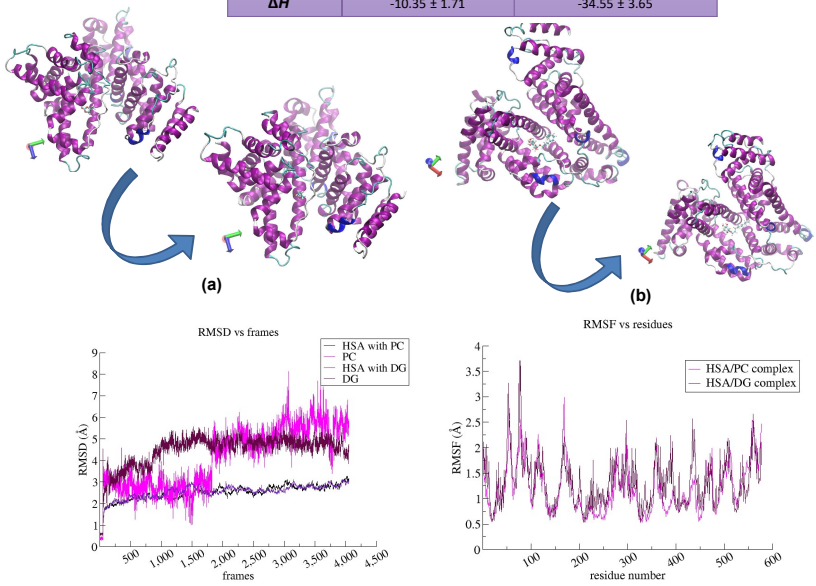


Fig. 1. (a), (b). Representative snapshots at 0 and 2 ns of the two MD simulations. Ligands are shown as sticks, waters are not shown for clarity, **(c), (d).** RMSD vs. frames plot and RMSF vs. protein residues plot.

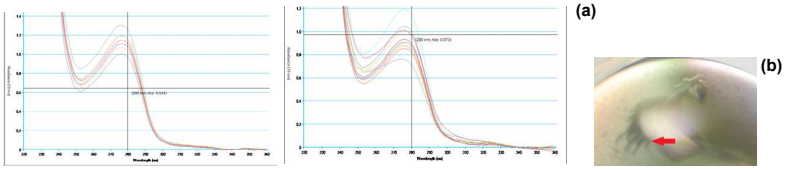


Fig. 2(a). The absorption spectra of HSA/PC and HSA/DG complexes and **(b).** Rhombohedral protein crystals of HSA

CONCLUSION

- This study investigates the binding affinity of two compounds (PC and DG), commonly found in commercial detergents, to human serum albumin using UV spectroscopy, molecular docking, and molecular dynamics simulations.
- The molecular binding results indicate that DG exhibits higher binding affinity to HSA compared to PC, as evidenced by the lower binding free energy values.
- Molecular dynamics simulations revealed that the HSA/DG complex exhibits remarkable stability, mainly due to the increased number of hydrogen bonds maintained throughout the simulation.

REFERENCES

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