THEORETICAL AND EXPERIMENTAL STUDY OF HEPARIN SODIUM SALT AND C-REACTIVE PROTEIN

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Abstract

In this work, we report a joint theoretical and experimental study on the molecular and vibrational structure of heparin sodium salt (heparin S) and C-reactive protein. Experimental techniques used in this investigation are FTIR, FT-Raman and SERS spectroscopies. In parallel, quantum chemical calculations, based on density functional theory (DFT) were performed in order to determine the geometrical, energetic and vibrational characteristics of the molecules with particular emphasis put on the interaction and adsorption geometry to the silver and gold colloidal surfaces. These studies elucidate the structure-activity relationship of the investigated systems.

Heparin is used as anticoagulant in the treatment of thrombosis. Therefore, a better understanding of its geometry and potential binding sites will help comprehend its impact on thrombosis. C-reactive protein, a protein secreted by cells as a first defense mechanism against inflammations, is known to bind to calcified plaques, the surfaces of atherosclerotic sites.

Heparin functionalized nanoparticles have been developed and used as transport vehicles for anticoagulation, but their full potential has not yet been exploited. We intend to investigate the binding mechanism on thrombotic tissue and to monitor the development of the treatment by using SORS (spatial offset Raman spectroscopy).

Experimental techniques

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FT-IR

heparin S powder samples, room temperature
Equinox 55 FT-IR spectrometer
InGaAs detector

FT-Raman

backscattering geometry
Bruker FRA 106/S Raman accessory, 1064 nm Nd:YAg laser, 400 mW, Resolution: 4 cm^{-1}

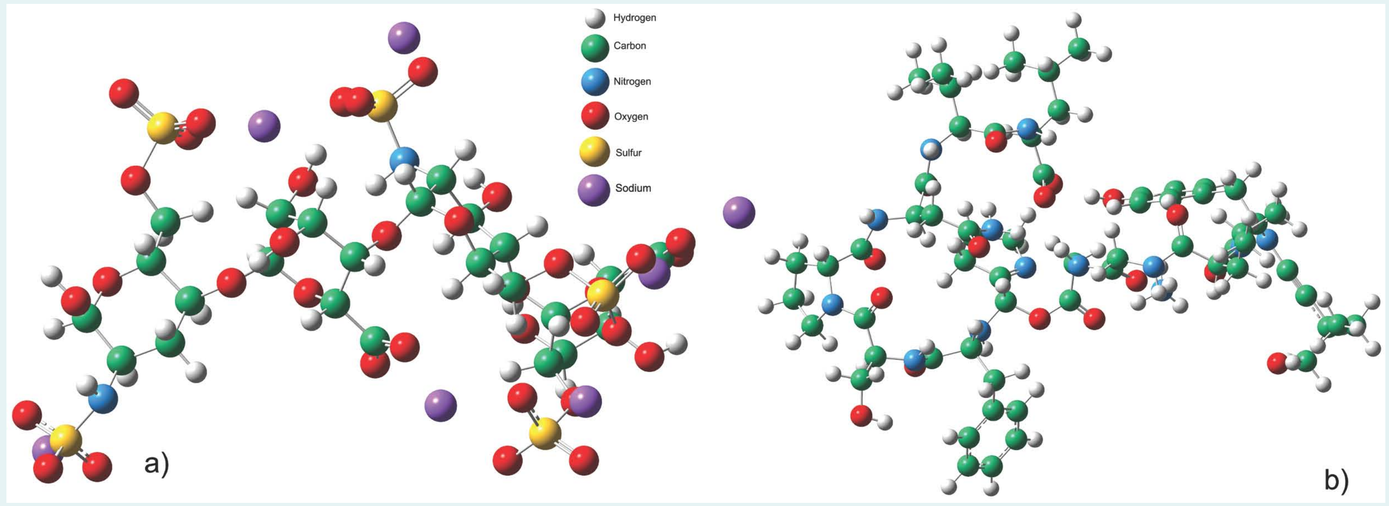
Raman/SERS

Multilaser confocal Renishaw InVia Reflex Raman spectrometer using the 632.8 nm laser line of a HeNe laser. The gold colloidal SERS substrate was prepared at room temperature using as reducing agent hydroxylamine.

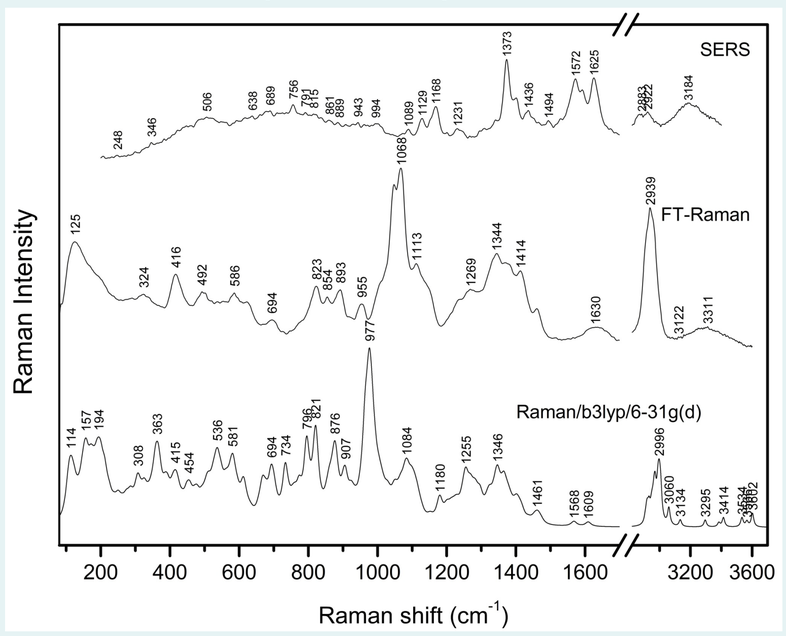
Computational methods

DFT Exchange-correlation functionals: B3LYP (for heparin S)
PM3, B3LYP (for C-reactive protein),
basis sets: "spectroscopic" 6-31G(d).

Chemical structure of heparin S (a) and C-reactive protein (b).



Calculated and experimental Raman spectra of heparin S

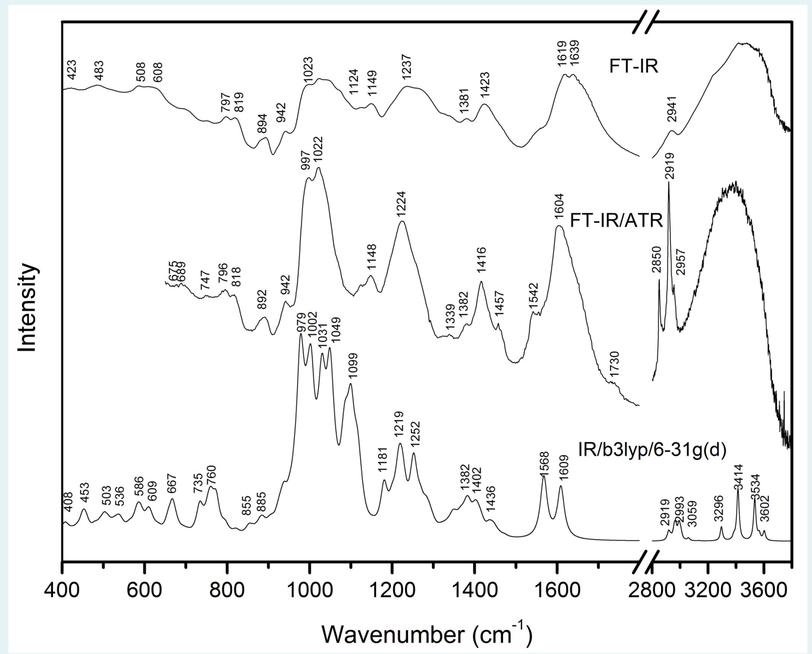


Selected SERS/Raman bands and calculated wavenumbers of heparin S

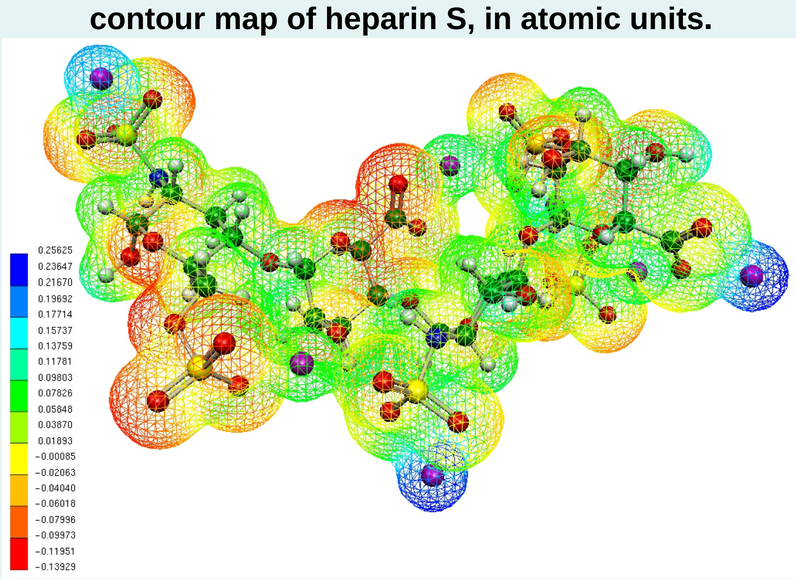
Experimental wavenumbers (cm^{-1})		Calculated wavenumbers (cm^{-1})	
SERS	FT-Raman	B3LYP/ 6-31g(d)	Band assignment
	125	114	δ(S_8O, $S_{16}O$, $S_{12}O$, $S_{43}O$) δ(CH (Ring2))
	416	415	δ($O_{39}H$, $N_{38}H$, $C_{35}H$, $C_{36}H$, $C_{37}H$, $S_{47}O$, $O_{59}H$)
	586	581	op. deformation of Ring1 δ(CH (Ring1), N_7H, $S_{16}O$)
815	823	821	ip. deformation of Ring1 and Ring2 δ_{ip}($C_{56}O$, $C_{25}O$)
1089	1068	1084	ν_{as}($S_{16}O_{17}O_{18}$) δ($C_{51}H$, $C_{54}H$, $C_{55}H$, $C_{22}H$, $C_{23}H$)
1168		1180	ν_{as}($S_{43}O_{44}O_{45}$) δ($O_{59}H$, $N_{38}H$, $C_{23}H$, $C_{34}H$, $C_{35}H$, $C_{36}H$, $C_{54}H$)
1373	1344	1346	δ_{op}(CH (Ring4) δ($O_{59}H$)
1572		1568	ν_{as}($C_{56}O$) δ($_{59}H$, $C_{55}H$)
1625	1630	1609	ν_{as}($C_{25}O$) δ($C_{24}H$, $C_{36}H$)
	3122	3134	ν($C_{51}H$)

ν-stretching, ν_{as}- asymmetric stretching, ν_s- symmetric stretching, δ-bending, ip-in plane, op.-out of plane Ring1-oxane(C_1-C_5,O_{10}), Ring2-oxane(C_{20}-C_{24},O_{29}), Ring3-oxane(C_{32}-C_{36},O_{41}), Ring4-oxane(C_{51}-C_{55},O_{60})

Calculated and experimental IR spectra of heparin S



B3LYP/6-31G(d) calculated 3D electrostatic potential contour map of heparin S, in atomic units.



Conclusions

The main conclusions of our work can be summarized as follows:

- The very good agreement between the experimental and calculated normal mode wavenumbers of heparin S allowed us to safely assign the vibrational spectrum of the molecule.
- Molecular electrostatic potential calculations indicate that the most suitable atomic sites for electrophilic attack or for metal coordination are the SO_3 and C-OH groups, while the most probable sites, which could be involved in nucleophilic processes, are the Na-SO_3 and NH groups in the heparin S molecule.

Selected FT-IR/ATR & FT-IR bands and calculated wavenumbers of heparin S

Experimental wavenumbers (cm^{-1})		Calculated wavenumbers (cm^{-1})	
FT-IR	FT-IR/ATR	B3LYP/ 6-31g(d)	Band assignment
585		586	δ(S16O, O26H, C6H, C20H, C3H, C33H, S12O, S47O, C5H, C3H98)
608		609	op. deformation Ring3
	675	667	δ($O_{26}H$, N_7H, $N_{38}H$, C_6H, O_8H)
894	892	885	ν_s(Ring4) ν($C_{55}C_{50}$) δ($C_{56}O$, $O_{59}H$, $N_{38}H$)
	997	979	ν_s($S_{12}O$, $S_{16}O$) ν($S_{12}N$) δ(N_7H, C_3H, $C_{24}H$, $N_{38}H$, $C_{22}H$)
1023	1022	1002	op.deformation Ring1 and Ring2 ν_{as}(O_9C) δ(CH (Ring1, Ring2))
	1224	1219	ν_{as}($S_{63}O_{64}O_{65}$) δ($C_{52}H$, $C_{54}H$, $O_{59}H$, $C_{23}H$, $C_{24}H$, $C_{22}H$)
1381	1382	1382	δ($C_{37}H$, $C_{36}H$, $C_{52}H$, $C_{53}H$, $O_{56}H$, $C_{34}H$, $C_{20}H$, C_6H)
1619	1604	1609	ν_{as}($C_{25}O_{31}O_{30}$) δ($C_{24}H$, $C_{36}H$)
	2919	2919	ν($C_{22}H$, $C_{34}H$, $C_{32}H$, $C_{21}H$)

ν-stretching, ν_{as}- asymmetric stretching, ν_s- symmetric stretching, δ-bending, ip-in plane, op.-out of plane Ring1-oxane(C_1-C_5,O_{10}), Ring2-oxane(C_{20}-C_{24},O_{29}), Ring3-oxane(C_{32}-C_{36},O_{41}), Ring4-oxane(C_{51}-C_{55},O_{60})

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