Experimental and theoretical investigation of heparin and C-reactive protein

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Abstract

In this work, a joint experimental and theoretical study on heparin and C-reactive protein is reported. The molecular vibrations of heparin and C-reactive protein were investigated by FT-IR, 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 on 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 to 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 [1], 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).

The heparin functionalized gold nanoparticles with popcorn shape were prepared at room temperature using as reducing agent hydroxylamine.

Experimental techniques

Biomedical Physics Department, Babeş-Bolyai University, Cluj-Napoca

FT-IR

heparin 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}

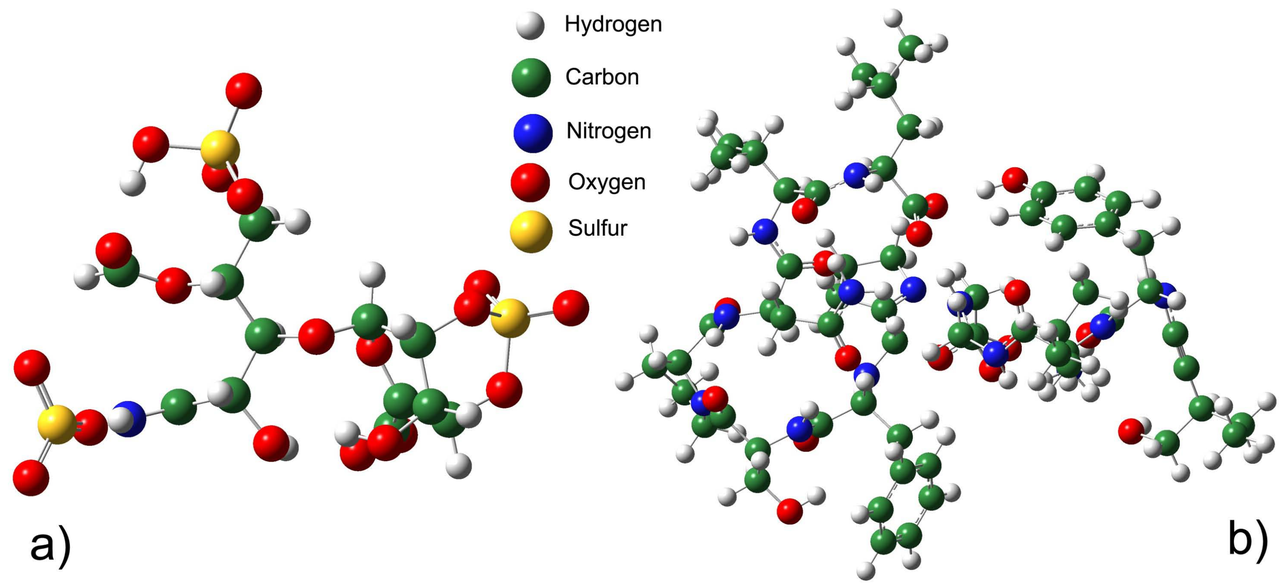
Raman/SERS

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.

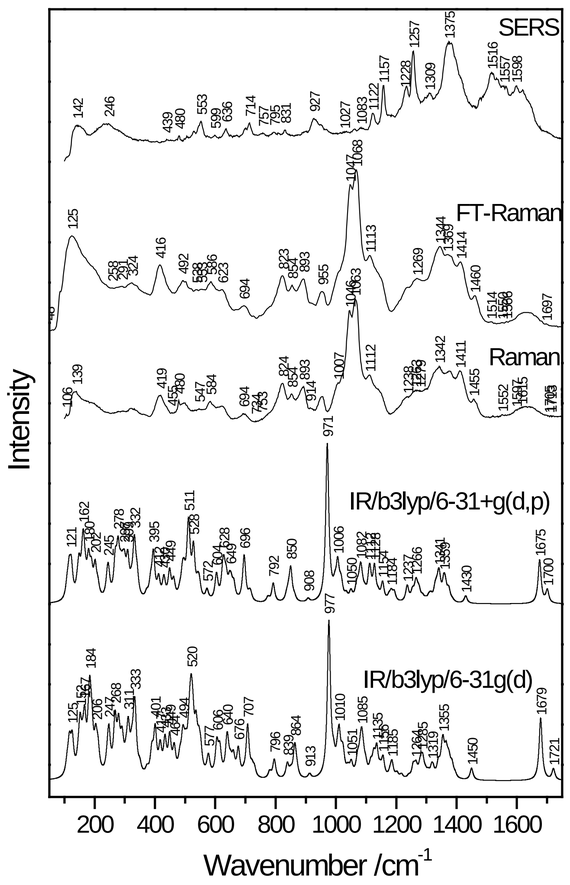
Computational methods

DFT Exchange-correlation functionals: B3LYP (for heparin)
PM3, BLYP (for C-reactive protein),
basis sets: “spectroscopic” 6-31G(d) and 6-31+G(d,p).

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



Calculated and experimental Raman spectra of heparin

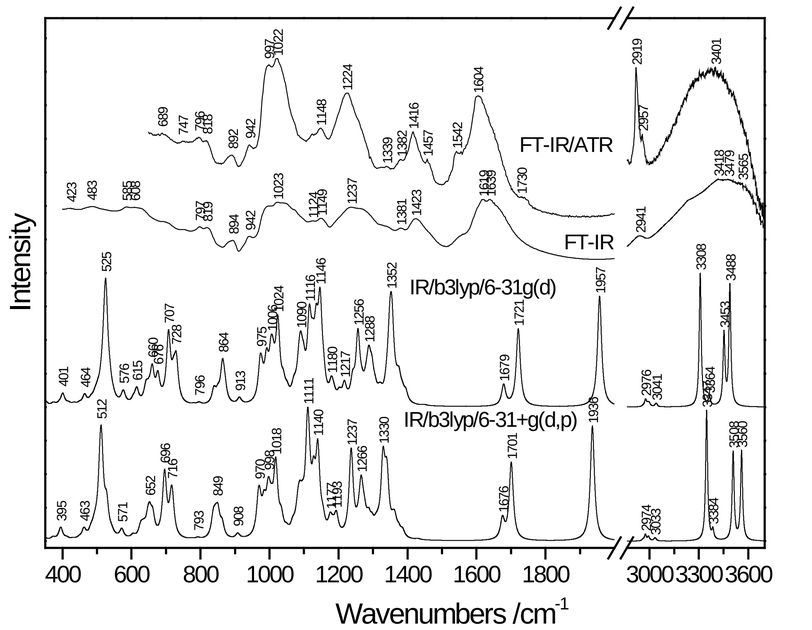


Selected SERS/Raman bands and calculated wavenumbers of heparin

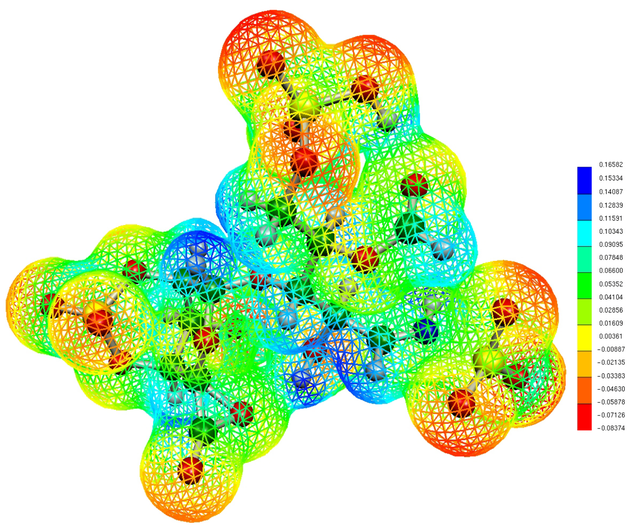
Experimental wavenumbers (cm^{-1})			Calculated wavenumbers (cm^{-1})		
SERS	**FT-Raman**	**Raman**	**B3LYP/ 6-31+g(d,p)**	**B3LYP/ 6-31g(d)**	**Band assignment**
142	125	139	121	125	$\delta(S_1O, S_2O, C_{27}H, C_{30}H, N_{23}H)$
	416	419	412	417	$\delta(S_2O_{15}O_{16}, O_{16}H, C_{30}H, C_{27}H, C_{28}H, N_{23}H)$
717	694	694	696	707	$\nu_s(S_2O)$, $\delta(O_{16}H, C_{30}H, C_{27}H, C_{24}H, C_{25}H)$
831	823	824	850	839	$\delta(N_{23}H, C_{26}H, C_{30}H, O_6H)$
	1047	1046	1050	1051	$\nu_{as}(C_{25}C_{26}O_6)$, $\nu_s(C_{24}C_{27}O_5)$, $\delta(C_{30}H, C_{25}H, C_{27}H, C_{26}H)$
1122	1113	1112	1082	1085	op. deformation(both rings), $\nu(O_4C_{27}, O_5C_{29}, O_6C_{25})$
1157			1154	1156	$\nu(C_{32}C_{33}, C_{34}C_{35})$, $\delta(C_{29}H, C_{31}H, C_{32}H, C_{33}H)$
1257	1269	1263	1266	1264	$\delta(C_{24}H, C_{25}H, C_{27}H, C_{30}H, C_{31}H, C_{32}H, C_{33}H, O_6H)$
	1344	1342	1341	1355	$\nu_{as}(S_3O_{18}O_{20})$, $\delta(C_{24}H, C_{25}H, C_{26}H, C_{29}H, C_{33}H, O_{11}H)$
	1460	1450		1450	$\delta(C_{30}H)$

ν-stretching, ν_{as}- asymmetric stretching, ν_s- symmetric stretching, δ-in-plane bending, op.-out of plane

Calculated and experimental IR spectra of heparin



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

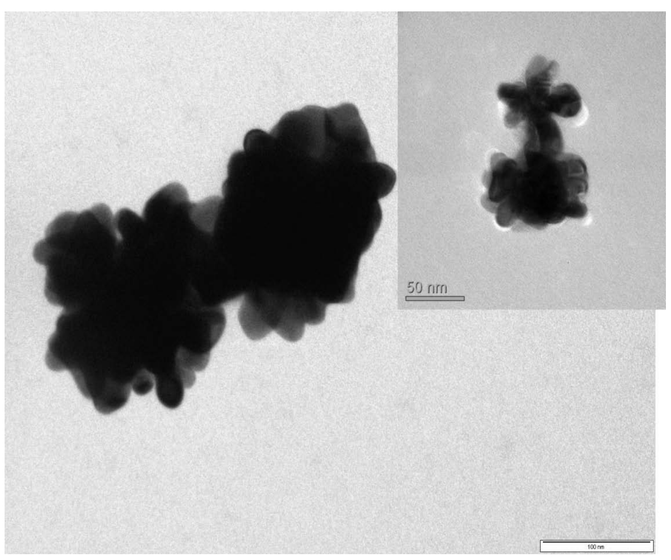


Selected FT-IR/ATR & FT-IR bands and calculated wavenumbers of heparin

Experimental wavenumbers (cm^{-1})		Calculated wavenumbers (cm^{-1})		
FT-IR/ATR	**FT-IR**	**B3LYP/ 6-31g(d)**	**B3LYP/ 6-31g+(d,p)**	**Band assignment**
	423	401	395	op. deformation(both rings), $\delta(O_{16}H, S_2O_{15}O_{16}, C_{30}H, C_{27}H, C_{26}H, C_{35}O)$
	483	464	463	$\delta(O_{11}H, O_6H, C_{24}H, C_{27}H, C_{30}H, C_{28}H, N_{23}H, S_1O_{12}, S_1O_{14})$
	585	576	571	op. deformation(both rings), $\delta(O_6H)$
796	797	796	793	op. deformation(both rings), $\delta_{ip}(C_{35}O_{21}O_{22})$
1022	1023	1024	1018	$\nu(O_9C_{30}, O_{10}C_{31}, O_{19}C_{33})$ $\delta(N_{23}H, C_{26}H, O_{11}H, C_{32}H, C_{29}H)$
	1124	1116	1111	$\nu(O_{11}C_{32})$ $\delta(C_{33}H, O_{11}H, C_{31}H)$
1148	1149	1146	1140	$\nu_{as}(O_4C_{27}C_{28})$ $\delta(O_{16}H, N_{23}H, C_{25}H, C_{26}H)$
1224	1237	1256	1237	$\nu_{as}(S_1O)$ $\delta(C_{25}H, C_{26}H)$
1382	1381	1352	1330	δ(C-H, $N_{23}H$)
	3479	3488	3508	$\nu(O_{11}H, O_5H)$

ν-stretching, ν_{as}- asymmetric stretching, ν_s- symmetric stretching, δ-in-plane bending, op.-out of plane, ip.-in plane

TEM images of hydroxylamine reduced gold nanoparticles.



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 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 OH groups, while the most probable sites, which could be involved in nucleophilic processes, are the CH and NH groups in the heparin molecule.
- By functionalizing the surface with highly biocompatible molecules, the proposed gold nanoparticles could become important transporting vehicles for drugs like heparin and C-reactive protein.

References

[1] Argyo, C., et al., Chemistry - A European Journal, 2012, 18(2): p. 428-432.

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