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Supporting Information

Large negatively charged organic host molecules as inhibitors of endonuclease enzymes

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Contents:

Experimental details:

Synthesis and characterization of calix[n]arenes

Para-sulphonato-calix[n]arenes have been synthesized as per the literature method of Coleman *et al* [1] and Calix[4]arene dihydroxyphosphorous acid as per the method of Markovsky and Kalchenko [2]

All the physical characteristics of the synthetized calixarenes correspond to the literature values.

Material and reagent

Sulphated β–cyclodextrin has been purchased from Sigma-Aldrich and restriction enzymes and rh DNAse I from Takara company.

Synthesis and characterization of sulphated β–cyclodextrin capped silver nanoparticles

10 mL of 10^{-2} M $AgNO_3$ solution was added to 80 mL of deionized water. To this solution, 10mL of 10^{-2} M of the sulphated β–cyclodextrin aqueous solutions were added as stabilizers with stirring for 30 min. And then, 44 mg of $NaBH_4$ was added to the solution. The colloidal silver suspensions were obtained after 5 minutes.

The sulphated β–cyclodextrin capped silver nanoparticles were then characterized by UV-Visible Absorption assays using a 96 well titre visible spectrometer (BioTek Power Wave 340). The presence of stable silver nanoparticles has been characterized by a maximum absorbance at 400nm.

Restriction enzymes inhibition assay for organic host molecules

100µM of each macrocyclic molecule β-CDsul, SC6 and SC8 (figure 1) have been mixed to 0.5µg of λ-DNA in a buffer at final concentration of 10mM Tris HCl pH 7.5, 50mM NaCl, 10 mM $MgCl_2$, 1mM DTT

The mixture was then mixed with the restriction enzymes and incubated 1 hour at 37°C.

The samples have been deposited on agarose gel 0.6% previously mixed with Ethidium bromide and run over 90 minutes at 75V. The gel was then scanned on ChemiDoc XRS system (Bio-Rad).

The digestion activity is then plotted as a matter of inhibitor concentration by quantifying the intensity of the digested bands with imageJ software.

Restriction enzymes inhibition assay for sulphated β–cyclodextrin capped silver nanoparticles DNAse I inhibition assay

A part of the β–CDsul capped silver nanoparticles (annotated Ag_NP_β–CDsul) solution prepared according to the method described above has been dialysed overnight in DI Water, using a dialysis cassette with a cut off of 10 000 Da (Slide-A-Lyzer Dialysis Cassettes, 10K MWCO, Pierce).

Then a varying concentration (from 100nM to 100µM) of β-CDsul, Ag_NP_β–CDsul not dialysed and Ag_NP_β–CDsul dialysed have been mixed to 0.5µg of λ-DNA in a buffer at final concentration of 10mM Tris HCl pH 7.5, 50mM NaCl, 10 mM $MgCl_2$, 1mM DTT.

The mixture was then mixed with the restriction enzymes NruI and incubated 1 hour at 37°C.

The samples have been deposited on agarose gel 0.8% previously mixed with Ethidium bromide and run over 45 minutes at 75V. The gel was then scanned on ChemiDoc XRS system (Bio-Rad).

The digestion activity is then plotted as a matter of inhibitor concentration by quantifying the intensity of the digested bands with imageJ software.

rh DNAse I inhibition assay for organic host molecules

The unit of rh DNase I and restriction enzymes are not equivalent. 1 unit for restriction will completely digest 1 µg of substrate DNA in 60 minutes, while 1 unit for rh DNase I corresponds to the amount of the enzyme that increases the absorbance at 260 nm by 0.001 per minute at 25 °C, pH5.0, with calf thymus DNA as the substrate.

IC_{50} of rh DNAse I for the macrocylclic molecules cannot be determined by agarose gel electrophoresis. Kinetic has been performed to compare the inhibition effect between the molecules.

100µM of each macrocyclic molecules SC4 and SC8 have been mixed to 0.5µg of λ-DNA in a buffer at final concentration of 10mM Tris HCl pH 7.5, 50mM NaCl, 10 mM $MgCl_2$, 1mM DTT

The mixture was then mixed with the rh DNase I (diluted 1000 time in the same buffer) at different incubation time of 1, 5, 10, 30 and 60 minutes, at 37°C.

The samples have been deposited on agarose gel 0.8% previously mixed with Ethidium bromide and run over 40 minutes at 75V. The gel was then scanned on ChemiDoc XRS system (Bio-Rad).

References and Note

[1] A. W. Coleman, S. Jebors, S. Cecillon, P. Perret, D. Garin, D. Marti-Battle, and M. Moulin, *New J. Chem.*, 2008, **32**(5), 780-782 ;

[2] V. I. Kalchenko, D. M. Rudkevich, L. N. Markovskii, *Zhurnal Obshchei Khimii,*1990, **60** (1-2), 2813-2814.

Supporting Figures:

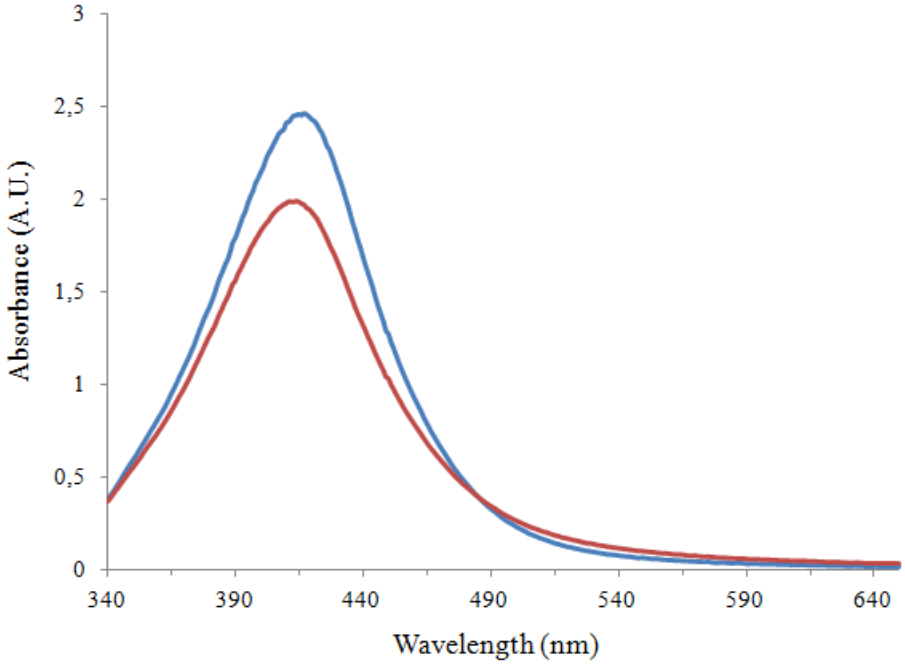

Fig. S1 UV-Visible spectra of β-cyclodextrin sulphate capped silver nanoparticles. Blue curve corresponds to nanoparticles before dialysis and Red curve represents nanoparticles after dialyseis. The dialysis was performed over night with a cut off of 10 000 Da against DI Water.