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Large negatively charged organic host molecules as inhibitors of endonuclease enzymes

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Three large negatively charged organic host molecules; β-cyclodextrin sulphate, para-sulphonato-calix[6]arene and para-sulphonato-calix[8]arene have been shown to be effective inhibitors of endonuclease in the low micromolar range, additionally para-sulphonato-calix[8]arene is a partial inhibitor of rhDNase I.

The endonucleases are a class of enzymes whose biological role is to digest DNA. As such, they play a role in human cell repair but also are key elements in viral infection. The endonucleases also act as protective elements in bacterial defense strategy against bacteriophages. They represent a valid target in drug design for anti-cancer, anti-viral and antibiotic treatments, however new compounds compatible with pharmaceutical criteria (high solubility, non-toxic) are needed.

A number of studies have pointed to endonucleases as potential targets for influenza treatment. The few anti-influenza medications currently available are often associated with severe side-effects. Commercial treatments target the viral membrane protein M2 (amantadine and rimantadine); or neuramidases, oseltamivir (Tamiflu) and zanamivir (Relenza). PA endonuclease is a domain belonging to the RNA-dependent RNA polymerase (RdRp) and it initiates the translation from viral mRNA to viral proteins. Its contribution is essential to viral production inside the infected cell. Pharmaceutically active soluble endonuclease inhibitors would thus appear to be excellent target as antiviral medications.

Secondly, human endonucleases present an interest as oncotherapeutic targets. AP endonuclease is a human enzyme involved in DNA lesion repairing system. This endonuclease is overexpressed in cancers such as glioblastoma leading to resistant to radio- and chemo-therapy. While development of AP endonuclease inhibitors is underway none are presently available due to their incompatibility with desirable clinical criteria (high solubility, non-toxic, low efflux transport, enzyme-resistant).

Supramolecular organic macrocycles, present considerable interest in biopharmaceutical science, the cyclodextrins are well known as transporters for bioactive compounds but are somewhat less well known for direct biological activity against proteins. The calix[n]arenes are well documented both as transporter molecules and also for their direct biological activity, particularly with regard to protein complexation.

In the current paper we describe the inhibitory activity of a series of organic host molecules, Scheme 1, with regard to four site specific endonucleases, Scheme 2, and the non-specific human rhDNase I enzyme.

Scheme 1. Structures of the organic host molecules evaluated as endonuclease inhibitors

The negatively charged organic host molecules, were chosen for the possible binding affinity for the DNA binding site and cleavage site, using the crystallographic information on the influenza virus PA endonuclease as the lead structure. The endonuclease enzymes were chosen for their known cleavage properties on the lambda phage DNA. Two, NruI, (CG site cleavage) and HindIII (AA) give rise to multisite cleavage of the DNA chain. The other two PdiI (CG) and Xbal (TT) cause cleavage at only a single site on the DNA chain. Our aim was to determine the factors which influence the inhibitory effects of the organic host molecules for possible use as therapeutic agents for influenza treatment.
In the inhibition experiments, the half maximal inhibitory concentration (IC_50) was measured. The digestion activity of endonuclease was evaluated using agarose gel electrophoresis at varying inhibitor concentrations. After quantifying the intensity of the digested bands on the gel, the concentration of inhibitor needed for 50% (IC_50) inhibition of the endonuclease activity, was determined. See SI Scheme 2. Sequence of cleavage site for different restriction enzymes and their positions on Lambda DNA phage.

The IC_50 concentrations for the six organic host molecules tested are given below in Table 1, of these three, β-CD, C4diP and SC4 show no inhibitory activity. The other three β-CDsul, SC6 and SC8 all show IC_50 values in the low micromolar range with regard to NruI and slightly lower values for HindIII. All three molecules are characterised by a combination of high negative charge and a size capable of spanning both the DNA binding site and the cleavage site in an endonuclease. As both these sites are characterised, in influenza PA Endonuclease by the presence of basic amino acids (DNA binding site, K34 and R124) and (Catalytic site, R84 and K184), blockage of the sites, by large anionic macrocycles, is not unexpected and is a requirement for enzyme inhibition.

Table 1. Half maximal inhibitory concentration (IC_50) of different organic host molecules determined for restriction enzymes NruI and HindIII. N.I. corresponds to an absence of endonuclease inhibition.

<table>
<thead>
<tr>
<th>Molecules</th>
<th>NruI (µM)</th>
<th>HindIII (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-CD</td>
<td>N.I.*</td>
<td>N.I.*</td>
</tr>
<tr>
<td>β-CDsul</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>C4diP</td>
<td>N.I.*</td>
<td>N.I.*</td>
</tr>
<tr>
<td>SC4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>SC6</td>
<td>1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>N.I.: No Inhibition</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results obtained can be compared to known inhibition activities, for example for small molecule inhibitors of Apurinic/apyrimidinic (AP) endonuclease 1 (Ape1) four were reported to have IC_50 values of less than 10µM and one, Ape1 repair inhibitor 03 [2,4,9-trimethylbenzo[b][1,8]naphthyridin-5-amine; AR03], inhibited cleavage of AP sites in SF767 glioblastoma cells, in whole cell extracts and inhibited purified human Ape1 in vitro. With regard to influenza PA Endonuclease inhibition, values are in the range high nanomolar to 10 µM for effective inhibitors. The observed values in this work are in the same range and IC_50 of SC8 with regard to HindIII is comparable to the best published value.

We have previously shown that supramolecular hybrid silver nanoparticles have anti-bacterial activity, thus it was of interest to investigate if such systems possess enzyme inhibitory activity. However, of the current systems only hybrid nanoparticles capped by β-CDsul proved stable under the conditions of the enzyme inhibition experiments.

The results are given in Figure 2 below. In order to ascertain that free β-CDsul was not responsible for the inhibitory effect the suspension was dialysed; the observed values decrease from an IC_50 of 3 µM for the free ligand to 3.8 µM for the β-CDsul capped silver nanoparticles. This decrease is similar to the small decrease in the plasmon resonance intensity observed, given in SI.
phosphodiester bonds. It is, also, to be noted that these enzymes by a need for divalent cations in the active sites. Thus rhDNase I has an active site with histidine, a sparagine and do not conserve the aminoacid geometry around the active site.

The inhibition experiments, using rhDNase I, were initially carried out at the same concentration as the Endonuclease been determined on the restriction enzyme NruI. capped on silver nanoparticles (dialysed in DI water or not) have been determined on the restriction enzyme NruI.

In conclusion we have demonstrated that large organic host molecules with a size above four units in the macrocycle and possessing strong negative charge are effective inhibitors of endonuclease enzymes. Work is currently underway to extend the work to influenza endonucleases and to study the cellular efficacy of the molecules.

Notes and references

† Electronic Supplementary Information (ESI) available: Full experimental values. See DOI: 10.1039/b000000x/

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