# DNAzymes for treatment of dengue fever

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KEY MESSAGES

1. Dengue virus is carried by mosquitoes and afflicts 50 to 100 million people each year.
2. DNAzymes are fragments of DNA that can destroy specific sequences of RNA.
3. We have discovered two DNAzymes that can destroy the RNA of dengue virus.
4. These DNAzymes might be developed into drugs to treat infection with dengue virus.

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## Introduction

Dengue virus is carried by mosquitoes and can cause dengue fever, dengue haemorrhagic fever, and dengue shock syndrome, afflicting 50 to 100 million people each year.[1] The genetic material of the virus consists of RNA.[1] DNAzymes are molecules that can cut RNA at certain places. DNAzymes are pieces of DNA.

DNA and RNA are strings of units made up of bases. There are four possible bases arranged in any order to make an infinite variety of DNA or RNA molecules. DNA has two strands. Bases can fit together with other bases like jigsaw puzzle pieces: A pairing with T, and C pairing with G.

DNAzymes are shaped like the Greek letter Ω, with the loop containing a series of bases that together have a special ability to cut RNA.[2] DNAzymes can be custom-made to cut a specific piece of RNA. To do this, one chooses bases in the horizontal part of the Ω that pairs with the specific fragment of RNA. DNAzymes have been used effectively in animals.[3] DNAzymes have the potential to attack the RNA in dengue viruses.[2] Therefore, we made and tested DNAzymes that may be used to treat dengue virus infection.

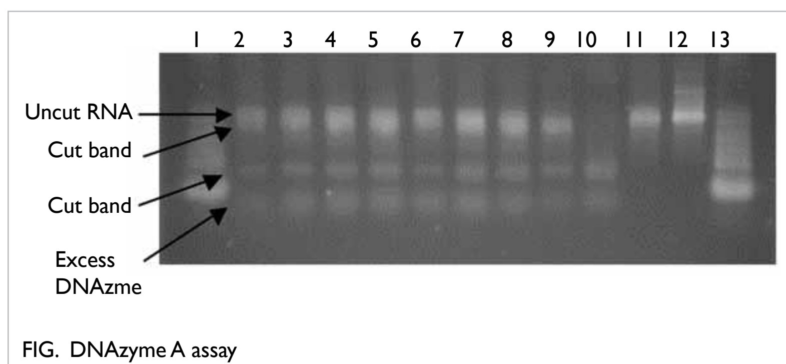


FIG. DNAzyme A assay

## Methods

This study was conducted from December 2006 to December 2007. We found 51 regions of dengue virus RNA that were the same in different strains. Within these regions, 26 good DNAzyme cutting sites were identified and 26 DNAzymes to cut these sites were made. To test the ability of each DNAzyme to cleave dengue RNA, we grew dengue virus, purified its RNA, mixed the RNA with a DNAzyme, and left the mixture for some time. To determine whether the RNA was cut, gel electrophoresis was performed to separate fragments of RNA or DNA according to their size.

## Results

Two DNAzymes (A and R) displayed cleavage activity. The Figure shows the gel separating the RNA and its fragments by their sizes, and the Table lists the contents of the mixtures that were separated on each lane of the gel. Magnesium chloride ($MgCl_2$) is required for optimal cleavage by DNAzymes. Increasing the $MgCl_2$ concentration increased cleavage by both DNAzymes. Complete fragmentation of RNA was only observed with DNAzyme A with the highest tested $MgCl_2$ concentration, but even the lowest concentration had at least a partial effect. Increasing the incubation period from 30 to 60 minutes did not affect the cleavage noticeably.

## Discussion

Two (8%) out of 26 DNAzymes (termed A and R) were found to cleave dengue RNA. This was comparable to that in a study showing that two out of 16 DNAzymes tested successfully cut the targeted RNA.[4] The two

TABLE. DNAzyme A assay

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RNA (relative units) | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| DNAzyme (relative units) | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - | - | - |
| $MgCl_2$ (relative units) | - | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 10 | 2 | - | - |
| Incubation time (min) | - | 30 | 30 | 30 | 30 | 60 | 60 | 60 | 60 | 60 | 60 | - | - |

DNAzymes that target different regions of dengue viral RNA may be used simultaneously to increase the chance of cleaving each strand of viral RNA.

Several obstacles need to be overcome before DNAzyme therapy can be realised: delivery across cell membranes, metabolism, digestion of DNAzymes, and optimising inhibitory and cleavage activity. Future work should involve testing modifications to increase the activity of DNAzymes A and R in serum and cultured cells infected with dengue virus. Delivery of DNAzymes to infected cells is a challenge, for which chemical modifications may enhance entry into cells.[5] Future work should also entail measuring the speed and efficiency by which DNAzymes cut RNA. In addition, variants of DNAzymes A and R (as negative controls) should be tested to demonstrate that the activity observed is specific.

Chemical modifications of DNAzymes can improve their activity.[6] Future work may involve testing modifications to increase the activity of DNAzymes A and R in cells infected with dengue virus. After optimising conditions for treating cells and animals and after ensuring the safety of treatment, human trials may proceed. If effective in treating dengue virus infection, DNAzymes could have the potential to save thousands of lives annually. Similarly, DNAzymes could be developed to treat other viral diseases, such as hepatitis, AIDS, and pandemic influenza.

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