External regulation of hairpin ribozyme activity by an oligonucleotide effector

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Supplement 1: Shiftgel analysis

Gel analysis was carried out to follow the formation of a ternary complex between HP-G25, HP-substrate and EF-01 or TW-G25, TW-substrate and EF-01, respectively. Bands were visualised by radiation with 254 nm UV-light after soaking the gel in ethidium bromide solution. In all experiments a non-cleavable analogue of HP-substrate or TW-substrate, respectively, containing 2'-deoxyadenosine instead of adenosine next to the cleavage site was used.


Conditions for formation of complexes were 2 μM HP-substrate, 2 μM HP-G25, 16 μM EF-01, 10 mM Tris-HCl, pH 7.5, 10 mM MgCl₂ (lane 4); 500 nM TW-substrate, 2 μM TW-G25, 10 μM EF-01, 10 mM Tris-HCl, pH 7.5, 10 mM MgCl₂ (lane 8). Ribozyme and EF-01 were mixed in buffer (10 mM Tris-HCl, pH 7.5), heated to 90 °C for 1 min followed by incubation at 37 °C for 15 min. MgCl₂ was added and the mixture incubated for another 15 min. The respective substrate was added and complex formation was allowed to proceed for 30 min at 37 °C. Then, reaction solutions were put on ice until subjecting individual samples on a 15 % native polyacrylamide gel.

Formation of a ternary complex between HP-G25, its substrate and EF-01 is clearly observed (lane 4, complex 2). For TW-G25 no resolution of the binary complex between TW-25 and its substrate and the ternary complex between TW-G25, its substrate and EF-01 could be obtained under the applied conditions. This is very likely due to the small size of EF-01 (18 nucleotides) compared to TW-G25 (78 nucleotides) and the TW-substrate (28 nucleotides). Therefore, both, the binary and the ternary complex are likely to have similar migration properties.
Supplement 2: Determination of $K_D$ und $k_1'$ for HP-G25 and TW-G25 with effector oligonucleotide EF-01.

\[ \text{[rib.sub] + EF} \xrightarrow{1 / K_D} \text{[rib.sub.EF]} \xrightarrow{k_1'} \text{products + rib + EF} \]

\[ k_{obs} = \frac{k_1' [EF]}{K_D + [EF]} \]  \hspace{1cm} (1)

Eadie-Hofstee equation:

\[ k_{obs} = -K_D \frac{k_{obs}}{[EF]} + k_1' \]  \hspace{1cm} (2)

a) Eadie-Hofstee plot for HP-G25

![Eadie-Hofstee plot for HP-G25](image)

b) Eadie-Hofstee plot for TW-G25

![Eadie-Hofstee plot for TW-G25](image)
Supplement 3: Determination of single turnover constants $K_1$ and $k_1$ for HP-WT and TW-WT.

\[
\text{rib} + \text{sub} \xrightarrow{1 / K_1} \text{[rib.sub]} \xrightarrow{k_1} \text{products} + \text{rib}
\]

\[
k_{\text{obs}} = \frac{k_1 [\text{rib}]}{K_1 + [\text{rib}]}
\]

Eadie-Hofstee equation:

\[
k_{\text{obs}} = -K_1 \frac{k_{\text{obs}}}{[\text{rib}]} + k_1
\]

a) Eadie-Hofstee plot for HP-WT

b) Eadie-Hofstee plot for TW-WT
Supplement 4: Determination of single turnover constants $K_i$ and $k_1$ for HP-G25 and TW-G25 with effector saturation.

\[
\text{[rib.EF]} + \text{sub} \xrightarrow{1 / K_i} \text{[rib.sub.EF]} \xrightarrow{k_1} \text{products} + \text{rib} + \text{EF}
\]

\[
[\text{[rib.EF]}] \approx [\text{rib}] \quad \text{equation (3)}
\]
\[
[\text{[rib.sub.EF]}] \approx [\text{[rib.sub]}] \quad \text{equation (4)}
\]

a) Eadie-Hofstee plot for HP-G25

![Eadie-Hofstee plot for HP-G25](image)

\[
k_{\text{obs}} \cdot [\text{Rib}]^{-1} / \text{min}^{-1}.\text{nM}^{-1}
\]

b) Eadie-Hofstee plot for TW-G25

![Eadie-Hofstee plot for TW-G25](image)

\[
k_{\text{obs}} \cdot [\text{Rib}]^{-1} / \text{min}^{-1}.\text{nM}^{-1}
\]