and thus allows investigation of the ability of an RNA-binding peptide to recognize various RNA sequences.

[1] C. Jain, J. G. Belasco, *Cell* **1996**, *87*, 115 – 125.
[2] H. Siomi, G. Dreyfuss, *Curr. Opin. Genet. Dev.* **1997**, *7*, 345 – 353.
[3] M. A. Weiss, N. Narayana, *Biopolymers* **1998**, *48*, 167 – 180.
[4] K. Harada, S. S. Martin, A. D. Frankel, *Nature* **1996**, *380*, 175 – 179.
[5] O. C. Uhlenbeck, *Nature* **1990**, *346*, 613 – 614.
[6] C. R. Woese, S. Winker, R. R. Gutell, *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8467 – 8471.
[7] R. T. Batey, R. P. Rambo, J. A. Doudna, *Angew. Chem.* **1999**, *111*, 2472 – 2491; *Angew. Chem. Int. Ed.* **1999**, *38*, 2326 – 2343.
[8] P. Legault, J. Li, J. Mogridge, L. E. Kay, J. Greenblatt, *Cell* **1998**, *93*, 289 – 299.
[9] a) H. Matsuno, K. Niikura, and Y. Okahata, *Chem. Eur. J.* **2001**, *7*, 3305 – 3312; b) Y. Okahata, K. Niikura, H. Furusawa, H. Matsuno, *Anal. Sci.* **2000**, *16*, 1113 – 1119; c) H. Matsuno, K. Niikura, Y. Okahata, *Biochemistry* **2001**, *40*, 3615 – 3622; d) A. Watanabe, H. Furusawa, Y. Okahata, *Nucleic Acids Symp. Ser.* **1999**, *42*, 193 – 194.
[10] G. Sauerbrey, *Z. Phys.* **1956**, *155*, 206 – 222.
[11] A. Watanabe, H. Furusawa, Y. Okahata, *Nucleic Acids Symp. Ser.* **1999**, *42*, 193 – 194.
[12] S. Fukusho, H. Furusawa, Y. Okahata, *Chem. Commun.* **2002**, 88 – 89.
[13] W. Xu, A. D. Ellington, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 7475 – 7480.
[14] C. Tnerk, S. MacDougal-Waugh, *Gene* **1993**, *137*, 33 – 39.
[15] L. Giver, D. Bartel, M. Zapp, A. Pawul, M. Green, A. D. Ellington, *Nucleic Acids Res.* **1993**, *21*, 5509 – 5516.
[16] D. Nieuwlandt, M. Weoker, L. Gold, *Biochemistry* **1995**, *34*, 5651 – 5659.

Received: September 19, 2002 [Z 491]

External Regulation of Hairpin Ribozyme Activity by an Oligonucleotide Effector

Stéphanie Vauléon and Sabine Müller*[a]

KEYWORDS:

enzymes · external regulation · oligonucleotides · ribozymes · RNA

The hairpin ribozyme belongs to the class of self-cleaving nucleases that are found in plant viroids, virussoids or viral satellite RNAs.[1] The 50-nucleotide-long minimal sequence (Figure 1) catalyses the reversible specific cleavage of a suitable 14-nucleotide-long RNA substrate. The secondary structure of the

[a] Dr. S. Müller, S. Vauléon
Humboldt-Universität zu Berlin
Institut für Chemie
Brook-Taylor-Strasse 2, 12489 Berlin (Germany)
Fax: (+49) 30-2093-7266
E-mail: s.mueller@chemie.hu-berlin.de

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

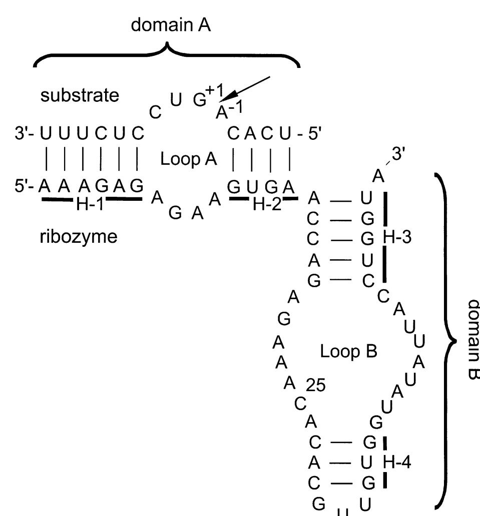

Figure 1. *Secondary structure of the hairpin ribozyme – substrate complex. The arrow denotes the cleavage site. The four Watson – Crick helices are marked (H-1 to H-4). Nucleotides discussed in the text are numbered.*

hairpin ribozyme – substrate complex is composed of two independently folded domains, A and B, each of which consists of an internal loop (loops A and B) flanked by two helices (H-1, H-2 in domain A; H-3, H-4 in domain B). The substrate is bound to the ribozyme by Watson – Crick base pairing, which generates helices 1 and 2, and cleavage takes place within loop A to produce characteristic products with a 2′,3′-cyclic phosphate group and a free 5′-OH group.

Herein we report on the external regulation of hairpin ribozyme activity by an oligonucleotide effector. RNA folding is a hierarchical process and thus formation of RNA secondary structure is an important prerequisite for tertiary folding into a functional conformation. Changes in RNA secondary structure may therefore influence the three-dimensional folding pattern and consequently the function of an RNA molecule. This fact allows for the development of activators or effectors, which assist the folding of an inactive ribozyme derivative into a catalytically competent conformation by restoring a required secondary structure. Over the past few years a number of allosteric RNA catalysts have been developed.[2] Most allosteric ribozymes described in the literature are derived from the hammerhead ribozyme, which, like the hairpin ribozyme, belongs to the group of small self-cleaving endonucleases. These hammerhead ribozymes usually contain an additional aptamer domain that can bind specific ligands, such as adenosine triphosphate,[3] flavin mononucleotide,[4] or Theophyllin.[4c, e, 5] Hammerhead ribozymes that are activated by oligonucleotide effectors have also been described. These ribozymes were developed either by rational design[6] or by in vitro selection from an RNA pool.[7] Ohtsuka and co-workers very recently introduced the first hairpin ribozyme that can be activated with short oligonucleotides.[8] In vitro selection was carried out to yield an allosteric hairpin ribozyme that showed cleavage activity only in the presence of an oligonucleotide that binds to an allosteric binding site and thus triggers a structural change of a hairpin loop to form an active conformation.

 © 2003 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

We used a different strategy to develop hairpin ribozymes to be externally regulated. Instead of introducing an additional allosteric binding site, we designed a hairpin ribozyme derivative that requires an oligonucleotide effector as a structural element for creation of the catalytic centre. Although this system is not truly allosteric, it represents a straight-forward strategy for the rational design of ribozymes that can be regulated by effector molecules. By simply adding an oligonucleotide, the catalytically competent conformation of a formerly inactive ribozyme is restored and ribozyme activity is switched on.

The active conformation of the hairpin ribozyme is based on an interdomain interaction between nucleosides in loop A and in loop B. A Watson–Crick base pair between the conserved guanosine base directly neighbouring the scissile phosphodiester bond (G + 1) and C25 in loop B (Figure 1) plays a major role in maintaining the tertiary interaction of the two domains.[9] Substitution of either nucleobase leads to loss of tertiary folding and consequently to inhibition of ribozyme activity.[10] This opens up the possibility of restoring activity of such a mutant by addition of an oligonucleotide effector that compensates for the mutated sequence. To this aim, we synthesised the hairpin ribozyme variant HP-G25, which has a point mutation in loop B (C25 → G, Figure 2). In addition, helix 3 and helix 4 in HP-G25 were destabilised by introduction of wobble base pairs as well as noncomplementary base pairs. The substrate RNA contains a fluorescence label at the 3′-end to allow us to analyse the reaction by the fluorescence assay that we developed previously.[11] Under standard conditions (10 mM tris(hydroxymethyl)aminomethane (Tris) – HCl, pH 7.5, 10 mM $MgCl_2$, 37 °C) HP-G25 did not show any activity (Figure 3, lane a). Addition of the oligonucleotide effector EF-01, however, restored activity (Figure 3, lane b). EF-01 contains the essential C25 and is fully complementary to one of the two strands in helices 3 and 4 of the ribozyme. Therefore, intermolecular interaction between effector and ribozyme to form the B domain should be preferred over intramolecular interaction in this region. For a ribozyme, the catalytically competent structure is mirrored by its activity. A ribozyme that cleaves its substrate must have undergone tertiary folding into the active conformation. Thus, the results shown in Figure 3 allow for the conclusion that EF-01 is indeed capable of restoring the catalytically competent conformation by replacing the mutated sequence in domain B. The formation of a ternary complex between EF-01, HP-G25 and hairpin (HP) substrate could also be demonstrated by gel shift analysis (See Supplement 1 in the Supporting Information).

To further evaluate the ability of EF-01 to invade domain B, we synthesised the hairpin ribozyme derivative TW-G25, which has the same point mutation (C25 → G) as HP-G25 as well as destabilised helices 3 and 4 (Figure 2). Furthermore, TW-G25 contains an additional helix (H-5), which forms a three-way junction with helices 2 and 3. This junction stabilises the structure of the B domain and compared with HP-G25 displace-

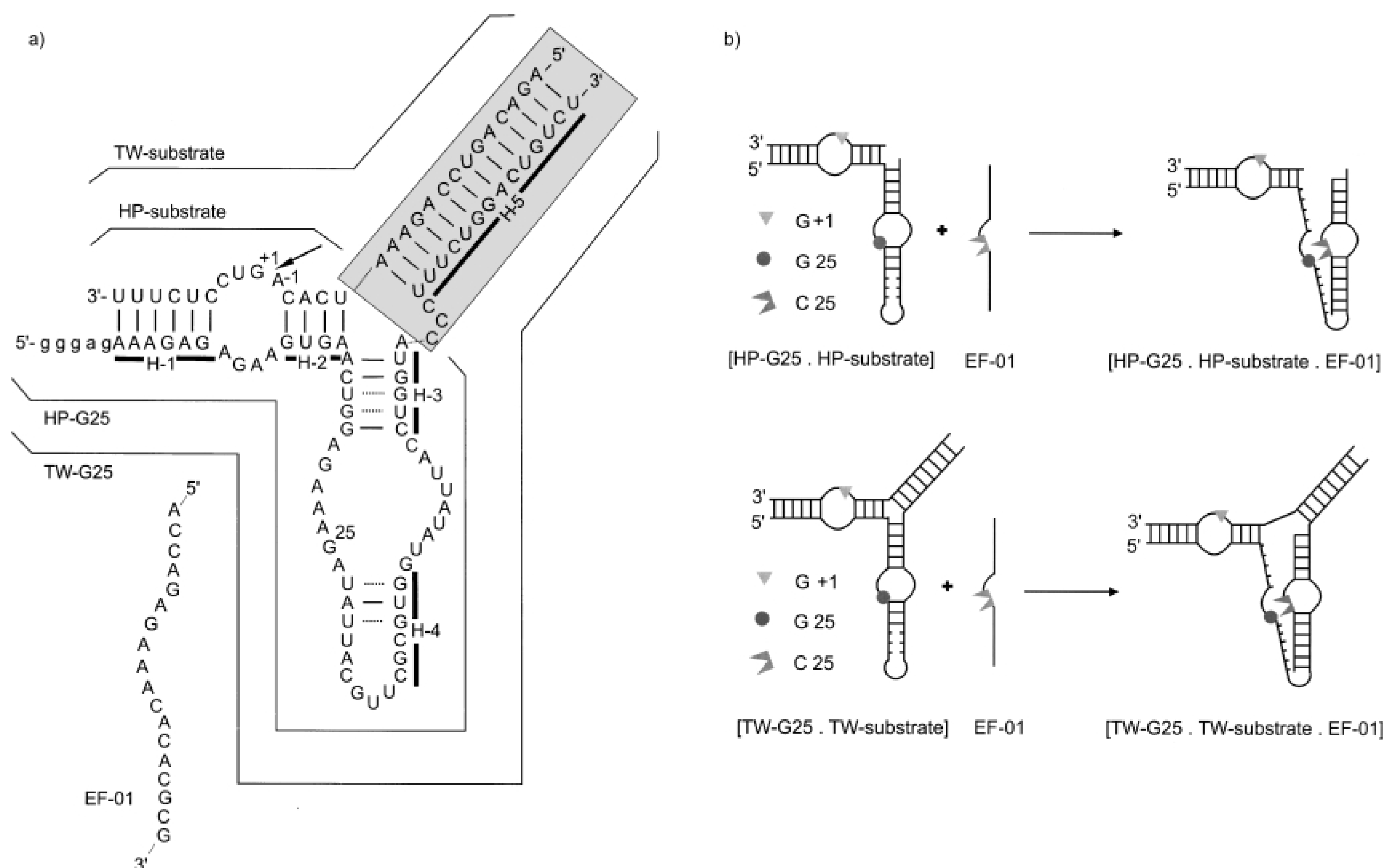

Figure 2. *a) Secondary structure of the hairpin ribozyme mutants HP-G25 and TW-G25 and the sequence of the oligonucleotide effector EF-01. The additional helix (H-5) in TW-G25 is shown in the box. b) Schematic presentation of activation by EF-01 for HP-G25 and TW-G25.*

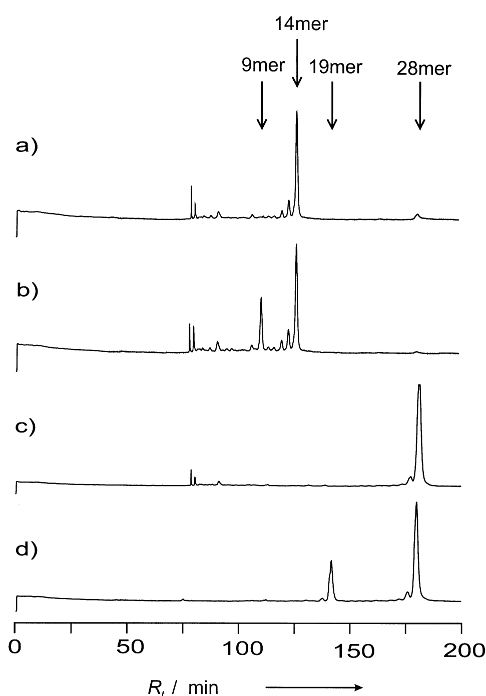

Figure 3. ALF DNA analyser primary data of cleavage reactions for HP-G25 und TW-G25 in the absence and presence of EF-01. Trace a: reaction of HP-G25 (250 nM) with HP substrate (25 nM) after 1 h; trace b: reaction of HP-G25 (250 nM) with HP substrate (25 nM) and EF-01 (250 nM) after 1 h; trace c: reaction of TW-G25 (250 nM) with TW substrate (25 nM) after 1 h; trace d: reaction of TW-G25 (250 nM) with TW substrate (25 nM) and EF-01 (250 nM) after 1 h.

ment of the mutated sequence by the oligonucleotide effector should be more difficult. We used a substrate with a fluorescence label at the 5′ end for activity tests to allow us to follow the reaction as described in ref. [13]. In the absence of the effector, no cleavage was observed for TW-G25 (Figure 3, lane c), while activity was again restored by addition of EF-01 (Figure 3, lane d). These results show that EF-01 is clearly also able to invade the three-way junction structure and as a result regenerates the catalytically competent conformation of TW-G25.

To kinetically characterise the activation process we investigated the dependence of ribozyme activation on the effector concentration. To rule out any influence of EF-01 on substrate association, fluorescein-labelled substrate RNA was prehybridised with an excess of ribozyme RNA in reaction buffer. The reaction was then started by adding the effector oligonucleotide in different concentrations (see the Experimental Section). The observed first-order rate constants were plotted against the effector concentration (Figure 4) and dissociation constants K_D for effector binding as well as maximum rate constants at effector saturation k'_1 were determined (see Table 1 and Supplement 2 in the Supporting Information). TW-G25 shows a somewhat higher affinity for the effector than HP-G25 although, within experimental error, both K_D values are in the same range. The observed first-order rate constants for HP-G25 and TW-G25 show a clear dependence on the effector concentration (Figure 4). The experiments were carried out under ribozyme saturation (see the Experimental Section) and thus the determined k'_1 values very likely mirror the rates of conforma-

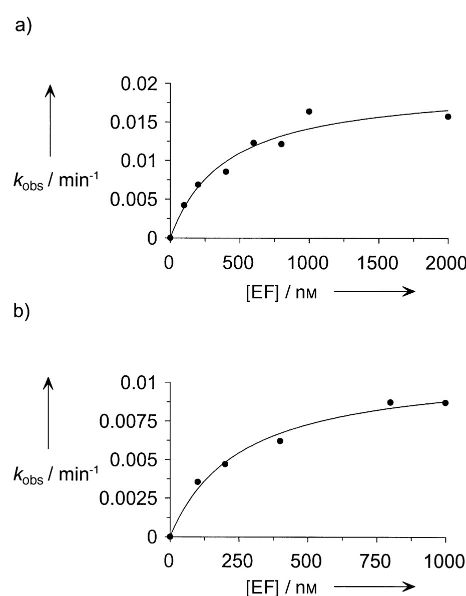

Figure 4. Dependence of the observed first-order reaction rates on the effector concentration for a) HP-G25 and b) TW-G25. Reactions were carried out under single turnover conditions. Ribozyme RNAs were prehybridised with substrate RNAs followed by addition of increasing concentrations of EF-01. Continuous lines represent the graph obtained from least square fitting of the experimental data according to Equation (1) given in Supplement 2 of the Supporting Information.

Table 1. Dissociation constants for EF-01 and cleavage rate constants at effector saturation.

Ribozyme	K_D [nM]	k'_1 [min^{-1}]
HP-G25	351 ± 71	0.019 ± 0.002
TW-G25	204 ± 39	0.010 ± 0.001

tional rearrangement of the ribozyme prior to substrate cleavage upon addition of the effector. To further substantiate this conclusion, we carried out single turnover experiments in the presence of saturating concentrations of the effector, a constant small substrate concentration and increasing ribozyme concentrations. The HP-G25/EF-01 or TW-G25/EF-01 ratio was kept constant for all measurements. A ribozyme/effector ratio of 1:8 was used for HP-G25 and of 1:5 for TW-G25, in accordance with the results shown in Figure 4. Single turnover rates were plotted as a function of ribozyme concentration in Eadie–Hofstee diagrams and the equilibrium constants K_1 as well as the cleavage rate constants k_1 were calculated (see Table 2 and Supplement 4 in the Supporting Information). The k_1 values of

Table 2. Single turnover reaction constants of ribozymes HP-WT, TW-WT and of HP-G25 and TW-G25 at effector saturation.

Ribozyme	K_1 [nM]	k_1 [min^{-1}]
HP-G25	41 ± 6	0.023 ± 0.001
TW-G25	93 ± 5	0.016 ± 0.001
HP-WT	71 ± 17	0.51 ± 0.05
TW-WT	23 ± 8	0.36 ± 0.04

 © 2003 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 1439-4227/03/04/2-3 $ 20.00+.50/0 ChemBioChem 2003, No. 2-3

0.023 min^{-1} (HP-G25) and 0.016 min^{-1} (TW-G25) found are in agreement with the k_1' values obtained from the experiments described above (0.019 min^{-1} for HP-G25; 0.01 min^{-1} for TW-G25). However, the k_1 values of both ribozymes are about twentyfold lower than the single turnover rate constants for the corresponding nonmutated ribozymes HP-WT and TW-WT (see Tables 1 and 2 and Supplement 3 in the Supporting Information). This result shows that the effector activates both ribozymes to the same extent, which corresponds well with the observed nearly equivalent affinity of both species for EF-01. As with the corresponding wild-type ribozymes, EF-01-activated HP-G25 and TW-G25 cleaved their substrate nearly to completion (end point of the reaction at 96% substrate cleavage, data not shown), although much more slowly than the wild-type ribozymes. The smaller k_1 values obtained for HP-G25 and TW-G25 compared to HP-WT and TW-WT further support the interpretation given above that the effector-induced conformational rearrangement is rate limiting in both HP-G25 and TW-G25. The obtained equilibrium constants K_1, however, are the same as for the corresponding wild-type ribozymes (Table 2), which allows for the conclusion that the process of substrate binding is not influenced by the effector oligonucleotide.

Within the hairpin ribozyme family, the constructs introduced here present the second example of externally labelled catalysts. In contrast to Komatsu et al., who recently developed allosteric hairpin ribozymes by in vitro selection,[8] we used a rational strategy for the design of hairpin ribozymes that can be regulated by an oligonucleotide effector. Even though our constructs are not allosteric in the classical sense,[7b, 12] they clearly respond to the addition of an external molecule that regulates activity.

The potential of external regulation for control of ribozyme activity is obvious. For example, Kuwabara et al. developed a hammerhead ribozyme for which a specific mRNA serves as both substrate and effector.[6b] Another interesting example was described by Wang and Sen, who designed an allosteric hammerhead ribozyme that is regulated by the HIV Tat protein and thus can be used as a diagnostic tool.[13] Recently, Vaish et al. reported the design of allosteric ribozymes to monitor post-translational modification of proteins.[14]

External regulation of ribozymes is also an elegant way to study their structural and functional properties. As we have shown in this work, an oligonucleotide effector can be used to reconstitute a functional domain within a previously functionally deficient ribozyme mutant and thus to switch activity in a defined way. The hairpin ribozyme can be inhibited by specific point mutations, which allows interruption of the catalytic cycle (folding/cleavage) at different stages. These mutants, however, can be reactivated by external effector molecules. The specific design of the effector determines the level of reactivation. This technique is of particular interest for investigation of the folding and catalytic cycle of large functional RNA molecules, for example the full-length Tobacco Ringsot Virus Satellite (−)RNA (sTRSV (−)RNA), from which the hairpin ribozyme is derived.[1] Instead of synthesising a series of distinct mutants, just one specifically designed mutant can be activated by different effectors to progress through single stages of the reaction pathway. This process allows analysis of structural and biochemical features of the sTRSV (−)RNA by methods like fluorescence resonance energy transfer or scanning force microscopy at the single-molecule level. Clearly, this technology can be extended to the study of other large RNAs containing the hairpin ribozyme motif, such as Arabis Mosaic Virus Satellite RNA or Chicory Yyellow Mottle Virus Satellite RNA.[15] Moreover, the described principle may be adapted to other catalytic RNA motifs and thus in general opens up attractive ways of carrying out RNA structure function analysis.

Experimental Section

Synthesis of ribozymes and substrates: Ribozyme RNAs HP-G25, TW-G25, HP-WT and TW-WT were transcribed from synthetic DNA templates with T7 RNA polymerase and purified by denaturing polyacrylamide gel electrophoresis.[11] Substrate RNAs and the effector oligonucleotide EF-01 were prepared by automated synthesis and purified as previously described.[14] The HP substrate was fluorescein labelled at the 3′ end by using 3′-fluorescein controlled pore glass (Glen research) as the solid phase in RNA synthesis. The TW-substrate was labelled by coupling FluorPrime fluorescein phosphoramidite (Amersham Biosciences) at the 5′ end of the oligonucleotide chain.[11]

Cleavage reaction with variation of effector concentration: Ribozyme RNAs HP-G25 and TW-G25 (final concentration: 250 nM) in reaction buffer (10 mM Tris–HCl, pH 7.5) were mixed with their substrates (final concentration: 25 nM), heated to 90 °C for 1 min and then allowed to cool down to 37 °C. After 15 min, $MgCl_2$ was added to give a concentration of 10 mM. After another 15 min had passed, individual reactions were started by addition of varying concentrations of EF-01 (0.1–2 μM). Aliquots of the reaction mixture were taken at suitable time points and immediately pipetted into StopMix (7 M urea, 50 mM ethylenediaminetetraacetate (EDTA), 0.04% Dextranblue) followed by cooling on ice. The samples were analysed with an ALF DNA sequencer and processed as described.[11] Kinetic constants K_D and k_1' were obtained from Eadie–Hofstee plots of k_{obs} against k_{obs}/[EF] (see Supplement 2 in the Supporting Information).

Single turnover kinetics: Ribozyme RNAs HP-G25 and TW-G25 (final concentration: 25–1000 nM) in reaction buffer (10 mM Tris–HCl, pH 7.5) were mixed with EF-01 (final concentration in the reaction with HP-G25: 0.2–8 μM; final concentration in the reaction with TW-G25: 0.12–5 μM), heated to 90 °C for 1 min and then allowed to cool to 37 °C. After 15 min, $MgCl_2$ was added to give a concentration of 10 mM. After another 15 min had passed, individual reactions were started by addition of the corresponding substrate RNA (25 nM). Aliquots of the reaction mixture were taken at suitable time points and immediately pipetted into StopMix (7 M urea, 50 mM EDTA, 0.04% Dextranblue) followed by cooling on ice. The samples were analysed with an ALF DNA sequencer and processed as described.[11] Kinetic constants K_1 and k_1 were obtained from Eadie–Hofstee plots of k_{obs} against k_{obs}/[Rib] (see Supplement 4 in the Supporting Information). Kinetic constants for the wild-type ribozymes HP-WT and TW-WT were obtained in the same way but in the absence of EF-01 (see Supplement 3 in the Supporting Information).

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

[1] a) A. C. Forster, R. H. Symons, *Cell* **1987**, *49*, 211 – 220; b) J. Haseloff, W. L. Gerlach, *Nature* **1988**, *334*, 585 – 591; c) P. A. Feldstein, J. M. Buzayan, G. Bruening, *Gene* **1989**, *82*, 53 – 61; d) A. Hampel, R. Tritz, *Biochemistry* **1989**, *28*, 4929 – 4933; e) M. B. DeYoung, A. M. Siwkowski, Y. Lian, A. Hampel, *J. Int. Biomed. Inf. Data* **1995**, *34*, 15785 – 15791.
[2] G. A. Soukoup, R. R. Breaker, *Curr. Opin. Struct. Biol*. **2000**, *10*, 318 – 325.
[3] a) M. Sassanfar, J. W. Szostak, *Nature* **1993**, *364*, 550 – 553; b) J. Tang, R. R. Breaker, *RNA* **1997**, *3*, 914 – 925.
[4] a) D. E. Ruffner, G. D. Stormo, O. C. Uhlenbeck, *Biochemistry* **1990**, *29*, 10659 – 10702; b) M. Araki, Y. Okuno, Y. Hara, Y. Sugiura, *Nucleic Acids Res*. **1998**, *26*, 3379 – 3384; c) G. A. Soukoup, R. R. Breaker, *Structure* **1999**, *7*, 783 – 791; d) P. Fan, A. K. Suri, R. Fiala, D. Live, D. J. Patel, *J. Mol. Biol*. **1996**, *258*, 480 – 500; e) M. P. Robertson, M. D. Ellington, *Nucleic Acids Res*. **2000**, *28*, 1751 – 1759.
[5] a) R. D. Jennison, S. C. Gill, A. Pardi, B. Polisky, *Science* **1994**, *263*, 1425 – 1429; b) G. R. Zimmermann, R. D. Jennison, C. L. Wick, J. P. Simorre, A. Pardi, *Nat. Struct. Biol.* **1997**, *4*, 644 – 649.
[6] a) H. Porta, P. M. Lizardi, *Biochemistry* **1995**, *13*, 161 – 164; b) T. Kuwabara, M. Warashina, T. Tanabe, K. Tani, S. Asano, K. Taira, *Cell*, **1998**, *2*, 617 – 627; c) Y. Komatsu, S. Yamashita, N. Kazama, K. Nobuoka, E. Ohtsuka, *J. Mol. Biol*. **2000**, *299*, 1231 – 1243; d) D. Y. Wang, B. H. Y. Lai, D. Sen, *J. Mol. Biol*. **2002**, *318*, 33 – 43; e) D. Y. Wang, B. H. Y. Lai, A. R. Feldman, D. Sen, *Nucleic Acids Res.* **2002**, *30*, 1735 – 1742.
[7] a) M. P. Robertson, A. D. Ellington, *Nat. Biotechnol.* **1999**, *17*, 62 – 66; b) G. A. Soukoup, R. R. Breaker, *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3584 – 3589; c) G. A. Soukoup, G. A. M. Emilsson, R. R. Breaker, *J. Mol. Biol*. **2000**, *298*, 623 – 632; d) M. Koizumi, G. A. Soukoup, J. N. Q. Kerr, R. R. Breaker, *Nat. Struct. Biol.* **1999**, *6*, 1062 – 1071.
[8] Y. Komatsu, K. Nobuoka, N. Karino-Abe, A. Matsuda, E. Ohtsuka, *Biochemistry* **2002**, *41*, 9090 – 9098.
[9] P. B. Rupert, A. R. Ferré-D'Amaré, *Nature* **2001**, *410*, 780 – 786.
[10] R. Pinard, D. Lambert, N. Walter, J. E. Heckmann, F. Major, J. M. Burke, *Biochemistry* **1999**, *38*, 16035 – 16039.
[11] C. Schmidt, R. Welz, S. Müller, *Nucleic Acids Res*. **2000**, *28*, 886 – 894.
[12] a) J. Monod, F. Jacob, *Cold Spring Harbor Symp. Quant. Biol.* **1961**, *26*, 389 – 401; b) J. Monod, J.-P. Changeux, F. Jacob, *J. Mol. Biol.* **1963**, *6*, 306 – 329; c) M. Perutz, *Mechanism of Cooperativity and Allosteric regulation in Proteins*, Cambridge University Press, New York, **1994**.
[13] D. Y. Wang, D. Sen, *Comb. Chem. High Throughput Screening* **2002**, *4*, 301 – 312.
[14] R. Welz, S. Müller, *Tetrahedron Lett*. **2002**, *43*, 795 – 797.
[15] M. B. DeYoung, A. M. Siwkowski, Y. Lian, A. Hampel, *Biochemistry* **1995**, *34*, 15785 – 15791.

Received: October 10, 2002 [Z 503]

Synthesis of Thioether-Linked Analogues of the 2,3-Sialyl-TF and MECA-79 Antigens: Mucin-Type Glycopeptides Associated with Cancer and Inflammation

Lisa A. Marcaurelle,[b] Matthew R. Pratt,[b] and Carolyn R. Bertozzi*[a]

KEYWORDS:

antigens · carbohydrates · chemoselective ligation · glycopeptides · sulfation

Interest in the synthesis of sulfated and sialylated oligosaccharides is motivated in part by their unique expression in tissues of certain disease states, including chronic inflammation and many types of cancer. In nature, sulfated and sialylated carbohydrates are abundantly expressed on mucins, such as GlyCAM-1, MAdCAM-1, CD34, and MUC-1.[1] To date, the synthesis of an *O*-linked glycopeptide containing a sulfated oligosaccharide has not been reported. This is most likely due to the acid-sensitive nature of carbohydrate sulfate esters[2] and a lack of suitable methods for their protection during peptide synthesis.[3] While syntheses of glycopeptides containing sialic acid have been described, the preparation of these compounds is still a difficult task.[4] Here we present an efficient method for the convergent assembly of *O*-linked glycopeptides bearing sulfated and sialylated oligosaccharides in a chemoselective fashion.

Previously, we reported the use of glycosyl amino acid **1** (Scheme 1) for the synthesis of glycopeptide analogues by thiol alkylation.[5] Building block **1** contains an unnatural 3-thio-GalNAc residue that can undergo condensation with *N*-bromoacetamido sugars, following its incorporation into a peptide by Fmoc-based solid-phase peptide synthesis (SPPS). The thiol

[a] *Prof. Dr. C. R. Bertozzi*[+]
Center for New Directions in Organic Synthesis
Departments of Chemistry and Molecular and Cell Biology
Howard Hughes Medical Institute, University of California
and
Materials Sciences Division
Laurence Berkeley National Laboratory
Berkeley, CA 94720 (USA)

[b] *Dr. L. A. Marcaurelle, M. R. Pratt*
Department of Chemistry
University of California
Berkeley, CA 94720 (USA)

[+] *Current address:*
Department of Chemistry
University of California
Berkeley, CA 94720 (USA)
Fax: (+1) 510-643-2628
E-mail: bertozzi@cchem.berkeley.edu

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

 © 2003 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim