remain intact during the steaming and acid-leaching steps. Furthermore, the pore-size distribution from the nitrogen-desorption isotherm of all three samples shows an additional peak at a pore diameter of 70 nm. That this peak, which is caused by the interstitial space between the crystals, is at the same position for all three samples indicates that the crystals of these samples are of the same order of magnitude. This is different from the results obtained by Beyerlein et al.,[7a–c] who claimed extensive fracturing of zeolite Y crystals.

Thus, the pores in a series of dealuminated zeolite Y have been imaged in order to gain insight in the shape and three-dimensional ordering of the mesopores in the zeolite crystallites. Based on the surprising result, that most of the mesopores are present as cavities rather than cylindrical pores connecting the external surface with the interior of the crystallite, a more detailed model for the generation of these mesopores is proposed. However, the shape of the mesopores also raises the question to what extent the accessibility and diffusion are enhanced by the formation of these cavities.

Experimental Section

Samples CBV100 (NaY), CBV400 (USY), and CBV780 (XVUSY) were obtained from Shell International Chemicals and Zeolyst. Nitrogen adsorption and desorption measurements (Micromeritics ASAP 2010) were performed at liquid nitrogen temperature. XPS measurements (Vacuum Generators XPS) were performed on a system using nonmonochromatic $Al_{K\alpha}$ radiation at an anode current of 20 mA at 10 keV. For electron microscopy, a droplet of a colloidal gold suspension (Sigma, 5 nm gold) was dried on a carbon-coated copper grid, thus providing markers for the alignment of the data set. Next, a droplet of a suspension of the sample in ethanol was dried on this grid. From a representative crystal, a tilt series of about 141 images was taken from about $+70^\circ$ to -70° at 1° intervals of magnification $\times 15$ k or $\times 20$ k on one of two microscopes (Philips CM 200 FEG or a Tecnai 20) at 200 kV and with software for automated electron tomography.[12] From the tilt series, a 3D-reconstruction of the crystal is calculated as a stack of thin (1–2 nm) slices.[9–12]

Received: October 20, 2000 [Z15971]

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Fluorescence Detection of Specific RNA Sequences Using 2′-Pyrene-Modified Oligoribonucleotides**

Kazushige Yamana,* Hirofumi Zako, Kentarou Asazuma, Reiko Iwase, Hidehiko Nakano, and Akira Murakami

Fluorescence detection of specific RNA sequences is an area of much current interest and activity. It is important for the investigation of the regulatory mechanisms of gene expression[1, 2] and diagnostic analysis of infectious organisms from viruses or bacteria.[3, 4] Recent works have focused on the design and synthesis of fluorescent oligonucleotides that exhibit an enhanced signal upon hybrid formation,[5–14] since these type of probes can be used in homogeneous, as well as heterogeneous, assays. The enhanced fluorescence has facilitated monitoring in vitro transcription,[15] ribozyme reaction,[16, 17] and RNA folding.[18]

Our research efforts have concentrated on the use of the sugar 2′-position as the site in the covalent attachment of several fluorophores.[19–22] In particular, the incorporation of pyrene, via a one carbon-atom linker, into the specified position of oligodeoxyribonucleotides has provided a probe that exhibits strong fluorescence upon binding to RNA.[21, 22] While the potential of these probes is appreciated, it has remained unfulfilled because the pyrene–DNA complexes are plagued by the sequence limitation of the probe or the positioning of C or G bases at the 3′-site of the pyrene modification to gain a measurably strong fluorescence.[22] We now disclose a new pyrene probe derived from RNA that overcomes this limitation. The pyrene fluorescence is signifi-

[*] Assoc. Prof. K. Yamana, H. Zako, K. Asazuma, Prof. H. Nakano
Department of Applied Chemistry
Himeji Institute of Technology
2167 Shosha, Himeji, Hyogo 671-2201 (Japan)
Fax: (+81) 792-67-4895
E-mail: yamana@chem.eng.himeji-tech.ac.jp
Dr. R. Iwase, Prof. A. Murakami
Department of Polymer Science and Engineering
Kyoto Institute of Technology
Matsugasaki, Sakyo-ku, Kyoto 606-8585 (Japan)

[**] We are very grateful to Professor Hiroshi Sugiyama, Dr. Tetsuji Yamaoka, and Dr. Takashi Morii for ion-spray mass spectrometric measurements and helpful comments on this research.

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

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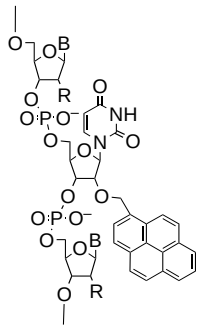
cantly enhanced upon hybridization to RNA, which is not dependent on the choice of probe sequence, but very sensitive to a mismatched base present in the target RNA. This is the first example of RNA base-mismatch recognition by pyrene fluorescence.[23]

We prepared RNA oligonucleotides possessing a pyrenylmethyl group at several different sugar residues and pyrene-modified DNA oligomers of the same sequences for comparison (Scheme 1). Binding properties for both the pyrene-



R = - OH
1 : 5′-rACAU(pyr)GCAGUGUUGAU
2 : 5′-rACAUGCAGU(pyr)GUUGAU
3 : 5′-rACAUGCAGUGU(pyr)UGAU
4 : 5′-rACAU(pyr)ACAGUGUUGAU
5 : 5′-rACAU(pyr)UCAGUGUUGAU
6 : 5′-rACAU(pyr)CCAGUGUUGAU

R = - H
7: 5′-dACAU(pyr)GCAGTGTTGAT
8 : 5′-dACATGCAGU(pyr)GTTGAT
9 : 5′-dACATGCAGTGU(pyr)TGAT

U(pyr) = 2′-(1-pyrenylmethyl)uridine

Scheme 1. RNA oligonucleotides with a pyrenylmethyl group located on various sugar residues.

modified RNA and DNA are shown in Table 1. The melting temperatures T_m of all the pyrene-modified duplexes differed slightly, by 0 to 5 °C, from those of the corresponding unmodified duplexes.

Table 1. Melting temperatures (260 nm) and fluorescence quantum yields of pyrene-modified oligonucleotide duplexes.

Oligomer	T_m[a] [°C]		Fluorescent Φ		
	duplex with DNA	duplex with RNA	single-stranded oligomer	duplex with DNA	duplex with RNA
RNA 1[b]	47.1	56.5			
1	50.0	53.3	0.005	0.007	0.098
2	48.6	52.9	0.007	0.004	0.113
3	46.9	52.4	0.006	0.004	0.119
RNA 2[c]		53.9			
4		50.6	0.008		0.102
RNA 3[d]		52.1			
5		49.5	0.007		0.162
RNA 4[e]		58.9			
6		54.0	0.007		0.237
DNA 1[f]	53.9	49.1			
7	53.6	46.9	0.006	0.005	0.006
8	50.5	45.3	0.004	0.006	0.007
9	48.2	44.2	0.005	0.007	0.006

[a] Monitored at 260 nm. [b] RNA 1 = 5′-rACAUGCAGUGUUGAU. [c] RNA 2 = 5′-rACAUACAGUGUUGAU. [d] RNA 3 = 5′-rACAUUCAGUGUUGAU. [e] RNA 4 = 5′-rACAUCCAGUGUUGAU. [f] DNA 1 = 5′-dACATGCAGTGTTGAT.

Table 1 summarizes the fluorescent quantum yields Φ of the pyrene-modified RNA and DNA before and after hybridization. The fluorescence spectra for RNA **2** and DNA **8** are shown as examples in Figure 1. The single-stranded probes exhibit typical pyrene-monomer emission, whose quantum efficiencies were very low. Upon binding with RNA, the fluorescence of the pyrene–RNA complex was significantly

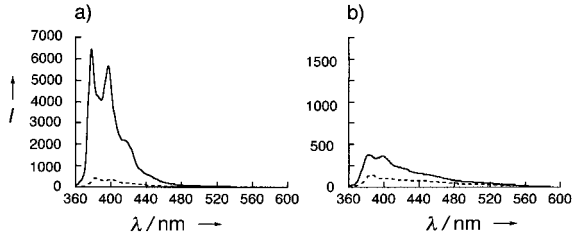


Figure 1. Fluorescence spectra of a) pyrene-modified RNA **2** (----) and its duplex with RNA (——) and b) pyrene-modified DNA **8** (----) and its duplexes with RNA (——).

enhanced, whereas the fluorescence enhancement of the pyrene–DNA complex was not high. All the pyrene-modified RNA/RNA duplexes displayed strong emissions, whose Φ values ranged from 0.1 to 0.23. These values are comparable to those for 1-pyrenebutyric acid ($\Phi = 0.30$) and 1-pyrenylmethanol ($\Phi = 0.25$) in an air-saturated aqueous solution. The extent of the fluorescence enhancement upon hybridization was not highly dependent on the probe sequence around the pyrene modification. This is in contrast to previous observations for pyrene-modified DNA.[21] Upon binding to DNA, the fluorescence of both the pyrene–RNA and –DNA complexes were not largely altered.

It is well documented that the nucleobases serve as an efficient quencher for pyrene fluorescence.[24–28] Fluorescence quenching of pyrene occurs in both noncovalent and covalent complexes of nucleosides and single-stranded oligonucleotides.[29–31] In a similar manner, introduction of the pyrene into the 2′-position of oligonucleotides resulted in strong fluorescence quenching. The quenched emission recovers in pyrene-modified RNA/RNA duplexes to a level that is as strong as the free pyrene compounds in an aqueous solution. The pyrene located outside of the RNA/RNA duplex is likely to be responsible for the strong emission, which was supported by the fluorescence excitation and CD studies.[22]

The fluorescence and melting data for hybrids of the pyrene-modified RNA with RNA possessing a single base

Table 2. Relative fluorescence intensity and T_m values for the duplexes of pyrene-modified RNA oligonucleotide **1** or **6** with RNA containing a single base mismatch.

pyrene-modified RNA	RNA[a]	Relative fluorescence intensity[b]	T_m [°C]
1	5′-rAUCAACACUGCAUGU	16	53.3
	5′-rAUCAACACUGC**G**UGU	1	45.2
	5′-rAUCAACACUGC**C**UGU	1	46.8
	5′-rAUCAACACUGC**U**UGU	1	44.7
	5′-rAUCAACACUG**G**AUGU	1	46.7
	5′-rAUCAACACUG**A**AUGU	1	44.7
	5′-rAUCAACACUG**U**AUGU	4	50.5
6	5′-rAUCAACACUGGAUGU	59	54.0
	5′-rAUCAACACU**U**GAUGU	2	44.1
	5′-rAUCAACAC**G**GGAUGU	52	43.3
	5′-rAUCAAC**G**CUGGAUGU	56	48.3
	5′-rAUCA**C**CACUGGAUGU	54	44.5
	5′-rAUC**U**ACACUGGAUGU	56	44.1

[a] The mismatch bases in RNA sequences are indicated by the bold font. [b] Relative fluorescence intensities at 380 nm were obtained at 23 °C based on the single-stranded pyrene-modified oligonucleotide **1** or **6**.

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mismatch are shown in Table 2. Little or no fluorescence changes were observed in binding of the pyrene probe to RNA possessing a mismatch facing the modified nucleoside. Similarly, the mismatch at the 5′-site near the pyrene modification in the target RNA affected the duplex fluorescence. In contrast, an RNA mismatch at the site separated more than three bases from the pyrene did not show significant effect on the duplex fluorescence. These observations strongly suggest that the base pairings at the neighboring positions of the modification are important for rearrangements of the local structural elements to yield a strongly emitting pyrene moiety in the duplex.

The structural basis for the different fluorescence properties between pyrene-modified RNA/RNA and pyrene-modifed DNA/DNA duplexes requires further investigation. With high quantum efficiency, sequence specificity, and binding affinity, RNA oligonucleotides labeled by a pyrenylmethyl group at the 2′-sugar residue provide a useful tool for monitoring RNA hybridization. The present labeling method should be applicable to investigation of RNA structures.[32]

Experimental Section

Synthesis of Pyrene-Modified RNA. Pyrene-modified oligoribonucleotides were synthesized by standard phosphoramidite chemistry using 5′-DMT-2′-(1-pyrenylmethyl)uridine phosphoramidite (DMT = 4,4′-dimethoxytrityl).[22] After removal of base and phosphate protective groups, the oligomers were purified with 20% denaturing polyacrylamide gel electrophoresis. The final deprotection of the 2′-blocking group (Fpmp = [1-(2-Fluorophenyl)-4-methoxypiperidinyl]) was done with HCl (0.1M) treatment. After neutralization, the solution was passed through a Sep-Pak C18 cartridge giving the desired oligonucleotides (8–10 O.D.; absorbance at 260 nm). The integrity of the purified oligonucleotides was verified with ion-spray mass-spectral analysis.

Physical Measurements. All measurements were carried out in Na_3PO_4 (10 mM) and NaCl (100 mM), adjusted to pH 7.0. Duplex melting curves were obtained at a common total strand concentration (4.3×10^{-5} M) containing a 1:1 molar ratio of oligonucleotides. Fluorescence spectra were obtained at an excitation wavelength of 338 nm. Fluorescence quantum yields were estimated at 23 °C on the basis of quinine sulfate in sulfuric acid (1.0 N) as a standard.

Received: September 1, 2000 [Z15741]

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Versatile Approaches to the Polymer-Supported Synthesis of Bidentate Phosphorus-Containing Ligands

George Y. Li,* Paul J. Fagan, and Patricia L. Watson

In recent years, combinatorial chemistry has been developed and applied by the pharmaceutical industry to rapidly synthesize and screen thousands of compounds in libraries for drug discovery and optimization.[1, 2] Combinatorial approaches have also led to the discovery of more efficient superconducting,[3] photoluminescent,[4] magnetoresistive,[5] dielectric,[6] and polymeric[7] materials, and the development of heterogeneous catalysts.[8] Moreover, promising results in the application of combinatorial methods to homogeneous

[*] Dr. G. Y. Li, P. J. Fagan, P. L. Watson
DuPont Company
Central Research & Development Department
Experimental Station
P.O. Box 80328, Wilmington, DE 19880-0328 (USA)
Fax: (+1) 302-695-3786
E-mail: george.y.li@usa.dupont.com

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