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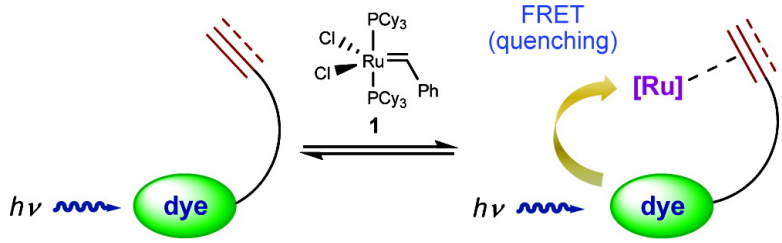

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Initial Catalyst−Substrate Association Step in Enyne Metathesis Catalyzed by Grubbs Ruthenium Complex Probed by Time-Dependent Fluorescence Quenching

Jeong-Hun Sohn,[†,*] Kyung Hwan Kim,[‡,§] Hee-Yoon Lee,[§] Zae Sung No,[†] and Hyotcherl Ihee[‡,§,*]

Institut Pasteur Korea, Seoul, Korea, Center for Time-Resolved Diffraction, Department of Chemistry (BK21), KAIST, Daejeon, Korea

Received September 30, 2008; E-mail: sohnjh@ip-korea.org; hyotcherl.ihee@kaist.ac.kr

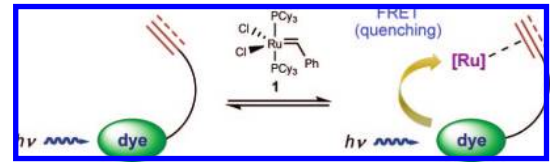
Herein we introduce a simple and efficient method for monitoring kinetics and thermodynamics of organic reactions based on fluorescence resonance energy transfer (FRET). We report its application to studies on the initial catalyst−substrate association step in the enyne metathesis catalyzed by a Grubbs ruthenium (Ru) complex to probe the reaction initiation on the alkyne versus the alkene.

The enyne metathesis catalyzed by the Grubbs Ru complex has attracted much attention from the synthetic chemistry community because of its ability to combine alkenes and alkynes to form 1,3-dienes in a single step under mild reaction conditions.[1,2] Two mechanistic interpretations of this reaction have been proposed, but the details of these proposals need experimental verification. In particular, the initial event of the reaction in ring-closing enyne metathesis has been a controversy.[3] Earlier work with group VI (Cr, Mo, W) metal-based catalysts commonly postulated the reaction initiation on an alkyne,[4] and presumably these results strengthen the interpretation of the reaction proceeding via this mechanism.[3d] On the other hand, the initiation on alkene was proposed by the studies using NMR,[3b,c] isotopic labeling,[3f] and IR[3g] and based on the plausible intermediates or regioselective product formation.[3a,e] Much of this controversy stems from the formation of the identical product from two equally plausible reaction pathways in the enyne metathesis reaction and the fact that none of the reports observed the very first event of the reaction.

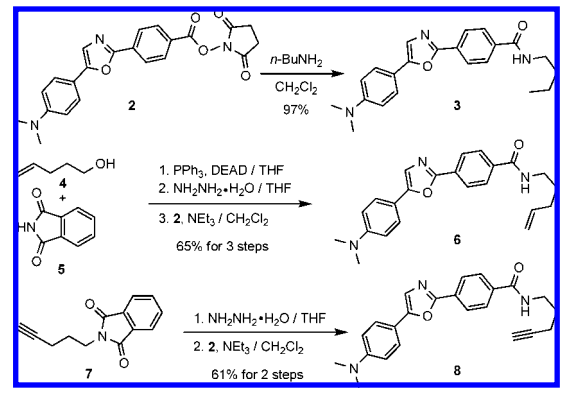
In transition metal catalyzed tandem reactions with multifunctionalities or multicomponent reactions such as the enyne metathesis catalyzed by the Grubbs Ru complex, determining the kinetic and thermodynamic parameters of the binding of each functionality to the catalyst is critical in characterizing the initial catalyst−substrate association step which may govern the overall reaction pathway. Toward this goal, we designed an FRET-based direct detection of the interaction between the Grubbs first generation Ru catalyst and a dye-sensitized alkene or alkyne and determined simultaneously both kinetic and thermodynamic parameters.

FRET is a distance-dependent interaction between the electronic excited states of the fluorescence donor and acceptor and is detected by the appearance of sensitized fluorescence from the acceptor or by quenching of donor fluorescence.[5] Since the Ru catalyst (**1**) has a broad absorbance at 527 nm with no fluorescence, we expected that this catalyst could act as a quencher of a fluorophore, of which the fluorescence emission band overlaps with the absorbance band of the catalyst.[6] By measuring and analyzing the time-dependent fluorescence quenching of a dye-conjugated alkene and alkyne after mixing each substrate with the catalyst under various reaction conditions, we determined k, k_{-1}, E_a, and ΔG of the binding of a substrate alkene or alkyne with the catalyst (Scheme 1).

Scheme 1. Schematic Illustration of Probing the Initial Catalyst−Substrate Association Step in the Enyne Metathesis Catalyzed by Grubbs Ru Complex Using FRET (Quenching)



Scheme 2. Synthesis of Dye-Conjugated Alkene, Alkyne, and Alkane



As an appropriate dye molecule, we chose Dapoxyl dye[5a] having a fluorescence emission band near the absorbance band of the Ru catalyst. A terminal alkene or alkyne was connected to the dye moiety through an amide bond with an identical three-carbon tether length (Scheme 2). As a control compound, dye-ane **3** was prepared by amide bond formation between activated dye **2** and n-$BuNH_2$. Dye-ene **6** was synthesized from alcohol **4** in 3 steps including a Mitsunobu reaction and deprotection of the amino group followed by amide bond formation. The other substrate, dye-yne **8**, was prepared from a commercially available compound **7**, via amino group deprotection followed by amide coupling. These three dye-conjugated compounds show identical fluorescence emission bands at 508 nm in CH_2Cl_2, which overlap with the broad absorbance band of the Ru catalyst.[6]

Time-dependent FRET data were obtained for dye-ene **6** and dye-yne **8** at various initial conditions.[6,7] For example, photoluminescence (PL) was measured as a function of time just after mixing the substrate at 18 μM with 1 equiv of the Ru catalyst in CH_2Cl_2 at 20 °C. Control experiments with the dye-ane **3** were also carried out under identical conditions to calibrate for quenching via nonspecific binding. Figure 1 displays the results. Apparently, the fluorescence quenching over time for alkene **6** is substantially faster than that for alkyne **8**. In the case of a higher concentration of the substrate (26.7 μM) and the catalyst (1 equiv), the quenching for both substrates was faster than those in the case of 18 μM. To quantitatively analyze the kinetic traces

[†] Institut Pasteur Korea.
[‡] Center for Time-Resolved Diffraction.
[§] Department of Chemistry (BK21), KAIST.

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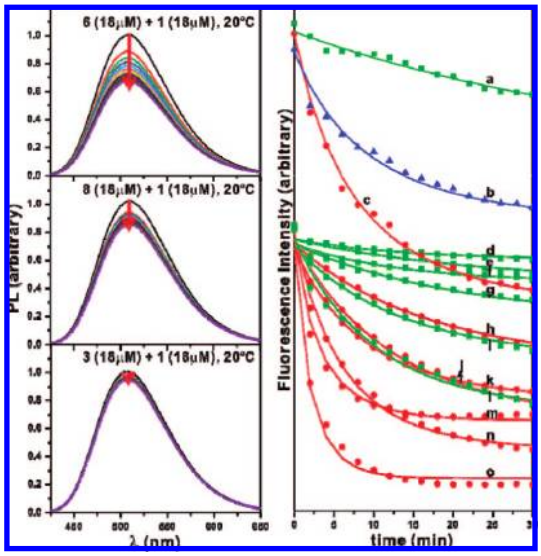

Figure 1. Quenching of the dye-conjugated alkene and alkyne by Grubbs' first generation Ru catalyst over time. (Left) PL spectra as a function of time for substrates alkene **6** (top), alkyne **8** (middle), and alkane **3** (bottom). (Right) Symbols are experimental time-dependent fluorescence traces at 15 different conditions (a−o)[6] and the curves are theoretical results from fitting 15 kinetic traces simultaneously. Red circles are from experiments with **6**, green squares with **8**, and blue triangles with a mixture of **6** and **8**.

Table 1. Kinetic and Thermodynamic Parameters for Alkene **6** and Alkyne **8** with Ru Catalyst **1**

substrate	T (°C)	k (M^{-1} s^{-1})	k_{-1} (s^{-1})	ΔG (kJ/mol)	E_a (kJ/mol)
alkene **6**	10	1.48 (±0.07) × 10^3	1.61 (±0.33) × 10^{-2}		
	20	3.71 (±0.13) × 10^3	2.12 (±0.23) × 10^{-2}	−29.4 × 0.2	64.2 × 1.9
	34	1.26 (±0.05) × 10^4	3.81 (±0.30) × 10^{-2}		
alkyne **8**	10	1.42 (±0.38) × 10^2	4.54 (±3.17) × 10^{-3}		
	20	2.60 (±0.41) × 10^2	9.81 (±8.15) × 10^{-3}	−24.8 × 0.4	84.7 × 6.1
	34	1.85 (±0.09) × 10^3	2.77 (±0.41) × 10^{-2}		

with a set of parameters and to determine both kinetic and thermodynamic parameters, we first increased the data content by using various initial conditions. For example, we collected more data with 2 equiv of the Ru catalyst at various temperatures, and in some cases both **6** and **8** were mixed together as an example of competing reaction conditions. In total, kinetic traces at 15 different conditions were measured as shown in Figure 1.

The 15 quenching traces of various reaction conditions were simultaneously fitted against a set of rate equations with kinetic parameters such as rate coefficients (k and k_{-1}).[6] In the fit, the differences between the theoretical and the experimental fluorescence decay curves were minimized, yielding optimal values of the rate constants for both substrates as listed in Table 1. The activation energy (E_a) for substrate binding was determined by using Arrhenius' equation, $k = A \exp(-E_a/RT)$. For dye−alkene **6** and dye−alkyne **8**, linear fits of $\ln k$ vs $-1/RT$ gave E_a values of 64.2 ± 1.9 and 84.7 ± 6.1 kJ/mol, respectively. The binding rate of dye−alkene **6** to the Ru complex is ~10-fold faster than that of dye−alkyne **8**. The change of Gibbs free energy was obtained using the equation $\Delta G = -RT \ln K$ and the plot $1/T$ vs $\ln K$ ($K = k/k_{-1}$). The Gibbs free energy changes determined for the alkene and the alkyne were −29.4 ± 0.2 and −24.8 ± 0.4 kJ/mol, respectively, meaning that the binding reaction of the alkene with the catalyst is thermodynamically 7-fold more favorable than that of the alkyne.

The kinetic and thermodynamic parameters determined in these measurements for the initial catalyst−substrate association step in the reaction strongly support the dominance of the reaction initiation on alkenes both kinetically and thermodynamically. It should be pointed out that scenarios where a particular reaction pathway leading to the final product is both kinetically and thermodynamically disfavored are rare.

In summary, we have introduced an FRET-based simple and efficient monitoring method applicable to probing the initial catalyst−substrate association step in the enyne metathesis catalyzed by Grubbs' Ru complex. Using this method, we directly determined both kinetic and thermodynamic parameters for the binding of an alkene and alkyne to Grubbs' first generation Ru catalyst, which strongly support the dominance of the reaction initiation on an alkene over alkyne in the enyne metathesis.

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Supporting Information Available: Experimental details, ^{1}H and ^{13}C NMR spectra of all compounds synthesized, and all FRET data at various reaction conditions such as the initial concentrations and temperatures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) See the Supporting Information.
(7) Monitoring of the initial association step for the second generation catalyst requires a better time resolution than presented in this work due to the fast reaction and intrinsic instability of the catalyst.

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