Rapid Fluorescence Determination of the Absolute Amount of Aldehyde and Ketone Groups on Resin Supports

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Received July 10, 1997

Synthetic combinatorial libraries[1] are becoming an indispensable tool for drug discovery in the pharmaceutical industry. Up to now, most of the small molecule combinatorial libraries have been constructed by solid-phase organic synthesis (SPOS) because SPOS has advantages in ease of purification and automation and the ability to drive reaction to completion. The major disadvantage compared with solution-phase methods is that a time-consuming reaction development period is generally required. This reaction development phase is often hindered by the lack of analytical methods, particularly quantitative methods, to assess the on-resin reaction yield in order to optimize successive synthetic steps. Researchers involved in solid-phase peptide synthesis have reported limited methods for quantitation of peptide related functional groups,[2] such as amine group. However, for various organic functional groups, quantitation can only be obtained by "cleave-and-weigh" method. The isolated yield, although indicative, is not the same as the on-resin yield due to complications from the cleavage reaction. In reality, it is difficult to determine the reaction yield for every step in a multistep synthesis by "cleave and weigh" method.

Here we present the proof of a general principle for direct quantitation of the absolute amount of an organic functional group in resin-bound starting materials, intermediates, and products. The method employs the sensitive fluorescence spectroscopy and the availability of a large number of fluorescent dyes, which can be attached to various organic functional groups, to quantify organic reactions directly on resin. As the first application of this principle, we report a novel fluorescence method for rapid determination of the absolute amount of aldehyde or/and ketone groups on polystyrene and TentaGel resins (Scheme 1).

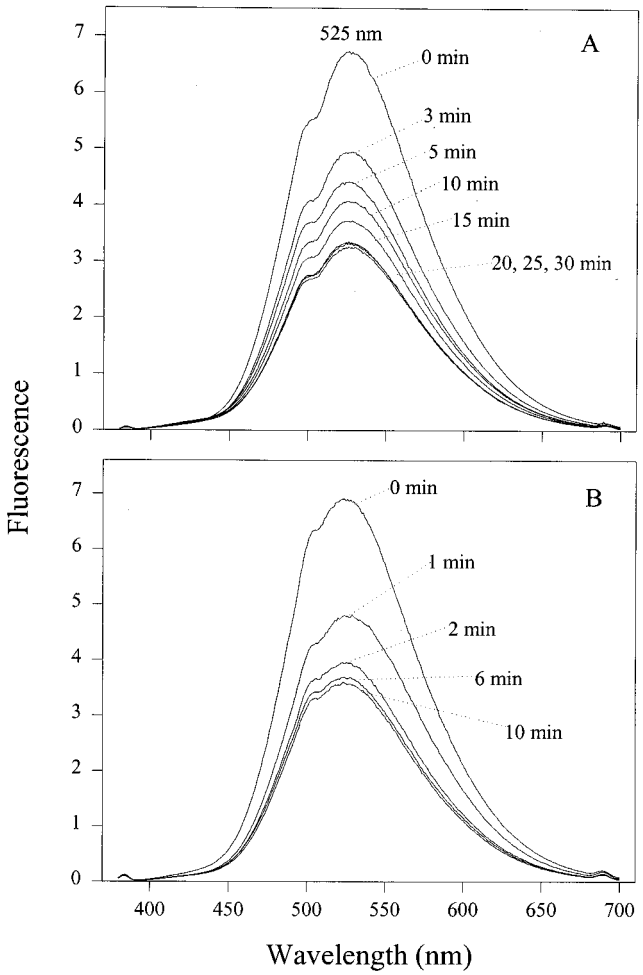
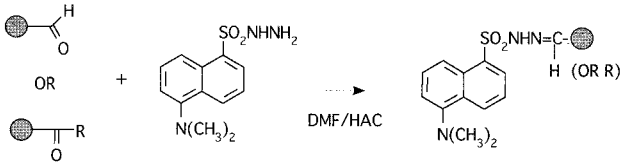


Figure 1. Fluorescence emission spectra of dansylhydrazine in the supernatant of the reaction mixture at various times for (A) formylpolystyrene resin and (B) TentaGel S-CHO resin.

Scheme 1



To a suspension of formylpolystyrene or TentaGel S-CHO resin in DMF was added an approximately 2-fold excess of dansylhydrazine. The fluorescence spectra shown in Figure 1A and Figure 1B show the decrease of dansylhydrazine concentration in the supernatant for reactions with formylpolystyrene and TentaGel S-CHO resins. After the reaction is completed, the comsumed amount of dansylhydrazine is equal to the absolute amount of dye molecules covalently and noncovalently bound to the resin beads. The amount of dansylhydrazine molecules noncovalently trapped in the resin matrix was corrected by subtracting the amount of dye molecules trapped in the same amount of 1% divinylbenzene−polystyrene or TentaGel S-Br resin under identical conditions. For TentaGel resins, the amount of nonco-

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S0022-3263(97)01251-6 CCC: $14.00

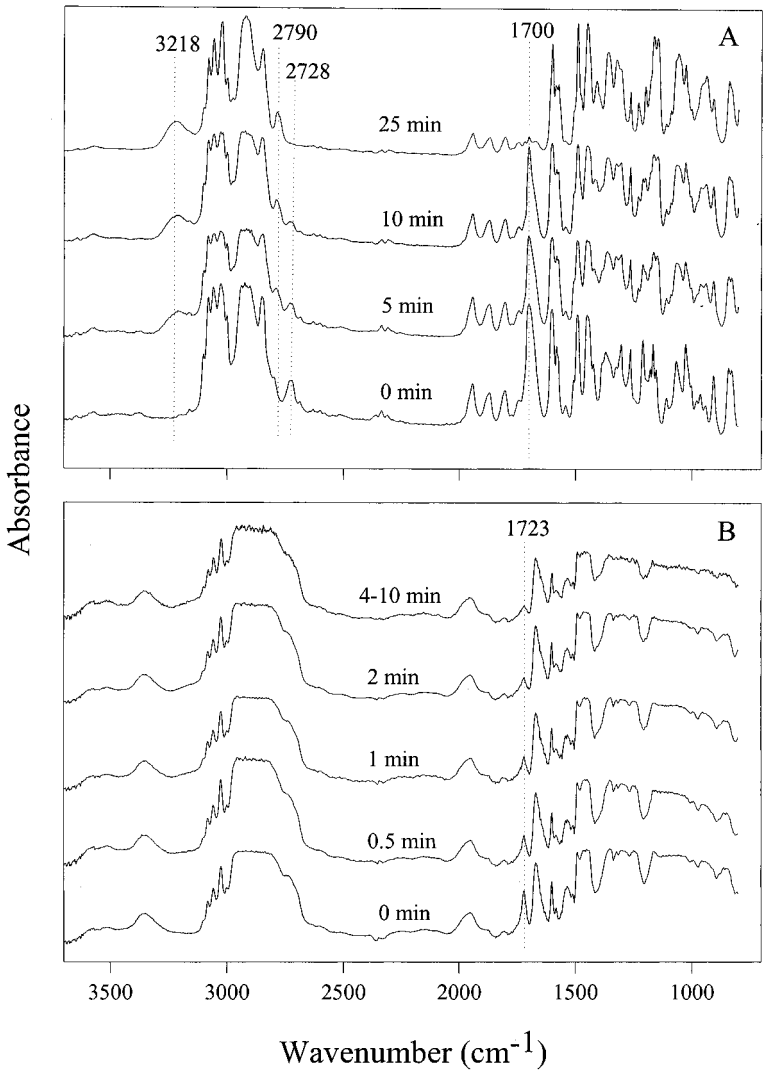

Figure 2. IR spectra of the single bead taken out of the reaction mixture at various times during the reaction for (A) formylpolystyrene resin and (B) TentaGel S-CHO resin.

valently trapped dye is negligible on the basis of six independent measurements.

To get the real-time reaction information during the time course of this reaction, we obtained single-bead IR spectra at various times during the reaction (Figure 2). For formylpolystyrene resin (Figure 2A), intensities of the C−H stretch band at 2728 cm^{-1} and the carbonyl band at 1700 cm^{-1} from the aldehyde decrease and intensities of the $N(CH_3)_2$ stretch band at 2790 cm^{-1} and the N−H band from the dansylhydrazone at 3218 cm^{-1} increase with time. These data unambiguously confirmed that the decrease in dansylhydrazine concentration in supernatant shown in Figure 1A is due to its reaction with the resin-bound aldehyde to form dansylhydrazone on resin. Similarly, reaction on TentaGel resin was followed by the decrease of the intensity of the aldehyde carbonyl band at 1723 cm^{-1} (Figure 2B).

The method was used to quantitate two batches of polystyrene-based aldehyde resins from NovaBiochem (San Diego, CA) and two batches TentaGel-based aldehyde resins from Rapp Polymere (Tubingen, Germany). The experimental results are shown in Table 1. The measured aldehyde loadings for formylpolystyrene resin are slightly higher than those given by the manufacturer (0.62 vs 0.58 and 0.50−0.53 vs 0.44). This discrepancy may be due to the different methods used by the manufacturer and by us. The manufacturer's values were obtained through a two-step reaction sequence. The aldehyde resin was first refluxed with Fmoc-Gly hydrazide in 2:1 THF/ethoxyethanol for several hours, and then Fmoc groups were cleaved for quantitation.[3] The harsh treatment, one more reaction step, and the prolonged reaction time may be responsible for their lower loading values. In the case of TentaGel aldehyde resin

Table 1. Absolute Amount of Aldehyde Groups on Resins[a]

sample	weight (mg)	no. of measure	this method (mmol/g)	method A/B[b] (mmol/g)
Ald-1[c]	6−11	8	0.62 ± 0.04	0.58[A]
Ald-2[d]	6−11	6	0.53 ± 0.03	0.44[A]
Ald-2[e]	3−6	10	0.50 ± 0.06	0.44[A]
S-CHO 1[f]	6−11	8	0.19 ± 0.02	0.23[B]
S-CHO 2[g]	5−11	6	0.24 ± 0.02	0.27[B]

[a] The reaction time was 30 min for all polystyrene-based resins. [b] Method from NovaBiochem (A) or Rapp Polymere (B) as described in the text. [c] Formylpolystyrene batch A15617. [d] Formylpolystyrene batch A16356. [e] Used a correction factor of 0.07 obtained with 5 mg of polystyrene beads as control. The reactiong time was 10 min for all TentaGel-based resins. [f] TentaGel S-CHO (batch 400S83.1). [g] TentaGel S-CHO (batch 100S2.34).

(3) Personal communication from Aubrey Mendonca, NovaBiochem.

Table 2. Absolute Amount of Aldehyde and Ketone Groups on Resin-Bound Products[a]

sample	weight (mg)	no. of measure	this method (mmol/g)	theoretical[b] (mmol/g)
Ald-1[c]	6	2	1.11 ± 0.03	1.14
Ald-2[d]	6	2	0.91 ± 0.03	0.97
Ald-3[e] (3 m)	3	1	0.11	N/A
Ald-3 (15 m)	4	1	0.53	N/A
Ald-3 (60 m)	5	1	0.62	N/A
Ald-4[f] (TG)	10	4	0.24 ± 0.02	0.27
ketone-1[g]	5−7	2	1.14 ± 0.02	1.17
ketone-2 (TG)[h]	8−11	4	0.26 ± 0.02	0.27

[a] The reaction time was 30−60 min for polystyrene-based resins and 25 min for TentaGel-based resins. [b] Based on the loading of the starting material and the percentage of conversion by single-bead IR or just the former. [c] The product of carboxybenzaldehyde and Wang resin. Single-bead IR showed that the conversion of the reaction is ~97%. [d] The product of the catalytic oxidation of Wang resin to a benzaldehyde by TPAP/NMO.[4] The loading of Wang resin is 1.0 mmol/g and single-bead IR showed a 97% of conversion. [e] The product of the catalytic oxidation of a primary alcohol by TPAP/NMO (resin-bound compound **5** in Scheme 1 in ref 4). This is the product after a three-step synthesis. The loading of the starting material is 1.0 mmol/g. [f] The product of the catalytic oxidation of a TentaGel-based alcohol (TentaGel S−PHB, loading 0.27 mmol/g) by TPAP/NMO. [g] Resin-bound product by coupling acetylbutyric acid to Wang resin. [h] TentaGel resin-bound product by coupling acetylbutyric acid to TentaGel S−OH resin.

(TentaGel S-CHO, Table 1), our results were obtained 5−8 months after the production of resins and are slightly lower than those measured by the manufacturer immediately after the production using ninhydrin test (Table 1). This fact suggests that the aldehyde resin can degrade with time and a quantitative analysis is highly desirable before synthesis. Finally, the sample weight for polystyrene-based resins could be lowered to 3 mg with no effect on the results (Table 1).

We have used this method to quantify aldehyde and ketone products for six solid-phase reactions. The first resin-bound compound (Ald-1 in Table 2) analyzed is the product of an esterification reaction between carboxybenzaldehyde and Wang resin (loading 1.17 mmol/g). Single-bead IR showed that the reaction is 97% complete on the basis of the disappearing hydroxyl IR band. We determined that the absolute amount of aldehyde on resin is 1.11 ± 0.03 mmol/g, equivalent to a yield of 95 ± 3%. The second product analyzed is the oxidation product (Ald-2 in Table 2) of Wang resin with a loading of 1.0 mmol/g by tetra-*n*-propylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO). Single-bead IR previously suggested a 97% conversion based on the disappearing hydroxyl IR band.[4] The average of two measurements is 0.91 ± 0.03 mmol/g which corresponds to an on-resin yield of 91 ± 3%. The third analysis (Ald-3 in Table 2) involves a time course study of a catalytic oxidation of a primary alcohol (resin-bound compound **4** in Scheme 1 in ref 4) by TPAP/NMO. The loading of the starting material is 1.0 mmol/g. The time course based on the absolute amount of aldehyde groups on resin after various reaction periods fits well with that previously obtained by single-bead IR.[4] The absolute amount of aldehyde on resin is determined to be 0.62 mmol/g, giving a yield of 62% for this three-step synthesis. The Ald-4 is the oxidation product of a TentaGel alcohol (TentaGel S-PHB, loading 0.27 mmol/g) by TPAP/NMO. The absolute amount of the aldehyde product was determined to be 0.24 ± 0.02 mmol/g (or a yield of 89 ± 7%).

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Table 3. Tests for the Interference from Resin-Bound Carbonyl Compounds[a]

sample	weight (mg)	no. of measure	loading (mmol/g)	this method (mmol/g)
carboxyl-ps	10	4	1.57	0 ± 0.02
HMBA AM	10	2	0.89	0 ± 0.03
ester resin[b]	10	1	1.17	0 ± 0.06
brominated Wang	10	6	1.05	0 ± 0.01
TG S-COOH	10	4	0.25	0 ± 0.02
TG S-HMB (amide)	10	2	0.23	0 ± 0.01
TG S-PHB-Gly Fmoc (ester and Fmoc)	10	4	0.26	0 ± 0.03

[a] The reaction time was 60 min for polystyrene-based resin and 25 min for TentaGel-based resin. [b] Resin-bound compound **5** in ref 5.

Ketone-1 and ketone-2 are products synthesized by coupling acetylbutyric acid to Wang resin or TentaGel S-OH resin. The absolute amount of ketone groups on both resins was quantitatively determined as shown in Table 2.

We further tested carboxyl acid, amide, and ester resins for an evaluation of potential interferences or complications. Our results (Table 3) showed that none of these functional groups posed a problem for polystyrene-based resin. To further confirm that there is no resin/dye reaction in these cases, we recorded IR spectra of these resins after reacting with the dye for 1 h. The hydrazone N−H band at 3218 cm^{-1} was not observed for any of these resins. The aromatic ketone group did not react with the dye as shown by brominated Wang resin (Table 3). These results indicate that, under the same conditions, the coexistence of these functional groups in the sample would not interfere with the quantitation of aldehyde or aliphatic ketones. For TentaGel resins, none of the amide, ester, Fmoc, or acid functional groups reacted with the dye (Table 3).

In summary, we have demonstrated a principle of quantitation of organic functional groups through dye-coupling reaction and dye consumption measurement. This method is simple to perform and accurately quantitated the absolute amount of aldehyde and aliphatic ketone groups on resin. It can be applied using 3−10 mg of resin sample (for loadings 1.0−0.3 mmol/g) containing low micromolar amounts of functional groups. With the excellent sensitivity of fluorescence spectroscopy and the ample availability of fluorescence dyes which can be attached to various organic functional groups, applications of the dye-coupling principle will provide chemists involved in SPOS an effective methodology to quantify reaction yields directly on resin.

Experimental Section

Solid-Phase Reactions. In a 1 mL Supelco filtration tube was added 3−11 mg (1.8−6.5 μmol) of weighed beads. The sealed tube was shaked with 0.5 mL of DMF for 30 min and then drained. The dansylhydrazine (approximately 2:1 molar ratio relative to the loading on resin) in 0.2 mL of DMF/HAc (2:1) was added to the resin, and the mixture was rotated on a Glas-Col Laboratory rotator (16 rpm) at room temperature. A fraction of the mixture was analyzed at various times. A 20 μL supernatant was diluted into 200 μL with DMF. Then 10 μL of this solution was further diluted into 2 mL of DMF for fluorescence measurements.

Fluorescence Spectroscopy. Fluorescence spectra were recorded on an Aminco Bowman Series 2 luminescence spectrometer (SLM Aminco, Rochester, New York). The wavelength of the excitation light was set at 345 nm. The concentration of the unreacted dye was quantitated through integrating the peak area from 400 to 680 nm or through the maximum emission at 525 nm.

Calculation. The loading of aldehyde (mmol/g) was calculated using

$$C = [(10 - M_s)/10 - (F_s - M_s F_c/10)(10 - kM_c)/(10F_c)]C_0/M_s \quad (1)$$

where M_c is the weight of control beads, F_c the fluorescence area or intensity at 525 nm for the control, M_s the sample weight, F_s the sample fluorescence area or intensity at 525 nm C_0 the total molar amount of the dansylhydrazine, and k the correction factor which is 0 for TentaGel resins and 0.11 for polystyrene resin using 10 mg beads.

Single-Bead IR. During the reaction, a drop of resin suspension was taken out of the reaction mixture at specified times. The mixture was washed with DMF (5 times), THF (5 times), and methylene chloride (5 times) and then vacuum-dried for 15 min. The measurement procedure is the same as previously reported.[4−6]

Acknowledgment. We gratefully acknowledge the technical assistance from Dr. Qun Sun and Ms. Catherine A. Astor.

JO9712512

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