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A new colorimetric test for detection of hydroxyl groups in solid-phase synthesis

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Abstract—A new colorimetric test (methyl red-DIC test) for monitoring the presence of hydroxyl groups on resins for use in solid-phase synthesis was developed. The resin became orange-red when the carboxyl group of methyl red formed ester linkages with the resin's hydroxyl groups. This new test can be used not only for qualitative analysis but also quantitative analysis by using pictures of the relationship between color depth and hydroxy loading rates.

In solid-phase synthesis, completion of protecting and de-protecting reactions is essential to ensure that only desired substances are produced. Particularly for selective synthesis of oligosaccharide, the detection of the presence of hydroxyl groups is very important for monitoring of hydroxy protecting and de-protecting reactions. If insufficient capping of carbohydrate hydroxyl groups occurs, or if the protecting group resists removal, non-desired compounds will be produced as impurity. Therefore, hydroxy protecting and de-protecting reactions should be monitored.[1] The alizarin–cyanuric chloride test,[2,3] methyl red-diphenyldichlorosilane test,[4] and PNBP test[5] are well-known colorimetric tests for detecting hydroxyl groups on resins. However, some of these tests are neither sensitive nor reliable enough.[6] In these tests, a highly reactive intermediate molecule (cyanuric chloride for the alizarin–cyanuric chloride test, diphenyldichlorosilane for the methyl red-diphenyldichlorosilane test, and *p*-TsCl for the PNBP test) binds to hydroxyl groups on a resin, before part or all of it is displaced by the dye. However, it would be more effective if a dye could be linked directly to the hydroxyl groups on the resin without the involvement of these intermediates.

We have developed a new colorimetric test, which we have called the 'methyl red-DIC test', for detecting hydroxyl groups on resins. The resins are stained orange-red when methyl red binds to the resin's hydroxyl groups directly. We chose methyl red because it is inexpensive, widely available, dark and deep red, and has a functional group that can bond readily to hydroxyl groups on the resin. To bind the methyl red's functional group, a carboxyl, to the resin's hydroxyl groups, we chose 1,3-diisopropylcarbodiimide (DIC) and 4-(dimethylamino) pyridine (DMAP) as coupling reagents. Typically, 20 mg of methyl red, 50 μl of DIC, and 5 mg of DMAP for about 1 mg of resin in 500 μl of dry dichloromethane was used. The mixture was agitated for 2 h at room temperature. Then, the resin was isolated by filtration, and washed with 10% water in DMF, DMF and chloroform until the washing solution became colorless.

The reactivity of this new test (methyl red-DIC test) on several types of resin with hydroxyl, carboxyl, chloride, and amino groups was examined (Fig. 1). The resin, which had a primary hydroxyl group (HMBA-AM resin, 1.16 mmol/g; Novabiochem: **1**), reacted well and changed to a dark red color. On the other hand, a trityl hydroxyl group resin (trityl alcohol resin SS, 1.30 mmol/g; Advanced ChemTech: **2**) did not react due to steric hindrance. Interestingly, resins with a carboxyl group (carboxypolystyrene HL, 1.44 mmol/g; Novabiochem: **3**), a chloride group (Merrifield resin LL, 0.50 mmol/g; Novabiochem: **4**), or an amino group (aminomethylated polystyrene LL, 0.45 mmol/g; Novabiochem: **5**) showed different color changes, becoming partially red, black, and red, respectively.

Keywords: Methyl red-DIC test; Detection of hydroxyl groups; Solid-phase synthesis; Colorimetric test.
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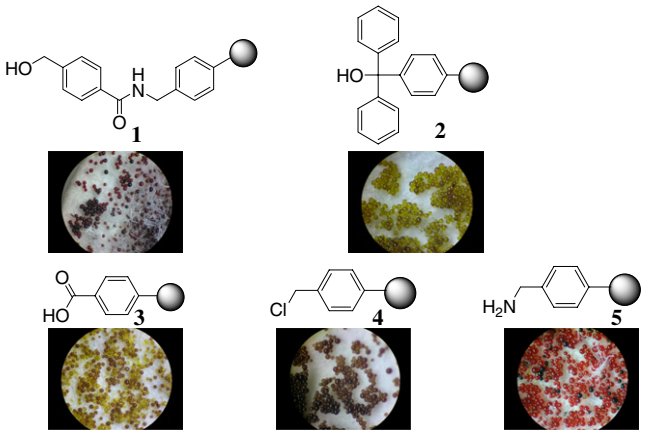

Figure 1. Reactivity of methyl red-DIC test against several types of resins viewed under a microscope.

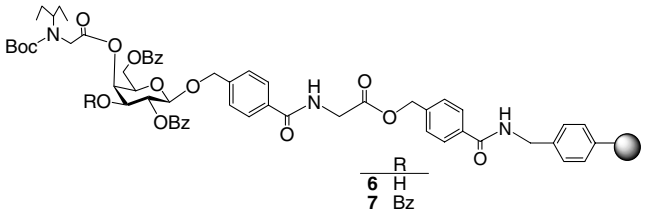

Figure 2. The structures of two kinds of resins.

We compared the methyl red-DIC test with traditional colorimetric tests (Fig. 3), that is, the alizarin–cyanuric chloride, PNBP and methyl red-diphenyldichlorosilane tests, by using two kinds of resins, HMBA-AM resin (**1**) and the carbohydrate connected resin (**6**, Fig. 2).[7,8] Inexplicably, the methyl red-diphenyldichlorosilane test gave unclear results for both resins even with large excess of reagents and when the reaction was allowed to proceed for longer than usual. The color of HMBA-AM resin changed to yellow (not red) from the white color of untreated resin. Burkett et al. showed the color picture of resin which was stained yellow color as a positive result.[4] So, we believe that we could exactly succeed in following Burkett's protocol. But the color

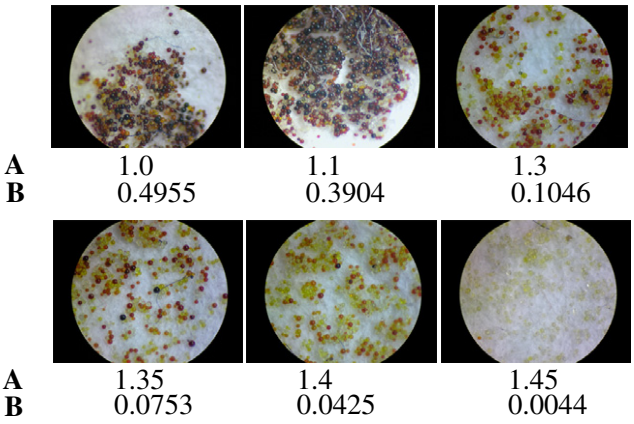

Figure 4. Relationship between loading rate and red color: (A) equivalent of total amount of acetic acid added; (B) OH loading (mmol/g).

was too light to determine the red color of methyl red. The color depth of such treated resin (theoretical loading is 0.83 mmol/g) is almost the same as the color of 0.0044 mmol/g loading resin which was treated by the methyl red-DIC test (Fig. 4). We wondered why the methyl red could connect to the hydroxyl resin almost below 1% in the case of the methyl red-diphenyldichlorosilane test. Neither the alizarin–cyanuric chloride test nor the PNBP test were able to detect hydroxyl groups on carbohydrate on the resin. On the other hand, the methyl red-DIC test clearly detected such hindered hydroxyl group on the carbohydrate. Moreover, the methyl red-DIC test gave a negative result after capping the hydroxyl group with a benzoyl group (**7**). For selective synthesis of oligosaccharide, the most important thing is to know the presence of hydroxyl groups on carbohydrate on solid support. Only the methyl red-DIC test could clearly detect the hydroxyl group on carbohydrate and also could show (negative result) that none of the hydroxyl group remained.

To examine the sensitivity of this new test, HMBA-AM resin was capped with different levels of an acetyl group to yield different loading rates.[9] After connecting Fmoc-Gly to the unacetylated (remaining) hydroxyl group,[10]

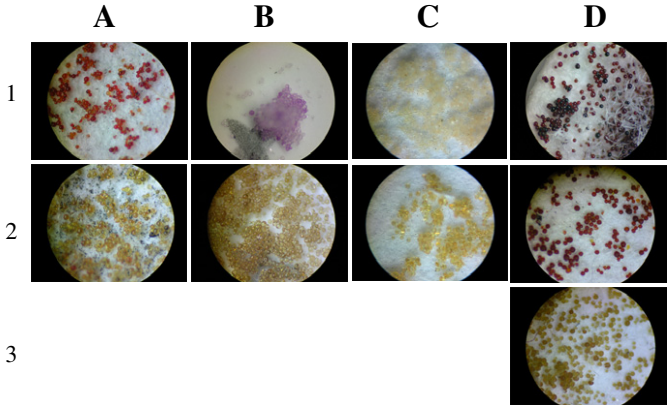

Figure 3. Results of four different resin tests: (A) alizarin–cyanuric chloride test; (B) PNBP test; (C) methyl red-diphenyldichlorosilane test; (D) methyl red-DIC test. (1) HMBA-AM resin (**1**). (2) Carbohydrate connected resin (**6**). (3) Bz Protected resin (**7**).

the loading value of the hydroxyl group was calculated by the Fmoc test.[11] Although quantitative analysis of hydroxyl group with Fmoc-Gly is reliable, it needs the long coupling time and the complicated manipulation. So, detecting of the hydroxyl group with Fmoc-Gly is not recommended for a frequent analysis of hydroxyl group. Thus, six types of resin with different loading rates, 0.4955, 0.3904, 0.1046, 0.0753, 0.0425, and 0.0044 mmol/g, were examined for sensitivity of the methyl red-DIC test (Fig. 4). The results indicated the minimum sensitivity to be around 0.042 mmol/g. This sensitivity was almost equivalent to those of previously reported methods.[4]

Finally, the minimum required reaction time was examined using the above six different loading resins. The reaction times were selected as 30 s, 2, 5, 10, and 30 m, and 2 h. The resin color was observed both by the naked eye and under a microscope (Fig. 5). Two minutes was sufficient to determine the presence of a hydroxyl group.

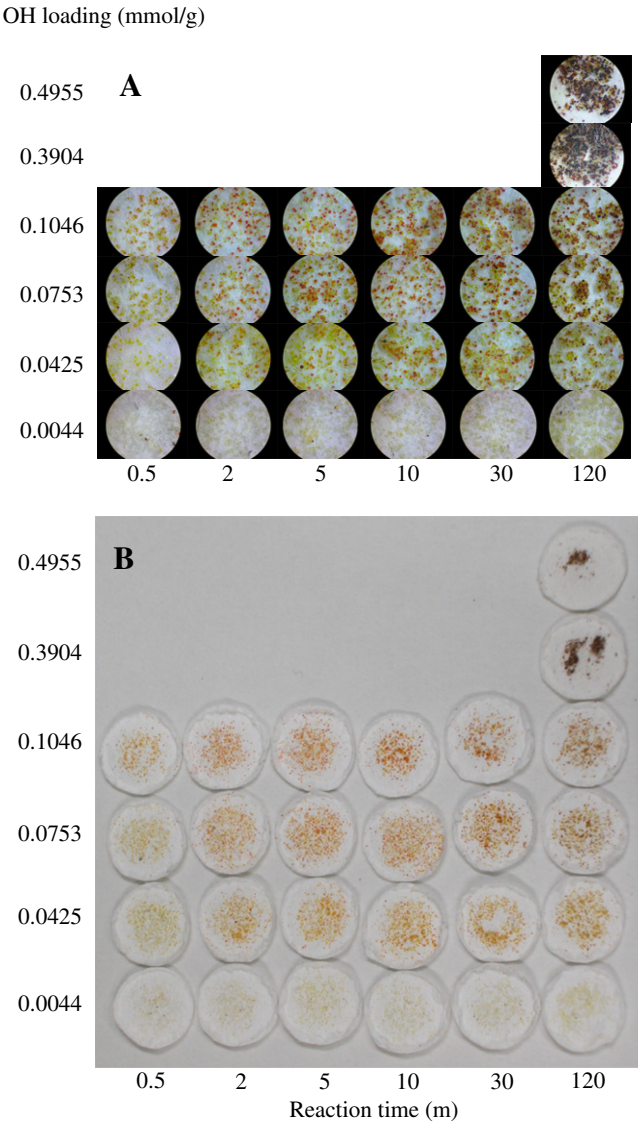

Figure 5. Reaction time of methyl red-DIC test: (A) as viewed under microscopy; (B) as viewed by naked eye.

Of course, the color depth was different between 2 h and 2 m. If you need to know the reliable data, 2 h is recommended. If you need to know the presence of a hydroxyl group as quickly as possible, 2 m is sufficient. By using the color change chart (relationship between red color and hydroxy loading rate) in Figures 4 and 5, not only we can presume the rough loading rate of the hydroxyl group, but also we can know the sufficiency of the hydroxy capping reaction or hydroxy de-protecting reaction.

In summary, the new colorimetric test (methyl red-DIC test) described here is very sensitive at detecting hydroxyl groups on resin, particularly carbohydrate. Also, by using the color change chart, we can presume the rough loading rate of hydroxyl groups on resin merely by the naked eye. In addition, this test is simple and rapid, yielding results in as little as two minutes. By using this test, we succeeded in synthesizing oligosaccharide selectively, and the results will be published elsewhere.

Acknowledgment

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Supplementary data

The high resolutions for Figures 1, 3, 4, 5 and both of the original resins and the reacted resins for methyl red-diphenyldichlorosilane test can be found, in the online version, at doi:10.1016/j.tetlet.2007.01.124.

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9. To synthesize six types of resin with different loading rate, the HMBA-AM resin (1.16 mmol/g) was repetitiously acetylated by six times. At first capping, HMBA-AM resin was treated with 1.0 equiv of acetic acid in the presence of DIC and DMAP in CH_2Cl_2 at room temperature for 1 h. At second capping, this acetylated resin was treated with 0.1 equiv of acetic acid at the same condition of previous capping. At third capping, this twice acetylated resin was treated with 0.2 equiv of acetic acid at the same condition

of previous capping. In this way, at fourth to sixth capping, the acetylated resin was treated with each 0.05 equiv of acetic acid, repetitiously. Thus, six types of resin with different capping rates, 1.0, 1.1, 1.3, 1.35, 1.4, and 1.45 equiv of total amount of acetic acid added, were examined for hydroxy loading rate.

10. The six types of resin with different capping rates were coupled with Fmoc-Gly in the presence of DIC and DMAP in CH_2Cl_2 at room temperature for 1 day.
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