

Notes & Tips

Microwave-assisted spectrophotometric estimation of functional groups on biochips using a universal reagent

Jyoti Choithani, Mayurika Goel, Ashwani K. Sharma, Kailash C. Gupta *

Nucleic Acid Research Laboratory, Institute of Genomics and Integrative Biology, Delhi University Campus, Delhi 110007, India

Received 8 March 2006

Available online 2 May 2006

Oligonucleotide microarray has emerged as a powerful tool to efficiently address various problems in molecular biology and medical sciences [1–3]. The technique allows simultaneous detection of many different targets in a sample based on base-pairing rules. The most commonly used approach for construction of oligonucleotide-based microarrays, the deposition method, involves the covalent immobilization of amino- or mercaptoalkyl probes on modified glass surface [4–6]. Of these, mercaptoalkylated probes have been immobilized on polymer surface carrying a variety of thiol group-specific functionalities, namely iodo-, bromo-, and maleimidoalkyl. Because the surfaces used for the construction of biochips are non-porous in nature, the functional group density is extremely low, therefore, sensitive methods are required to estimate the accessible functional group density so as to determine the exact concentration of the ligand (expensive material) to be immobilized. Some reagents employing the dimethoxytrityl group have already been reported by the authors' laboratory for the estimation of aldehyde-, amino-, carboxylic-, epoxy-, hydroxyl-, and mercaptoalkyl groups on polymer surface [7–13]. Unfortunately, there is no sensitive method available for the estimation of polymer-bound iodo-, bromo-, and maleimidoalkyl groups. In connection with our ongoing research program on the development of biochips for diagnostics, it was deemed necessary to devise a method involving a universal reagent for the estimation of thiol group-specific functionalities, viz., iodo-, bromo-, and maleimidoalkyl, on polymer supports, including glass microslides and polypropylene (PP)¹ films.

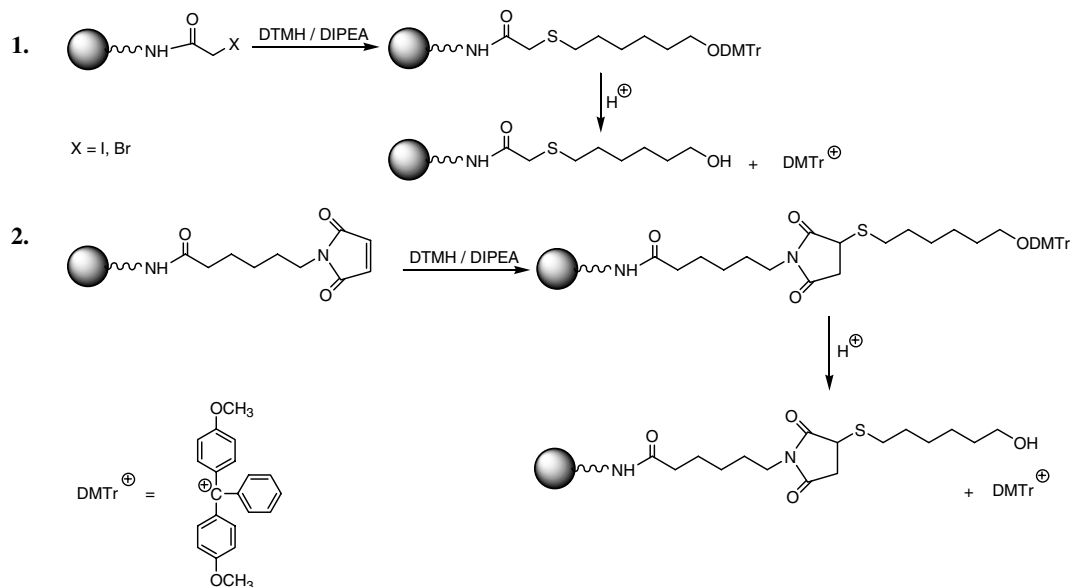
In the current article, we propose a spectrophotometric method employing a universal reagent, 1-*O*-(4,4'-dimethoxytrityl)-6-mercaptohexanol (DTMH, already reported by the authors' laboratory [14]), for the estimation of polymer-supported iodo-, bromo-, and maleimidoalkyl groups under microwaves. The reaction completes in 10 min (30 × 20 s) in case of iodo- and bromoalkyl functions, whereas it takes 15 min (45 × 20 s) with the maleimidoalkyl group on the support. The polymer capacity was quantified spectrophotometrically by monitoring the amount of 4,4'-dimethoxytrityl cation released on treatment of an accurately weighed amount of DTMH-treated support with an acid. The sequence of reactions involved in estimation of polymer-supported functional groups using DTMH is shown in Scheme 1.

To find the optimal time for the reaction of DTMH with functionalized polymer supports (iodo-, bromo-, and maleimidoalkyl) under microwaves, a kinetic study was performed (Fig. 1A). In brief, a weighed amount of iodoalkylated controlled pore glass (CPG) 500 Å polymer support (~4–5 mg) was taken in five different vials, 1 ml solution of DTMH (0.01 M) in acetonitrile (MeCN) containing 1% diisopropylethylamine (DIPEA) was added to each of them, and exposed to microwaves in a domestic microwave oven operating at 800 W. No exposure was longer than 20 s, and after each exposure, the vial was cooled in an ice–water mixture to room temperature. At different time intervals (5, 10, 15, 20, and 25 min), vials containing support were withdrawn and washed with MeCN containing 1% DIPEA and were subjected to drying

* Corresponding author. Fax: +91 11 27667471.

E-mail address: kcgupta@igib.res.in (K.C. Gupta).

¹ Abbreviations used: PP, polypropylene; DTMH, 1-*O*-(4,4'-dimethoxytrityl)-6-mercaptohexanol; CPG, controlled pore glass; MeCN, acetonitrile; DIPEA, diisopropylethylamine; TCA, trichloroacetic acid; EDC, ethylene dichloride; DMF, dimethylformamide; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide.



Scheme 1. Estimation of polymer-supported functional groups using DTMH.

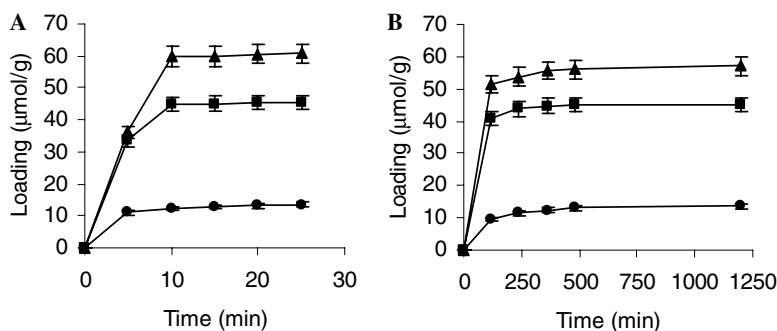


Fig. 1. Kinetics plots of the estimation reaction on CPG 500 Å supports bearing iodoalkyl (▲), bromoalkyl (■), and maleimidoalkyl (●) groups under microwaves (A) and thermal conditions (B).

under vacuum. The extent of reaction was determined by treating an accurately weighed amount (~1–2 mg) of DTMH-treated support with 3% trichloroacetic acid (TCA) in ethylene chloride (reagent A) for 5 min. The released DMTr cation was measured spectrophotometrically at 505 nm taking reagent A as a control. Similarly, loading on bromo- and maleimidoalkylated polymer supports was determined.

Likewise, loading on glass microslide/film was calculated by subjecting DTMH-treated glass microslide/film (1 × 1 cm²) to reagent A. The functional group density was calculated according to the following formula:

Amount of Functional Groups

$$\begin{aligned} & (\mu\text{mol/g of support or } \mu\text{mol/m}^2 \text{ of microslide/PP film}) \\ &= \frac{12.8 \times \text{Volume (ml)} \times A_{505\text{nm}}}{\text{Weight of Support (mg) or Area of Slide/Film (mm}^2\text{)}} \end{aligned}$$

The kinetic studies of DTMH reaction with all three functionalized supports were carried out under thermal conditions for comparison purposes (Fig. 1B). The results of the kinetic study performed under microwaves reveal

that the reaction of DTMH with iodo- and bromoalkylated support is completed in 10 min, whereas the reaction takes 15 min with maleimidoalkylated support. Table 1 shows a comparison of functional group loadings obtained using DTMH reaction under microwaves and the thermal conditions with those attained through the reported method.

Because medium plays a significant role in microwave-assisted reactions, it was considered necessary to study the effect of different solvents on the reaction of DTMH with polymer-supported functionalities. To investigate the solvent effect, functionalized polymer supports were taken in five vials and treated with DTMH reagent in different solvents, namely MeCN, EDC, dimethylformamide (DMF), tetrahydrofuran (THF), and dimethyl sulfoxide (DMSO) (data not shown). MeCN gave the best results among all of the solvents tried for this purpose except for PP films. Therefore, the estimation reactions were performed in MeCN only. Because MeCN is not compatible with PP films because of its hydrophobic nature, estimation reactions with PP films were carried out in EDC, a lipophilic solvent.

In conclusion, a simple and rapid method employing a universal reagent, DTMH, has been developed for the esti-

Table 1
Estimation of polymer-supported functional groups under microwave and thermal conditions

Support number	Polymer supports	Loading of support			Iodoalkyl support			Bromoalkyl support			Maleimidoalkyl support		
		Microwave \pm SD ^a	Thermal \pm SD ^a	Reported method ^b \pm SD ^a	Microwave \pm SD ^a	Thermal \pm SD ^a	Reported method ^b \pm SD ^a	Microwave \pm SD ^a	Thermal \pm SD ^a	Reported method ^b \pm SD ^a	Microwave \pm SD ^a	Thermal \pm SD ^a	Reported method ^b \pm SD ^a
1	CPG 500 Å ^c	62.00 \pm 1.86	60.77 \pm 2.43	61.08 \pm 2.44	45.92 \pm 1.83	44.90 \pm 2.69	44.53 \pm 2.02	45.92 \pm 1.83	44.90 \pm 2.69	44.53 \pm 2.02	13.62 \pm 0.72	13.53 \pm 0.67	8.22 \pm 0.35
2	Fractosil 500 Å ^c	30.08 \pm 0.87	24.05 \pm 0.84	30.54 \pm 0.94	35.48 \pm 1.17	34.19 \pm 1.19	33.24 \pm 0.86	35.48 \pm 1.17	34.19 \pm 1.19	33.24 \pm 0.86	10.21 \pm 0.29	8.05 \pm 0.22	8.58 \pm 0.23
3	Polypropylene films ^d	10.51 \pm 0.63	4.88 \pm 0.24	2.08 \pm 0.08	11.50 \pm 0.33	4.07 \pm 0.24	5.05 \pm 0.21	11.50 \pm 0.33	4.07 \pm 0.24	5.05 \pm 0.21	5.00 \pm 0.17	3.87 \pm 0.13	2.28 \pm 0.09
4	Glass microslides ^d	0.59 \pm 0.047	0.52 \pm 0.046	0.36 \pm 0.030	0.48 \pm 0.046	0.37 \pm 0.032	0.21 \pm 0.016	0.48 \pm 0.046	0.37 \pm 0.032	0.21 \pm 0.016	0.26 \pm 0.022	0.20 \pm 0.017	0.10 \pm 0.012

^a $n = 3$.

^b To a weighed amount of iodo-, bromo-, or maleimidoalkyl supports (~ 5 mg), 2-mercaptoethylamine (500 μ l, 0.01 M) dissolved in methanol was added. The reaction was allowed to swirl overnight at room temperature. Then the support was washed with methanol (2 ml \times 5) and diethyl ether (2 ml \times 2). Then the amino groups generated were estimated by the reported method (cf. Ref. [11]).

^c Loadings are expressed in μ mol/g.

^d Loadings are expressed in pmol/cm².

mation of iodo-, bromo-, and maleimidoalkyl groups, commonly used in the preparation of biochips (oligonucleotide microarrays). We hope that the proposed method will be useful for laboratories engaged in construction of microarrays.

Acknowledgments

Financial support from the CSIR Task Force Project (NNIOSB, COR010) is gratefully acknowledged. One of the authors (J. Choithani) is thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for the award of a senior research fellowship to carry out her research work.

References

- [1] R.J. Cho, M. Fromont-Racine, L. Wodicka, B. Feierbach, T. Stearns, P. Legrain, D.J. Lockhart, R. Davis, Parallel analysis of genetic selections using whole genome oligonucleotide arrays, *Proc. Natl. Acad. Sci. USA* 95 (1998) 3752–3757.
- [2] A.N. Drobyshev, N. Mologina, V. Shick, D. Pobedinskaya, G. Yershov, A.D. Mirzabekov, Sequence analysis by hybridization with oligonucleotide microchip: identification of β -thalassemia mutations, *Gene* 188 (1997) 45–52.
- [3] G. Yershov, V. Barsky, A. Belgovskiy, E. Kirillov, E. Kreindlin, I. Ivanov, S. Parinov, D. Guschin, A. Drobishev, S. Dubiley, A.D. Mirzabekov, DNA analysis and diagnostics on oligonucleotide microchips, *Proc. Natl. Acad. Sci. USA* 93 (1996) 4913–4918.
- [4] H. Seliger, M. Hinz, E. Happ, Arrays of immobilized oligonucleotides - contributions to nucleic acids technology, *Curr. Pharm. Biotechnol.* 4 (2003) 379–395.
- [5] P. Kumar, J. Choithani, K.C. Gupta, Construction of oligonucleotide arrays on a glass surface using a heterobifunctional reagent, N-(2-trifluoroethanesulfonatoethyl)-N-(methyl)-triethoxysilylpropyl-3-amine (NTMTA), *Nucl. Acids Res.* 32 (2004) e80.
- [6] P. Kumar, K.C. Gupta, A rapid method for the construction of oligonucleotide microarrays, *Bioconjugate Chem.* 14 (2003) 507–512.
- [7] R.K. Gaur, K.C. Gupta, A spectrophotometric method for the estimation of amino groups on polymer supports, *Anal. Biochem.* 180 (1989) 253–258.
- [8] R.K. Gaur, P. Sharma, K.C. Gupta, 4, 4'-Dimethoxytrityl chloride: a reagent for the spectrophotometric determination of polymer-supported amino groups, *Analyst* 114 (1989) 1147–1150.
- [9] P. Sharma, S. Sathyanarayana, P. Kumar, K.C. Gupta, A spectrophotometric method for the estimation of polymer-supported sulfhydryl groups, *Anal. Biochem.* 189 (1990) 173–177.
- [10] R.K. Gaur, S. Paliwal, P. Sharma, K.C. Gupta, A simple and sensitive spectrophotometric method for the quantitative determination of solid supported amino groups, *J. Biochem. Biophys. Methods* 18 (1989) 323–329.
- [11] N.S. Rao, S.K. Agrawal, V.K. Chauhan, D. Bhatia, A.K. Sharma, P. Kumar, B.S. Garg, K.C. Gupta, Microwave-assisted spectrophotometric estimation of polymer-supported functional groups using a universal reagent, *Anal. Chim. Acta* 405 (2000) 247–254.
- [12] S. Mahajan, A. Garg, M. Goel, P. Kumar, K.C. Gupta, Spectrophotometric estimation of functional groups on microslides for preparation of biochips, *Anal. Biochem.* 351 (2006) 273–281.
- [13] S. Mahajan, P. Kumar, K.C. Gupta, S-(4, 4'-dimethoxytrityl)-3-mercapto-propionic acid (DMPA), an efficient reagent for estimation of epoxy functionalities on microslides used for biochips, *Anal. Biochem.* 353 (2006) 299–301.
- [14] K.C. Gupta, P. Sharma, P. Kumar, S. Sathyanarayana, *Nucl. Acids Res.* 19 (1991) 3019.