Notes & Tips

Spectrofluorometric assay for monoamine-preferring phenol sulfotransferase (SULT1A3)

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ABSTRACT

A continuous and real-time fluorometric assay for monoamine-preferring phenol sulfotransferase (SULT1A3) was developed. The methodology was based on the coupling of SULT1A1 to regenerate 3′-phosphoadenosine-5′-phosphosulfate (PAPS) using 4-methylumbelliferyl sulfate (MUS) as a sulfuryl group donor. The fluorophore product (4-methylumbelliferone, MU) was continuously produced and monitored when SULT1A3 catalyzed dopamine sulfation with PAPS. The optimal conditions of this turnover reaction and substrate inhibition of SULT1A3 were also determined. This coupled-enzyme assay allows the continuous measurement of initial reaction velocity and the sensitivity is comparable to that of end-point radioactive isotope assay.

The sulfate conjugation of endogenous molecules is a prevalent biological phenomenon, and the physiological functions, however, are not always easily understood. For example, the sulfated form for total content of norepinephrine and dopamine is at least 70% and 95%, respectively, in plasma circulation [1]. The significance of the existence of sulfated dopamine, however, remains obscure. Human SULT1A3 (also known as monoamine (M)-form phenol sulfotransferase, M-PST) is the enzyme responsible for the sulfation of monoamine neurotransmitters, such as dopamine, tyrosine, adrenaline, noradrenaline, and 5-hydroxytryptamine [2], and SULT1A3 is found to distribute in brain, gastrointestinal tract, and platelet [3]. Unlike all other known members of the human sulfotransferase family, SULT1A3 exhibits a high degree of selectivity for catecholamines, especially for dopamine, and interestingly, the orthologs of human SULT1A3 have not been identified in other mammalian species yet [4].

The traditional assays for sulfotransferase activity mostly relied on radioactive isotope labeling using [35S]PAPS,[1] high-performance liquid chromatography (HPLC), and electrophoresis [5–7], all of which were end-point analyses. The radioactive method was relatively cumbersome and expensive, and the sulfated products isolated from chromatography and electrophoresis were too imprecise to estimate enzyme kinetics accurately. In this study, a convenient fluorometric assay with continuous data processing for the sulfation of dopamine was developed to evaluate SULT1A3 activity.

This assay was conducted by the coupling of rat SULT1A1 and human SULT1A3 as delineated in Fig. 1A. SULT1A1 and SULT1A3 were subcloned into pET-3c (Novagen) and pGEX-2TK (GE Healthcare) vector, respectively, and purified to near homogeneity [8,9]. SULT1A1 was a K65ER68G mutant, which was PAP free [8], and it catalyzes the transfer of a sulfuryl group from MUS to PAP [10]. The wild-type SULT1A1 catalyzes a similar reaction but it contains tightly bound PAP, which forms a ternary dead-end complex of SULT1A1–PAP–MU, and perturbs the transfer reaction [11,12]. Previous study showed that the K65ER68G mutant of SULT1A1 efficiently catalyzed the production of PAPS and MU and can serve as an auxiliary enzyme to monitor the steady-state production of initial product catalyzed by another sulfotransferase [13,14]. The reaction conditions including the excitation wavelengths of MU and MUS, buffer system, and the corresponding concentrations of MUS, PAPS, and SULT1A1 mutant were optimized previously [13]. This reaction was initially set out by a preincubation to transform residual PAP to PAPS in the absence of SULT1A3 because commercial PAPS is known to contain a significant amount of PAP [10]. The dopamine sulfation was triggered as soon as SULT1A3 was added in the reaction mixture and monitored by fluorescence of MU as shown in Fig. 1B. The change of fluorescence can be observed only in the presence of complete reaction mixtures. Control experiments, in the absence of PAPS, MUS, SULT1A1 mutant, or SULT1A3 gave stable backgrounds. Data shown in Fig. 1B indicated that the activity of SULT1A3 could be reliably monitored by the scheme illustrated in Fig. 1A and would not be affected by the reagents used in the reaction mixture.

The linear range of the coupled-enzyme assay (0.15–0.47 μg/ml SULT1A3 under complete reaction conditions as described in Fig. 1B) is shown in Fig. 2A and some properties of SULT1A3 were characterized as shown in Fig. 2B–D. The optimal pH and working

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   [1] Abbreviations used: MU, 4-methylumbelliferone; MUS, 4-methylumbelliferyl sulfate; PAPS, 3′-phosphoadenosine-5′-phosphosulfate.
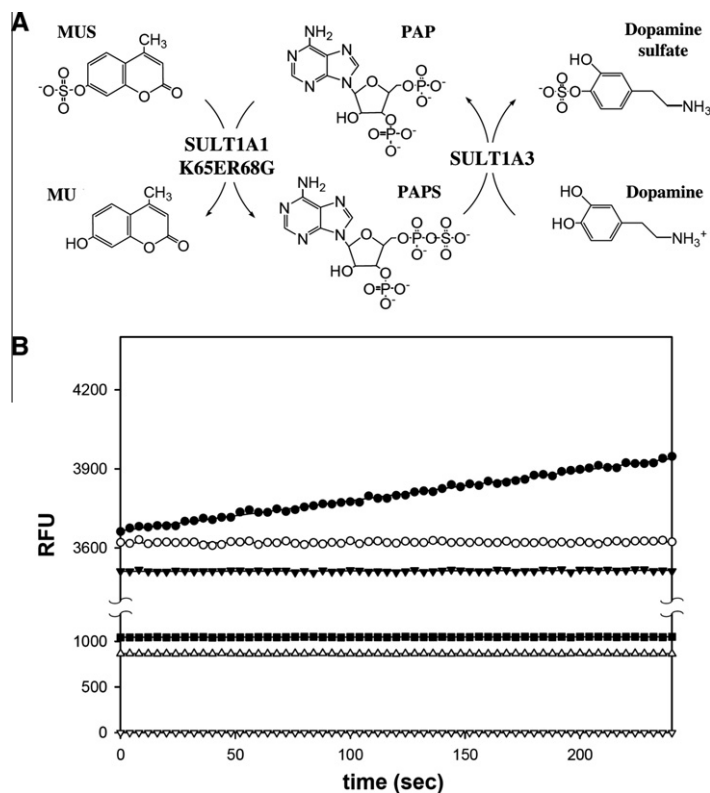
Fig. 1. Fluorometric assay for the determination of SULT1A3 activity. (A) Coupled-enzyme system for the regeneration of PAPS and sulfation of dopamine. PAPS regenerated from PAP through the catalysis by rat SULT1A1 (K65ER68G mutant) was used as a sulfuryl group donor for dopamine sulfation catalyzed by SULT1A3. MUS was utilized to generate PAPS, and the product, MU, was used as a fluorescent indicator during enzyme turnover. (B) Progress curves of coupled-enzyme assay for SULT1A3. The complete reaction (●) mixture included 100 mM potassium phosphate buffer at pH 7.0, 5 mM 2-mercaptoethanol, 20 µM PAPS, 1 mM MUS, 10 µM dopamine, 0.3 µg SULT1A3, and 1.5 mU K65ER68G of rat SULT1A1 (one unit was defined as 1 µmol PAP converted to PAPS per minute with MUS as sulfate group donor) at 37 °C. The control reactions were in the absence of PAPS (■), dopamine (○), MUS (▽), K65ER68G of rat SULT1A1 (△), or SULT1A3 (▼), respectively. All reactions were monitored by fluorescence of MU with excitation and emission wavelengths at 355 and 460 nm, respectively. SULT1A3 was not inhibited by MUS. However, higher concentrations of MUS will contribute to the higher background due to slight contamination of MU. The optimal concentration of MUS (1 mM) was much higher than the K_m value (183 µM for MUS) of mutated rat SULT1A1 (so that near V_{max} can be obtained) and gave low enough MU background for the assay.

temperature was 7.0 (Fig. 2B) and 37 °C (Fig. 2C), respectively. Substrate inhibition was observed (Fig. 2D) at higher dopamine concentrations (more than 30 µM). V_{max}, K_m, and K_i values of SULT1A3 using dopamine as substrate were 623 nmol/min/mg, 6.8 µM, and 174 µM, respectively, when dopamine was used as substrate. These

results were consistent with previous research using radioactive assay procedures [9].

In conclusion, this fluorometric assay allowed the continuous measurement of initial reaction velocity of SULT1A3. The sensitivity of this fluorometric assay was also comparable to that of the
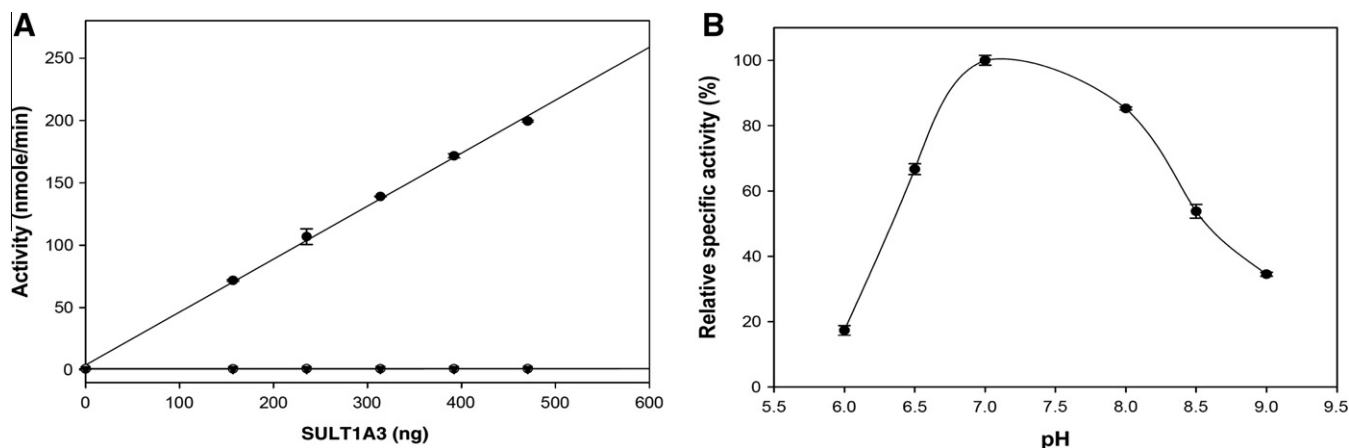

Fig. 2. Effective assay range and characterization of SULT1A3. (A) Linear range for effective SULT1A3 fluorometric assay. Activity of the complete (●) reaction was linearly enhanced with the increased amount of SULT1A3. The control reactions without dopamine (○) or K65ER68G (▼), respectively, gave no SULT1A3 activity. (B) The pH profile was determined in complete reaction mixture described in Fig. 1B with various buffer systems: 100 mM citric acid–phosphate at pH 6.0–6.5, potassium phosphate at pH 7.0, and Tris–base at pH 8.0–9.0. (C) Thermal effect was demonstrated by preincubating SULT1A3 alone in the buffer including 20 mM Tris (pH 8.0), 150 mM NaCl, and 10% glycerol at various temperatures (25–50 °C) for 5 min before the enzyme assay. (D) The enzymatic assay was carried out in complete reaction mixture described in Fig. 1B with varied dopamine concentrations. Kinetic parameters were evaluated from the substrate inhibition equation by SigmaPlot 2001 V7.0 and Enzyme Kinetics Module, V1.1 (SPSS Inc., Chicago, IL). Each data point was obtained from triplicate assay data and the error bars shown in these graphs represent standard deviation.
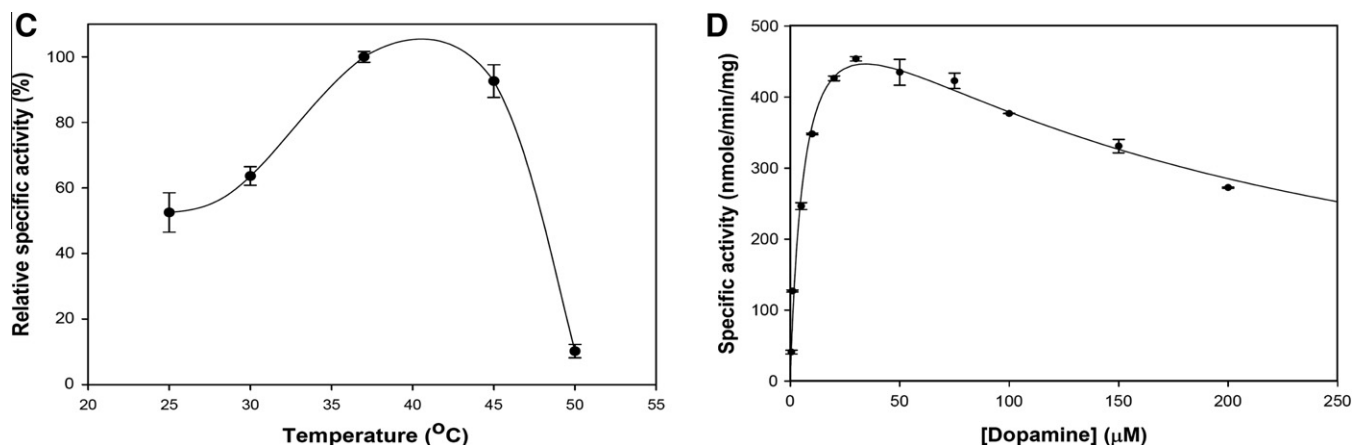
Fig. 2 (*continued*)

radioactive analysis previously reported for SULT1A3 [9]. Using this fluorometric assay, the activity of SULT1A3 can be determined at 150 ng and can be further improved (Fig. 2A). This assay could also be adapted for high-throughput analysis, such as microplate reader, to quickly detect monoamine sulfation qualitatively and quantitatively.

Acknowledgments

References

[1] C.A. Strott, Sulfonation and molecular action, Endocr. Rev. 23 (2002) 703–732.
[2] M.W. Coughtrie, S. Sharp, K. Maxwell, N.P. Innes, Biology and function of the reversible sulfation pathway catalysed by human sulfotransferases and sulfatases, Chem. Biol. Interact. 109 (1998) 3–27.
[3] G.L. Rubin, S. Sharp, A.L. Jones, H. Glatt, J.A. Mills, M.W. Coughtrie, Design, production and characterization of antibodies discriminating between the phenol- and monoamine-sulphating forms of human phenol sulphotransferase, Xenobiotica 26 (1996) 1113–1119.
[4] G. Eisenhofer, M.W. Coughtrie, D.S. Goldstein, Dopamine sulphate: an enigma resolved, Clin. Exp. Pharmacol. Physiol. Suppl. 26 (1999) S41–S53.
[5] R.D. Sekura, C.J. Marcus, E.S. Lyon, W.B. Jakoby, Assay of sulfotransferases, Anal. Biochem. 95 (1979) 82–86.
[6] J. Reinen, E. Vriese, H. Glatt, N.P. Vermeulen, Development and validation of a fluorescence HPLC-based screening assay for inhibition of human estrogen sulfotransferase, Anal. Biochem. 357 (2006) 85–92.
[7] Z.L. Wu, C.M. Ethen, S. Larson, B. Prather, W. Jiang, A versatile polyacrylamide gel electrophoresis based sulfotransferase assay, BMC Biotechnol. 10 (2010) 11.
[8] Y.S. Yang, A.D. Marshall, P. McPhie, W.X. Guo, X. Xie, X. Chen, W.B. Jakoby, Two phenol sulfotransferase species from one cDNA: nature of the differences, Protein Expr. Purif. 8 (1996) 423–429.
[9] T.G. Pai, K. Ohkimoto, Y. Sakakibara, M. Suiko, T. Sugahara, M.C. Liu, Manganese stimulation and stereospecificity of the Dopa (3,4-dihydroxyphenylalanine)/tyrosine-sulfating activity of human monoamine-form phenol sulfotransferase. Kinetic studies of the mechanism using wild-type and mutant enzymes, J. Biol. Chem. 277 (2002) 43813–43820.
[10] E.S. Lin, Y.S. Yang, Colorimetric determination of the purity of 3′-phospho adenosine 5′-phosphosulfate and natural abundance of 3′-phospho adenosine 5′-phosphate at picomole quantities, Anal. Biochem. 264 (1998) 111–117.
[11] T.M. Su, Y.S. Yang, Mechanism of posttranslational regulation of phenol sulfotransferase: expression of two enzyme forms through redox modification and nucleotide binding, Biochemistry 42 (2003) 6863–6870.
[12] L.Y. Lu, Y.C. Hsieh, M.Y. Liu, Y.H. Lin, C.J. Chen, Y.S. Yang, Identification and characterization of two amino acids critical for the substrate inhibition of human dehydroepiandrosterone sulfotransferase (SULT2A1), Mol. Pharmacol. 73 (2008) 660–668.
[13] W.T. Chen, M.C. Liu, Y.S. Yang, Fluorometric assay for alcohol sulfotransferase, Anal. Biochem. 339 (2005) 54–60.
[14] M.D. Burkart, C.H. Wong, A continuous assay for the spectrophotometric analysis of sulfotransferases using aryl sulfotransferase IV, Anal. Biochem. 274 (1999) 131–137.