

Fluorometric assay for alcohol sulfotransferase

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Abstract

A sensitive fluorometric assay was developed for alcohol sulfotransferase (AST). This was the first continuous fluorometric assay reported for AST. It used 3'-phosphoadenosine 5'-phosphosulfate regenerated from 3-phosphoadenosine 5'-phosphate by a recombinant phenol sulfotransferase (PST) using 4-methylumbelliferyl sulfate as the sulfonyl group donor. The recombinant PST did not use the alcohol substrate under the designed condition, and the sensitivity for AST activity was found to be comparable to that of radioactive assay as reported in the literature. The change of fluorescence intensity of 4-methylumbelliferone corresponded directly to the amount of active AST and was sensitive enough to measure nanogram or picomole amounts of the enzyme activity. This fluorometric assay was used to determine the activities of AST as purified form and in crude extracts of pig liver, rat liver, and *Escherichia coli*. Some properties of human dehydroepiandrosterone sulfotransferase were determined by this method and were found to be comparable to published data. Under similar assay conditions, the contaminated activities of arylsulfatase in crude extracts were also determined. This method not only is useful for the routine and detailed kinetic study of this important class of enzymes but also has the potential for the development of a high-throughput procedure using microplate reader.

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Sulfotransferases (STs)¹ represent a large family of enzymes that catalyze the transfer of sulfonyl group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to numerous endogenous and exogenous compounds [1]. Cytosolic STs catalyze the sulfonation of steroid hormones, catecholamines/neurotransmitters, drugs, and environmental chemicals, and they are involved in hormone homeostasis and metabolic detoxication/acti-

vation of xenobiotics [2–6]. In contrast, Golgi membrane-bound STs catalyze the sulfonation of macromolecules, including glycosaminoglycans and proteins, and play important roles in the modulation of receptor binding, intercellular communication, and signaling processes [7,8].

As a member of cytosolic STs, alcohol sulfotransferase (AST) catalyzes the sulfonation of various steroids and their derivatives as well as many xenobiotic alcohols [8,9]. Substrates of human AST include hydroxysteroids such as dehydroepiandrosterone (DHEA), testosterone, β -estradiol, and many other endogenous steroids [10,11]. Steroid sulfonation has been recognized as an important function for maintaining steroid hormone level in vivo [12]. A prominent example is that the bulk of DHEA produced by adrenal glands is sulfonated and secreted into circulation, serving as a precursor to the androgenic and estrogenic steroids in extraadrenal tissues [13,14].

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¹ Abbreviations used: ST, sulfotransferase; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; AST, alcohol sulfotransferase; DHEA, dehydroepiandrosterone; PAP, 3'-phosphoadenosine 5'-phosphate; PST, phenol sulfotransferase; MUS, 4-methylumbelliferyl sulfate; hDHEA-ST, human dehydroepiandrosterone sulfotransferase; MU, 4-methylumbelliferone; MES, 2-[N-morpholino]ethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; EDTA, [ethylenedinitrilo]tetraacetic acid; DTT, dithiothreitol; SDS, sodium dodecyl sulfate.

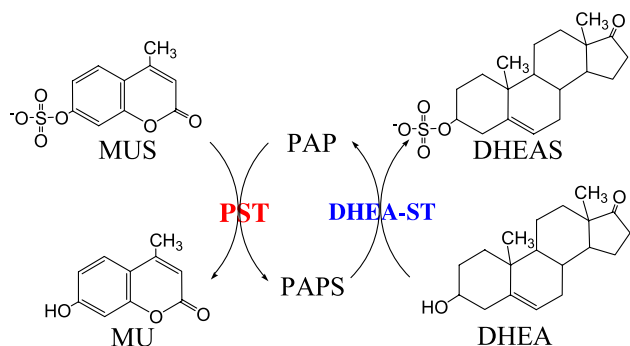


Fig. 1. Scheme for the determination of AST activity. This assay was based on the regeneration of PAPS from PAP catalyzed by a recombinant PST (K65ER68G) using MUS as the sulfonyl group donor. In coupled enzyme assay, PST represented an auxiliary enzyme and the product, MU, was used as a fluorescent indicator of enzyme turnover.

Suitable assays for STs are essential for investigation into their physiological functions. To date, the most common assays for AST activities are usually conducted by monitoring the transfer of radioisotopic sulfate from [^{35}S]PAPS to specific products [15] and involve stopping the reactions by heat treatment after a fixed time interval, centrifuging to remove precipitates formed, and changing the solvent system prior to thin-layer or paper chromatography. These procedures are tedious for routine and detailed kinetic studies of AST enzymes. Other reported AST assays are also end-point analyses requiring the determination of 3'-phosphoadenosine 5'-phosphate (PAP) using high-performance liquid chromatography [16]. Routine spectrophotometric assays, however, have been available only for phenol sulfotransferase (PST) [15]. Despite the considerable progress made on AST enzymes during recent years, several fundamental issues concerning the role of neurosteroids in neuron morphogenesis, regulation, and physiological involvement still remain to be fully elucidated. The current study was prompted by an attempt to develop a convenient assay to address these important issues.

Here we report the development of a fluorometric assay for AST (Fig. 1). In this assay, the regeneration of PAPS from PAP with 4-methylumbelliferyl sulfate (MUS) as the sulfonyl group donor was catalyzed by PAP-free PST in a reverse physiological reaction [17]. This reaction was coupled to AST, where purified human dehydroepiandrosterone sulfotransferase (*hDHEA-ST*) was used. The product, 4-methylumbelliferone (MU), served as fluorometric indicator to monitor *hDHEA-ST* activity.

Materials and methods

Materials

MUS, MU, DHEA, PAP, PAPS, Tris, 2-[*N*-morpholino]ethanesulfonic acid (MES), phenylmethylsulfonyl

fluoride (PMSF), [ethylenedinitrilo]tetraacetic acid (EDTA), glutathione (reduced form), and dithiothreitol (DTT) were purchased from Sigma (St. Louis, MO, USA). Potassium phosphate (dibasic), glycine, and sodium dodecyl sulfate (SDS) were obtained from J.T. Baker (Phillipsburg, NJ, USA). Glutathione *S*-transferase Sepharose fast flow was obtained from Amersham-Pharmacia Biotech Asia Pacific (Hong Kong). All other chemicals were of the highest purity commercially available.

Preparation of PAP-free PST

The β -form of recombinant PST [18] was used as the PAP-free enzyme. Recombinant mutant PST, K65ER68G, was cloned into an expression vector, pET3c, and transformed into *Escherichia coli* BL21 (DE3) [19]. The enzyme isolation procedure was the same as described previously [18], resulting in a homogeneous protein as determined by SDS-polyacrylamide gel electrophoresis [20].

Preparation of *hDHEA-ST*

Recombinant *hDHEA-ST* was cloned into an expression vector, pGEX-2TK, and transformed into *E. coli* BL21 (DE3). The expression and purification of *hDHEA-ST* was described previously [21], and a homogeneous protein was obtained as determined by SDS-polyacrylamide gel electrophoresis [20].

Determination of protein concentration

Protein concentration of the homogeneous form of K65ER68G and *hDHEA-ST* was estimated on the basis of absorbency at 280 nm (1.7 and 2.4 ml/mg/cm, respectively) [22] with a UV/Vis spectrophotometer (Hitachi UV/Vis-3300, Japan).

PST assay

The activity of PAP-free PST, K65ER68G, was determined based on the change of fluorescence due to the production of MU from MUS as measured using a spectrofluorometer (Hitachi F-4500). The excitation and emission wavelengths were 360 and 450 nm, respectively. The reaction mixture included 5 mM 2-mercaptoethanol, 4 mM MUS, 20 μM PAP, 100 mM potassium phosphate buffer (pH 7.0), and 0.5–2 μg K65ER68G. This assay was also referred to as a reverse physiological reaction catalyzed by K65ER68G. One unit was defined as 1 μmol of PAP converted to PAPS per minute with MUS under the reaction conditions described above.

Coupled enzyme assay for AST

Reaction mixture (1 ml) contained 100 mM potassium phosphate buffer (pH 7.0), 5 mM 2-mercaptoethanol,

20 μ M PAPS, 4 mM MUS, 5 μ M DHEA, and 3.2 mU (5.4 μ g) K65ER68G. AST (*h*DHEA–ST or crude extract) was added following a preincubation period so as to start the reaction at 37°C. Because commercial PAPS contained significant amounts of PAP, which is an inhibitor of sulfotransferase [18,23], the assay mixture was preincubated for 15 min prior to the addition of DHEA to ensure that all PAP had been converted to PAPS by K65ER68G before the complete enzymatic reaction was started. The production of MU was monitored by fluorescence as described previously. The change of fluorescence was linear for more than 20 min of initial reaction time.

Preparation of biological samples for enzymatic assay

The liver extracts were prepared from 20 g each of frozen rat or pig liver that was mixed with 20 ml buffer A (10 mM Tris–HCl (pH 7.4) plus 125 mM sucrose, 10% glycerol, 1 mM DTT, 1.5 mM PMSF, and 1 mM EDTA) and homogenized with liquid nitrogen. Bacterial cell extract was prepared from approximately 1 g *E. coli* pelleted from 250 ml cell culture, which was mixed with 20 ml buffer A and sonicated three times before supernatants were collected.

Results and discussion

K65ER68G as catalyst for the regeneration of PAPS

In this proposed coupled enzyme assay, PST was used to regenerate PAPS from PAP and MUS as illustrated in Fig. 1. Under similar conditions, not only could arylsulfatase activity be determined in the presence of only MUS, but also PST activity could be determined in the absence of AST or its substrate. Two characteristics of wild-type PST, however, might prevent the effective production of PAPS. It has been shown that wild-type PST contains tightly bound PAP [18], a sulfotransferase inhibitor that exists in all sulfotransferase-catalyzed reactions. Moreover, phenols have also been shown to be inhibitors of PST [23]. Under reducing conditions, the activity of PST could be significantly affected by the presence of PAP and/or phenol in the proposed PAPS regenerating system. A ternary complex of PST, PAP, and phenol might form to slow down the regeneration of PAPS from PAP [24–26]. Fortunately, previous studies demonstrated that a PST mutant, K65ER68G, was free of the above-mentioned complications [18]. Preliminary studies showed that K65ER68G could efficiently catalyze the production of PAPS, and PAP and MU did not inhibit the reaction under the conditions adopted in this study. As shown in Table 1, K_m for MUS determined was not significantly affected by the pH of the solution. In contrast, V_{max} of the same reaction was significantly

Table 1

Production of PAPS from PAP and MUS catalyzed by K65ER68G

pH	K_m (μ M)	V_{max} (nmol/min/mg)
6.0	161 \pm 14	204 \pm 7
7.0	183 \pm 15	399 \pm 9
8.0	196 \pm 21	142 \pm 5
9.0	167 \pm 51	2.9 \pm 0.2

Note. The reaction mixture included 5 mM 2-mercaptoethanol, 20 μ M PAP, and 50 μ M to 3.2 mM MUS plus 0.55 μ g enzyme in 100 mM buffer (MES at pH 6.0, potassium phosphate at pH 7.0, and Tris base at pH 8.0 and pH 9.0). The K_m and V_{max} values were obtained using nonlinear regression by SigmaPlot 2001 (version 7.0) and Enzyme Kinetics Module (version 1.1, SPSS, Chicago, IL, USA).

decreased at pH 9.0. These data were useful for the design of coupled enzyme assay.

Selection of excitation and emission wavelengths for the coupled enzyme assay

To bring about a virtually irreversible auxiliary reaction with regard to the initial product [27] in the proposed coupled enzyme assay, a high concentration (4 mM) of MUS was used to saturate the PAPS regenerating system based on data compiled in Table 1. A suitable excitation wavelength was determined to prevent the “inner filter effect” [28] caused by the absorption of MUS. The excitation wavelength, therefore, was selected according to the absorption spectra of MU and MUS, as shown in Fig. 2. The suitable excitation wavelength was chosen by comparing the absorption spectra of the two compounds. Therefore, AST activity was, in effect, determined by the increase of fluorescence of MU at 450 nm on excitation at 360 nm. The relative emission coefficient of the fluorescence at 450 nm was determined under different conditions, as shown in Table 2. Thus, the sensitivity of this method could reach the nanomolar range because the picomole amount of MU could be reliably determined. The intensity of the fluorescence was significantly dependent on the pH value, ranging from 6 to 10, primarily due to the deprotonation of MU at alkaline pH (pK_a of MU = 7.8) [29]. The temperature effect was less significant, as shown in Table 2.

Coupled enzyme assay

Based on the data compiled in Table 2, it is expected that the rate of AST, which was in the range of sub- μ mol/min/mg [21], could be easily monitored using this coupled enzyme system. It is worth pointing out that the major requirements of this assay were the adequate amount of MUS, an excess of K65ER68G activity, and a saturating PAPS concentration.

Fig. 3 shows the time course of the changes in fluorescence for the coupled *h*DHEA–ST/K65ER68G reaction and corresponding controls, where specific components

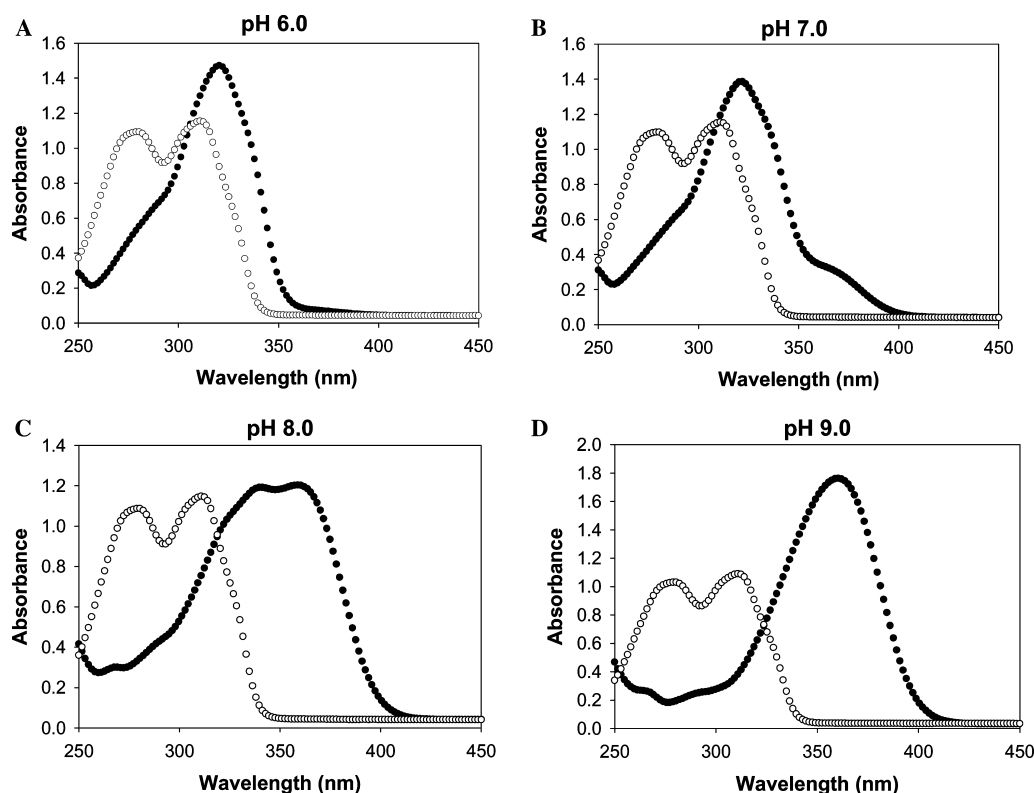


Fig. 2. Absorption spectra of MUS and MU. The spectra were obtained in 1 ml aqueous solutions that contained 100 μ M MUS (○) or MU (●) and 100 mM buffer (MES at pH 6.0, potassium phosphate at pH 7.0, and Tris base at pH 8.0 and pH 9.0).

Table 2
Relative emission coefficients (cm^{-1}/nM) of MU at 450 nm

pH value	Temperature ($^{\circ}\text{C}$)						
	25	30	35	37	40	45	50
6.0	—	—	—	0.12	—	—	—
7.0	0.4	0.43	0.45	0.48	0.50	0.52	0.55
7.5	—	—	—	0.64	—	—	—
8.0	—	—	—	1.14	—	—	—
9.0	—	—	—	2.14	—	—	—
10.0	—	—	—	4.10	—	—	—

Note. The fluorescence of MU (100, 200, and 400 nM) in 100 mM buffer (MES at pH 6.0, potassium phosphate at pH 7.0 and pH 7.5, Tris base at pH 8.0 and pH 9.0, and glycine at pH 10.0) was determined with a spectrofluorometer (Hitachi F-4500). The excitation and emission wavelengths were at 360 and 450 nm, respectively.

of the reaction mixture were omitted. Only for the complete reaction did we observe an increase in fluorescence, reflecting the enzymatic activity of *h*DHEA-ST. Some background fluorescence due to the auxiliary reaction was observed. This was probably a result of the presence as an impurity of PAP in commercially available PAPS [18,23]. The fluorescence background was, however, low in the absence of PAPS, K65ER68G, or MUS. Under these conditions, the auxiliary reaction did not take place. No effects were observed on the measured rates by raising the concentration of PAPS (data not shown), indicating that PAPS concentration was saturating enough for the coupling system to reach its maximum activity. Taking together the results shown in Fig. 3, it was concluded that

the continuous changes in fluorescence were specifically attributable to *h*DHEA-ST activity.

To determine the linear range of AST assay at a given K65ER68G concentration (3.2 μ M), the rate of MUS reduction was measured using a concentration range (0.07–1.35 μ g) of *h*DHEA-ST, as shown in Fig. 4. Although this linear range and sensitivity of *h*DHEA-ST assay could be further extended, it was suitable for our needs under the current situation.

To test our coupled enzyme assay and compare the results with the data appearing in the literature, the *h*DHEA-ST was characterized by this fluorometric assay, as shown in Figs. 5–7. These results fitted perfectly with previously reported data using radioactive assay

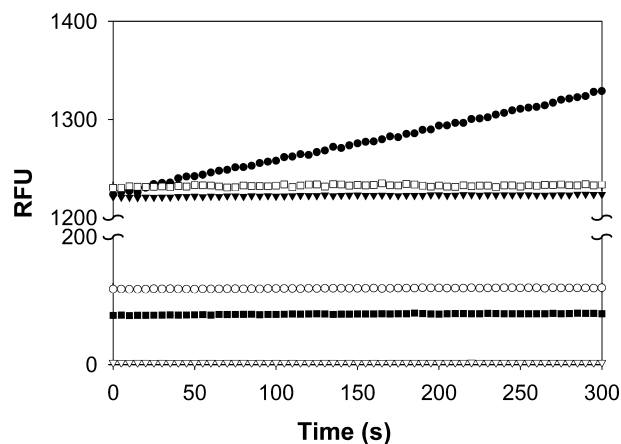


Fig. 3. Progress curves of the coupled enzyme assay for AST. Complete reaction (●) and control reactions without PAPS (○), DHEA (▼), MUS (▽), K65ER68G (■), or *hDHEA-ST* (□) of coupled enzyme assay for AST were conducted at pH 7.0 and 37 °C. RFU, relative fluorescent units. Detailed procedures are described under “coupled enzyme assay for AST” in Materials and methods.

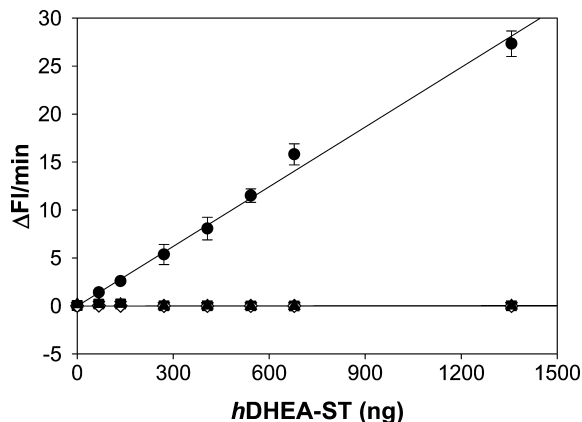


Fig. 4. Effective range of fluorometric assay for *hDHEA-ST*. Complete reaction (●) and control reactions without PAPS (○), DHEA (▼), MUS (▽), or K65ER68G (■) of coupled enzyme assay were run in a total volume of 1000 μ l at 37 °C. Detailed procedures are described under “coupled enzyme assay for AST” in Materials and methods. Each point was determined from triplicate assay data, and standard error was obtained by SigmaPlot 2001.

procedures [21]. Fig. 5 shows the pH dependency of the DHEA sulfonating activity. The optimal pH spanned from 7 through 9. The enzyme activity at pH 6.0 was approximately 50% of that in the optimal pH range. Virtually no activity was detected at pH 10.0. The effects of temperature on the DHEA sulfonating activity was examined over 25–50 °C, as shown in Fig. 6. Maximum sulfonating activity was observed at approximately 40 °C. The enzyme activities at 25 and 50 °C were approximately 50% that of maximal activity at 45 °C. At 55 °C or higher, *hDHEA-ST* became inactive. Fig. 7 shows the effects of DHEA concentration on the DHEA sulfonating activity. Significant substrate inhibition was observed as reported previously [12,21]. Substrate inhibition leading to the formation of a nonproductive

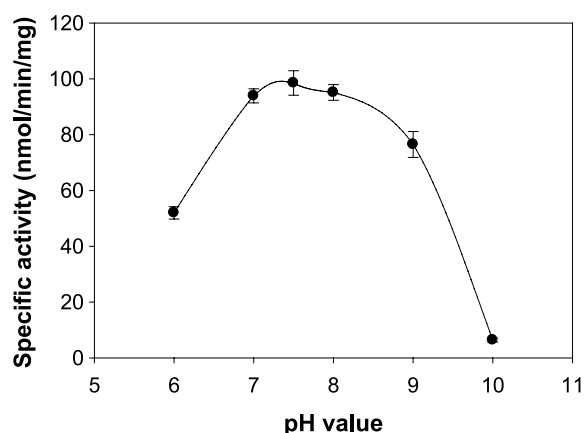


Fig. 5. pH profile of recombinant *hDHEA-ST*. The enzymatic assays were carried out with approximately 0.5 μ g recombinant *hDHEA-ST* under the condition of coupled enzyme assay for AST using different buffer systems (100 mM MES at pH 6.0, potassium phosphate at pH 7.0, Tris base at pH 8.0 and pH 9.0, and glycine at pH 10.0). Each point was determined from triplicate assay data, and standard error was obtained by SigmaPlot 2001.

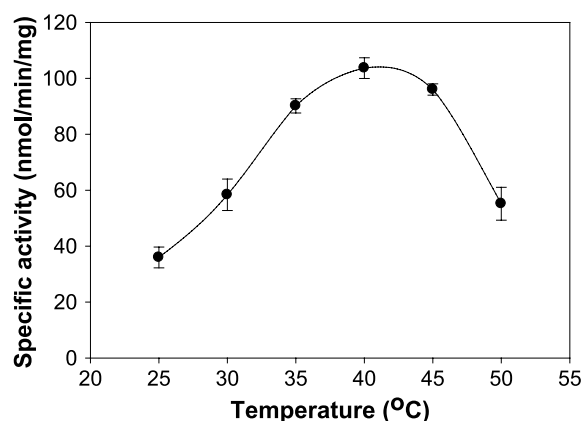


Fig. 6. Temperature effect on the activity of recombinant *hDHEA-ST*. The enzymatic assays were carried out with approximately 0.5 μ g recombinant *hDHEA-ST* under the condition of coupled enzyme assay for AST at pH 7.0 in different temperatures. Each point was determined from triplicate assay data, and standard error was obtained by SigmaPlot 2001.

enzyme–PAP–substrate complex [24–26], in fact, is a rather common feature among members of the sulfotransferase family [30–32]. These results compare favorably with published values determined by a noncoupled, radioisotopic, thin-layer chromatography assay [12,21] where K_m and K_{is} values of DHEA were 2.1 and 3.8 μ M, respectively. According to the coupled enzyme assay, these values were determined to be 4.7 and 4.3 μ M, respectively. The discrepancy between these values should be within the inherent inaccuracy of the radioisotopic assay for enzyme activity. This coupled enzyme assay allows the continuous measurement of initial reaction velocity and should be more accurate than end-point assays. Besides, it was found that the sensitivity of this fluorometric assay was

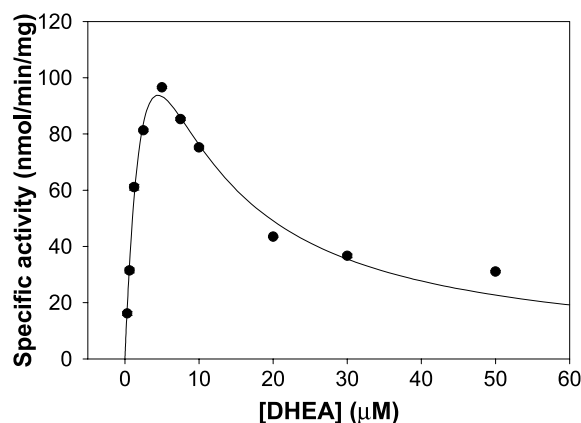


Fig. 7. Substrate inhibition of DHEA on recombinant *hDHEA-ST*. The enzymatic assays were carried out under the condition of coupled enzyme assay for AST at pH 7.0 with approximately 0.5 μ g recombinant *hDHEA-ST* and different DHEA concentrations (0.3–50.0 μ M). The kinetic parameters were obtained using the substrate inhibition equation $v = V[S]/\{K_m + [S] (1 + [S]/K_{is})\}$ by SigmaPlot 2001 and Enzyme Kinetics Module.

comparable to that of the radioactive assay for AST reported in the literature [12,21]. The activity of the amount of enzyme used previously (100 ng) could be easily determined by the current method, as shown in Fig. 4.

Determination of AST activity in biological samples

We further demonstrated the feasibility of the current assay for measuring AST activity in biological samples. In addition, several other enzyme activities associated with sulfonation/desulfonation could be determined under similar conditions. Sulfatase, alcohol, and phenol sulfotransferase activities might all exist in biological samples, and we could take advantage of the coupled enzyme system to determine all of these enzyme activities. As shown in Table 3, the complete system (reaction condition 1) could detect not only AST but also arylsulfatase activities due to the presence of MUS. The sulfotransferase activity could not be observed in the absence of PAPS; therefore, reaction condition 2 gave a background activity exhibited mainly by arylsulfatase. The sulfatase activity was further confirmed and quantified under the reaction conditions that contained only MUS and buffer (or in the absence of DHEA and PAPS) (data not shown). PST activity could also be determined using a similar procedure (data not shown because this activity did not interfere with the AST activity). In the absence of MUS, the fluorescence observed was close to the experimental error (data not shown) and represented the background derived from the interference of biological samples. Thus, the AST activity could be calculated simply by subtracting arylsulfatase activity from that measured in the complete coupled enzyme reaction. Based on this method, the AST activity in rat liver was found to be significantly higher than that in pig liver or *E. coli*. Moreover, the AST activity

Table 3
Activities of AST and arylsulfatase in biological samples

Reaction condition	Enzyme activity involved	Rat liver		Pig liver		<i>E. coli</i>		<i>E. coli</i> (with pGEX-2TK)	
		Specific activity (pmol/min/ A_{280})	Total activity (nmol/min/g)	Specific activity (pmol/min/ A_{280})	Total activity (nmol/min/g)	Specific activity (pmol/min/ A_{280})	Total activity (nmol/min/g)	Specific activity (pmol/min/ A_{280})	Total activity (nmol/min/g)
1. Complete	AST + sulfatase	245.0 \pm 4.0	39.6 \pm 0.7	132.0 \pm 4.0	7.2 \pm 0.2	6.1 \pm 1.0	2.9 \pm 0.7	280.0 \pm 10.0	236.0 \pm 8.1
2. PAPS ^a 1–2	Sulfatase	34.5 \pm 1.5	5.6 \pm 0.2	21.2 \pm 5.1	0.8 \pm 0.3	0.4 \pm 0.1	0.6 \pm 0.1	5.0 \pm 1.3	0.8 \pm 0.2
	AST	210.0 \pm 6.0	34.0 \pm 0.9	113.0 \pm 2.0	6.2 \pm 0.4	5.6 \pm 1.0	2.3 \pm 0.7	274.0 \pm 11.0	231.0 \pm 9.0

Note. Detailed procedures are described under “coupled enzyme assay for AST” in Materials and methods except that purified *hDHEA-ST* was replaced by the extract of biological sample. Specific activity refers to MU produced following the addition of extract whose protein concentration was determined by absorption at A_{280} . Total activity refers to MU produced with 1 g of wet cell or liver.

^a AST activity was eliminated in the absence of PAPS, as shown in Fig. 3.

extracted from transformed *E. coli* cells was approximately 100 times that in untransformed cells.

Conclusion

In this study, we developed a continuous fluorometric AST assay whose sensitivity is comparable to that of the end-point radioactive assay reported in the literature. This method was demonstrated to be useful for the determination of AST activities associated with homogeneous AST or those present in crude extracts from biological samples. This new assay procedure could be adapted for high-throughput assay using a microplate reader.

Acknowledgments

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References

- [1] W.B. Jakoby, D.M. Ziegler, The enzymes of detoxication, *J. Biol. Chem.* 265 (1990) 20715–20718.
- [2] G.J. Mulder, W.B. Jakoby, in: G.J. Mulder (Ed.), *Conjugation Reactions in Drug Metabolism*, Taylor & Francis, London, 1990, pp. 107–161.
- [3] C. Falany, J.A. Roth, in: E.H. Jeffery (Ed.), *Human Drug Metabolism: From Molecular Biology to Man*, CRC Press, Boca Raton, FL, 1993, pp. 101–115.
- [4] R. Weinshilboum, D. Otterness, in: F.C. Kaufmann (Ed.), *Conjugation–Deconjugation Reactions in Drug Metabolism and Toxicity*, Springer-Verlag, Berlin, 1994, pp. 45–78.
- [5] M.W.H. 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.
- [6] M.W. Duffel, in: F.P. Guengerich (Ed.), *Comprehensive Toxicology*, Elsevier Science, Oxford, UK, 1997, pp. 365–383.
- [7] K.G. Bowman, C.R. Bertozzi, Carbohydrate sulfotransferases: mediators of extracellular communication, *Chem. Biol.* 6 (1999) R9–R22.
- [8] R.M. Weinshilboum, D.M. Otterness, I.A. Aksoy, T.C. Wood, C. Her, R.B. Raftogianis, Sulfation and sulfotransferases: I. Sulfotransferase molecular biology—cDNAs and genes, *FASEB J.* 11 (1997) 3–14.
- [9] C.N. Falany, Sulfation and sulfotransferases: III. Enzymology of human cytosolic sulfotransferases, *FASEB J.* 11 (1997) 206–216.
- [10] G. Chen, E. Banoglu, M.W. Duffel, Influence of substrate structure on the catalytic efficiency of hydroxysteroid sulfotransferase STa in the sulfation of alcohols, *Chem. Res. Toxicol.* 9 (1996) 67–74.
- [11] Y. Kakuta, L.C. Pedersen, K. Chae, W.C. Song, D. Leblanc, R. London, C.W. Carter, M. Negishi, Mouse steroid sulfotransferases: substrate specificity and preliminary X-ray crystallographic analysis, *Biochem. Pharmacol.* 55 (1998) 313–317.
- [12] H.J. Chang, R. Shi, P. Rehse, S.X. Lin, Identifying androsterone (ADT) as a cognate substrate for human dehydroepiandrosterone sulfotransferase (DHEA-ST) important for steroid homeostasis: structure of the enzyme–ADT complex, *J. Biol. Chem.* 279 (2004) 2689–2696.
- [13] F. Labrie, A. Belanger, J. Simard, V. Luu-The, C. Labrie, DHEA and peripheral androgen and estrogen formation: intracrinology, *Ann. NY Acad. Sci.* 774 (1995) 16–28.
- [14] P.D. Kroboth, F.S. Salek, A.L. Pittenger, T.J. Fabian, R.F. Frye, DHEA and DHEA-S: a review, *J. Clin. Pharmacol.* 39 (1999) 327–348.
- [15] S.G. Ramaswamy, W.B. Jakoby, Sulfotransferase assays, *Methods Enzymol.* 143 (1987) 201–207.
- [16] J.J. Sheng, M.W. Duffel, Bacterial expression, purification, and characterization of rat hydroxysteroid sulfotransferase STa, *Protein Expr. Purif.* 21 (2001) 235–242.
- [17] 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.
- [18] 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.
- [19] X. Chen, Y.-S. Yang, Y. Zheng, B.M. Martin, M.W. Duffel, W.B. Jakoby, Tyrosine-ester sulfotransferase from rat liver: bacterial expression and identification, *Protein Expr. Purif.* 3 (1992) 421–426.
- [20] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* 227 (1970) 680–685.
- [21] H.J. Chang, M. Zhou, S.X. Lin, Human dehydroepiandrosterone sulfotransferase: purification and characterization of a recombinant protein, *J. Steroid Biochem. Mol. Biol.* 77 (2001) 159–165.
- [22] S.C. Gill, P.H. von Hippel, Calculation of protein extinction coefficients from amino acid sequence data, *Anal. Biochem.* 182 (1989) 319–326.
- [23] M.W. Duffel, W.B. Jakoby, On the mechanism of aryl sulfotransferase, *J. Biol. Chem.* 256 (1981) 11123–11127.
- [24] R.M. Whittemore, L.B. Pearce, J.A. Roth, Purification and kinetic characterization of a dopamine-sulfating form of phenol sulfotransferase from human brain, *Biochemistry* 24 (1985) 2477–2482.
- [25] A.D. Marshall, P. McPhie, W.B. Jakoby, Redox control of aryl sulfotransferase specificity, *Arch. Biochem. Biophys.* 382 (2000) 95–104.
- [26] E. Vakiani, J.G. Luz, J. Buck, Substrate specificity and kinetic mechanism of the insect sulfotransferase, retinol dehydratase, *J. Biol. Chem.* 273 (1998) 35381–35387.
- [27] F.B. Rudolph, B.W. Baugher, R.S. Beissner, Techniques in coupled enzyme assays, *Methods Enzymol.* 63 (1979) 22–43.
- [28] J.R. Lackowicz, *Principles of Fluorescence Spectroscopy*, Plenum, New York, 1983.
- [29] W.C. Sun, K.R. Gee, R.P. Haugland, Synthesis of novel fluorinated coumarins: excellent UV-light excitable fluorescent dyes, *Bioorg. Med. Chem. Lett.* 8 (1998) 3107–3110.
- [30] C.N. Falany, M.E. Vazquez, J.M. Kalb, Purification and characterization of human liver dehydroepiandrosterone sulfotransferase, *Biochem. J.* 260 (1989) 641–646.
- [31] D.M. Otterness, E.D. Wieben, T.C. Wood, W.G. Watson, B.J. Madden, D.J. McCormick, R.M. Weinshilboum, Human liver dehydroepiandrosterone sulfotransferase: molecular cloning and expression of cDNA, *Mol. Pharmacol.* 41 (1992) 865–872.
- [32] C.J. Marcus, R.D. Sekura, W.B. Jakoby, A hydroxysteroid sulfotransferase from rat liver, *Anal. Biochem.* 107 (1980) 296–304.