HUMAN 3′-PHOSPHOADENOSINE 5′-PHOSPHOSULFATE SYNTHETASE: RADIOCHEMICAL ENZYMATIC ASSAY, BIOCHEMICAL PROPERTIES, AND HEPATIC VARIATION

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This paper is available online at http://dmd.aspetjournals.org

ABSTRACT:

Sulfation is a major pathway in the biotransformation of many drugs and other xenobiotic compounds. The sulfotransferase (SULT) enzymes that catalyze these reactions use 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as a sulfate donor cosubstrate. The synthesis of PAPS from inorganic sulfate and ATP is catalyzed by PAPS synthetase (PAPSS). We previously cloned the genes for human PAPSS1 and PAPSS2 as a step toward pharmacogenetic studies of these enzymes. We have now developed a sensitive PAPSS radiochemical enzymatic assay for use in genotype-phenotype correlation analyses. This coupled assay uses the sulfation of 17β-[³H]estradiol catalyzed by recombinant human SULT1E1 to measure PAPS, which has been generated by PAPSS during the initial step of the assay. SULT1E1 proved to be ideal for this application both because of its relative resistance to inhibition by ATP,

a substrate for the PAPSS-catalyzed step, and because of its low K_m values for both PAPS (58 nM) and estradiol (29 nM). After optimal PAPSS assay conditions had been established, substrate kinetic studies were performed with cytosol preparations from human liver and cerebral cortex, two tissues with very different expression patterns for PAPSS1 and PAPSS2 mRNA. Brain and liver cytosol PAPSS activities had apparent K_m values for ATP of 0.26 and 0.62 mM, respectively, and for SO_4^{2-} of 0.08 and 0.31 mM, respectively. PAPSS activity was then measured in 83 human liver biopsy samples to determine the nature and extent of individual variation in this enzyme activity. An 18-fold variation was observed. This sensitive new radiochemical assay can now be used in pharmacogenetic studies of PAPSS in humans.

Sulfate conjugation is an important pathway in the metabolism of many drugs, other xenobiotics, neurotransmitters, and hormones (Weinshilboum and Otterness, 1994; Falany, 1997). PAPS[1] is the high-energy sulfate donor cosubstrate for the sulfotransferase (SULT) enzymes that catalyze these reactions (Weinshilboum and Otterness, 1994; Klaassen and Boles, 1997). In prokaryotes, PAPS is synthesized from 2 moles of ATP and 1 mole of SO_4^{2-} by two separate enzymes, ATP sulfurylase and adenosine 5′-phosphate kinase (Farooqui, 1980; Klaassen and Boles, 1997). However, in higher organisms these reactions are catalyzed by a single bifunctional cytosolic enzyme, PAPS synthetase (PAPSS) (Fig. 1, step A) (Geller et al., 1987; Lyle et al., 1994). Two isoforms of PAPSS have been identified in both humans and mice (Li et al., 1995; Girard et al., 1998; Kurima et al., 1998; ul Haque et al., 1998; Venkatachalam et al., 1998). We recently

cloned the genes for the two known PAPSS isoforms in humans, *PAPSS1* and *PAPSS2*, as a step toward pharmacogenetic studies (Xu et al., 2000). Each of these genes consisted of 12 exons, with virtually identical exon-intron splice junction locations (Kurima et al., 1999; Xu et al., 2000). However, Northern blot analysis demonstrated different patterns of tissue expression. For example, PAPSS1 mRNA was highly expressed in human brain, whereas PAPSS2 mRNA was not, but the opposite was true of human liver (Xu et al., 2000). Finally, although both isoforms have usually been considered cytosolic enzymes, there has been a recent report that PAPSS1 can be nuclear in its subcellular localization as can PAPSS2 when coexpressed with PAPSS1 (Besset et al., 2000).

Rare mutations of *PAPSS2* that result in inactive enzyme have been associated with congenital skeletal disorders in both humans and mice (Kurima et al., 1998; ul Haque et al., 1998). However, no common polymorphisms that alter the biochemical properties or levels of activity of these enzymes have been described in humans. As the next step toward pharmacogenetic studies of human PAPSS1 and PAPSS2, we have developed a sensitive radiochemical enzymatic assay suitable for use in genotype-phenotype correlation analyses. This assay uses the sulfation of [³H]E2 by SULT1E1 (Fig. 1, step B) to measure PAPS formed from ATP and inorganic sulfate by PAPSS. Although assays based on a similar principle have been described previously (Hazelton et al., 1985; Vargas, 1988; Wong et al., 1990), we found their sensitivity inadequate for many purposes. The increased sensitivity of our new assay resulted from the relative resistance of human

[1] Abbreviations used are: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SULT, sulfotransferase; PAPSS, 3′-phosphoadenosine 5′-phosphosulfate synthetase; E2, estradiol; DHEA, dehydroepiandrosterone; DTT, dithiothreitol; HSS, high-speed supernatant.

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Step A

$$2 \text{ ATP } + \text{ SO}_4^{2-} \xrightarrow{\textbf{PAPSS}} \text{PAPS } + \text{ ADP } + \text{ PP}_i$$

Step B

17β-Estradiol   17β-Estradiol 3-O-Sulfate
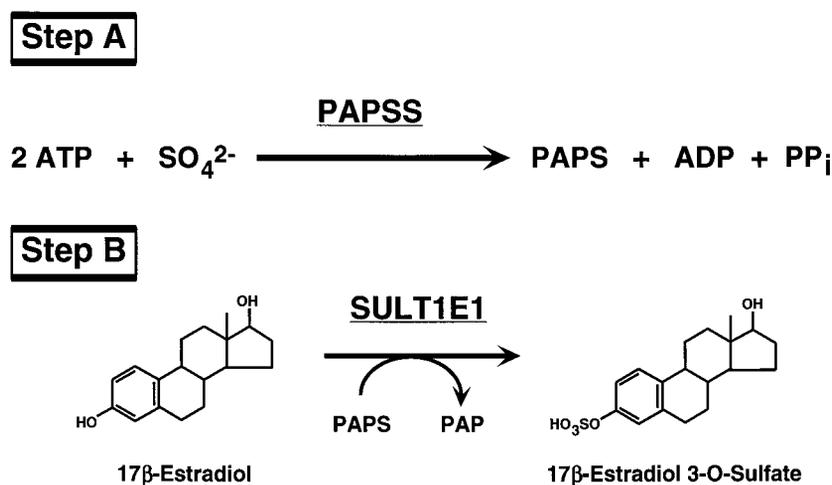
Fig. 1. *PAPSS-coupled assay.*
Step A, PAPSS catalyzed synthesis of PAPS from ATP and SO_4^{2-}. Step B, SULT1E1 catalyzed, PAPS-dependent sulfate conjugation of 17β-estradiol.

SULT1E1 to inhibition by ATP, the low K_m values of SULT1E1 for both E2 and PAPS, and the commercial availability of high specific activity radioactively labeled E2. We have used this new assay both to determine selected biochemical properties of PAPSS in human tissue preparations and to measure PAPSS activity in 83 human hepatic biopsy samples to study the nature and extent of individual variation of this activity in an important drug-metabolizing organ, the liver.

Materials and Methods

Chemicals and Reagents. 4-Nitrophenol, dopamine, E2, dehydroepiandrosterone (DHEA), ATP, Na_2SO_4, PAPS (80% purity), dithiothreitol (DTT), bovine serum albumin, and $NaClO_3$ were purchased from Sigma Chemical Co. (St Louis, MO). Purified PAPS (>95% purity) was obtained from Dr. S. Singer, Dayton University, Dayton, OH. [^{35}S]PAPS (2.52 Ci/mmol) and [2,4,6,7-^{3}H]E2 (72 Ci/mmol) were purchased from New England Nuclear (Boston, MA).

Tissue Acquisition and Preparation. Human liver ($N = 83$, 43 samples from men and 40 from women) and temporal lobe cerebral cortical ($N = 6$, two samples from men and four from women) surgical biopsy samples were obtained from the operating rooms at St. Mary's and Rochester Methodist hospitals in Rochester, MN. These tissue samples were removed in the course of clinically indicated surgical procedures under guidelines reviewed and approved by the Mayo Clinic Institutional Review Board. Portions of liver or cerebral cortical "waste tissue" distant from regions affected by disease and that appeared visually normal were stored at −80°C. Before enzyme assay, frozen tissue was weighed and homogenized in 5 mM potassium phosphate buffer, pH 7.5. The homogenates were centrifuged at 15,000g for 15 min, and the resulting supernatant preparations were centrifuged at 100,000g for 1 h. All steps were performed at 4°C. The final 100,000g "high-speed supernatant" (HSS) preparations were stored at −80°C. Pooled samples that included equal volumes of HSS from 10 liver or six brain biopsy samples were used to determine optimal conditions for the enzyme assay as well as the biochemical properties of PAPSS activity in these two tissues.

Recombinant SULTs. cDNAs for SULT1A1 (GenBank accession no. L19999), SULT1A3 (GenBank accession no. U08032), SULT1E1 (GenBank accession no. U08098), and SULT2A1 (GenBank accession no. U08024) were cloned into the mammalian expression vectors p91023(B) or pCR3.1. Each of these expression constructs was then used to transfect COS-1 cells with the DEAE-dextran method (Luthman and Magnusson, 1983). After the transfected cells were harvested, cell pellets were washed with 5 ml of phosphate-buffered saline and were homogenized for 30 s in 2 ml of 5 mM potassium phosphate buffer, pH 6.5. The homogenates were centrifuged for 15 min at 15,000g at 4°C, and supernatants from that step were centrifuged at 100,000g for 1 h at 4°C. The final HSS preparations were used as a source of recombinant human SULTs.

SULT Assays. The activities of recombinant human SULT1A1, SULT1A3, SULT1E1, and SULT2A1 were determined with the method of Foldes and Meek (1973) modified to measure the activities of each of these isoforms under optimal conditions with isoform-specific "prototypic" substrates (Anderson and Weinshilboum, 1980; Campbell et al., 1987; Sundaram et al., 1989; Otterness et al., 1992; Aksoy et al., 1994). All of these SULT assays used 0.4 μM [^{35}S]PAPS as a sulfate donor cosubstrate. Blanks were samples that did not contain a sulfate acceptor substrate. Reactions were terminated by the precipitation of unreacted PAPS with $Ba(OH)_2$. The sulfate acceptor substrates used in these reactions were 4 μM 4-nitrophenol for SULT1A1, 60 μM dopamine for SULT1A3, 0.1 μM E2 for SULT1E1, and 5 μM DHEA for SULT2A1. These assays were used to study the inhibition of recombinant human SULTs by ATP.

A different assay was used to measure apparent K_m values for SULT1E1. This assay used radioactively labeled E2, the sulfate acceptor cosubstrate, rather than radioactively labeled PAPS (Falany et al., 1995). Unconjugated E2 was then removed by organic solvent extraction, as described subsequently, and the radioactivity of the sulfate-conjugated steroid that remained in the aqueous phase was measured.

Protein Assay. Protein concentrations were measured by the dye-binding method of Bradford (1976) with bovine serum albumin as a standard.

PAPSS Assay. The first step in the PAPSS assay involved the generation of PAPS from ATP and Na_2SO_4 (Fig. 1, step A), followed by measurement of the PAPS that had been formed by using it as a substrate for the sulfate conjugation of [^{3}H]E2 in a reaction catalyzed by recombinant human SULT1E1 (Fig. 1, step B). Specifically, PAPS was generated in the presence of 1 mM ATP, 4 mM Na_2SO_4, 1 mM $MgCl_2$, and 2 mM DTT dissolved in 60 mM glycine-NaOH buffer, pH 8.6. The reaction was initiated by the addition to a 100-μl volume that contained the other reagents of a 50-μl enzyme source that contained either 50 or 100 μg of tissue cytosol protein. Blanks were samples that contained 50 μl of tissue cytosol that had been heated at 100°C for 5 min. This final 150-μl reaction mixture was incubated at 37°C for 20 min, and the PAPSS reaction was terminated by heating at 100°C for 1 min. Subsequently, 150 μl of 50 mM potassium phosphate buffer, pH 5.5, was added to lower the pH to 7.0. This mixture was then centrifuged for 5 min at 16,000g in an Eppendorf model 5415C desktop centrifuge (Brinkmann Instruments, Westbury, NY) to precipitate protein. One hundred microliters of the supernatant after centrifugation was added to 400 μl of 5 mM potassium phosphate buffer, pH 6.5, to adjust the pH to that required for the second stage reaction. A 50-μl aliquot of this 500-μl volume was used as a source of PAPS during the second step in the assay.

That second step (Fig. 1, step B) involved a 20-min incubation at 37°C in a final volume of 160 μl that contained 10 mM potassium phosphate buffer, pH 6.5, 27 nM [^{3}H]E2 (the radioactively labeled sulfate acceptor substrate), 8 mM DTT, and 1.25 mM $MgCl_2$. The 160-μl volume included 50 μl of recombinant

human SULT1E1. The SULT1E1-catalyzed sulfate conjugation reaction was terminated by the addition of 1 ml of 10 mM KOH, and organic solvent extraction was performed twice with 3 ml of chloroform. Five hundred and fifty microliters of the aqueous phase remaining after the two chloroform "washes" was aspirated and mixed with 5 ml of Biosafe II to make it possible to measure the radioactivity of the sulfate-conjugated [^{3}H]E2 formed during the second stage reaction. To quantitate PAPS, a series of known concentrations that ranged from 7.8 to 62.5 nM, a range chosen on the basis of PAPSS activity present in hepatic tissue samples, was incubated under the same conditions as those present in the second step of the assay. This standard curve was used to determine the concentration of PAPS formed during the first step of the assay. In addition, a single pooled human liver cytosol preparation was included with each set of assays as a "positive control". We observed less than a 5% day-to-day variation in PAPSS activity measured in this control sample.

Data Analysis. Apparent K_m values were calculated with the method of Wilkinson (1961) using a computer program written by Cleland (1963). IC$_{50}$ values for the inhibition of enzyme activity were estimated from least-squares best fit polynomial equations by using the GraphPad Inplot program, version 3.1 (GraphPad Software Inc., San Diego, CA). Statistical analyses involved the use of unpaired Student's t test, the Mann-Whitney U test, and correlation analysis performed with the StatView program, version 4.5 (Abacus Concepts, Berkeley, CA).

Results

These studies involved three sets of experiments. The first set was designed to determine whether it would be possible to identify a recombinant human SULT with optimal properties for use as a reagent in a sensitive coupled radiochemical PAPSS assay. Once such a SULT had been identified, assay conditions were optimized with human tissue preparations as an enzyme source, and the assay was used to determine selected biochemical properties of PAPSS activity in two human tissues with differing patterns of PAPSS isoform mRNA expression—liver and brain. Tissue preparations were used to perform these experiments because tissue, not purified enzyme, will be used to perform future genotype-phenotype correlation analyses. The third and final set of experiments involved a determination of the nature and extent of individual variation in level of PAPSS activity in an important human drug-metabolizing organ, the liver.

Recombinant Human SULT Selection. The strategy used in the final coupled assay is depicted schematically in Fig. 1. For this strategy to succeed required the identification of a SULT with optimal properties for use in the PAPS measurement step (Fig. 1, step B). All SULTs in higher organisms use PAPS as a cosubstrate (Klaassen and Boles, 1997). Therefore, any SULT-catalyzed reaction could potentially be used for this purpose, and similar coupled assays have been described previously, usually using cytosol from organs that predominantly express phenol SULTs such as SULT1A1 (Hazelton et al., 1985; Vargas, 1988; Wong et al., 1990; Wong and Wong, 1994). Unfortunately, the sensitivity of previous coupled PAPSS assays was limited. That was true in part because ATP, one of the cosubstrates for PAPSS (Fig. 1, step A), inhibits all SULTs (Rens-Domiano and Roth, 1987). Therefore, we set out to identify a SULT that was both relatively resistant to inhibition by ATP and that also had high affinity for its substrates—PAPS and the sulfate acceptor cosubstrate. Low K_m values for substrates would mean that it would be possible to add only a small aliquot of the PAPS-generating reaction to the second, coupled reaction. As a result, the inhibitory effect of ATP could be further minimized. As described subsequently, human SULT1E1 proved to have ideal properties for use in a coupled PAPSS assay.

We began by comparing the inhibition of four different recombinant human SULTs by ATP (Fig. 2). Human SULTs belong to two large families: phenol SULTs and hydroxysteroid SULTs (Weinshilboum et al., 1997). We selected three well characterized members of the phenol SULT family: SULT1A1, SULT1A3, and SULT1E1,
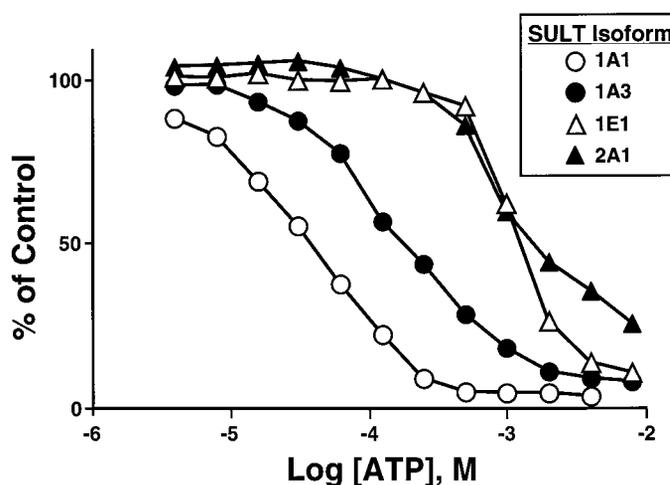

FIG. 2. *Inhibition of recombinant human SULT isoforms by ATP.*

The activities of recombinant human SULT1A1, 1A3, 1E1, and 2A1 were measured under optimal assay conditions in the absence of ATP and in the presence of a series of concentrations of ATP using the prototypic substrates 4 μM 4-nitrophenol (SULT1A1), 60 μM dopamine (SULT1A3), 0.1 μM E2 (SULT1E1), and 5 μM DHEA (SULT2A1). Activities shown are expressed as a percentage of control values.

and one hydroxysteroid SULT, SULT2A1, for study. The activities of these four recombinant human SULT isoforms were then assayed using prototypic substrates for each isoform in the presence of a series of concentrations of ATP that ranged from 4 to 8000 μM (Fig. 2). IC$_{50}$ values for the inhibition of SULT1A1, SULT1A3, SULT1E1, and SULT2A1 by ATP were 36, 205, 1300, and 2056 μM, respectively. These results demonstrated that human SULT1E1 and SULT2A1 were much more resistant to ATP inhibition than were SULT1A1 and SULT1A3. Therefore, SULT1E1 and SULT2A1, rather than the phenol SULTs that have been used in previous coupled PAPSS assays (Hazelton et al., 1985; Vargas, 1988; Wong et al., 1990; Wong and Wong, 1994), appeared to be superior for this application. However, because a sensitive assay for SULT1E1 had been described that was based on the use of commercially available [^{3}H]E2 as a sulfate acceptor substrate (Falany et al., 1995), we began by evaluating SULT1E1.

The next step in the evaluation involved a determination of apparent K_m values of SULT1E1 for PAPS and E2. Concentrations of [^{3}H]E2 (18 Ci/mmol) that ranged from 0.8 to 100 nM were used to determine the apparent K_m value of SULT1E1 for E2 in the presence of 0.4 μM PAPS. Figure 3A is a double inverse plot of those data that demonstrates that SULT1E1, like most human SULTs (Weinshilboum and Otterness, 1994), displays substrate inhibition. The apparent K_m value was 29 nM. A series of PAPS concentrations that ranged from 8 to 500 nM was then tested in the presence of 27 nM [^{3}H]E2, and an apparent K_m value for PAPS of 58 nM was calculated. The high affinity of SULT1E1 for both E2 and PAPS, when combined with the availability of high specific activity radioactively labeled E2 and the relative resistance of SULT1E1 to inhibition by ATP, resulted in our selection of SULT1E1 for use in the second step of the assay.

PAPSS Assay Optimization and Enzyme Characterization. The next series of experiments was designed to determine optimal conditions for the coupled PAPSS assay and to use the assay to study selected biochemical characteristics of PAPSS activity in human liver and cerebral cortical preparations, tissue preparations like those that will be used in future genotype-phenotype correlation analyses. When the coupled assay was performed with both tissues as an enzyme source, the reaction was linear for at least 20 min (data not shown).
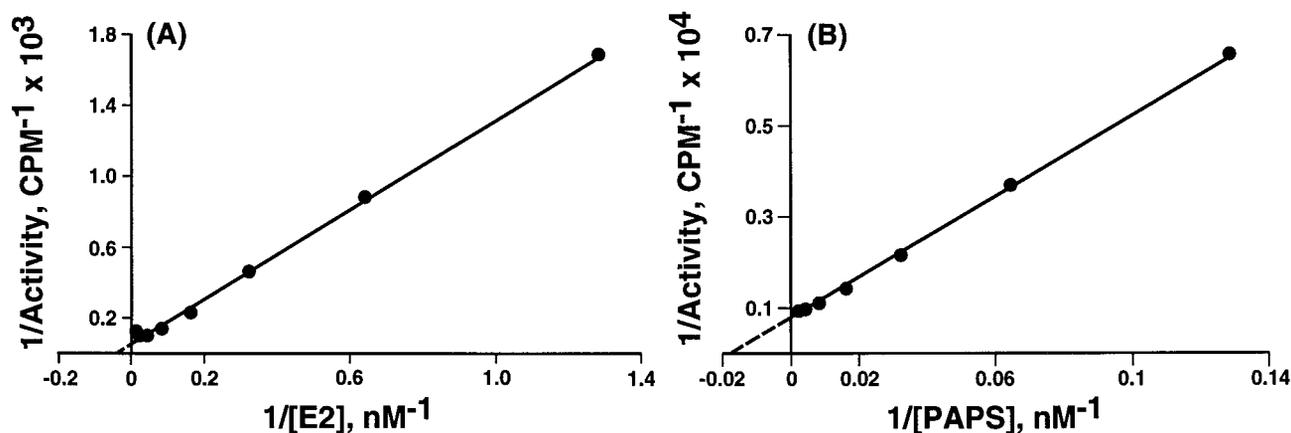
FIG. 3. *Human SULT1E1 substrate kinetics.*

SULT1E1 catalyzed sulfate conjugation of E2. Double reciprocal plots of data obtained with E2 (A) or PAPS (B) as the varied substrate are shown. Eight concentrations of E2 that varied from 0.8 to 100 nM were used, as were seven concentrations of PAPS that ranged from 8 to 500 mM. See text for details.

The formation of PAPS by liver and cerebral cortex preparations was also linear with regard to quantity of cytosol protein over a range from 12.5 to 100 μg of protein (data not shown). Finally, the optimal pH for PAPS formation was approximately 8.6 with both cytosol preparations as enzyme sources (data not shown). Therefore, either 50 or 100 μg of cytosol protein, a 20-min incubation time, and a pH of 8.6 were the assay conditions used to study selected biochemical properties of PAPSS in human liver and brain cytosol. In addition, during each assay a standard curve that used final concentrations of PAPS that ranged from 7.8 to 62.5 nM was also measured in the second stage reaction mixture. This PAPS was dissolved in a mixture that included all components of the first-step assay since we found that failure to do so resulted, on average, in an increase of approximately 5% in the apparent concentration of PAPS. We also compared commercially available PAPS that was 80% pure with PAPS that was more than 95% pure as standards. There was no significant difference between results obtained with the two preparations as long as correction was made for the degree of purity. However, all of our data were obtained using highly purified PAPS (>95% purity) as a standard.

PAPSS1 is the major isoform expressed in human brain on the basis of Northern blot analysis, whereas PAPSS2 is the predominant isoform expressed in liver (Xu et al., 2000). Therefore, we used both brain and liver cytosol as enzyme sources to study PAPSS substrate kinetics in tissue preparations. It should be emphasized that this was done with the understanding that kinetic constants obtained with tissue preparations represent only estimates. However, an important future application of this assay will involve genotype-phenotype correlation analyses performed with tissue preparations. A series of ATP (0.125–8 mM) and Na_2SO_4 (0.125–32 mM) concentrations was used to estimate apparent K_m values for these two cosubstrates in liver and brain preparations. When ATP was the varied substrate, the concentration of Na_2SO_4 used was 4 mM, and when Na_2SO_4 was the varied substrate, the ATP concentration was 1 mM. The experimental basis for the selection of these concentrations will be described subsequently. Double inverse plots of the effect of varying ATP concentration for both tissues are shown in Fig. 4, and apparent K_m values, values calculated using only data that did not display substrate inhibition, are listed in Table 1. The value for brain was significantly lower than that for liver. However, both values are very similar to those reported previously for human liver PAPSS (Wong et al., 1990). The 1 mM ATP concentration used in the assay was selected because it resulted in maximal activity under these assay conditions.

Double inverse plots of data for liver and cerebral cortex with Na_2SO_4 as the varied substrate are shown in Fig. 5. These data seemed to indicate the presence of both "high"- and "low"-affinity components of the double inverse plots. Apparent K_m values for these two kinetic components are listed in Table 1. In each case, the contribution of the high-affinity component was subtracted before calculation of the apparent K_m value for the low-affinity component. To ensure that the biphasic kinetics did not result from the effects of increasing concentrations of Na_2SO_4 on the second step of the assay (the step catalyzed by SULT1E1), an experiment was performed during which 15.6 nM PAPS was added to the second stage reaction in the presence of six different concentrations of Na_2SO_4 that were the equivalent of from 1 to 32 mM in the first stage reaction. These concentrations of Na_2SO_4 had no effect on the second stage reaction.

The underlying molecular mechanism responsible for the biphasic kinetics observed with Na_2SO_4 remains unclear. These curves could indicate either the existence of two separate enzymes with very different K_m values for Na_2SO_4, or the presence of substrate-dependent variation in the kinetic properties of a single enzyme. However, the biphasic kinetics probably does not result from the effects of the two known isoforms of PAPSS because the low-affinity component, with an apparent K_m of >20 mM, could not be of physiological importance. Therefore, an Na_2SO_4 concentration of 4 mM, one that would measure only the high-affinity component of the curves shown in Fig. 5, was selected for use in the assay. It should also be noted that apparent K_m values for the high-affinity components of the Na_2SO_4 curves differed significantly in the two tissues studied, with values of 0.31 ± 0.031 mM (mean ± S.D.) in the liver and 0.08 ± 0.004 mM in brain ($p < 0.0001$ by Student's t test). Use of 1 mM ATP and 4 mM Na_2SO_4, a 20-min incubation time, a pH of 8.6, and either 50 or 100 μg of cytosol protein resulted in "signal-to-noise" ratios for the assay of approximately 7 for a pooled human liver cytosol sample and approximately 5 for a pooled cerebral cortical cytosol preparation.

Since we had observed significant differences in apparent K_m values for SO_4^{2-} between the two tissues studied, we also studied the inhibition of PAPSS activity in these two tissues by chlorate, a competitive inhibitor of PAPSS with regard to SO_4^{2-} (Baeuerle and Huttner, 1986). When a series of concentrations of sodium chlorate was tested, this compound, as anticipated, inhibited PAPSS activity in both pooled human hepatic and cerebral cortical cytosol preparations (Fig. 6). Furthermore, chlorate inhibition of PAPSS activity in brain and liver differed significantly, with IC_{50} values of 6.57 ± 0.26 (mean ± S.D.) and 3.26 ± 0.13 mM for these two tissues, respectively
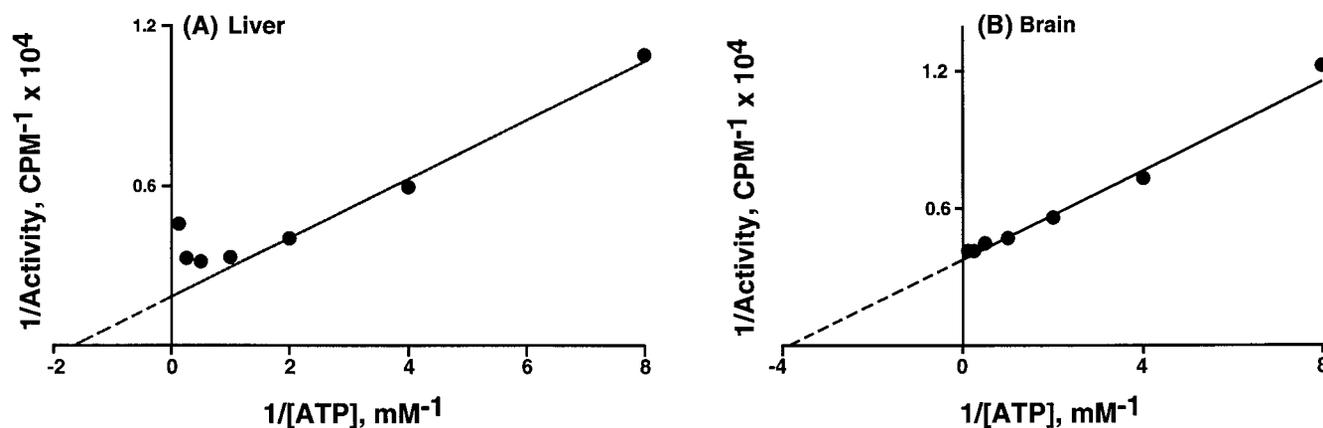
FIG. 4. *Human PAPSS substrate studies.*

Effect of ATP concentration on the PAPSS-catalyzed formation of PAPS with either human liver (A) or cerebral cortical (B) cytosol as an enzyme source. Double reciprocal plots for both tissues are shown for data obtained with seven concentrations of ATP that varied from 0.125 to 8 mM. The concentration of Na_2SO_4 in all assays was 4 mM. See text for details.

TABLE 1

PAPSS apparent K_m values for ATP and SO_4^{2-} measured with pooled human liver or brain cytosol as an enzyme source

Values are mean ± S.D.

Tissue	mRNA Expression Pattern	ATP K_m	SO_4^{2-} K_m	
			High-Affinity	Low-Affinity
		mM	*mM*	
Liver	PAPSS2 ≫ PAPSS1	0.62 ± 0.03*	0.31 ± 0.031*	21.9 ± 7.3
Brain	PAPSS1 ≫ PAPSS2	0.26 ± 0.01	0.08 ± 0.004	25.2 ± 4.5

* Differs from the value for brain with $p < 0.0001$.

($p < 0.0001$) (Fig. 6). The next studies, experiments that represented a step toward future genotype-phenotype analyses, involved use of this coupled assay to determine the nature and extent of individual variation of PAPSS activity in an important human drug-metabolizing organ, the liver.

Hepatic PAPSS Activity. PAPSS activity was measured in 83 surgical biopsy samples of hepatic tissue obtained from 43 men and 40 women. These samples had been collected over the past 3 years during clinically indicated surgical procedures. The coefficient of variation for assay of the same sample daily for 7 days was 4.3%, whereas the intra-assay coefficient of variation for a single sample assayed 12 times was 1.8%. The average level of PAPSS activity in these 83 samples was 8.43 ± 5.85 nmol/h/mg protein (mean ± S.D.), with an 18-fold interindividual variation (Fig. 7). The frequency distribution shown in Fig. 7 was skewed, with a small number of samples having relatively high levels of enzyme activity, greater than 20 nmol/h/mg protein. We attempted to determine whether this variation in level of PAPSS activity might be systematically related to the time of tissue storage at −80°C. There was not a significant correlation between time of tissue storage and level of enzyme activity ($r_s = 0.145$, $p = 0.196$). We next asked whether subject age or gender might be related to level of hepatic PAPSS activity. There was also not a significant relationship between subject age and PAPSS activity ($r_s = 0.130$, $p = 0.245$). Finally, even though the average level of activity in hepatic tissue from men was higher than that for women, 9.52 ± 6.33 nmol/h/mg protein (mean ± S.D.) versus 7.46 ± 5.20, this difference was not statistically significant ($p = 0.19$ by Mann-Whitney U test).
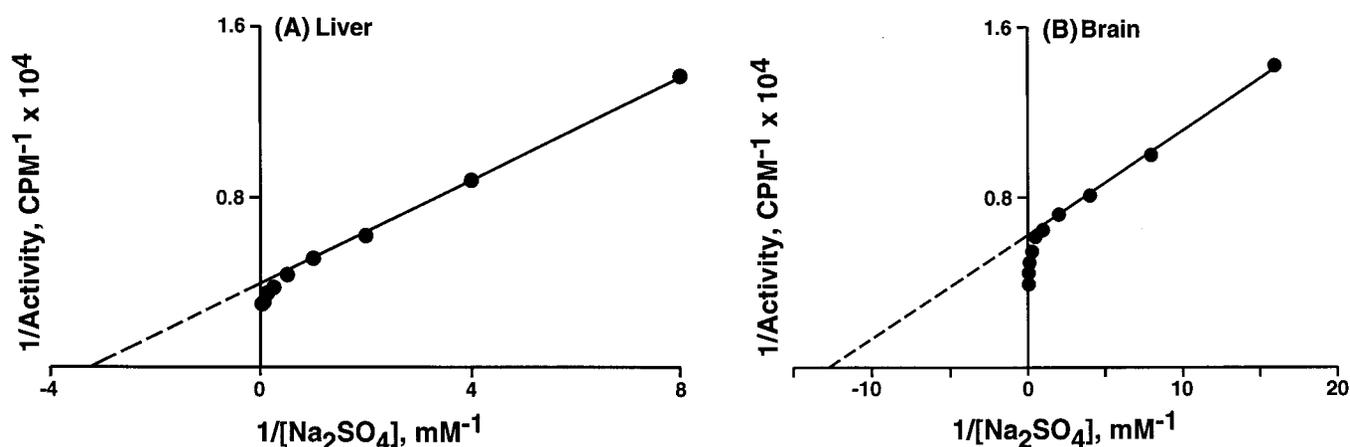
Discussion

We have developed a sensitive radiochemical enzymatic assay for the measurement of PAPSS activity that is suitable for use in genotype-phenotype correlation analyses as a step toward future pharmacogenetic studies. The principle underlying this coupled, two-step radiochemical assay is depicted schematically in Fig. 1. ATP is a substrate for PAPSS, but it is also a potent competitive inhibitor of SULTs (Rens-Domiano and Roth, 1987). Therefore, we initially determined whether there might be significant differences among recombinant human SULTs in their sensitivity to inhibition by ATP. We found that SULT1E1 and SULT2A1 were much more resistant to ATP inhibition than were the phenol SULTs studied (Fig. 2). In addition, SULT1E1 had low K_m values for both of its cosubstrates, E2 and PAPS. As a result of these observations, and because of the commercial availability of high specific activity [^{3}H]E2, SULT1E1 was selected for use in the PAPS measurement step of the coupled assay. The final concentration of ATP present in that step, after dilution of the reaction mixture from the first step, was approximately 30 μM, a concentration that does not inhibit SULT1E1 (Fig. 2). By taking this approach, we were able to eliminate a significant problem that has been encountered in the course of previously described coupled PAPSS assays, since ATP that is "carried over" from the initial PAPS-generating step inhibited the second stage reaction when phenol SULTs or tissue cytosol was used as an enzyme source (Hazelton et al., 1985; Vargas, 1988; Wong et al., 1990; Wong and Wong, 1994).

Human liver and brain have very different patterns of expression for PAPSS isoforms on the basis of Northern blot analysis (Xu et al., 2000). Liver displayed a high level of expression of PAPSS2 mRNA relative to PAPSS1 mRNA, whereas the reverse was true of human brain. We found that PAPSS activity in liver cytosol had slightly higher apparent K_m values for both ATP and SO_4^{2-} than did brain cytosol (Table 1). In addition, substrate kinetic studies performed with Na_2SO_4 as the varied cosubstrate displayed biphasic behavior in both tissues (Fig. 5). It is unclear whether the high- and low-affinity activities with regard to SO_4^{2-} result from the effects of two separate enzymes or merely a single enzyme with kinetic characteristics that vary with substrate concentration. However, it is clear that the apparent low-affinity activity cannot be of physiological significance since serum SO_4^{2-} concentrations are estimated to be approximately 0.4 mM (Morris and Levy, 1983), well below the apparent K_m value for

FIG. 5. *Human PAPSS substrate kinetic studies.*

Effect of Na_2SO_4 concentration on the PAPSS-catalyzed formation of PAPS with either human liver (A) or cerebral cortical (B) cytosol as an enzyme source. Double reciprocal plots for both tissues are shown for data obtained with a series of concentrations of Na_2SO_4. The concentration of ATP in all assays was 1 mM. See text for details.
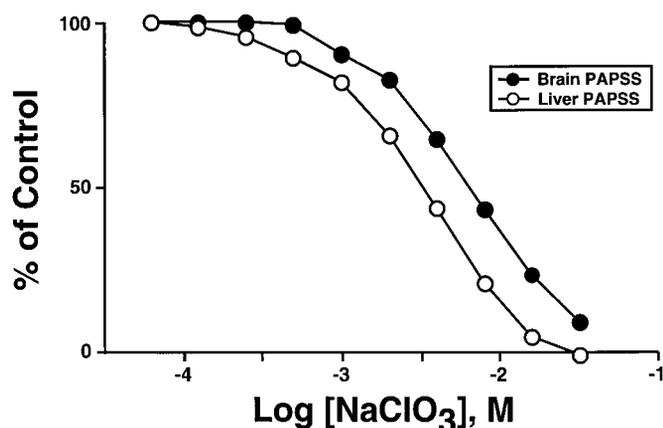

FIG. 6. *Chlorate inhibition of PAPSS in human liver or cerebral cortical cytosol.*

PAPSS activity was measured in the absence of sodium chlorate and in the presence of a series of concentrations of sodium chlorate. ATP (1 mM) and Na_2SO_4 (4 mM) were present in all assays.

FIG. 7. *Human liver PAPSS activity.*

Frequency distribution histogram of PAPSS activity in hepatic biopsy samples from 83 patients (43 males and 40 females). See text for details.

the low-affinity activity. Therefore, we used an SO_4^{2-} concentration of 4 mM in the assay. As a result, the low-affinity component with regard to Na_2SO_4 would not contribute significantly to the activity measured. It should be emphasized that biochemical characteristics measured in tissue preparations must be viewed with caution, and cannot substitute for values measured with purified and/or recombinant enzyme. A further complicating factor that will have to be considered in future studies of PAPSS activity in tissues is the recent report of the nuclear localization of PAPSS1 (Besset et al., 2000). The present studies were performed primarily to optimize the assay, an assay that could be used, among other applications, to measure PAPSS activity both in recombinant enzyme and in tissue preparations. However, future experiments performed with tissue preparations will have to take into account potential differences in the subcellular localization of PAPSS isoforms.

Finally, in an attempt to begin the process of characterizing the nature and extent of individual variation in PAPSS activity in human tissues, we phenotyped 83 human liver surgical biopsy tissue samples for level of activity. Levels of PAPSS activity in these 83 tissue biopsy samples varied over an 18-fold range (Fig. 7). That variation
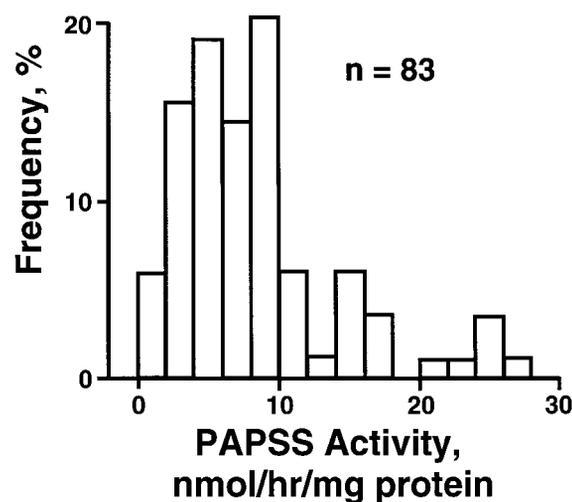
was not significantly related to either time of tissue storage or subject age. The molecular mechanism or mechanisms responsible for this wide variation in level of activity among individuals will be the subject of future studies.

In summary, we have developed and optimized a sensitive, coupled radiochemical enzymatic assay for the determination of PAPSS activity. The selection of recombinant SULT1E1 for use in the PAPS measurement step represented a critical factor for assay sensitivity. We then used the assay to study selected biochemical characteristics of PAPSS activity in two important human tissues—liver and brain. We also used the assay to perform an initial study of the nature and extent of individual variation in human hepatic PAPSS activity. All of these experiments represent steps toward an eventual study of mechanisms, including pharmacogenetic mechanisms, that might participate in the regulation of PAPSS in humans.

References

Anderson RJ and Weinshilboum RM (1980) Phenolsulphotransferase in human tissue: Radiochemical enzymatic assay and biochemical properties. *Clin Chim Acta* **103:**79–90.

Aksoy IA, Wood TC and Weinshilboum R (1994) Human liver estrogen sulfotransferase: Identification by cDNA cloning and expression. *Biochem Biophys Res Commun* **200:**1621–1629.

Besset S, Vincourt J-B, Amalric F and Girard J-P (2000) Nuclear localization of PAPS synthetase 1: A sulfate activation pathway in the nucleus of eukaryotic cells. *FASEB J* **14:**345–354.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72:**248–254.

Baeuerle PA and Huttner WB (1986) Chlorate—a potent inhibitor of protein sulfation in intact cells. *Biochem Biophys Res Commun* **141:**870–877.

Campbell NRC, Van Loon JA, Sundaram RS, Ames MM, Hansch C and Weinshilboum R (1987) Human and rat liver phenol sulfotransferase: Structure-activity relationships for phenolic substrates. *Mol Pharmacol* **32:**813–819.

Cleland WW (1963) Computer programmes for processing enzyme kinetic data. *Nature (Lond)* **198:**463–465.

Falany CN (1997) Enzymology of human cytosolic sulfotransferases. *FASEB J* **11:**206–216.

Falany CN, Krasnykh V and Falany JL (1995) Bacterial expression and characterization of a cDNA for human liver estrogen sulfotransferase. *J Steroid Biochem Mol Biol* **52:**529–539.

Farooqui AA (1980) 3′-Phosphoadenosine-5′-phosphosulfate metabolism in mammalian tissues. *Int J Biochem* **12:**529–536.

Foldes A and Meek JL (1973) Rat brain phenolsulfotransferase: Partial purification and some properties. *Biochim Biophys Acta* **327:**365–374.

Geller DH, Henry JG, Belch J and Schwartz NB (1987) Co-purification and characterization of ATP-sulfurylase and adenosine-5′-phosphosulfate kinase from rat chondrosarcoma. *J Biol Chem* **262:**7374–7382.

Girard J-P, Baekkevold ES and Amalric F (1998) Sulfation in high endothelial venules: Cloning and expression of the human PAPS synthetase. *FASEB J* **12:**603–612.

Hazelton GA, Hjelle JJ, Dills RL and Klaassen CD (1985) A radiometric method for the measurement of 3′-phosphoadenosine-5′-phosphosulfate in rat and mouse liver. *Drug Metab Dispos* **13:**30–34.

Klaassen CD and Boles JW (1997) The importance of 3′-phosphoadenosine-5′-phosphosulfate (PAPS) in the regulation of sulfation. *FASEB J* **11:**404–418.

Kurima K, Singh B and Schwartz NB (1999) Genomic organization of the mouse and human genes encoding the ATP sulfurylase/adenosine 5′-phosphosulfate kinase isoform SK2. *J Biol Chem* **274:**33306–33312.

Kurima K, Warman ML, Krishnan S, Domowicz M, Krueger RC Jr, Deyrup A and Schwartz NB (1998) A member of a family of sulfate-activating enzymes causes murine brachymorphism. *Proc Natl Acad Sci USA* **95:**8681–8685.

Li H, Deyrup A, Mensch JR Jr, Domowicz M, Konstantinidis AK and Schwartz NB (1995) The isolation and characterization of cDNA encoding the mouse bifunctional ATP sulfurylase-adenosine 5′-phosphosulfate kinase. *J Biol Chem* **270:**29453–29459.

Luthman H and Magnusson G (1983) High efficiency polyoma DNA transfection of chloroquine treated cells. *Nucleic Acids Res* **11:**1295–1308.

Lyle S, Stanczak J, Ng K and Schwartz NB (1994) Rat chondrosarcoma ATP sulfurylase and adenosine 5′-phosphosulfate kinase reside on a single bifunctional protein. *Biochemistry* **33:**5920–5925.

Morris ME and Levy G (1983) Serum concentration and renal excretion by normal adults of inorganic sulfate after acetaminophen, ascorbic acid, or sodium sulfate. *Clin Pharmacol Ther* **33:**529–536.

Otterness DM, Wieben ED, Wood TC, Watson WG, Madden BJ, McCormick DJ and Weinshilboum RM (1992) Human liver dehydroepiandrosterone sulfotransferase: Molecular cloning and expression of cDNA. *Mol Pharmacol* **41:**865–872.

Rens-Domiano SS and Roth JA (1987) Inhibition of M and P phenol sulfotransferase by analogues of 3′-phosphoadenosine 5′-phosphosulfate. *J Neurochem* **48:**1411–1415.

Sundaram RS, Szumlanski C, Otterness D, Van Loon JA and Weinshilboum RM (1989) Human intestinal phenol sulfotransferase: Assay conditions, activity levels and partial purification of the thermolabile form. *Drug Metab Dispos* **17:**255–264.

ul Haque MF, King LM, Krakow D, Cantor RM, Rusiniak ME, Swank RT, Superti-Furga A, Haque S, Abbas H, Ahmad W, Ahmad M and Cohn DH (1998) Mutations in orthologous genes in human spondyloepimetaphyseal dysplasia and the brachymorphic mouse. *Nat Genet* **20:**157–162.

Vargas F (1988) Preparation and quantification of 3′-phosphoadenosine-5′-phospho[35S]sulfate with high specific activity. *Anal Biochem* **172:**82–88.

Venkatachalam KV, Akita H and Strott CA (1998) Molecular cloning, expression and characterization of human bifunctional 3′-phosphoadenosine 5′-phosphosulfate synthase and its functional domains. *J Biol Chem* **273:**19311–19320.

Weinshilboum RM and Otterness DM (1994) Sulfotransferase enzymes, in *Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity* (Kaufmann FC ed), chapter 22, Handbook of Experimental Pharmacology series, vol 112, pp 45–78, Springer Verlag, Berlin.

Weinshilboum RM, Otterness DM, Aksoy IA, Wood TC, Her C and Raftogianis RB (1997) Sulfotransferase molecular biology: cDNAs and genes. *FASEB J* **11:**3–14.

Wilkinson GN (1961) Statistical estimation in enzyme kinetics. *Biochem J* **80:**324–332.

Wong KO and Wong KP (1994) Biosynthesis of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) in rat skin. *Biochem Pharmacol* **47:**477–483.

Wong KP, Khoo BY and Sit KH (1990) Biosynthesis of PAPS in vitro by human liver: Measurement by two independent assay procedures. *Biochem Pharmacol* **41:**63–69.

Xu ZH, Otterness DM, Freimuth RR, Carlini EJ, Wood TC, Mitchell S, Moon E, Kim UJ, Xu JP, Siciliano MJ and Weinshilboum RM (2000) Human 3′-phosphoadenosine 5′-phosphosulfate synthetase 1 (PAPSS1) and 2 gene cloning, characterization and chromosomal localization. *Biochem Biophys Res Commun* **268:**437–444.