FEBS 24579 FEBS Letters 490 (2001) 39–43

The dimerization motif of cytosolic sulfotransferases

Evgeniy V. Petrotchenko[a], Lars C. Pedersen[a], Christoph H. Borchers[b], Kenneth B. Tomer[b], Masahiko Negishi[a,*]

[a] *Pharmacogenetics Section, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA*
[b] *Laboratory of Structural Biology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA*

Received 9 November 2000; revised 20 December 2000; accepted 5 January 2001

First published online 23 January 2001

Edited by Hans Eklund

Abstract Cytosolic sulfotransferases sulfate steroids such as estrogens and hydroxysteroids. The enzymes, including human estrogen sulfotransferase (hEST) and hydroxysteroid sulfotransferase (hHST), are generally homodimers in solution with mouse estrogen sulfotransferase (mEST) being one of few exceptions. To identify the amino acid residues responsible for the dimerization, eight residues on the surface of hEST were mutated to their counterparts in mEST and mutated hESTs were then analyzed by gel filtration chromatography. A single mutation of Val^{269} to Glu was sufficient to convert hEST to a monomer and the corresponding mutation of Val^{260} also altered hHST to a monomer. The hHST crystal structure revealed a short stretch of peptide with the side-chains from two hHST monomers forming a hydrophobic zipper-like structure enforced by ion pairs at both ends. This peptide consisted of 10 residues near the C-terminus that, including the critical Val residue, is conserved as KXXXTVXXXE in nearly all cytosolic sulfotransferases. When mEST underwent the double mutations Pro269Thr/Glu270Val dimerization resulted. Thus, the KXXXTVXXXE sequence appears to be the common protein–protein interaction motif that mediates the homo- as well as heterodimerization of cytosolic sulfotransferases.

Key words: Sulfotransferase; Protein–protein interaction; Mass spectrometry; Crystal structure; Site-directed mutagenesis

1. Introduction

Cytosolic sulfotransferases catalyze the transfer of the sulfuryl group from the ubiquitous cofactor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to numerous substrates including steroids, bioamines (e.g. dopamine and catecholamine), therapeutic drugs, and environmental chemicals. While sulfation is generally considered to be deactivation and secretion processes of biological signal molecules and xenochemicals, sulfation/desulfation may also be involved in maintaining physiological homeostasis such as steroid hormones [1,2]. Cytosolic sulfotransferases consist of around 300 amino acid residues, and are generally present as dimers in solution. Interestingly, sulfotransferases appear to be capable of forming not only homodimers but also heterodimers. For example, the sulfation activity of *N*-hydroxy-2-acetylaminofluorene is associated with three different dimers of sulfotransferases from rat liver cytosol: ASTIV dimer, ST1C1 dimer, and ASTIV/STC1 heterodimer [3]. However, protein–protein interaction of sulfotransferases has not been well studied and the functional significance of the dimerization process is not known at the present time.

The crystal structures of three cytosolic sulfotransferases, mouse estrogen sulfotransferase (mEST), human hydroxysteroid sulfotransferase (hHST), and human aryl sulfotransferase (hAST, SULT1A3), have recently been solved [4–8]. These structures reveal a conserved PAPS-binding site and, also, an in-line displacement transfer of the sulfuryl group as the reaction mechanism. The binding site and the reaction mechanism are also conserved in Golgi membrane-bound sulfotransferases such as heparan sulfate *N*-deacetylase/*N*-sulfotransferases [9,10]. In addition to these structural features, the hHST as well as the hAST structures have substantially different properties near the substrate-binding site. This surface has been suggested as a possible dimerization interface based on buried surface area calculations and the involvement of this surface in protein–protein interactions in the crystals [4]. The question has arisen whether or not this crystallographic contact represents the physiological dimer interface of sulfotransferases in solution. Our present site-directed mutagenesis and mass spectrometric analyses on proteolytic peptides generated from hHST have failed to support the crystallographic interface as being the true dimer interface of hHST in solution. To identify residues that might regulate the dimerization, we have utilized the fact that human estrogen sulfotransferase (hEST) and mEST are highly homologous, yet the former enzyme is dimer and the latter is monomer. Comparing the primary and tertiary structures of two enzymes, surface residues of hEST are mutated to the correspondents in mEST. Various mutated hESTs are then expressed in bacterial cells and analyzed by gel filtration chromatography for their dimerization capability. Here we present the experimental considerations that lead us to define a short peptide of 10 residues near the C-terminus as the novel protein–protein interaction motif that mediates both homo- and heterodimerizations of cytosolic sulfotransferases in solution.

*Corresponding author. Fax: (1)-919-541 0696.
E-mail: negishi@niehs.nih.gov

PII: S0014-5793(01)02129-9

2. Materials and methods

2.1. Plasmids, mutations and bacterial expression

All enzymes were cloned into pGEX-4T3 vectors. hEST was subcloned using *Bgl*II and *Xho*I sites from pMAL-C2 vector kindly provided by C. Falany [11]. hHST DNA was amplified from human liver cDNA library using specific primers designed from the known sequence (GenBank accession, Q06520), and was cloned using *Eco*RI and *Xho*I sites, while mEST plasmid was previously constructed [12]. Mutations were introduced using QuickChange kit (Stratagene) and verified by DNA sequencing. Enzymes were expressed as glutathione S-transferase fusion proteins in *Escherichia coli* BL21DE3 cells, adsorbed on glutathione-Sepharose (Amersham Pharmacia Biotech), and subsequently eluted by thrombin cleavage (Sigma).

2.2. Gel filtration chromatography

25 μl of various samples was applied on a Superdex 75 HR10/30 column (Amersham Pharmacia Biotech) that was equilibrated with 20 mM phosphate buffer pH 7.4 containing 0.15 or 0.5 M NaCl. Proteins were eluted with the same buffer at a flow rate of 1.0 ml/min. Low molecular weight gel filtration calibration markers (Amersham Pharmacia Biotech) were used to calibrate the column.

2.3. Limited proteolysis, cross-linking and mass spectrometry

For limited proteolysis, 1 ml of 1 mg/ml hHST-His_6 in 20 mM phosphate buffer pH 7.4 containing 0.15 M NaCl was digested by 4 μl of 1 mg/ml of sequencing grade trypsin (Boehringer Mannheim Biochemical). The digest was treated with 1% AcOH for 15 min at 25°C, and was directly loaded on and separated by a C4 reversed phase high performance liquid chromatography (RP-HPLC) column (Hewlett Packard). Lyophilized elutes were analyzed by 15% SDS–PAGE to select the fractions containing digested peptides for matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analysis on a PE Biosystem Voyager System 4159 spectrometer (Applied Biosystems). For cross-linking, 300 μl of protein solution (1 mg/ml in 20 mM phosphate buffer pH 7.4 containing 0.15 M NaCl) was reacted with 10 μl 1 M ethyl (dimethyl aminopropyl) carbodiimide (EDC, Pierce) in water at 25°C for 2 h. Reaction mixtures were centrifuged. Then the supernatants were separated by a C4 RP-HPLC to obtain the cross-linked dimers, lyophilized, and analyzed by 15% SDS–PAGE. Subsequently, these dimers were digested by trypsin in 50 mM NH_4HCO_3 containing 30% methanol at 37°C for 4 h and tryptic peptides were applied to a MALDI-TOF PE Biosystem Voyager System 4159 spectrometer (Applied Biosystems). Cross-linked peptides were assigned based on additive calculated molecular mass (minus 19 for water molecule), and based on the presence of complementary Lys-Glu or Lys-Asp pair in all possible combinations of tryptic peptides.

2.4. Heterodimerization assay

10 μl aliquots of glutathione-Sepharose-bound GST-hEST, GST-hHST, GST-$hEST_{V269E}$ were mixed with 10 μl of hEST, hHST, or $hEST_{V269E}$ in 20 mM phosphate buffer pH 7.4 containing 0.5 M NaCl, then incubated for 2 h at 25°C with several gentle mixings. Settled samples were decanted, repetitively washed by 400 μl of the same buffer, and directly analyzed by a 15% SDS–PAGE

3. Results and discussion

3.1. Mutational and proteolytic analysis of hHST

Previous analysis of the hHST crystal structure showed protein–protein interactions near the substrate-binding site entrance [4]. The interface is a relatively flat hydrophobic surface of the molecule. In an attempt to examine whether this lattice contact may also provide the physiological dimer interface in solution, we mutated Leu^{233} of hHST, one of the central hydrophobic residues on the interface, to the negatively charged Glu. Since the interface is symmetrical in nature, the Leu233Glu mutation would be equivalent to the introduction of two repulsive negative charges in the center of hydrophobic interface of hHST. Although the mutation was thought to destabilize hHST dimer in solution, the mutated enzyme still remained in its dimer form based on gel filtration chromatography (data not shown).

We then performed trypsin digestion on hHST protein with and without chemical cross-linking. After the digest was separated by HPLC, the fractions were analyzed by MALDI-TOF mass spectrometry. First, peptides were generated from the non-cross-linked hHST by limited proteolysis and average isotopic molecular masses of these peptides were calculated to identify trypsin cleavage sites (Fig. 1A). These sites are mapped on the hHST structure (Fig. 2). One of the prominent cleavage sites was Lys^{227} that lies on the surface of the previous crystallographic dimer interface.

hHST protein was cross-linked by EDC at a 2 to 1 ratio of hHST molecule, followed by complete trypsin digestion. Again the digest was subjected to HPLC and MALDI-TOF mass spectrometry. Of all possible average isotopic molecular masses obtained, no peptide was consistent with masses predicted from possible cross-links between residues on the pre-

A.

Observed Mass	Corresponding Peptide	Cleavage Site
5233.82	248-292	Arg^{247}
5107.15	249-292	Lys^{248}
10142.05	34-121	Arg^{33}, Arg^{121}
9253.55	194-273	Arg^{193}, Lys^{273}
7537.43	228-292	Lys^{227}
6014.42	95-144	Arg^{94}, Lys^{144}
26921.01	1-227	Lys^{227}

B.

Mass		Residue			Residue		
Observed	Calculated	Start	End	Peptide A	Start	End	Peptide B
2900.91	2898.19	249	255	(K)GVSGDWK(N)	256	273	(K)NHFTVAQAEDFDKLFQEK(M)
3111.19	3110.40	248	255	(R)KGVSGDWK(N)	249	268	(K)GVSGDWKNHFTVAQAEDFDK(L)
3581.95	3582.06	249	255	(K)GVSGDWK(N)	256	279	(K)NHFTVAQAEDFDKLFQEKMADLPR(K)
3692.20	3692.12	243	255	(K)AQLLRKGVSGDWK(N)	249	268	(K)GVSGDWKNHFTVAQAEDFDK(L)

Fig. 1. MALDI-TOF analysis for tryptic peptides generated from hHST. Peptides generated by limited proteolysis (A) and EDC cross-linked peptides (B) were identified as described in Section 2. Residues with underline represent apparent sites for cross-links.

vious crystallographic interface. Instead, we obtained the peptides that represent cross-links between Asp^{253} in one monomer and Lys^{268} in the other monomer or Asp^{265} and Lys^{248} (Fig. 1B). These residues are located on the surface of hHST molecule away from the crystallographic interface. Therefore, both limited proteolysis and cross-linking experiments do not support our previous hypothesis that the surface near the substrate-binding site may constitute the physiological dimer interface. Consequently, we turned to mEST and hEST so as to utilize the fact that while these two share 77% amino acid sequence identity, the former is monomer, whereas the latter is a dimer.

3.2. *Mutation screening of hEST for dimer–monomer conversion*

To proceed, we constructed a model structure of hEST using homology-based modeling from the crystal structure of mEST (not shown), and selected eight surface residues that differ between the two EST enzymes for site-directed mutagenesis studies: residues 87, 96, 98, 111, 208, 269, 272, and 277. Then each of these residues of hEST was mutated to the corresponding residue in mEST: Asn87Asp, Asp96Lys, Met98Lys, Glu111Lys, His208Glu, Val269Glu, Asn272Arg, and Lys277Glu. The eight mutated enzymes were expressed in *E. coli* cells and analyzed through gel filtration chromatography for a dimerization profile. Only the Val269Glu mutation led the conversion of hEST dimer to a monomer (Fig. 3A). Thus, the chemical nature of the residue at position 269 may determine dimerization of hEST enzyme in solution.

Multiple amino acid sequence alignments showed that Val^{269} of hEST is conserved in a consensus sequence of KXXXTVXXXE residing near the C-terminus in almost all cytosolic sulfotransferases (Fig. 4). First of all, Val^{269} of hEST is conserved at position 260 in hHST, consistent with the fact that both enzymes are homodimers. The mutation of Val^{260} to Glu also converted hHST to a monomer (data not shown). On the other hand, mEST is monomeric in solution. This enzyme has Pro^{269} and Glu^{270} rather than Thr^{260}–Val^{270} as the consensus requires. When Pro^{269} and Glu^{270} of mEST were simultaneously mutated to Thr and Val, respectively, the mutated mEST behaved as the homodimer on a gel filtration chromatography (Fig. 3B). These results provide strong evidence that the KXXXTVXXXE sequence functions as the common protein–protein interaction motif that mediates the dimerization of cytosolic sulfotransferases.

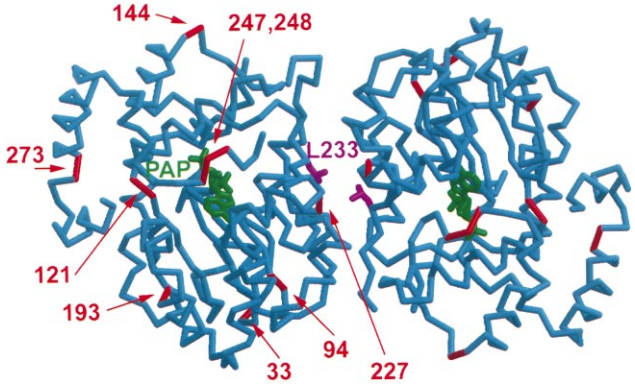

Fig. 2. Trypsin cleavage sites on the surface of hHST. The hHST X-ray crystal structure is depicted from our previous work [4] for which the numbers in red represent residues in the protein susceptible to trypsin digestion. Also shown in red is the side-chain of Leu^{233} that was subjected to site-directed mutagenesis to test for distribution of the physiological dimer. The PAP molecule is shown in green. This figure was created using MOLSCRIPT [18] and RASTER3D [19].

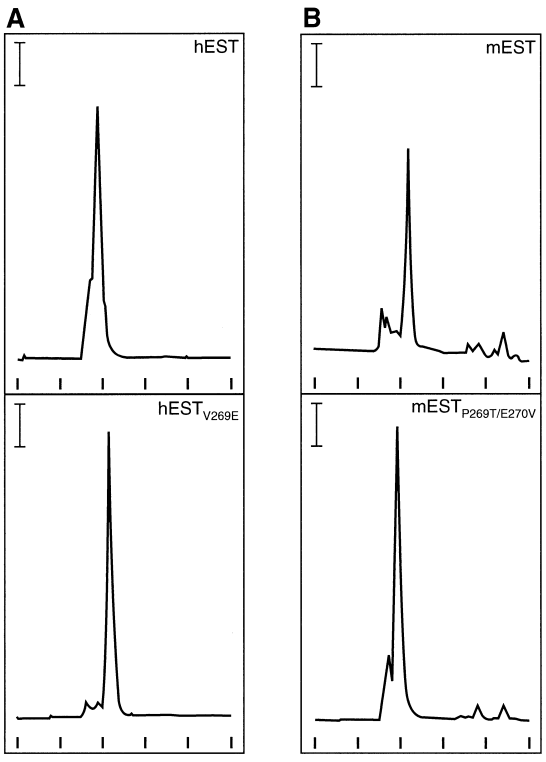

Fig. 3. Gel filtration patterns of monomeric and dimeric ESTs. A: The wild-type hEST and mutant hESTV269E were chromatographed on a Superdex 75 HR10/30 column as described in Section 2. Protein concentration was monitored through flow cell using UV absorption at 280 nm. Sticks on the horizontal axis indicate retention times 5 min intervals from left to right. Vertical bars correspond to 0.01 AU. B: The wild-type mEST and mutant $mEST_{P269T/E270V}$ were analyzed accordingly.

3.3. *Examination of hHST crystal structure for the new interface*

Subsequently, the hHST crystal structure revealed that Val^{260}, the mutation of which disrupts hHST dimer, is located on a loop region consisting of residues from Trp^{254} to Glu^{264} near the C-terminal end of the molecule away from the substrate-binding site (Fig. 5A). This loop is involved in a small lattice contact between two monomers. Despite the small area of the interface, the overall interactions within this region are extensive. The hydrophilic interactions are composed of four backbone hydrogen bond interactions and two side-chain interactions (Fig. 5B). The carbonyl oxygen of residue Lys^{255} of one molecule is positioned at 2.8 Å from atom N of Ala^{261} of the other molecule. Residues Phe^{258} and Val^{260} form near anti-parallel β-sheet with the same two residues of the second molecule. Atom O of Phe^{258} is 3 Å from the N of Val^{269} in both molecules. These backbone interactions are flanked at both ends by side-chain interactions between atom NZ of Lys^{255} and OE2 of Glu^{264} of both molecules. The main hydrophobic interaction comes from residue Val^{269} of one molecule extending into a hydrophobic pocket comprised of the carbon side-chain atoms of Lys^{255}, Trp^{254}, Phe^{258}, Ala^{263}, and Val^{260} of the other molecule. Consequently, these side-chains from

two hHST monomers form a hydrophobic zipper-like structure that is enforced by ion pairs at each end of the loop. The buried surface area of the interface is 715 Å^2 for the dimer, which is approximately 3% of the total surface. The surface complementarity score (Sc) between the two monomers at the interface involving loop Trp^{254}–Glu^{264} of hHST is 0.83. This value is substantially larger than the Sc value (only 0.64) of the surface near the substrate-binding site. The Sc of 0.83 is also larger than the values reported for antibody/antigen interfaces (around 0.66) or for protease/peptide inhibitor interfaces (around 0.75) [13]. This hydrophobic zipper-like structure is also found in the hAST structure. Thus, the KXXXTVXXXE sequence appears to be a conserved structural motif for which we designate as the KTVE motif in cytosolic sulfotransferases.

Enzyme	Spec	Code	Sequence
ST1A3	h	AAA02935	K T T F T V A Q N E
ST1A2	h	AAB09658	K T T F T V A Q N E
ST1A9	mo	BAA12822	K T T F T V A Q N E
ST1A5	h	AAA02943	K T T F T V A Q N E
ST1A6	b	AAA85510	K S T F T V A Q N E
ST1A7	d	BAA06190	K T T F T V A Q N E
ST1A1	r	CAA37065	K N T F T V A Q N E
St1a4	m	P52840	K N T F T V A Q S E
St1d1	m	AAC69919	K N Q F T V A Q Y E
ST1D2	r	AAC99890	K N Q F T V A Q Y E
ST1B1	r	AAB31318	K N Y F T M T Q S E
St1b3	m	AAD09249	K N Y F T M T Q T E
ST1B2	h	BAA24547	K N Y F T V A Q N E
ST1C1	r	A49098	K N Y F T V A Q S E
St1c4	m	AAC17740	K N Y F T V A Q S E
ST1C3	h	AAC95519	K K H F T V A Q N E
ST1C2	h	AAC00409	K N H F T V A Q N E
ST1C5	ra	AAC00410	K N H F T V A Q S H
ST1C6	r	CAB41460	K N H F T V A Q N D
ST1C7	r	CAB41461	K N H F T V A Q N E
ST1E3	gp	AAA18495	K N H F T V A L N E
ST1E4	h	AAA82125	K N H F T V A L N E
ST1E2	r	AAA41128	K N H F P E A L R E
ST1E6	r	AAB33442	R N H F P E A L R E
St1e5	m	AAB34320	K N H F P E A L R E
ST1E1	b	AAA30679	K N H F T V A L N E
ST2A1	r	A34822	K N H F T V A Q A E
ST2A2	r	BAA03632	K N H F T V S Q A E
ST2A5	r	BAA03634	K N H F T V A Q A E
St2a4	m	2021282A	K N H F T V A Q A E
St2a6	m	2021282B	K N H F T V A Q A E
SMP-2	r	A26136	K N H F T V A Q A E
ST2A6	gp	AAA19588	K N H F T V A Q A E
ST2A7	gp	AAB07868	K H H F T V A Q A E
ST2A10	mo	BAA12823	K N H L T V A Q A E
ST2A3	h	2021281A	K N H F T V A Q A E
ST2A8	ra	BAA25387	K N H F T V T Q A E
St4a1	m	AAB82292	R E Y F T P E L N E
ST3A1	ra	BAA24994	K H H M T V E Q S E
St3a2	m	AAB82293	K H Y L T V D Q S E
ST5A2	h	CAB09788	K D I F T V S M N E
St5a1	m	AAC63999	K D I F T V S M N E
ST2B1	h	AAC78498	K N H F T V A Q S E
St2b2	m	AAC69918	K N H F T V A Q S E

Fig. 4. The KTVE motif: sequence alignment of cytosolic sulfotransferases. The conserved residues are highlighted. Sulfotransferases are listed according to the classification used in a recent review article [20]. Species (Spec) are represented as h, human; m, mouse; r, rat; b, bovine; d, dog; gp, guinea pig; mo, monkey; ra, rabbit.

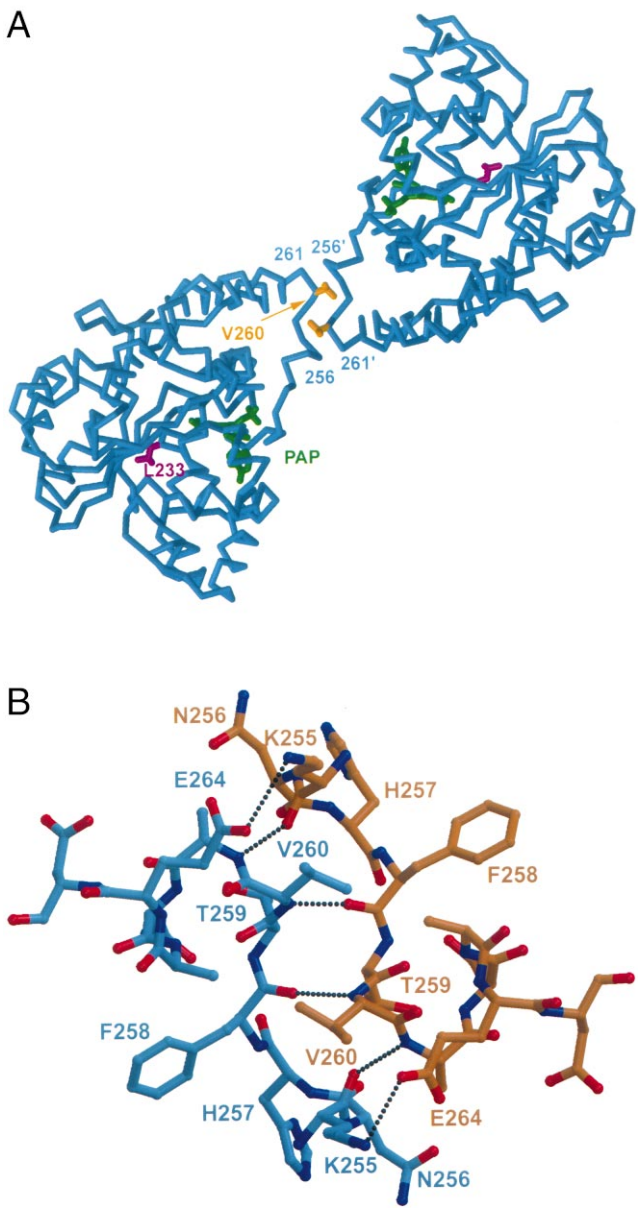

Fig. 5. Structure of physiological dimerization motif of hHST. A: The loop region from residues 256 to 264 in each monomer forms a small crystal contact between two monomers in the HST crystal. Val^{260} (in yellow), Leu^{233} (in red), PAP molecule (in green) are also shown. B: Detailed diagram of the small interface in the HST crystals. The two monomers are related by a two-fold axis running perpendicular to the plane of the page. Residues Gln^{256} to Glu^{264} from each monomer form four backbone hydrogen bonds, and are flanked by ionic interactions between side-chains Glu^{264} and Lys^{255} at both ends of the loop. Hydrogen bonds are represented by dotted lines. These figures were created using MOLSCRIPT [18] and RASTER3D [19].

3.4. Heterodimerization

The KTVE motif may mediate not only homodimerization but also heterodimerization of sulfotransferases. To investigate this possibility, we bound GST-tagged hHST to glutathione-Sepharose followed by incubation with either the wild-type hEST or its monomer mutant (Val270Glu). After being

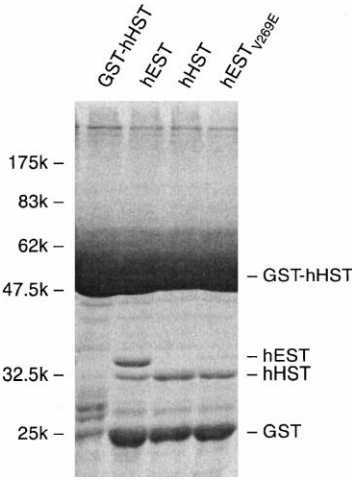

Fig. 6. Heterodimerization of hEST with hHST. Following incubation with GST-hHST-bound glutathione-Sepharose and washing, the wild-type hEST, the wild-type hHST, and the mutant $hEST_{V269E}$ were eluted with SDS sample buffer from the Sepharose. The recombinant enzymes used for dimerization partners are shown on the top of each well, except that the left-side well contained GST-hHST. Protein Marker Broad Range (New England Biolabs) was used as the molecular mass marker. hHST in the eluates might be generated by a residual protease used for the preparation of the recombinant sulfotransferases. Gel was stained by Coomassie blue G250.

washed repeatedly with high salt buffer, the proteins that bound to the Sepharose were eluted and subjected to SDS gel electrophoresis. Only the wild-type hEST was found in the eluted fraction (Fig. 6), suggesting that hEST forms a heterodimer with hHST through the KTVE motifs. The conservation of the KTVE motif in nearly all cytosolic sulfotransferases and the capability of forming the heterodimers suggest that the dimerization may play a role in regulating the enzyme activity and/or other physiological functions yet to be known, although these are totally elusive at the present time.

Consequently, the KTVE motif mediates both homo- and heterodimerizations of cytosolic sulfotransferases. Gel filtration chromatography of the wild-type hHST suggested that the motif might be strong enough to hold the dimer at about 10 nM although the equilibrium shifted toward the monomer under the experimental conditions used here (data not shown). The fact that specific protein–protein interaction can be mediated by short segments of polypeptide chains provides an exciting challenge for the exploration of the structural principles of complex formation that are pivotally important for many physiological processes. For example, upon phosphorylation, ERK2 (a MAP-kinase) forms a homodimer that translocates into the nucleus (this dimerization is mediated by a flexible loop of ERK2 molecule) [14]. The key contact surface consists of 12 residues that constitute the hydrophobic zipper-like dimer interface enforced by ion pairs at each end of the zipper [15,16]. Another example is the dimer interface of the PAK-1 [17]. Structural analysis (PDB accession code 1F3M) shows that the interface consists of a short segment with five residues, like the KTVE motif of sulfotransferases, which also contains a Thr-Ile sequence at its center. These short peptides from two monomers form a pair of anti-parallel β-strand reinforced by the ion (Glu/His) pairs at both ends. The KTVE motif exhibits structural features similar to the ERK2 and PAK-1 zippers, thus providing a new example of a short peptide that can constitute the interface of protein–protein interaction.

4. Conclusion

We have delineated the physiological dimerization interface of cytosolic sulfotransferases to the conserved sequence KXXXTVXXXE that occurs near the C-terminus. The KTVE motifs of two given monomers appear to form a zipper-like, anti-parallel interface, leading to the dimerization of two monomers. The main feature of the interface can be described as complementary hydrophobic interactions and backbone hydrogen bonds in the central portion of motif that are reinforced by an ion pair at each end of the motif. The KTVE motif is conserved in nearly all cytosolic sulfotransferases, and may mediate their homo- as well as heterodimerization, except in rat and mouse ESTs in which the central TV residues are substituted with PE. The physiological significance of the homo- and/or heterodimerization of cytosolic sulfotransferases is yet to be defined. The present identification of the physiological dimer interface KTVE motif should help us by prompting our investigation to find such significance.

Acknowledgements: We thank Dr. C. Falany for providing us with a bacterial expression plasmid of hEST.

References

[1] Falany, C.N. (1997) FASEB J. 11, 206–216.
[2] Hobkrik, R. (1993) Trends Endocrinol. Metab. 4, 69–73.
[3] Kiehlbauch, C.G., Lam, Y.F. and Ringer, D.P. (1995) J. Biol. Chem. 270, 18941–18947.
[4] Pedersen, L.C., Petrotchenko, E.V. and Negishi, M. (2000) FEBS Lett. 475, 61–64.
[5] Kakuta, Y., Pedersen, L.G., Carter, C.W., Negishi, M. and Pedersen, L.C. (1997) Nat. Struct. Biol. 4, 904–908.
[6] Bidwell, L.M., McManus, M.E., Gaedigk, A., Kakuta, Y., Negishi, M., Pedersen, L. and Martin, J.F. (1999) J. Mol. Biol. 293, 521–530.
[7] Dajani, R., Cleasby, A., Neu, M., Wonacott, A.J., Jhoti, H., Hood, A.M., Modi, S., Hersey, A., Taskinen, J., Cooke, R.M., Manchee, G.R. and Coughtrie, M.W.H. (1999) J. Biol. Chem. 274, 37862–37868.
[8] Kakuta, Y., Pedersen, L.G., Pedersen, L.C. and Negishi, M. (1998) Trends Biochem. Sci. 23, 129–130.
[9] Kakuta, Y., Sueyoshi, T., Negishi, M. and Pedersen, L.C. (1999) J. Biol. Chem. 274, 10673–10676.
[10] Sueyoshi, T., Kakuta, Y., Pedersen, L.C., Wall, F.E., Pedersen, L.G. and Negishi, M. (1998) FEBS Lett. 433, 211–214.
[11] Falany, C.N., Krasnykh, V. and Falany, J.L. (1995) J. Steroid Biochem. Mol. Biol. 52, 529–539.
[12] Kakuta, Y., Pedersen, L.C., Chae, K., Song, W.-C., Leblanc, D.L., London, R., Carter, C.W. and Negishi, M. (1998) Biochem. Pharmacol. 55, 313–317.
[13] Lawrence, M.C. and Colman, P.M. (1993) J. Mol. Biol. 234, 946–950.
[14] Khokhlatchev, A.V., Canagarajah, B., Wilsbacher, J., Robinson, M., Atkinson, M., Goldsmith, E.J. and Cobb, M.H. (1997) Cell 93, 605–615.
[15] Scheufler, C., Brinker, A., Boroder, L., Pagoraro, S., Moroder, L., Bartunik, H., Hartl, F.U. and Moarefi, I. (2000) Cell 101, 199–210.
[16] Cobb, M.H. and Goldsmith, D.J. (2000) Trends Biol. Sci. 25, 7–9.
[17] Hoffman, G.R. and Cerione, R.A. (2000) Cell 102, 403–406.
[18] Kraulis, P.J. (1991) J. Appl. Cryst. 24, 940–950.
[19] Merritt, E.A. and Bacon, D.J. (1997) Methods Enzymol. 277, 505–524.
[20] Nagata, K. and Yamazoe, Y. (2000) Annu. Rev. Pharmacol. Toxicol. 40, 159–176.