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Effects of 3′-phosphoadenosine 5′-phosphate on the activity and folding of phenol sulfotransferase

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Abstract

Known spectroscopic and kinetic data are used to formulate pathways of the physiological and transfer reactions and the substrate inhibition of phenol sulfotransferase. Kinetic mechanisms indicate that release of PAP from enzyme complex is required for the physiological reaction but not for the transfer reaction. The pathways explain rate difference between the physiological and transfer reactions since the release of PAP is the rate-limiting step of the former reaction. Two enzyme species of phenol sulfotransferase which distinguish the physiological and transfer reaction were found to involve the binding of PAP. Differences between two forms of phenol sulfotransferase, α and β, indicate that they assemble through different folding process. It is demonstrated that only α enzyme renatures in the presence of PAP and β enzyme renatures only in the absence of PAP in vitro. In the over-expressed system, formation of α and β phenol sulfotransferase is also dependent on the availability of PAP in *Escherichia coli*. It is concluded that folding of phenol sulfotransferase is assisted by PAP to form α enzyme. In the absence of PAP, β form of phenol sulfotransferase is produced.

Keywords: Phenol sulfotransferase IV; 3′-phosphoadenosine 5′-phosphosulfate; 3′-phosphoadenosine 5′-phosphate; Protein folding; Physiological reaction; Transfer reaction

Abbreviations: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PAP, 3′-phosphoadenosine 5′-phosphate; STIV, phenol sulfotransferase IV or aryl sulfotransferase IV or tyrosine-ester sulfotransferase; GNHCl, guanidine hydrochloride; pNP, *p*-nitrophenol.

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1. Introduction

Physiological reaction catalysed by phenol sulfotransferases is generally believed to be sulfuryl group transfer from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to phenols or other substrates [1]. Phenol sulfotransferase also catalyses sulfuryl group transfer between two phenols [2]. PAPS or 3′-phosphoadenosine 5′-phosphate (PAP) is acting as co-enzymes instead of as substrate or product in the transfer reaction. Reversible sulfuryl group transfer [1] indicates that the physiological reaction and the transfer reaction go through the same chemical mechanism and both reactions were catalysed by purified rat liver [2] and recombinant [3] phenol sulfotransferase IV (STIV). However, the transfer reaction which requires more reaction steps is a much faster reaction than that of the physiological reaction [2]. This is because the release of PAP which is mandatory for the physiological reaction becomes the rate-limiting step. Loosen PAP binding to sulfotransferase can eliminate this rate-limiting step and thus improve its catalytic efficiency. This is demonstrated by a mutant of STIV [4]. Specific activity of the enzyme is better (120 and 480 nmol/mg min for the wild type and mutant, respectively; unpublished data) when its binding to PAP is less tight ($K_d = 0.02$ and 270 μM for the wild type and mutant, respectively [4]). On the contrary, phenol sulfotransferase would not catalyse the physiological reaction if the binding of PAP to enzyme is too tight to release. Without looking for another mutant, it was found recently that a form of phenol sulfotransferase, α, catalyses only the transfer reaction but not the physiological reaction [4]. The α form of phenol sulfotransferase contains a tightly bound PAP that can not be diffused away from enzyme by gel filtration or dialysis. Detailed analysis reveals that enzyme conformation which is caused by the binding of PAP co-factor is the only difference between the two forms of recombinant protein. However, addition of PAP to β enzyme does not turn it to α and removal of PAP from α enzyme requires denaturation of the protein [4]. To explain the above observations, it is proposed that PAP binds to α enzyme during protein folding producing a PAP binding site that is not the same as that of β enzyme. In this presentation, it is demonstrated how two forms of recombinant phenol sulfotransferase is produced in vitro and in *Escherichia coli*. It was determined that presence of PAP is required for the formation of α enzyme. Since all the PAP in the over-expressed system is tightly bound to α enzyme, the α activity can be related to the amount of PAP. Production of two enzyme species from a single cDNA [4] can be explained by the effect of PAP on protein folding.

2. Materials and methods

2.1. Materials

All chemicals were commercially available and purchased at highest purity possible. pET3c11 transformed *E. coli* [3] is a gift of Dr W.B. Jakoby and A.D. Marshall of NIH, Bethesda, MD. Recombinant enzyme was over-expressed, purified and separated as described earlier [3].

2.2. Denaturation/renaturation of phenol sulfotransferase

The α enzyme (60 μl, 25 mg/ml) was denatured by mixing with equal volume of 5.2 M guanidine hydrochloride (GnHCl). Enzyme activity was monitor by standard assay [4]. A total of 95% of activity was lost within 5 min and no enzyme activity can be observed after 20 min of incubation with GnHCl. Partial enzyme activity was recovered by dialysis of the denatured enzyme in 500 ml solution (10 mM potassium phosphate at pH 7, 10% glycerol and 250 mM sucrose) overnight in the presence or absence of 50 μM PAP. In a separate experiment, enzyme in 2.6 M GnHCl was precipitate by mixing with 500 μl water. Precipitated protein was collected by centrifugation and redissolved in 5.2 M GnHCl. Renaturation of enzyme in 5.2 M GnHCl is the same as described above.

2.3. Cell growth condition for the expression of phenol sulfotransferase

Glycerol preserved pET3c11 transformed cell [3] from -80°C freezer was first activated as described by Wright and Crease [5] in 50 ml LB broth containing ten times the normal amount of ampicillin (250 μg/ml). This mixture was incubated at 37°C, 200 rpm shaking incubator until cell density reached O.D. between 1.0 and 1.2 measured at 600 nm by UV/vis spectrophotometer. Activated cell culture of 1 ml was then used for the inoculation at different growth conditions in 50 ml LB broth containing a normal amount of ampicillin (25 μg/ml).

2.4. Extraction of enzyme

Enzyme was extracted from cells as described before [3] with slight modification to ensure that the enzyme was not inactivated during process of sonication and all activity was recovered. A 2 ml buffer (10 mM Tris–HCl at pH 8, 125 mM sucrose, 10% glycerol, 1 mM PMSF, 1 mM EDTA and 1 mM dithiothreitol) was mixed with pellet of 50 ml cell culture prepared as described above. Power of sonicator (550 Sonic Dismembrator, Fisher Scientific) was set at 35–40% and a small tip was placed 0.5 cm above the bottom of a 10 ml tube. Sonicator was programmed for a 2 min cycle with the period of 2 s on and 1 s off. At least four cycles of sonication were performed for each extraction.

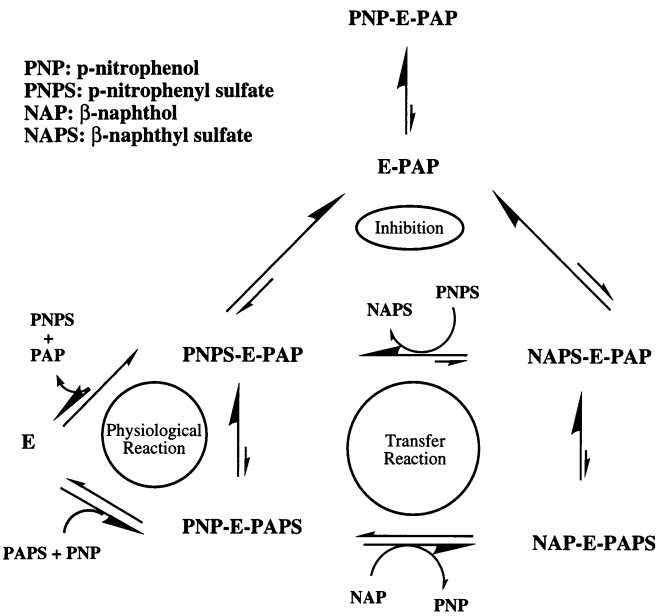
2.5. Enzyme assay and the determination of two forms of enzyme

Standard assay is used to determine total enzyme activity [3]. The change of absorbency at 400 nm due to the production of pNP ($\varepsilon = 10\,500$/M per cm at pH 7) was measured in the presence of 1 mM pNPS, 2 μM PAP, 0.05 mM 2-naphthol, 5 mM 2-mercaptoethanol, 100 mM bis-tris propane at pH 7 and 2–15 μg of phenol sulfotransferase. The α and β enzyme are distinguished with or without tightly bound PAP [4]. Free PAP is not available in a system with over-expressed phenol sulfotransferase and thus α enzyme activity can be determined in the absence of exogenous PAP. For the denaturation and folding experiments, free PAP was

removed by extensive dialysis or by the precipitation of the protein. The β enzyme activity is the difference of total enzyme and α enzyme activity.

3. Results and discussion

Reaction and inhibition pathways shown in Scheme 1 are constructed based on known kinetic and spectroscopic data. Kinetic mechanisms for physiological reaction were found to be Bi-Bi random [2] for STIV and Bi-Bi order [6,7] for other sulfotransferases. Both mechanisms indicate that sulfuryl group can be transferred directly from PAPS to phenols and the release of PAP is mandatory to complete the cycle of the physiological reaction as shown in Scheme 1. The transfer reaction uses PAP/PAPS as co-enzymes which can be regenerated in each reaction cycle by sulfotransferase. Thus, the release of co-factor is not necessary to complete the transfer reaction cycle as shown in Scheme 1. As expected, Ping-Pong mechanism is observed for the transfer reaction using *p*-nitrophenyl sulfate as sulfuryl group donor and *p*-methoxy phenol as acceptor (data not shown). Above, kinetic mechanisms explain why the rate of transfer reaction, even requires more reaction steps, is faster than that of the physiological reaction since release of PAP is the



Scheme 1. Pathways of reaction and inhibition of phenol sulfotransferase. Different pathways for the physiological and transfer reactions were proposed to account for their difference in reaction rate. Release of PAP is required for the physiological reaction but not for the transfer reaction. A ternary complex which is formed following ordered binding of PAP and phenol leads to the inhibition of phenol sulfotransferase.

Table 1
Folding of denatured phenol sulfotransferase in vitro[a]

Denaturant (GnHCl)	2.6 M		5.2 M	
	α	β	α	β
% recovered activity				
+PAP[b]	100	0	100	0
−PAP[c]	38	62	33	67

[a] Denaturation and refolding procedures were described in Section 2. Total recovered enzyme activity of the denatured phenol sulfotransferase was about 4% in the presence of PAP and 2% in the absence of PAP relative to the native enzyme. Enzyme activity is determined as described in Section 2. Data presented are the percentage of total recovered enzyme activity.
[b] A total of 50 μM of exogenous PAP was added into dialysis buffer.
[c] Contain PAP from denatured α enzyme before dialysis.

rate-limiting step for the physiological reaction. E-PAP binary complex ($K_d = 0.02$ μM) can bind to *p*-nitrophenol (pNP, $K_d = 0.6$ μM) or other phenols and cause the substrate inhibition for both physiological and transfer reactions as shown in the inhibition cycle of Scheme 1. The inhibition pathways are proposed based on the binding order of PAP and pNP to form E-PAP-pNP ternary complex observed by UV/vis and circular dichroism spectroscopies [4].

Conformational difference between α and β enzymes and PAP-induced conformational change of phenol sulfotransferase was observed by UV/vis and circular dichroism spectroscopies [4]. The other difference between α and β enzymes is how they bind to PAP. Addition of PAP to β does not produce α and removal of PAP from α requires denaturation of the protein [4]. This leads to the hypothesis that binding of PAP to α enzyme must emerge before the completion of protein folding. As shown in Table 1, refolding of denatured phenol sulfotransferase in the presence of exogenous PAP produces only α but not β enzyme. On the contrary, β enzyme was obtained only in the absence of exogenous PAP. GnHCl (2.6 or 5.2 M) was used to denature α enzyme and to remove PAP [4]. However, PAP (1 molecule of PAP from 1 dimer of α enzyme) was present and may not be removed completely by dialysis. This is why α enzyme was obtained (Table 1) in the absence of exogenous PAP. This result shows that PAP is involved in the folding of phenol sulfotransferase in vitro and the formation of two enzyme species is PAP dependent.

Two phenol sulfotransferase species are not artifact due to over-expressed rat liver enzyme in *E. coli*. Both enzyme activities are observed from its nature source [4]. Free PAP in the extract of rat liver, pig liver and *E. coli* has been determined (unpublished data). Thus, folding of phenol sulfotransferases with PAP to form α enzyme is possible in their native environments. Fig. 1 shows the production of over-expressed phenol sulfotransferase in *E. coli*. Two cells growth conditions were chosen to produce significantly different amounts of total phenol sulfotransferase. At low level of enzyme expression, almost all the recombinant phenol sulfotrans-

ferase is α form (open circles versus triangles in Fig. 1). This observation is consistent with the in vitro experiment (Table 1) that in the presence of PAP only α enzyme refolded. At high levels of enzyme expression, β enzyme becomes the majority (closed squares in Fig. 1) while α enzyme increases only slightly compared to low level expression condition (closed versus open triangles in Fig. 1). This result is consistent with the in vitro experiment that the β form of phenol sulfotransferase is produced when PAP is sufficient (Table 1). Similar results were obtained from separate experiments (data not shown) that, while the amount of total enzyme can be very different, the amount of α enzyme remains relatively stable at various growth conditions. Since normal PAP concentration is lower than that of over-expressed phenol sulfotransferase, formation of α enzyme is a good indicator for the available PAP in *E. coli*.

In the over-expressed system [3], free PAP is not found from the cell extract. However, the amount of enzyme bound PAP (as calculated from data of Fig. 1) can be 50 times more than that of free PAP in untransformed cells (unpublished data). This is more evidence to show that available PAP is folding with α enzyme so that it is protected from metabolism. In summary, the data shows that two phenol sulfotransferase species are produced in the presence or absence of PAP and PAP is assisting folding of phenol sulfotransferase to form the α enzyme.

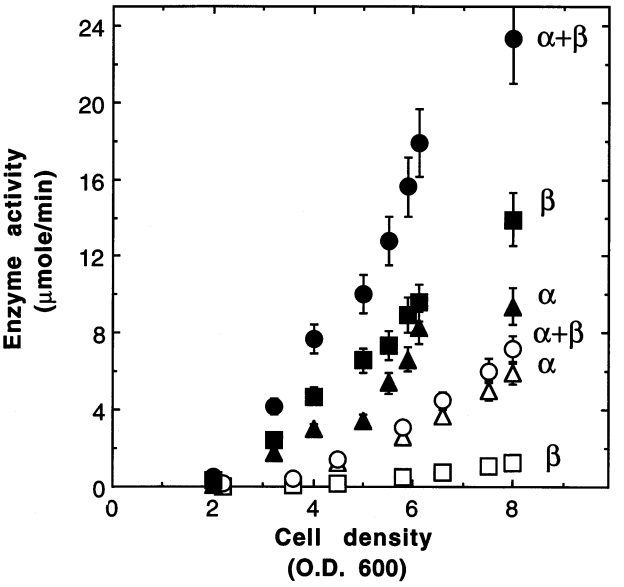


Fig. 1. Effect of cell growth condition on the expression of phenol sulfotransferase. A total of 50 ml cell cultures were incubated at 37°C and harvested at a different stage of cell density as described in Section 2. Oxygen supply to the cell cultures was controlled by a thermostated shaking incubator at 100 rpm (closed symbols) or at 300 rpm (open symbols). Circles designate total enzymatic activity, squares are β enzyme activity and triangles are α enzyme activity. The α and β enzyme activities were determined as described in Section 2. Each data is the average of six measurements from two different batches of cell growth at the same condition. The variation of each measurement from the same batch of cell is within 10% error. Error bars of the figure reflect 10% error of the co-ordinate.

Acknowledgements

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References

[1] W.B. Jakoby, D.M. Ziegler, The enzymes of detoxication, J. Biol. Chem. 265 (1990) 20715–20718.
[2] M.W. Duffel, W.B. Jakoby, On the mechanism of aryl sulfotransferase, J. Biol. Chem. 256 (1981) 11123–11127.
[3] 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 Express. Purific. 3 (1992) 421–426.
[4] Y.-S. Yang, A.D. Marshall, P. McPhie, W.-X.A. Guo, X. Xie, X. Chen, W.B. Jakoby, Two phenol sulfotransferase species from one cDNA; nature of the difference, Protein Express. Purific. 8 (1996) 423–429.
[5] A.-D.G. Wright, T.J. Crease, Detection of 'lost' plasmids from *Escherichia coli* using excess ampicillin, Anal. Biochem. 236 (1996) 181–182.
[6] S. Barnes, R. Waldrop, J. Crenshow, R.J. King, K.B. Taylor, Evidence of an ordered reaction mechanism for bile salt: 3′-phosphoadenosine-5′-phosphosulfate: sulfotransferase from Rheus monkey liver that catalyses the sulfation of the hepatotoxin glycolithocholate, J. Lipid Res. 27 (1986) 1111–1123.
[7] O. Frerot, F. Vargas, Cholecystokinin activation: evidence for an ordered reaction mechanism for the tyrosyl protein sulfotransferase responsible for the peptide sulfation (1991) Biochem. Biophys. Res. Commun. 181 (1991) 989–996.

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