PHENOL-SULFOTRANSFERASE INACTIVATION BY 2,3-BUTANEDIONE AND PHENYLGLYOXAL: EVIDENCE FOR AN ACTIVE SITE ARGINYL RESIDUE*

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Received August 22,1977

SUMMARY: In an attempt to determine if arginyl residues play a role in sulfate transfer reactions, we studied the effects of 2,3-butanedione and phenylglyoxal, both chemical modifying agents for arginyl residues, on phenol-sulfotransferase. Both reagents produced rapid inactivation of the enzyme, with the inactivation following pseudo-first order kinetics. The rate of inactivation was dependent upon the concentration of the chemical modifier. Competition studies showed that inclusion of 3'-phosphoadenosine-5'-phosphosulfate during the preincubation step protected the enzyme from inactivation. The results suggest a possible role for arginyl residues as anionic recognition sites for sulfate transfer reactions.

Riordan et al. (1) have shown that arginyl residues are anion recognition sites on enzymes which catalyze phosphate transfer reactions in the glycolytic pathway. An analogous enzyme catalyzed process would be that of a sulfate transfer reaction, which in our study is represented by the enzyme phenol-sulfotransferase (PST) (EC 2.8.2.1). PST plays an important role in the detoxification of xenobiotic phenol-containing compounds, as well as the metabolism of endogenous neurotransmitters (e.g. dopamine, norepinephrine, serotonin, etc.) (2-5). PST catalyzes the transfer of a sulfate group from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to a phenolic acceptor substrate. To determine whether arginyl residues exist at the active site of PST and whether they play a role as anionic recognition sites for the binding of PAPS, we have studied the effects of 2,3-butanedione

*The abbreviations used are: PST, phenol-sulfotransferase; PAPS, 3'-phosphoadenosine-5'-phosphosulfate.

ISSN 0006-291X

and phenylglyoxal, both chemical modifying agents for arginyl residues (6,7), on PST activity. Both reagents were shown to inactivate PST and evidence is provided here to support a role for an arginyl residue as an anionic recognition site on PST for the sulfate donor PAPS.

Materials and Methods

PST was purified from rat liver (male, Sprague-Dawley, 150-175 gr) according to a previously reported procedure (8). The enzyme was purified through the $(NH_4)_2SO_4$ fractionation step resulting in a preparation which contained 42 mg of protein per milliliter with a specific activity of 0.1 pmol product formed/mg of protein/min using p-nitrophenol as a substrate. Enzyme activity was determined using [^{35}S]PAPS (New England Nuclear, 1.02-2.03 Ci/mmole) and p-nitrophenol by a modification of a previously described radiochemical assay (9). The assay is based on the transfer of $^{35}SO_4$ from [^{35}S]PAPS to p-nitrophenol, then precipitation of unreacted [^{35}S]PAPS with $Ba(OH)_2$ and $ZnSO_4$, leaving the product p-nitrophenyl[^{35}S]sulfate in solution. Aliquots (0.5-1 ml) of the resulting supernatant were counted by liquid scintillation counting. The results were corrected using appropriate p-nitrophenol blanks. Unlabeled PAPS was prepared using the enzymatic method of Roy (10) and purification was performed by chromatography on Ecteola-cellulose (Sigma) (11).

A typical inactivation experiment with 2,3-butanedione consisted of the following components added in this sequence in a total volume of 0.4 ml: 50 mM borate buffer, pH 7.40; 2,3-butanedione (Aldrich Chemical Co.) (variable concentration) which was prepared in 50 mM borate buffer, pH 7.40; and the enzyme preparation. Phenylglyoxal inactivation experiments were carried out in a total volume of 0.4 ml using 125 mM bicarbonate buffer, pH 7.9;

phenylglyoxal (Sigma) (variable concentration) prepared in the same buffer; and the enzyme preparation. All preincubation experiments were initiated by addition of enzyme and incubation was carried out at 25°C. Samples were withdrawn at various times and assayed for residual sulfotransferase activity using the radiochemical assay. In the competition experiments p-nitrophenol (variable) or PAPS (variable) were included during the preincubation step. After the appropriate preincubation period aliquots were removed, dialyzed against 5 mM phosphate buffer, pH 7.40, and then assayed for residual activity. The percent activity remaining at any given time was calculated relative to zero-time activity.

Results and Discussion

In preliminary experiments 2,3-butanedione was shown to rapidly inactivate PST. A detailed time course for this inactivation is shown in Figure 1. The inactivation followed pseudo-first order kinetics with respect to active enzyme remaining and the rate of inactivation was dependent upon the concentration of 2,3-butanedione. Increasing concentrations of 2,3-butanedione resulted in increasing rates of inactivation. The inactivation was observed to be reversible upon dialysis, which is consistent with the proposed mechanism of 2,3-butanedione modification of arginyl residues (2). When borate buffer was replaced with phosphate buffer no inactivation of PST was observed, providing further evidence for the product-stabilizing borate complex proposed earlier by Riordan *et al.* (2).

To provide further evidence that the PST inactivation by 2,3-butanedione was due to arginyl residue modification, similar experiments were performed using phenylglyoxal, another chemical modification reagent specific for arginyl residues (7). In Figure

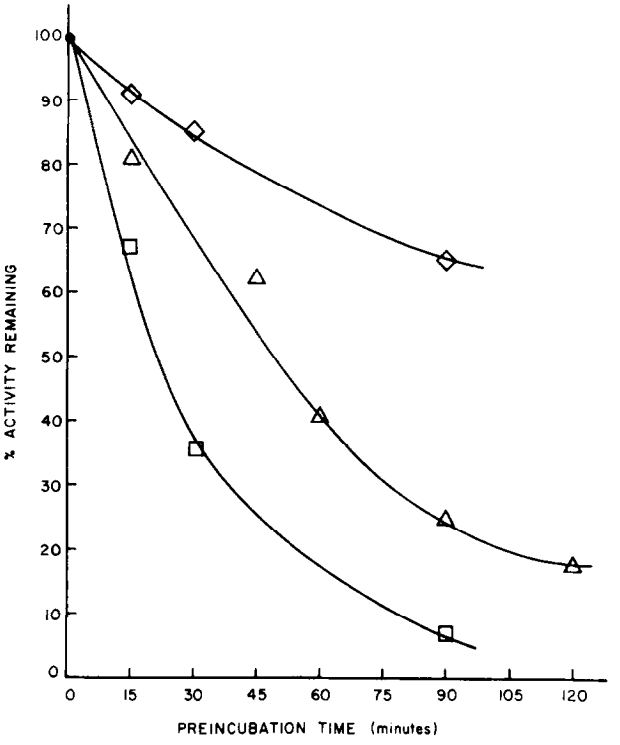

Figure 1: Effect of varying concentrations of 2,3-butanedione on PST activity as a function of preincubation time. PST was incubated with 10 mM (◇), 20 mM (△) or 40 mM (□) 2,3-butanedione. The PST activities were determined as described in the Materials and Methods Section.

2 is shown a plot of the log of the percentage PST activity remaining vs. preincubation time in the presence of varying concentrations of phenylglyoxal. The inactivation again follows pseudo-first order kinetics with respect to active enzyme remaining. The enzyme activity could be partially recovered after dialysis, consistent with the general properties of phenylglyoxal as an arginyl modifying reagent (7).

Evidence that an arginyl residue on PST is acting as an anion recognition site was obtained from protection experiments using the enzymes two substrates p-nitrophenol and PAPS (Table 1). Inclusi

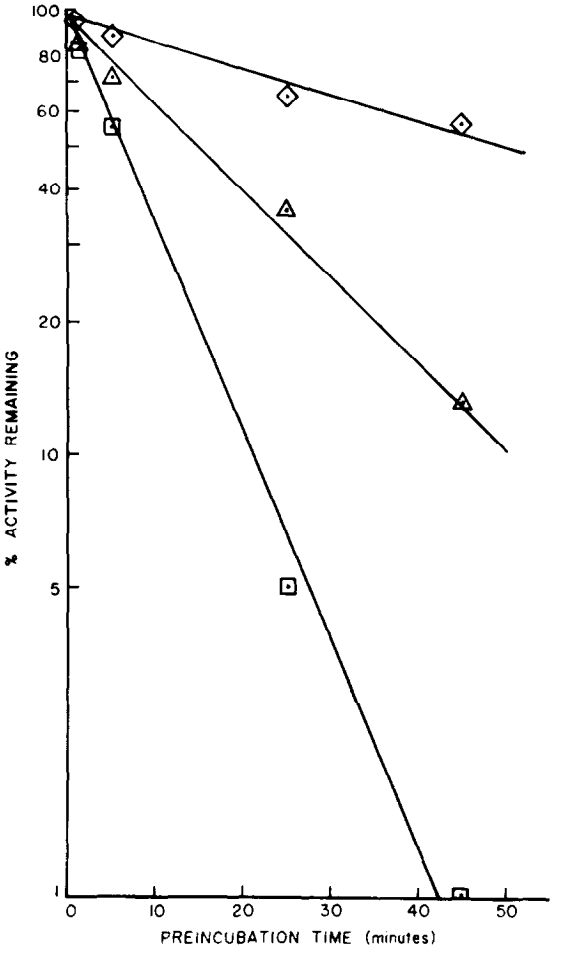

Figure 2: Effect of varying concentrations of phenylglyoxal on PST activity as a function of preincubation time. PST was incubated with 1 mM (◇), 2 mM (△) or 5 mM (□) phenylglyoxal as described in the Materials and Methods Section.

of p-nitrophenol in the preincubation step with PST and 2,3-butane dione failed to afford any protection of the enzyme from inactivation. However, when PAPS, the negatively charged sulfate donor, was included in the preincubation mixture, complete protection of PST from inactivation by 2,3-butanedione was observed.

Recent experiments using 2,3-butanedione and phenylglyoxal have shown that certain enzymes contain arginyl residues as anion

Table 1

Substrate Protection of PST from Inactivation by 2,3-Butanedione

Additions[a]	Concentration mM	Residual Activity after 90 min, 25°[b] %
None	--	37
p-Nitrophenol	4	34
p-Nitrophenol	40	30
PAPS	0.48	98
PAPS	4.84	95

[a]The standard preincubation mixture consisted of 2,3-butanedione (40 mM); borate buffer, pH 7.40 (50 mM); and the enzyme preparation in a total volume of 0.4 ml. The preincubation was carried out for 90 min at 25° after which the samples were dialyzed against phosphate buffer, pH 7.40 (5 mM) for 4-5 hours. The samples were then assayed for residual activity using the methods described in the text.

[b]PST activity remaining was calculated using as a reference a preincubation mixture treated under similar conditions except the 2,3-butanedione was not included.

recognition sites. For example, these recognition sites are thought to be crucial in the binding of peptides to carboxypeptidase A (12); NADH binding to alcohol dehydrogenases (13); RNA template binding to the reverse transcriptases from type C RNA viruses; and substrate binding to creatine kinase (14), hexokinase (1), fructose-6-phosphate kinase (1), 3-phosphoglycerate kinase (1), and pyruvate kinase (1). It would appear from the results reported here that sulfotransferases which utilize PAPS as a sulfate donor could be added to the list of enzymes which have arginyl residues as anionic recognition sites. The data reported here is, to our knowledge, the first evidence that arginyl residues participate in sulfate-transfer enzymes as recognition sites for the negatively charged sulfate donor PAPS.

Acknowledgements: The authors gratefully acknolwedge support for this project by grants from the National Institutes of Health (GM-22357) and the Kansas Heart Association. This work was done during the tenure of an Established Investigatorship of the American Heart Association.

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