Tyrosylprotein sulfotransferase (TPST) catalyzes the sulfation of proteins at tyrosine residues. We have analyzed the substrate specificity of TPST from bovine adrenal medulla with a novel assay, using synthetic peptides as substrates. The peptides were modeled after the known, or putative, tyrosine sulfation sites of the cholecystokinin precursor, chromogranin B (secretogranin I) and vitronectin, as well as the tyrosine phosphorylation sites of \( \alpha \)-tubulin and pp60\(^{c-s} \). Varying the sequence of these peptides, we found that (i) the apparent \( K_m \) of peptides with multiple tyrosine sulfation sites decreased exponentially with the number of sites; (ii) acidic amino acids were the major determinant for tyrosine sulfation, acidic amino acids adjacent to the tyrosine being more important than distant ones; (iii) a carboxyl terminally located tyrosine residue may be sulfated. Moreover, TPST catalyzed the sulfation of a peptide corresponding to the tyrosine autophosphorylation site of pp60\(^{c-s} \) (Tyr-416) but not of a peptide corresponding to the non-autophosphorylation site of pp60\(^{c-s} \) (Tyr-527). These results experimentally define structural determinants for the substrate specificity of TPST and show that this enzyme and certain autophosphorylating tyrosine kinases have overlapping substrate specificities in vitro.

Sulfation is the most abundant post-translational modification of tyrosine residues in metazoan cells (1). As an increasing number of tyrosine-sulfated proteins have been identified, there is growing interest in the biochemistry, cell biology, and function of protein tyrosine sulfation. This post-translational modification is catalyzed by tyrosylprotein sulfotransferase (TPST)\(^4\) (2), an integral membrane protein of the trans-Golgi (3, 4), and occurs in soluble and membrane proteins residing in, or passing through, this compartment (1). Although TPST activity has been found in various cell-free systems (for review, see Ref. 1), little information is available on the detailed biochemical properties and characteristics of this enzyme (3, 5-7).

Various types of substrates have previously been used to detect TPST activity. These include: (i) physiologically tyrosine-sulfated proteins and peptides that were desulfated prior to use; (ii) physiologically tyrosine-sulfated proteins isolated in unsulfated or partially sulfated form; (iii) proteins that are not tyrosine-sulfated physiologically but serve as substrates for TPST in vitro; (iv) synthetic, randomly condensed, tyrosine-containing polymers; and (v) synthetic peptides (for review, see Ref. 1). While these substrates are all appropriate for demonstration of the presence of TPST in various cells and tissues, they are not equally suited for the purification of TPST and characterization of its protein substrate specificity. For the purification of TPST, the substrate should be easily available in large quantities; for the characterization of TPST, the amino acid sequence of the substrate should be easily modifiable. Only synthetic peptides meet both requirements.

Here we describe the use of a variety of synthetic peptides in a novel TPST assay. This has allowed us to experimentally test previously proposed, and to identify new, structural determinants of substrate proteins for TPST.

**MATERIALS AND METHODS**

**Chemicals**

"Carrier-free" \( [^{35}\text{S}] \)PAPS was synthesized from ATP and carrier-free inorganic \( {^{35}}\text{SO}_4 \) using highly purified APS kinase (kindly provided by Dr. Irwin H. Segel, University of California, Davis), ATP-sulfurylase (Sigma A 5791), and inorganic pyrophosphatase (Sigma 14503) according to Daley et al. (8) with modifications that will be described elsewhere.\(^5\) For sulfation reactions, this \( [^{35}\text{S}] \)PAPS was adjusted to the indicated concentration by addition of unlabeled PAPS. Peptides were synthesized as described (9) and purified by HPLC. Concentrations of peptide stock solutions were determined spectrophotometrically at 240 nm. Varsol 145/200 was obtained from ESSO.

**TPST Assay**

Golgi-enriched membranes from bovine adrenal medulla were prepared by sucrose density gradient centrifugation, peripheral membrane proteins were removed by carbonate treatment at pH 11.0, and TPST was solubilized with Triton X-100 as described in detail elsewhere (42). This partially purified TPST preparation (<50-fold enriched in specific activity over homogenate) was used for all experiments except those shown in Figs. 1, 2, 4A, and 9, and Figs. 1-3 of this Miniprint, in which TPST solubilized from non-carbonate-treated membranes was used.

**Sulfation Reaction**—The standard reaction mixture consisted of, in final concentrations, 50 mM MES, pH 6.5, 5 mM MnCl\(_2\), 5 mM MgCl\(_2\), 5 mM 2-mercaptoethanol, 1 mM NaF, 1% (w/v) Triton X-100, TPST preparation (10-50 \( \mu \)g of protein), 5 \( \mu \)M \( [^{35}\text{S}] \)PAPS (0.4 TBq/\( \mu \)mol), and synthetic peptides as indicated in the figure legends. In those experiments in which the PAPS concentration was <5 \( \mu \)M, the
specific activity varied accordingly (0.4-37 TBq/mmol). Some reactions also contained 1 mM 5'-AMP (see figure legends). The final reaction volume was 50 µl. Reactions were started by the addition of [35S]PAPS and placing the samples at 30 °C. After various times, reactions were terminated by chilling the samples for 1 min in a boiling water bath. After chilling on ice, the samples received 2 µl of a phenol red solution that served as an indicator in the subsequent paper electrophoresis. The samples were concentrated to ~30 µl in a Speedvac concentrator followed by centrifugation for 3 min in an Eppendorf benchtop centrifuge to remove the precipitated proteins.

**Paper Electrophoresis and Quantitation of Sulfated Peptides**—The supernatants were applied to a 10 × 24-cm sheet of Whatman 3MM chromatography paper that had been preretted with electrophoresis buffer (7.8% acetic acid, 2.2% formic acid, pH 1.9) and blotted onto Whatman 3MM paper to remove excess liquid. Samples were spotted at 2-cm distance from each other on a line running at a 4-cm distance in parallel to one of the 24-cm edges of the sheet; this edge subsequently became the anodic side. After spotting, up to two such wet paper sheets (with up to 12 samples/sheet) were placed into an electrophoresis tank (see Fig. 1 of Miniprint for details) and subjected to electrophoresis at 4 °C. In the case of peptide CCK-1, electrophoresis was carried out until the front of the phenol red marker had migrated ~2 cm out of the origin toward the anode (~12 min at 400 V, 40 V/cm). For the other peptides, the time of electrophoresis was adjusted according to the respective R value (see Table I of Miniprint). After electrophoresis, the paper sheet was dried at 120 °C for 10 min. For each peptide, the electrophoretic migration was examined in initial experiments by autoradiography. All peptides used in this study, unsulfated or sulfated, migrated toward the cathode; the unsulfated peptides were present in sufficient amounts to be detected by ninhydrin staining; the sulfated peptides migrated slower than the unsulfated peptides and were not formed in sufficient quantities to be detected by ninhydrin staining.

Radioactive sulfate incorporation into the peptides was quantitated by liquid scintillation counting of the area extending from 0.8 cm to ~4.8 cm on the cathodic side of the origin; this area included the sulfated peptide (as judged by autoradiography) and most of the ninhydrin-stained spot corresponding to the unsulfated peptide. Samples lacking added peptide served as blank, the value of which was subtracted from that obtained in the presence of peptide. Unless indicated otherwise, initial rate values (v) of sulfate transfer by TPST were determined from a linear fit of at least three reaction time points between 5 and 45 min or from duplicate or triplicate reactions carried out for 20 min. Data are represented as micromoles/mg protein if not indicated otherwise. 1 microunit corresponds to the transfer of 1 pmol [35SO4]/min at 30 °C.

**Tyrosine Sulfate Analysis**

[35S]CCK-1 separated by paper electrophoresis was eluted from the paper with 2 ml of H2O, lyophilized, and subjected to alkaline hydrolysis followed by two-dimensional cellulose thin layer electrophoresis as described (10).

**Protein Determination**

Protein was determined using the dye binding assay (Bio-Rad) or a turbidometric assay (11).

**RESULTS**

**Synthetic Peptides as Substrates for TPST**—The goal of the present study was to investigate the substrate specificity of TPST using a variety of synthetic peptides. In developing a suitable assay for this purpose, we encountered the problem (particularly when crude membranes were the source of TPST) that the sulfated peptides were incompletely freed from the sulfate donor [35S]PAPS and/or endogenous sulfate-labeled compounds. It seemed likely that not only PAPS but any material with faster electrophoretic mobility than PAPS was most likely inorganic [35SO4]. Both [35S]APS and free [35SO4] probably are breakdown products generated by nucleotidases typically present in membrane preparations (13). One unidentified sulfate-labeled compound was present on the cathodic side after incubation for 30 min irrespective of the presence of CCK-1. This endogenous product was not observed when TPST solubilized from carbonate-treated, Golgi-enriched membranes was assayed (data not shown). [35S]CCK-1 was clearly separated from all of these radioactive compounds.

Since CCK-1 contains serine and threonine residues, it was necessary to ascertain that its sulfation occurred only on tyrosine. When [35S]CCK-1 was subjected to alkaline hydrolysis followed by two-dimensional thin layer electrophoresis, 65% of the starting radioactivity was recovered in the tyrosine sulfate spot (Fig. 2). This recovery was in the same range as that of authentic tyrosine [35S]sulfate standard (typcally ~71%, (14)), showing that the sulfation of CCK-1 occurred only on tyrosine. Comparison of the amount of sulfate-labeled CCK-1 and sulfate-labeled endogenous proteins showed that CCK-1 was the major acceptor for sulfate on tyrosine residues (data not shown).

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The apparent \( K_m \) of TPST for the cosubstrate PAPS was 2 \( \mu M \) (see Fig. 3A of Miniprint), which is similar to the reported value of 5 \( \mu M \) for TPST using EAV as sulfate acceptor (3). We therefore used 5 \( \mu M \) PAPS in the following experiments in which the substrate specificity of TPST was investigated.

The apparent \( K_m \) of TPST from adrenal medulla for SgI-1, a peptide containing a single tyrosine residue and corresponding to an identified tyrosine sulfation site of a protein from the same tissue (secretogranin I (chromogranin B) (15)), was 43 \( \mu M \) (Table I).

**Peptides with Multiple Tyrosine Sulfation Sites**—The apparent \( K_m \) of TPST for CCK-1 was 35 \( \mu M \) (Table II). Since this peptide contains 2 tyrosine residues, corresponding to position 111 and 113 in prepro-cholecystokinin, both of which are sulfated in vivo (12), we tested two variants in which either tyrosine residue was replaced by a phenylalanine residue. The K\(_m\) of the latter two peptides decreased exponentially with the number of sulfation sites present in the peptide, reaching values as low as 44 \( \mu M \) (Table I).

The apparent \( K_m \) of TPST for CCK-1, which like SgI-4 contains only 1 tyrosine residue, and 113 in prepro-cholecystokinin, both of which are sulfated in vivo (12), we tested two variants in which either tyrosine residue was replaced by a phenylalanine residue. The K\(_m\) of the latter two peptides decreased exponentially with the number of sulfation sites present in the peptide, reaching values as low as 44 \( \mu M \) (Table I). In contrast, the V\(_{max}\) was largely unaffected (Table I). Both SgI-2 and SgI-3 appeared to be converted to the monosulfated form, as judged by the decrease in their electrophoretic mobility compared with the respective unsulfated form.

To investigate whether this remarkable decrease in K\(_m\) was due to an increase in the number of sulfation sites or in some other parameter, we compared SgI-3 with the peptide SgI-4, in which 2 of the 3 tyrosine residues of SgI-3 are replaced by phenylalanines. The K\(_m\) of SgI-4 was one order of magnitude higher than that of SgI-3 and two orders of magnitude lower than that of the peptide SgI-1, which like SgI-4 contains only 1 tyrosine residue. This indicated that both the tyrosine residue itself as well as the surrounding amino acid residues contributed to the decrease in K\(_m\) observed for SgI-3.

The Role of Acidic Residues—Which features of the amino acid residues surrounding a tyrosine residue are important for sulfation to occur? A hallmark of the peptides SgI-1 and CCK-1 and of tyrosine sulfation sites in general appears to be the presence of acidic amino acid residues near the tyrosine residue to be sulfates (1, 16). To test the influence of flanking acidic residues on tyrosine sulfation in vivo, we investigated the sulfation of three CCK-1 variants (Fig. 4A). The acidic amino acid residues surrounding the tyrosines (DYEY) were replaced by the respective amides DYQY (CCK-4), NYEY (CCK-5), and NYQY (CCK-6). Fig. 4A shows that the rate of sulfate transfer to these variants decreased markedly as a function of increasing substitution of acidic amino acids. A 6- and 12-fold decrease of sulfation was observed when CCK-4 and CCK-5, respectively, were compared with CCK-1. Sulfation of the doubly substituted CCK-6 was even less than that of CCK-5.

The influence of acidic amino acids distant from the tyrosine was investigated using variants of the peptide SgI-1. In these variants (SgI-5, SgI-6, and SgI-7), distant acidic amino acids were cumulatively exchanged for alanine residues (Fig. 4B). When tested at 0.1 mm, the sulfate transferred to the peptides gradually decreased 2-, 3-, and 6-fold with increasing replacement of acidic amino acids.

The Role of Turn-inducing Amino Acids—In addition to the presence of acidic amino acid residues, CCK-1 and SgI-1 as well as tyrosine sulfation sites in general contain turn-inducing amino acids, in particular proline and glycine residues. To test for the influence of these amino acids in sulfation sites in vivo, we investigated the sulfation of an SgI-1 variant in which the proline and glycine residues were replaced by alanines (SgI-8) (Fig. 5). The sulfation of SgI-8 was about 2-fold less than that of SgI-1.

It has been noted that aspartate residues, which have a greater turn conformational preference than glutamate residues (17), occur more frequently adjacent to sulfated tyrosine residues than glutamates (1, 16). We therefore compared the sulfation of SgI-1 with that of the variant SgI-9, which had an Asp for Glu replacement in position -1 of the tyrosine. Fig. 5 shows that there was little difference in the sulfation of these peptides.

In contrast to the results obtained with SgI-1 versus SgI-8, replacement of the proline and glycine residues in SgI-9 by alanine residues (SgI-10) resulted in a 2-fold increase (rather

---

**TABLE I**

Sulfation of SgI-1 variants containing multiple sulfation sites

| Peptide | Sequence | \( K_m \) | V\(_{max}\) |
|---------|----------|---------|---------|
| SgI-1   | K KE EPEYEG E | 43.0 | 9.0 |
| SgI-2   | K KE (EPEYEG E)\(_2\) | 1.7 | 7.7 |
| SgI-3   | K KE (EPEYEG E)\(_2\) | 0.044 | 6.2 |
| SgI-4   | K KE EPEYEG E(EPEFEG E)\(_2\) | 0.34 | 7.7 |

The sequences of the synthetic peptides are given in the single-letter code. TPST assays were carried out in duplicate with six concentrations of each peptide. Kinetic constants were determined from a double reciprocal plot by linear regression.
Tyrosylprotein Sulfotransferase

TABLE II

Sulfation of CCK-2 variants containing only 1 tyrosine

The sequences of the synthetic peptides are given in the single-letter code. Dashes indicate amino acid residues that are identical to the top sequence. TPST assays were carried out in duplicate with 11, 7, and 7 different concentrations of CCK-1, CCK-2, and CCK-3, respectively. Kinetic constants were determined from a double reciprocal plot by linear regression.

| Peptide | Sequence | K_m (μM) | V_max (microunits/mg) |
|---------|----------|----------|-----------------------|
| CCK-1   | K K S A E D Y E Y P S M G | 35        | 3.1                   |
| CCK-2   | -- -- -- -- -- -- -- -- F -- -- -- -- | 150       | 7.5                   |
| CCK-3   | -- -- -- -- -- -- F -- -- -- -- -- -- | 120       | 5.2                   |

Fig. 3. The K_m of TPST for synthetic peptides decreases exponentially with the number of tyrosine sulfation sites. The K_m values of TPST for Sgl-1, Sgl-2, and Sgl-3 shown in Table I are expressed semilogarithmically as a function of the number of tyrosine sulfation sites.

Fig. 4. TPST recognizes peptides containing acidic amino acids. The sequences of the synthetic peptides are given in the single-letter code. Dashes indicate amino acid residues that are identical to the top sequence. CCK-1 and CCK-3 were assayed at three time points at 0.1 mM concentration.

Fig. 5. Sulfation of peptides with varying numbers of turn-inducing amino acids. The sequences of the synthetic peptides are given in the single-letter code. Dashes indicate amino acid residues that are identical to the top sequence. Sgl-1 and three variants of this peptide (Sgl-8, Sgl-9, Sgl-10) were assayed in triplicate at 0.1 mM concentration.

Fig. 6. Sulfation of terminal tyrosine residues by TPST. The sequences of the synthetic peptides are given in the single-letter code. Dashes indicate amino acid residues that are identical to the respective top sequence. Vtn-1, Vtn-2, Vtn-3, and Vtn-4 were assayed in triplicate at 0.1 mM concentration.

Plots for西洋語を踏まえて日本語に翻訳しました。
The sequences of the synthetic peptides are given in the single-letter code. Dashes indicate amino acid residues that are identical to the top sequence. Sgl-1 and two variants of this peptide (Sgl-11, Sgl-12) were assayed in triplicates at 0.1 mM concentration.

The tyrosine kinase substrate-related peptides Tub-1, srcds, and srcslr and, for comparison, CCK-1 were assayed at three time points at 1 mM concentration.

The tyrosine phosphorylation site of a-tubulin (Tub-1) (19), the tyrosine autophosphorylation site of pp60^src (srcds, 20), and (iii) the non-autophosphorylation site of pp60^src (srcslr, 31). As shown in Fig. 9, Tub-1 and CCK-1 were sulfated to a similar extent, consistent with the apparent K_m of TPST for these two peptides being of the same order of magnitude (140 and 35 μM, Fig. 3B of Minpun and Table II, respectively). The srcds peptide, which has been shown to be tyrosine-phosphorylated by the epidermal growth factor receptor kinase in vitro (22), was also sulfated by TPST, but with a 6-fold lower transfer rate compared with CCK-1. No sulfation was detectable for the peptide srcslr in which the acidic amino acids are not adjacent to the tyrosine residue.

**DISCUSSION**

The purification of TPST and the characterization of its substrate specificity requires a rapid and versatile TPST assay. We here describe such an assay using synthetic peptides as substrates. Although it cannot be excluded that the presence of 2 basic residues at the amino terminus of the peptides, facilitating their separation from other sulfate-labeled compounds, might be a potential limitation, the present assay has been found to be very versatile. It allowed the use of peptides with widely varying sequences and, hence, the characterization of the substrate specificity of TPST.

**Single Versus Multiple Sulfation Sites**—The apparent K_m for peptides containing only 1 tyrosine (Sgl-1, CCK-2, CCK-3, and Tub-1) was in the range of 10^{-4} to 10^{-5} M, with the notable exception of peptide Sgl-4 (apparent K_m = 10^{-7} M) which is discussed below. The former values are in good agreement with the observed apparent K_m of 10^{-3} M for the peptide tert-butoxy carbonyl-CKK-8 sulfated by non-solubilized TPST from rat brain (5). It remains to be investigated whether the moderate affinity of TPST observed in vitro toward small synthetic peptides containing only 1 tyrosine sulfation site reflects the affinity of this enzyme in vivo toward the larger physiological protein substrates in the lumen of the trans-Golgi. In the case of the 65-residue, tyrosine-sulfated protein hirudin, the kinetic constants of leech TPST toward the full-length polypeptide were found to be similar to those toward a nonapeptide corresponding to the single tyrosine sulfation site of hirudin (23).

However, in the case of larger proteins and peptide precursors, which may contain multiple recognition sites for TPST, the apparent K_m may well turn out to be lower than 10^{-5} M: we found remarkably low apparent K_m values for peptides with multiple sulfatable tyrosines. The apparent K_m values of the CCK-1 variants increased 3-4-fold as a result of the presence of 2 sulfatable tyrosine residues and decreased ex-
ponentially in Sg1-1 variants with increasing number of sulfation sites, reaching 44 nm for the peptide Sg1-3 which contains three tandemly repeated tyrosine sulfation sites. One possible explanation for this increase in affinity may be the presence of binding subsites in TPST, in analogy to other enzymes (24). Since the peptide Sg1-4, which like Sg1-1 contains one tyrosine sulfation site but three times the number of acidic amino acids, had a >100-fold higher affinity for TPST than Sg1-1, it is likely that binding subsites in TPST recognize acidic amino acids in substrate proteins. This, however, does not fully explain the increase in affinity for the peptide Sg1-3, which differed from Sg1-4 by the presence of 2 additional tyrosines (as opposed to 2 phenylalanines) and by a further >10-fold increase in affinity.

Previously, TPST from bovine adrenal medulla (3) and rat brain (25) was found to have a very low apparent Km (0.3 μM) toward the random synthetic polymer EAY, which may contain multiple tyrosine sulfation sites per single polymer molecule. Our findings might explain this low apparent Km in light of the above observations.

The increased affinity of TPST for substrates with multiple sulfation sites may be of physiological importance since a number of proteins containing such sites are known, e.g., prepro-cholecystokinin (12, 26), clonin (27), complement C4 (28), and heparin cofactor II (29). The presence of multiple sulfation sites might promote stoichiometric sulfation.

Acidic Amino Acids Are A Major Structural Requirement for Tyrosine Sulfation—Our data demonstrate that the previously noted presence of acidic amino acid residues in the vicinity of sulfated tyrosine residues is not merely a correlation and useful for the prediction of potential tyrosine sulfation sites (1, 16), but is indeed a structural requirement for the sulfation reaction per se. Both adjacent and distant acidic amino acids contributed to the sulfation of the peptides, adjacent acidic amino acids being more important. It will be interesting to determine whether acidic amino acid residues are only necessary for substrate recognition or whether they are actively involved in catalysis, e.g., by forming an acv enzyme intermediate.

Although turn-inducing amino acids are frequently found in tyrosine sulfation sites of proteins (1, 16), we observed little effects of such residues on the sulfation of synthetic peptides. The replacement of two turn-inducing amino acids by alanine residues resulted in a 2-fold reduction in the rate of sulfation in one case and in a 2-fold increase in another case. The substitution of a glutamate by an aspartate, which has a higher turn-conformational preference than the former, did not affect the sulfation of the peptide markedly. One interpretation of these results is that the high frequency of turn-inducing amino acids in tyrosine sulfation sites of proteins is coincidental, reflecting the fact that both tyrosine sulfation sites and loops (which are rich in turn-inducing amino acids, Ref. 30) are preferentially located on the protein surface. Alternatively, the presence of turn-inducing amino acids may promote tyrosine sulfation of proteins, but not of peptides since the latter are less restrained in adopting an optimal conformation for recognition by TPST.

We found that the COOH-terminal tyrosine residue in the peptide Vtn-2 could be sulfated by TPST, and, surprisingly, with a 5-fold increase in sulfate transfer when compared with the parent peptide which contained two additional COOH-terminal amino acids. This increase may be interpreted as a positive effect exerted by the carboxyl group of the tyrosine, which provides a close-by negative charge and thus might mimic an adjacent acidic amino acid residue. In contrast, a peptide containing an NH2-terminal tyrosine residue could not be sulfated significantly by TPST.

Variants of the peptide Sg1-1 which were extended by three hydrophobic amino acids were worse substrates for TPST than the parent peptide. This decrease in sulfate transfer might be due to the stabilization of a conformer unfavorable for recognition by TPST or to an increased hydrophobicity.

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**RESULTS**

The electrophoresis tank used to separate the synthetic peptides from [35S]PAPS after sulfation is shown in Fig. 1. The Rf values of the various synthetic peptides upon paper electrophoresis at pH 1.9 are shown in Table I.

![Diagram of the paper electrophoresis system used to separate sulfated peptides from PAPS.](image)

**Table I**

| Protein                | Peptide | Rf value |
|------------------------|---------|----------|
| Cholecystokinin (111-113) | CCK-1   | 1.0      |
|                        | CCK-2   | 0.9      |
|                        | CCK-3   | 0.8      |
|                        | CCK-4   | 0.6      |
|                        | CCK-5   | 0.9      |
|                        | CCK-6   | 0.9      |
| Secretinogen (321)     | SgI-1   | 1.0      |
|                        | SgI-2   | 0.7      |
|                        | SgI-3   | 0.6      |
|                        | SgI-4   | 0.1      |
|                        | SgI-5   | 1.2      |
|                        | SgI-6   | 1.3      |
|                        | SgI-7   | 1.4      |
|                        | SgI-8   | 1.2      |
|                        | SgI-9   | 1.2      |
|                        | SgI-10  | 1.2      |
|                        | SgI-11  | 1.0      |
|                        | SgI-12  | 1.1      |
| Vimentin (56)          | Vn-1    | 1.1      |
|                        | Vn-2    | 1.2      |
| Vimentin (59)          | Vn-3    | 1.1      |
|                        | Vn-4    | 1.2      |
| α-Tubulin (432)        | Tα-1    | 1.1      |
| pp60-csrc (416)        | pp60-csrc | 1.3     |
| pp46-csrc (527)        | pp46-csrc | 1.0    |

Using CCK-1 as substrate, we found the sulfation reaction to be linear with time of incubation (Fig. 2 A) and protein concentration (Fig. 2 B). The standard deviation of these independent assays was found to be 10% (not shown).

The authors also discuss the separation of sulfated peptides from PAPS and the recovery of [35S]PAPS after electrophoresis in Table I and Fig. 1. The signal-to-noise ratios of the TPST assays ranged from 2 to 50. The apparent K_m values for PAPS and Tab-1 were 2 μM and 140 μM, respectively (Fig. 3).
FIG. 2. Linearity of the TPST assay with time and protein concentration. Top (A): TPST assays were carried out for the indicated times with (+) and without (-) 280 μM CCK-1 in the presence of 2.5 μM [35S]PAPS and 1 mM S-AMP. Data points are single determinations. Bottom (B): TPST assays were carried out with increasing amounts of TPST preparation in the presence of 1 μM [35S]PAPS and 280 μM CCK-1.

FIG. 3. Apparent K_m values of TPST for PAPS and Tub-1. Top (A): TPST assays were carried out with increasing concentrations of [35S]PAPS in the presence of 100 μM CCK-1. Bottom (B): TPST assays were carried out with increasing concentrations of Tub-1 (KAAEAEOYEEV) in the presence of 0.1 μM [35S]PAPS. Data are expressed as double reciprocal plots.
Analysis of the substrate specificity of tyrosylprotein sulfotransferase using synthetic peptides.
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