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SULFATION OF TYROSINE RESIDUES DOES NOT INFLUENCE SECRETION OF α_2-ANTIPLASMIN OR C4

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SUMMARY: Sulfation of tyrosine residues is a biosynthetic modification of many secretory proteins. The function of this modification is not known, but it has been proposed that tyrosine sulfate residues may act as sorting signals to direct proteins along the secretory pathway. We tested this hypothesis by examining the effect of sulfation inhibitors on the kinetics of secretion of proteins by HepG2 cells. The inhibitors induced no change in the rate of secretion of α_2-antiplasmin and C4 (fourth component of complement), both of which contain tyrosine sulfate residues. Sulfation of tyrosine residues does not contribute to secretion of these proteins.

Recently, many proteins have been found to contain tyrosine-O^4-sulfate residues (1-5). The sulfation occurs as an enzymatic modification of proteins along the secretory pathway in the Golgi apparatus (6). The reaction is highly site-specific (4) and efficiently modifies a subset of proteins passing along the secretory pathway. Most proteins containing tyrosine sulfate are secretory proteins, but some membrane proteins may be modified as well (7). Although this modification of proteins is common and widely distributed among organisms, its function has not been established. One proposed function of the sulfation of tyrosine residues is that it may assist in directing proteins along the secretory pathway (2), i.e., tyrosine sulfate may act as a targeting signal, with a role analogous to that of mannose-6-phosphate in directing proteins to lysosomes (8). According to this hypothesis, secretion of the sulfate-containing proteins should be either blocked or slowed if sulfation did not occur. We tested this hypothesis by exposing cells to sulfation inhibitors that efficiently block the sulfation of tyrosine residues (9) and then examining the secretion of two proteins, α_2-antiplasmin and C4, that are known to contain tyrosine sulfate (1, 10).

MATERIALS AND METHODS

[^{3}H]Leucine and [^{35}S]sulfate were purchased from ICN Radiochemicals. Antiserum versus human C4 and α_2-antiplasmin were obtained from Miles Laboratories and from Accurate Chemical. Chemicals were bought from Sigma Chemical, except for $NaClO_3$, which was bought from EM Science. Protein A-Sepharose was from Pharmacia.

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HepG2 cells were cultured in 75 cm^2 flasks in Earle's minimal essential medium supplemented with 10% fetal bovine serum and with glutamine. Cells were used after reaching confluence. Labeling experiments were performed using the same medium without sulfate, leucine, and serum, and 10 mM Hepes was added to stabilize pH. Ten ml of medium with 0.5 mCi [^{35}S]sulfate or 0.4 mCi [^{3}H]leucine were added for labeling of cells. For chase incubations, fresh medium containing 1 mM leucine was added. Incorporation of labels into total secreted products was assessed by precipitation with 10% trichloroacetic acid (9). Incorporation of label into C4 and α_2-antiplasmin were determined by specific immunoprecipitation (1).

RESULTS AND DISCUSSION

In accord with previous studies (9), a combination of two inhibitors of sulfation very efficiently blocked the incorporation of sulfate into total secreted products and into proteins containing tyrosine sulfate (Table 1). In this experiment the combination of 2 mM $NaClO_3$ and 0.2 mM guaiacol inhibited the incorporation of [^{35}S]sulfate into C4 and α_2-antiplasmin by well over 90% during a period of 6 hours. Incubations with 2 mM Na_2SO_4 dilute the [^{35}]sulfate several hundred-fold, nearly completely blocking the incorporation of labeled sulfate and, thus, providing a measure of nonspecific binding of label during the immunoprecipitations. Estimates of the inhibition of sulfation can be made by subtracting the nonspecific binding from label recovered in the presence of inhibitors. This yielded estimates of 95% inhibition of sulfation of C4 and greater than 99% inhibition of sulfation of α_2-antiplasmin. Sites of sulfation of these proteins have been characterized in detail (1, 10), and all of the sulfate on these proteins is linked to tyrosine residues.

More detailed analysis of the effects of sulfation on the secretion of C4 and α_2-antiplasmin was performed by use of pulse-chase experiments. These studies examined

Table 1. Effect of inhibitors on incorporation of [^{35}S]sulfate into C4 and α_2-antiplasmin

PROTEIN	TREATMENT	[^{35}S]SULFATE INCORPORATION	RELATIVE TO CONTROL
C4	No addition (Control)	3,184 dpm	100%
	$NaClO_3$ + Guaiacol	255	8.0%
	Na_2SO_4 + $NaClO_3$ + Guaiacol	112	3.5%
α_2-Antiplasmin	No addition (Control)	786	100%
	$NaClO_3$ + Guaiacol	52	2.7%
	Na_2SO_4 + $NaClO_3$ + Guaiacol	51	2.5%

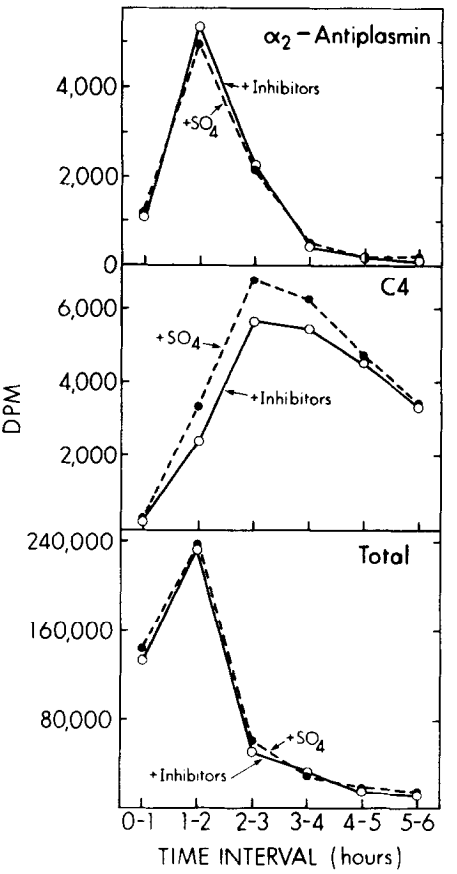

Figure 1. Affect of sulfation inhibitors on the kinetics of secretion of [^{3}H]leucine-labeled α_2-antiplasmin, C4, and total protein.

whether sulfation influenced the kinetics of protein secretion. Cells were pulse-labeled for 1 hour with [^{3}H]leucine in medium containing the inhibitors 2 mM $NaClO_3$ and 0.2 mM guaiacol or a control incubation with the inhibitors plus 2 mM Na_2SO_4 (which prevents action of the inhibitors). Medium was changed at one hour intervals over a period of 6 hours. Chase medium contained 1 mM leucine to block further incorporation of label. Labeled C4 and α_2-antiplasmin were isolated from culture medium and quantified by specific immunoprecipitation. The specificity of the immunoprecipitations has been demonstrated previously (1, 10). Total secretion of leucine-labeled products was assessed by acid precipitation.

The sulfation inhibitors did not affect the kinetics of the secretion of C4 and α_2-antiplasmin (Fig. 1). Secretion of C4 was relatively slow compared to that of α_2-antiplasmin. Maximal secretion of labeled C4 continued up to 6 hours, while secretion of α_2-antiplasmin peaked within the first two hours like the pattern of total protein secretion. No significant difference was noted between incubations with normal and inhibited sulfation

(dashed and solid line in Fig. 1, respectively). This lack of effect of sulfation on secretion of C4 and α_2-antiplasmin was confirmed in multiple experiments. Also, no difference in the secretion of C4 occurred in experiments in which the control consisted of incubations without the inhibitors rather than inhibitors plus sulfate (not shown).

C4 and α_2-antiplasmin serve as useful models for examining the effects of sulfation on protein secretion. Sulfation occurs exclusively on tyrosine residues-- on 3 tyrosine residues of C4 and a single tyrosine residue of α_2-antiplasmin (1, 10)-- and they provide examples with very different rates of secretion under normal circumstances. The transit time from synthesis to secretion of different proteins in hepatocytes varies markedly (11, 12), but the reason for this difference in rate of secretion is not known. Our results suggest that sulfation of tyrosine residues is not a major factor contributing to the variable secretory rate of proteins. Sulfation of tyrosine residues also is not likely to contribute to the segregation of proteins into constitutive and regulated pathways of secretion (13). Products containing tyrosine sulfate exit from cells via both pathways. C4, α_2-antiplasmin, and other hepatic proteins exit via a constitutive pathway while other products such as gastrin and cholecystokinin utilize regulated pathways.

The physiological function of the sulfation of tyrosine residues in proteins remains unclear. Here, we discount one hypothesis, that tyrosine sulfate is a targeting signal (2). What then is the function of this modification of proteins? The sulfation of tyrosine residues is a widely distributed process among higher organisms (2), requiring a specific sulfotransferase and expending energy for the activation of sulfate. Such a process should not be developed and conserved through evolution unless it has adaptive value. One potential function of this modification is to protect highly exposed tyrosine residues from oxidants and other chemical reactants in the extracellular environment. Analysis of known sites of sulfation suggests that they all consist of hydrophilic, highly accessible regions of proteins (4). This proposed raison d'etre for sulfation of tyrosine residues does not exclude the possibility that in some proteins the sulfate group may subserve specific structural roles required for full activity or stability of the proteins. In the case of some peptides such as cholecystokinin (14), phyllokinin (15), and leucosulfakinin (16), sulfation of a tyrosine residue dramatically augments activity.

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REFERENCES

1. Hortin, G., Sims, H., and Strauss, A.W. (1986) J. Biol. Chem. 261, 1786-1793.
2. Huttner, W.B. (1987) Trends Biochem. Sci. 12, 361-363.

3. Liu, M.-C., Yu, S., Sy, J., Redman, C.M., and Lipmann, F. (1985) Proc. Natl. Acad. Sci. USA 7160-7164.
4. Hortin, G., Folz, R., Gordon, J.I., and Strauss, A.W. (1986) Biochem. Biophys. Res. Commun. 141, 326-333.
5. Hortin, G., Tollefsen, D.M., and Strauss, A.W. (1986) J. Biol. Chem. 261, 15827-15830.
6. Baeuerle, P.A. and Huttner, W.B. (1987) J. Cell Biol. 105, 2655-2664.
7. Liu, N. and Baenziger, J.U. (1986) J. Biol. Chem. 261, 856-861.
8. Kornfeld, S. (1987) FASEB J. 1, 462-468.
9. Hortin, G.L., Schilling, M., and Graham, J.P. (1988) Biochem. Biophys. Res. Commun. (In press)
10. Hortin, G., Fok, K.F., Toren, P.C., and Strauss, A.W. (1987) J. Biol. Chem. 262, 3082-3085.
11. Lodish, H.F., Kong, N., Snider, M., and Strous, G.J.A.M. (1983) Nature 304, 80-83.
12. Parent, J.B., Bauer, H.C., and Olden, K. (1985) Biochim. Biophys. Acta 846, 44-50.
13. Kelly, R.B. (1985) Science 230, 25-32.
14. Jensen, S.L., Holst, J.J., Nielsen, O.V., and Rehfeld, J.F. (1981) Digestion 22, 305-309.
15. Anastasi, A., Bertaccini, G., and Erspamer, V. (1966) Br. J. Pharmacol. Chemother. 27, 45-49.
16. Nachman, R.J., Holman, G.M., Haddon, W.F., and Ling, N. (1986) Science 234, 71-73.