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INTERACTION OF PROTEASE INHIBITORS WITH THE CATALYTIC SUBUNIT OF cAMP-DEPENDENT PROTEIN KINASE

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Summary

The inactivation of the catalytic subunit from rabbit muscle cAMP-dependent protein kinase by the chloromethyl ketones from lysine and phenylalanine (TLCK and TPCK; A. Kupfer et al. (1979) Proc. Natl. Acad. Sci. USA 76, 3073) has been confirmed for the same enzyme from rat muscle. However, other structurally not related protease inhibitors, antipain and leupeptin, did not inhibit the catalytic subunit from rat muscle. Thus it seems to be critical to attribute the interference of protease inhibitors with complex biological phenomena like tumorigenesis etc. generally to the inhibition of protein kinases.

Introduction

The ability of the protease inhibiting chloromethyl ketones from lysine and phenylalanine [TLCK (N-α-p-tosyl-L-lysine chloromethylketone) and TPCK (N-α-tosyl-L-phenylalanine chloromethylketone), originally used (1) to label active sites of trypsin and chymotrypsin, respectively] as well as of the microbial anti-proteases antipain and leupeptin (2) to interfere with complex biological processes like tumorigenesis (3-7), growth and fertilization (for reviews see ref. 8) led to the conclusion that proteases play an important role in these phenomena. However, a recent report on the inactivation of the catalytic subunit of cAMP-dependent protein kinase by TLCK and TPCK (9) raised the possibility that the interference with this enzyme may also be a key part of the antimetabolic activity of protease inhibitors in general. This idea seems to be rather attractive in view of the central role of cAMP-dependent protein kinases for the "translation" of the "second message" (10, 11).

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For this reason we tested antipain and leupeptin in comparison with TPCK and TLCK for their ability to interfere directly with the catalytic subunit of the cAMP-dependent protein kinase from rat muscle.

Materials and Methods

Antipain and leupeptin were a gift from Dr. W. Troll, New York University (U.S.-Japan Cooperative Cancer Research Program). [γ^{32}P]ATP (specific activity > 20 Ci/mmol; New England Nuclear), TLCK, TPCK, Triton X100 (Serva, Heidelberg), calf thymus histone (Sigma, cat. number H 9250), dimethyl sulfoxide (DMSO), and methanol (Merck, Darmstadt) were obtained from the quoted sources. Pure catalytic subunit of the cAMP-dependent protein kinase from rat muscle was isolated as reported (12, 13).

Incubation with protease inhibitors was carried out with high enzyme concentrations (20 µg/ml; to prevent adsorbtion effects) at different pH. For this purpose enzyme probes were dialysed against 50 mM N-morpholino-3-propanesulfonic acid buffer (MOPS), pH 6, 7 or 8 containing 1 mM ethylene diamine tetraacetate (EDTA). The reaction mixture contained 50 µl of pure catalytic subunit and 1 µl protease inhibitor (0.1 M in DMSO, methanol, 10% Triton X100, or 50 mM MOPS buffer) resulting in 2 mM final inhibitor concentration. The mixture was incubated for 40 min at room temperature, cooled and diluted with 450 µl 50 mM MOPS buffer, pH 6.8, containing 1 mM EDTA and 0.76 mg bovine serum albumin in order to prevent enzyme adsorbtion to the vessel. Three times 25 µl of this mixture was assayed at pH 6.8 for protein kinase activity as reported (13) using calf thymus histone as the substrate.

Results and Discussion

An about 4×10^3 fold molar excess of the active-site-directed alkylating reagents TLCK and TPCK (solubilized in 3 or 2 different solvents) effectively inhibited the catalytic subunit from rat muscle protein kinase as shown in Table 1. The inactivation of the enzyme by TLCK occurred at pH 7 and 8, whereas no inactivation was observed at pH 6 under the conditions employed. The corresponding inhibition by TPCK was also expressed at pH 6. These data confirm observations reported by Kupfer et al. (9) for the catalytic subunit of rabbit muscle protein kinase. The protein kinase associated with $p60^{src}$ (14) in cells transformed with avian sarcoma virus has been also shown to be inhibited by TLCK (15).

On the other hand, the structurally unrelated antipain and leupeptin, protease inhibitors of microbial origin (2), did not

Table 1: Protein kinase activity after pre-incubation with different protease inhibitors solubilized in different solvents; expressed as percent of the control with according solvent ± S.D.

	MOPS buffer	methanol			DMSO			Triton X100 (10%)		
pH	8	6	7	8	6	7	8	6	7	8
TLCK	-	107±37	20± 3	5± 1	158±79	22± 6	36± 3	579±60	12± 1	2± 1
TPCK	-	11± 2	6± 1	5± 1	17± 1	22± 3	83±40	-	-	-
antipain	107±30	168±59	100± 8	109±34	91±19	37± 7	426± 6	171± 3	126± 2	110±11
leupeptin	103±32	265±13	169± 5	80±10	117±24	82±16	783±60	263±24	91± 6	108±16

cause a general decrease in protein kinase activity (Table 1). Since antipain and leupeptin do also interfere with the complex biological phenomena mentioned earlier, direct inhibition of protein kinases can not play a major part in this at least in their case. It seems that the ability of chloromethyl ketones to inhibit protein kinases is an incidental property unconnected with the general ability of protease inhibitors to disrupt tumorigenesis, fertilization and growth. This does not exclude the possibility that protein phosphorylation may be indirectly influenced by protease inhibition, as for instance via control of the level of other enzymes or modulatory proteins (16). But the wider effects of protease inhibition cannot be shown to work through the protein kinase system as a general mechanism.

It should be noted that the direct inhibition of protein kinase by TLCK and TPCK may be one reason for the cytotoxicity of these compounds (15, 17-19), which are probably involved in a number of other unwanted side reactions, particularly at -SH groups (1).

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