Uridylate Kinase Activity: Effect of Isoproterenol

Abstract. *Isoproterenol stimulates cell proliferation in mouse salivary glands. Prior to the stimulation of DNA synthesis, ^{3}H-uridine incorporation into RNA is decreased. This decreased incorporation results from a depression of uridylate kinase activity.*

A single burst of DNA synthesis and mitosis occurs in mouse salivary glands about 24 hours after a single injection of isoproterenol (*1*). Between 2 and 8 hours after such injection there is a decreased ^{3}H-uridine incorporation into salivary gland RNA followed by an increased incorporation between 8 and 20 hours (*2*). These changes are not due to alterations in rates of RNA synthesis, however, since they are associated with a comparable decrease and increase in the precursor pool of phosphorylated uridine (*2*). The present study demonstrates that the initial decrease in ^{3}H-uridine incorporation results from a decreased activity of uridylate kinase. Furthermore, these changes in ^{3}H-uridine incorporation appear to be relevant to subsequent DNA synthesis.

Table 1 shows that there is a decrease in ^{3}H-uridine incorporation into salivary gland RNA at 4 hours after isoproterenol injection and an increased incorporation at 20 hours, but these changes do not occur in liver, a tissue which does not respond to isoproterenol with increased DNA synthesis. Furthermore, with a lower dose of isoproterenol, which does not stimulate DNA synthesis, there is no decrease at 4 hours and no increase at 20 hours. This lower dose is sufficient to produce salivation and a decrease in alpha amylase activity (*3*). These findings suggest that the changes in uridine metabolism occur only under conditions which induce DNA synthesis, namely, in the salivary gland after a high dose of isoproterenol.

The observed changes could be the result of alterations in the kinase enzymes which phosphorylate nucleosides prior to incorporation into RNA. Uridine kinase catalyzes the phosphorylation of uridine to uridine monophosphate (UMP), while uridylate kinase activity is considered here as the total formation of uridine diphosphate (UDP) and uridine triphosphate (UTP). To study the activity of these enzymes, salivary glands were homogenized in sucrose-tris-KCl, *p*H 7.8 (*4*), and centrifuged at 105,000*g* for 60 minutes. An aliquot of the supernatant containing 0.2 to 0.4 mg of protein was incubated in the following reaction mixture at 37°C for 20 minutes: 20 μmole of tris-HCl, 20 μmole of $MgCl_2$, 2 μmole of adenosine triphosphate, 8 mμmole of uridine or UMP, and 2 μc of ^{3}H-5-uridine or ^{3}H-5-UMP (specific activity, 19.5 and 6.6 c/mmole) in a total volume of 0.4 ml. To determine total phosphorylation of uridine, 25 μl of the reaction mixture was pipetted onto diethylaminoethanol cellulose discs (*5*). Nonphosphorylated uridine was washed off with water, and discs were transferred to alcohol, air-dried, and counted in a liquid scintillation counter with an efficiency of 8.5 percent. Protein determinations were carried out by the method of Lowry *et al.* (see *6*). The reaction was linear for at least 30 minutes at protein concentrations from 0.2 to 1.4 mg.

Table 1. Effect of isoproterenol (IPR) on ^{3}H-uridine incorporation into salivary gland and liver RNA. Male Swiss mice were given a single intraperitoneal injection of IPR, at the dosage indicated per gram of body weight. ^{3}H-uridine (20 μc) was injected subcutaneously 45 minutes before killing. RNA specific activity was determined by the method of Scott *et al.* (*9*) as modified by Hinrichs *et al.* (*10*). Values are given as mean ± S.D. for three mice.

Treatment	Count/min in RNA per milligram of RNA	
	Salivary gland	Liver
^{3}H-uridine only	1798 ± 309	1053 ± 268
IPR, 4 hr, 1 μmole	778 ± 173	931 ± 200
IPR, 20 hr, 1 μmole	3997 ± 885	1205 ± 554
IPR, 4 hr, 3 mμmole	1762 ± 404	
IPR, 20 hr, 3 mμmole	1468 ± 439	

Total phosphorylation of uridine was decreased 20 to 25 percent at 4 and 7 hours after injection of isoproterenol. By 20 hours after this injection, phosphorylation of uridine returned to control levels. Sodium fluoride (40 μg/ml), a selective inhibitor of 5′-nucleotidase (*7*), inhibited control and isoproterenol groups to the same extent, suggesting that the decreased phosphorylation was not due to increased nucleotidase activity.

Chromatography with 4*N* formic acid–0.1*N* ammonium formate for 6 hours resolved the reaction mixture into UTP, UDP, UMP, and uridine with R_F values of .09, .24, .60, and .90, respectively. At 7 hours after isoproterenol injection there was no decrease in UMP, while UTP formation was depressed to about 60 percent of the

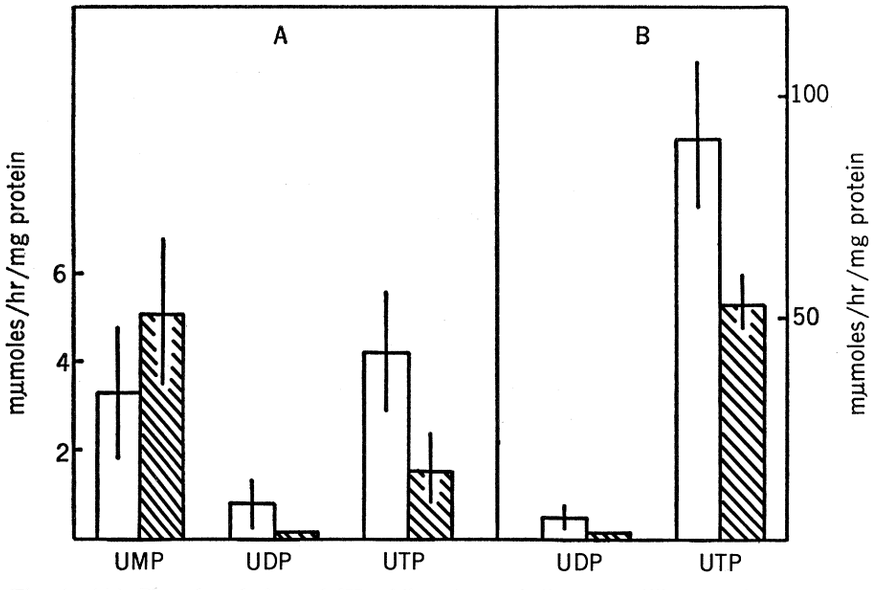

Fig. 1. (A) Phosphorylation of ^{3}H-uridine (mμmole/hr per milligram of protein) in mouse salivary glands. Enzyme reactions were carried out as described in the text, using ^{3}H-uridine as substrate, with preparations from control mice (unshaded bars) and animals killed 7 hours after injection of isoproterenol (shaded bars) (1 μmole/g of body weight). The reaction mixture was resolved by chromatography. Vertical markers indicate standard deviation. There were seven mice in each group. (B) Phosphorylation of ^{3}H-uridine monophosphate in mouse salivary glands. The experiment was the same as in (A), except that the substrate was ^{3}H-UMP. There were four mice in each group. Chromatography was carried out on diethylaminoethyl cellulose strips.

control value (Fig. 1A). Since this finding suggested that the decrease was at the level of uridylate kinase, we repeated the experiment with ^{3}H-5-UMP as substrate. The decrease of ^{3}H-UTP formation amounts to about 40 percent of the control value (Fig. 1B), a figure comparable to the inhibition of ^{3}H-uridine incorporation into RNA.

We have demonstrated that uridylate kinase activity is decreased in mouse salivary glands after injection of isoproterenol. The magnitude of this decrease is sufficient to account for the decreased incorporation of ^{3}H-uridine into RNA. In conjunction with earlier findings that there is a decreased specific activity of the phosphorylated uridine pool (*2*), it becomes clear that there is no change in the rate of RNA synthesis detectable with the methods employed at the times studied. The significance of a decreased uridylate kinase activity is not clear at the present time. Since the changes occur only under conditions which stimulate DNA synthesis, they may be involved in metabolic alterations which precede cell proliferation. The situation in isoproterenol-stimulated salivary glands clearly differs from that found in another model of stimulated cell proliferation, phytohemagglutinin-stimulated lymphocytes. In this latter model there is a stimulation of ^{14}C-uridine incorporation into RNA between 10 and 50 hours after stimulation (*8*). This stimulation is accompanied by an increase in uridine kinase activity, with no change in uridylate kinase (*8*).

DANIEL MALAMUD
RENATO BASERGA

Fels Research Institute and
Department of Pathology,
Temple University School of Medicine,
Philadelphia, Pennsylvania 19140

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Hydrolysis at Arginylproline in Polypeptides by Clostridiopeptidase B

Abstract. *Clostridiopeptidase B, a sulfhydryl protease from* Clostridium histolyticum, *hydrolyzes the arginylproline bond in the synthetic polypeptide methionyllysyl-bradykinin. Hydrolysis also occurs at a reduced rate at the lysylarginine bond, further delineating the environment necessary for the minor proteolysis seen with this enzyme at lysine residues. The specificity of clostridiopeptidase B differs from trypsin, which hydrolyzes this synthetic polypeptide only at the lysylarginine bond.*

Clostridiopeptidase B (E.C. 3.4.4.20), a sulfhydryl protease from *Clostridium histolyticum*, has been obtained recently from a commercial collagenase preparation in a state of apparent homogeneity (*1*). The enzyme has a narrow specificity range against synthetic esters and amides as well as against polypeptide substrates. Hydrolysis of polypeptides of known sequence has always been less than that obtained with trypsin. The major specificity is directed at the carboxyl side of arginine. Lysyl bonds are also attacked, but at rates generally much slower than hydrolysis of arginyl bonds. The unique specificity of clostridiopeptidase B and the judicious use of controlled enzymatic hydrolysis should facilitate sequence analysis by allowing the isolation of larger peptide fragments without chemical modification of the substrate. This communication extends the specificity data for this bacterial protease.

Synthetic methionyllysyl-bradykinin NH_2-Met-Lys-Arg-Pro-Pro-Gly-Phe-Pro-Phe-Arg-COOH (*2*) was hydrolyzed by clostridiopeptidase B and trypsin (~ 1:50, enzyme : substrate) for 1 hour at 30°C in sodium phosphate buffer (*p*H 7.7, ionic strength, 0.025) containing 1 m*M* dithiothreitol. The products were analyzed by paper electrophoresis in formate buffer (*3*) at *p*H 2.0 at 2000 volts on Whatman 3 MM paper (46 by 57 cm) for 75 minutes. Peptides were identified by ninhydrin tests, and arginine-containing peptides were identified with the Sakaguchi stain (*4*). A lysine standard was used for reference positions. Peptides adjacent to the ninhydrin and Sakaguchi markers were eluted with water (overnight), hydrolyzed for 18 hours in 6*N* HCl, and analyzed with a Technicon amino acid analyzer; the peptide fragments were identified by the molar ratios of component amino acids.

The difference in specificity of trypsin and clostridiopeptidase B for the synthetic peptide methionyllysyl-bradykinin is shown in Fig 1. The parent peptide, represented in column V, showed a single spot that was both Sakaguchi- and ninhydrin-positive ($\varepsilon f = 0.72$).

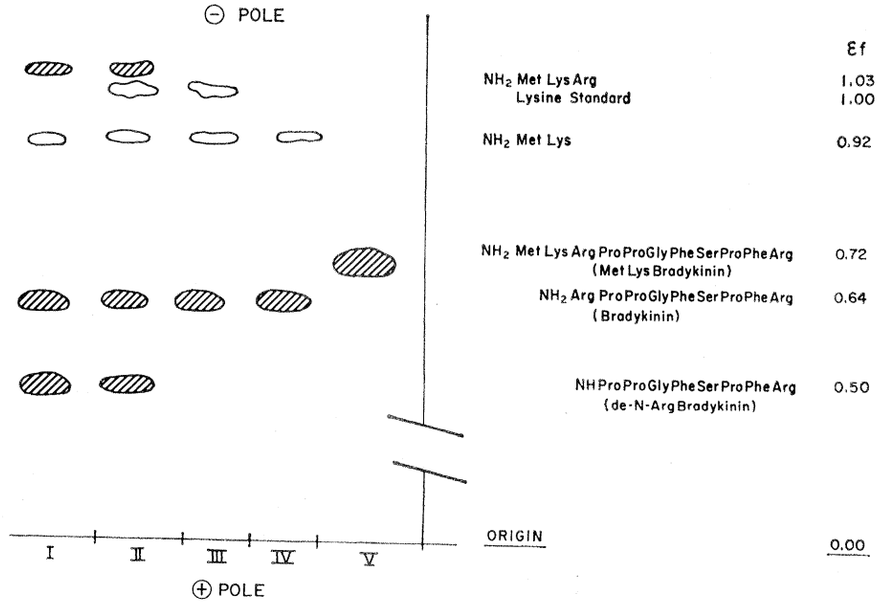

Fig. 1. Paper electrophoresis of metlysyl-bradykinin and degradation products. All spots are ninhydrin-positive. Shaded spots indicate Sakaguchi-positive peptides. Column I, hydrolysis by clostridiopeptidase B; II, I plus lysine standard; IV, hydrolysis by trypsin; III is IV plus lysine standard; V is metlysyl-bradykinin, the parent substrate. Content of the fragments is indicated on the right as judged by mobility, staining reactions, and apparent amino acid content after elution from paper. Mobility relative to lysine (εf) is given at the far right. Arginine has an εf of 0.92.