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Endogenous Activity of Phospholipases A and C in *Ureaplasma urealyticum*

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The results of recent studies support the concept that *Ureaplasma urealyticum* may be a major cause of perinatal infection in both term and preterm infants. It has been postulated that phospholipase degradation of placental phospholipids by microorganisms triggers the onset of premature labor. Since the presence of ureaplasmas in placentas is associated with pregnancy loss, prematurity, and neonatal morbidity, we assayed *U. urealyticum* for the presence of phospholipase A_1, A_2, and C activities. Phospholipase A_1 activity was low in lysates of exponential-phase cells of *U. urealyticum*. Phospholipase A_2 activity was present and was 100-fold higher than the activity of phospholipase A_1 in serotypes 3, 4, and 8. The total activity and specific activity of phospholipase A_2 in serotype 8 were nearly threefold higher than the activities in serotypes 3 and 4. Cell lysates of all three serotypes showed the presence of phospholipase C activity during the exponential phase of growth, and no significant difference in activity was observed among the three serotypes. In stationary-phase cells the phospholipase C activity was 10-fold lower than the activity in exponential-phase cells. Our results demonstrate that phospholipase activities are present in *U. urealyticum* cells and that the specific activities of phospholipase A_2 differed among the three serotypes tested, while the activities of phospholipases A_1 and C were similar.

Studies in the past few years demonstrated a significant association among spontaneous abortion, stillbirth, prematurity, perinatal morbidity, and mortality in pregnancies in which the placentas or fetuses were infected with *Ureaplasma urealyticum* and, in a few cases, with *Mycoplasma hominis* (7, 13, 18, 24). It has also been argued that bacterial infections of the amniotic fluid and the placenta may trigger premature labor during pregnancy, leading to premature delivery (2). One possible mechanism in the initiation of premature labor may be the effect of microbial phospholipase hydrolysis of placental membrane phospholipids to produce an increase in the amount of free arachidonic acid and, consequently, an increase in the synthesis of prostaglandins. However, no clear picture has emerged regarding the exact mechanism of action of phospholipases in the infectious process.

The importance of phospholipases as catabolic enzymes in the phospholipid metabolism of most cells has been clearly defined (10). Phospholipases A_1 and A_2 are responsible for the hydrolysis of the acyl ester bonds at the *sn*-1 and *sn*-2 positions of the glycerol backbone, resulting in the production of the corresponding lysophospholipids and fatty acids. Phospholipase C is a phosphorylhydrolase that acts on phospholipids and liberates the corresponding 1,2-diglycerides and phosphorylesters. In addition to its function as a catabolic enzyme in phospholipase turnover (3, 26), phospholipase A_2 plays a role in the resynthesis of phospholipase via the deacylation-reacylation pathway (14) and in the production of prostaglandin precursors (26). Phospholipase C activity has been detected mainly as a secreted phosphorylhydrolase of many microorganisms, such as *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus* (3), and *Legionella* species (1). Phospholipase C activities are also elevated in perinatal pathogens, such as *Bacteroides fragilis*, *Streptococcus viridans*, and *Fusobacterium* species (2). Phospholipase C secretion by these organisms may play a significant part in the physiological effects of infection (3). The role of bacterial phospholipases as components of secreted toxins in infections is not well understood.

Genital mycoplasmas, especially *U. urealyticum*, are found in placental tissues and fetal lungs (7, 18). These organisms have no cell wall and limited metabolic capabilities, and the presence of phospholipases in them may be important in the pathogenesis of human diseases. Enzymes associated with lipid metabolism have been studied in other mycoplasma species (21, 27), but the presence of such enzymes in *U. urealyticum* has not been demonstrated previously. It is also possible that mycoplasma phospholipases may play a role as components of toxic agents in infectious processes. In this paper we describe the total and specific activities of phospholipases A and C in whole-cell lysates of *U. urealyticum* serotypes 3, 4, and 8.

MATERIALS AND METHODS

Materials. 1-Palmitoyl-2-[2-^{14}C]oleoylphosphatidylcholine (specific activity, 57 mCi/mmol) and L-α-dipalmitoylphosphatidylcholine-[choline-methyl-^{3}H] (specific activity, 40.5 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass. Phospholipase A_2 (from *Naja naja* venom) and phospholipase C (from *Clostridium welchii*) were purchased from Sigma Chemical Co., St. Louis, Mo. The purity of substrates was checked by thin-layer chromatography in silica gels, using chloroform-methanol-water (65:35:4, vol/vol) as the developing solvent. All lipid standards and thin-layer chromatography plates were obtained from Supelco Chromatography Supplies, Bellefonte, Pa. All other chemicals were of reagent grade and were purchased from Fisher Scientific Co., Toronto, Ontario, Canada.

Organism and growth. Pure cultures of *U. urealyticum* were originally obtained from R. Purcell, National Institutes of Health, Bethesda, Md., in 1967. The strains used were serotype 3 strain 27, serotype 4 strain 58, and serotype 8 strain T960-CX3. The serotype stock strains were cultured in

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Trypticase soy broth base (BBL Microbiology Systems, Cockeysville, Md.) medium at pH 6.0 supplemented with 20% donor horse serum (KC Biologicals, Lenexa, Kans.), 10% fresh yeast extract (25%, wt/vol), 0.1% urea, 0.002% bromothymol blue, and 1,000 IU of penicillin per ml (TS medium) (29). Ureaplasma serotypes regularly grow to titers of 10^8 to 10^{10} color-changing units per ml in this medium. The three serotypes were adapted to lean TS medium containing 5% fetal calf serum (Bocknek Organic Matters, Rexdale, Ontario, Canada) and 5% fresh yeast extract (25%, wt/vol) by a single passage to prepare stock pools in lean medium. Stocks were stored at −70°C in polypropylene vials (Nunc, GIBCO Canada, Burlington, Ontario, Canada). Thawed contents of vials containing 10^4 to 10^5 color-changing units of each serotype per ml were sonicated in a water bath for 30 s (100 W; MSE Scientific Instruments, Toronto, Ontario, Canada). Growth curves were done to determine the time to harvest exponential phase organisms growing in lean TS medium. Each serotype was inoculated into 500 ml of lean TS medium and incubated at 37°C. At intervals, 0.2-ml samples were titrated by examining serial 10-fold dilutions to assay the growth pattern.

Preparation of ureaplasma cell lysate. To obtain a whole-cell lysate (homogenate), ureaplasma cells were harvested by centrifugation at 35,000 × *g* for 45 min in a model RC-2B centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.) and washed once in 30 ml of 0.25 M NaCl. The resulting pellet was resuspended in 2 ml of 0.25 M NaCl and used for the preparation of a cell lysate by the standard method of Razin (19). Since mycoplasma medium contained yeast and serum, 500 ml of incubated medium was separately centrifuged, washed, and suspended in 0.25 M NaCl. This preparation was used as the medium control for the enzyme assays. All fractions were stored at −70°C in plastic vials. The protein content of each fraction was determined by the method of Lowry et al. (15).

Phospholipase A. The assays for phospholipases A_1 and A_2 were performed by the methods of Ferber et al. (8) and Heath and Jacobson (12), with a few modifications. The substrate was lecithin labeled specifically in the 2 position (i.e., 1-palmitoyl-2-[2-^{14}C]oleoylphosphatidylcholine) and was prepared as unsonicated liposomes. The reaction mixture (1.05 ml) contained 18.3 nmol of labeled substrate diluted with egg phosphatidylcholine (Sigma), 30 to 50 μg of cell lysate protein, and 0.1 M Tris buffer (pH 7.4). After 1 h of incubation at 37°C, the reaction was terminated by adding 2 ml of chloroform-methanol (2:1, vol/vol) containing 50 μg of oleate and 50 μg of lysophosphatidylcholine. The chloroform layer was separated by centrifugation, and phospholipids were extracted by the procedures of De Silva (4). The lipids were separated by thin-layer chromatography (6) on silica gel G (Redi-Coat G; Supelco), using acetone-acetic acid-water (100:2:1, vol/vol) followed by petroleum ether (50-70%)–diethyl ether (80:20, vol/vol) (solvent system 1) or chloroform-methanol-water (65:35:5, vol/vol) (solvent system 2). The lipids were located by using I_2 vapor; the spots corresponding to free fatty acids, phosphatidylcholine, and lysophosphatidylcholine were scraped off, and their levels of radioactivity were measured directly by liquid scintillation in a model LS-100C counter (Beckman Instruments, Inc., Fullerton, Calif.) with 10 ml of Aquasol 2. Phospholipase A_1 activities were calculated from the yield of labeled lysophosphatidylcholine, and phospholipase A_2 activities were calculated from the yield of labeled free fatty acid. The positional specificity of ^{14}C in lecithin was determined by using purified phospholipase A_2 by the technique described above; more than 98% of the counts were recovered in the lysophosphatidylcholine, and 1 to 2% of the counts were recovered in the fatty acid fraction. Boiled enzyme preparations (100°C, 5 min) were used as controls. Zero-time (substrate only) controls and medium controls were also included.

Phospholipase C assay. Phospholipase C assays (23) were performed with the following modifications. The substrate was a lecithin that was labeled specifically in the choline moiety, such as L-α-dipalmitoylphosphatidylcholine-[choline-methyl-^{3}H], and prepared as unsonicated liposomes. Each reaction mixture (1.1. ml) contained 0.18 to 0.36 nmol of labeled substrate, 30 to 50 μg of enzyme protein (cell lysate), 10 mM $CaCl_2$, and 0.1 M Tris buffer (pH 7.4). After 2 h of incubation at 37°C, the reaction was terminated by adding 2 ml of chloroform-methanol (2:1, vol/vol), and the mixture was centrifuged to separate the phases. An aliquot from the water-soluble phase (glycerophosphorylcholine) was removed, and radioactivity was determined directly by liquid scintillation counting. The positional specificity of ^{3}H in the lecithin (the substrate) was checked by using phospholipase C. An analysis of the hydrolytic products indicated that less than 0.05% of the counts were in the diglycerides and more than 90% of the counts were in the water-soluble component (glycerophosphorylcholine).

All of the protein estimates and hence the specific activities of the enzymes for ureaplasma cell lysates were corrected for contamination by culture medium components. All values for radioactivity in controls (boiled control, medium control, and zero-time control) were subtracted from the values for radioactivity in experimental samples before the total and specific activities of enzymes were calculated.

RESULTS

Growth characteristics of *U. urealyticum* serotypes. In order to assure that the phospholipase activities were measured in actively metabolizing organisms, growth curves were determined over a period of 50 h at 37°C using lean TS medium (Fig. 1). Starting with an initial inoculum of 10^2 to 10^3 color-changing units per ml, serotype 8 grew very rapidly during the first 20 h to 10^{10} color changing units (CCU) per ml and reached a short stationary phase at 25 to 30 h of growth, and then the cells died. Similarly, serotype 4 grew rapidly during the first 28 h, reached a stationary phase at 30 to 37 h of growth, and then entered a decline phase. However, serotype 3 grew more slowly, reaching a stationary phase at 45 h of growth, and then declined rapidly. The difference in the growth rate of serotype 3 compared with the growth rates of the other serotypes may be attributed to the poor adaptation of serotype 3 to the minimal concentrations of fetal calf serum (5%) and yeast extract (5%) used in the growth medium. Ureaplasma cells were harvested in the exponential phase (14 to 15 h for serotype 8, 24 to 25 h for serotype 4, and 38 to 40 h for serotype 3) (Fig. 1). For stationary-phase cultures the ureaplasma cells were harvested at 28 h (serotype 8), 35 h (serotype 4), or 47 h (serotype 3).

Detection and estimation of phospholipase A activity. Phospholipase A activities were assayed in lysates of ureaplasma cells harvested in exponential phase at a titer of 10^6 to 10^7 CCU/ml for all three serotypes. Lecithin labeled specifically in the fatty acid moiety at the *sn*-2 position only was used as the substrate. Thus, any phospholipase A_1 activity present in the lysate would have hydrolyzed lecithin to liberate labeled lysophospholipid and unlabeled fatty acid as the reaction products. In these experiments phospholipase A_1 activities were estimated by the amount of radioactivity in the liber-

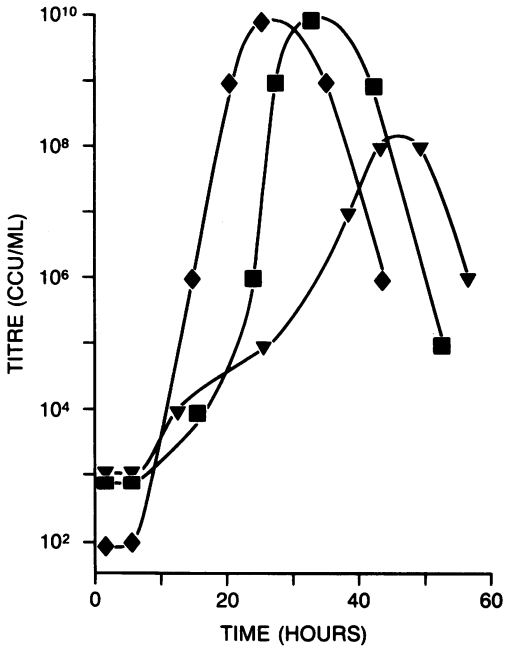

FIG. 1. Growth curves of *U. urealyticum* serotypes 3 (▼), 4 (■), and 8 (◆) in lean Trypticase soy medium.

ated lysophospholipid. More than 80% of the radioactivity (as [2-oleoyl-^{14}C]lecithin) added to the incubation vessels was recovered as products and unreacted substrate following incubation, extraction of reaction products, thin-layer chromatography, etc. Initial studies to determine the time course of hydrolysis and the effect of protein concentration indicated that the rate of hydrolysis was linear up to 90 min of incubation and up to about 100 μg of protein. Only minimal amounts of the recovered radioactivity (1 to 2%) were associated with the glycerophosphorylcholine fraction. In the zero-time control, only about 1% of the added radioactivity from the substrate was recovered in either fatty acid or lysophosphatidylcholine. These experiments showed that a low but detectable level of phospholipase A_1 activity is present in *U. urealyticum* (Table 1). Both the total activity and the specific activity of phospholipase A_1 were higher in *U. urealyticum* serotype 4 than in serotypes 3 and 8.

The phospholipase A_2 activities in ureaplasma lysates were assayed by using the same substrate labeled specifically in the fatty acid moiety in the *sn*-2 position only. Thus, the presence of any phospholipase A_2 activity in a cell lysate would have hydrolyzed lecithin to liberate labeled fatty acids and unlabeled lysolipid as the products of the reaction. In these experiments the labeled fatty acids were isolated by thin-layer chromatography, using solvent system 2, and measured directly by using liquid scintillation spectrometry. These experiments demonstrated that there was a high level of phospholipase A_2 activity in *U. urealyticum* (Table 2). The total phospholipase A_2 activity in serotype 8 was threefold higher than the total activities in serotypes 3 and 4. Similarly, the specific activity of serotype 8 was threefold higher than the specific activities of serotypes 3 and 4. Since the same substrate was used to measure the activities of phospholipases A_1 and A_2, the relative activities of these enzymes could be compared (Tables 1 and 2). Our results indicated that phospholipase A_2 activity may be as much as 100-fold higher than phospholipase A_1 activity in all three serotypes.

TABLE 1. Phospholipase A_1 activities in whole-cell lysates of exponential-phase cells of *U. urealyticum* serotypes

Serotype	Total activity (pmol/ml of lysate per min)	Sp act (pmol/mg of protein per min)
3	0.30 ± 0.1 (3)[a]	5.2 ± 0.5 (3)
4	0.70 ± 0.2 (3)	8.7 ± 1.2 (3)
8	0.10 ± 0.06 (3)	3.1 ± 0.6 (3)

[a] The values are means ± standard deviations. The numbers in parentheses are the numbers of replicate incubations.

TABLE 2. Phospholipase A_2 activities in whole-cell lysates of exponential-phase cells of *U. urealyticum* serotypes

Serotype	Total activity (pmol/ml of cell lysate per min)	Sp act (pmol/mg of protein per min)
3	60.0 ± 6 (3)[a]	300.0 ± 28 (3)
4	68.9 ± 7 (3)	401.0 ± 18 (3)
8	176.7 ± 10 (3)	1077.0 ± 20 (3)

[a] The values are means ± standard deviations. The numbers in parentheses are the numbers of replicate incubations.

Detection and estimation of phospholipase C activity. The substrate used for the phospholipase C assay in ureaplasma lysates was specifically labeled in the choline moiety (choline-methyl-^{3}H). Therefore, the enzymatic activities in lysates were monitored by the cleavage of the choline base to liberate radiolabeled phosphorylcholine and unlabeled diglyceride (3). The phospholipase C activity was estimated as the amount of radioactivity in the liberated phosphorylcholine (water-soluble phase). Furthermore, no radioactivity was detected in the diglyceride isolated in the lipid phase, which indicated that phospholipase C activity was present in the lysates. Initial studies to determine the time course of hydrolysis and the effect of protein concentration showed that the hydrolytic action of the enzyme was linear up to 120 min of incubation and up to 100 μg of protein. The cell lysates of all three serotypes showed the presence of phospholipase C activity during the exponential phase of growth (Table 3). The specific activities and total activities of phospholipase C were comparable among the three ureaplasma serotypes.

Phospholipase C activity of stationary-phase cells of *U. urealyticum*. Since the growth of ureaplasmas is characterized by a rapid exponential phase, a short stationary phase, and a rapid decline phase, it was important to determine whether the phospholipase C activity varied during these phases of the growth cycle. To examine this possibility, phospholipase C activities were determined for stationary-phase cells of serotype 8 (at 28 h), serotype 4 (at 35 h), and

TABLE 3. Phospholipase C activities in whole-cell lysates of exponential-phase cells of *U. urealyticum* serotypes

Serotype	Total activity (pmol/ml of lysate per min)	Sp act (pmol/mg of protein per min)
3	2.8 ± 2 (3)[a]	9.0 ± 2 (3)
4	3.2 ± 1 (3)	10.0 ± 2 (3)
8	3.7 ± 2 (4)	12.3 ± 3 (4)

[a] The values are means ± standard deviations. The numbers in parentheses are the numbers of replicate incubations.

TABLE 4. Phospholipase C activities in whole-cell lysates of stationary-phase cells of *U. urealyticum* serotypes

Serotype	Total activity (pmol/ml of lysate per min)	Sp act (pmol/mg of protein per min)
3	0.6 ± 0.5 (3)[a]	1.5 ± 0.8 (3)
4	1.1 ± 0.8 (3)	1.4 ± 1.0 (3)
8	1.3 ± 0.6 (3)	1.6 ± 0.6 (3)

[a] The values are means ± standard deviations. The numbers in parentheses are the numbers of replicate incubations.

serotype 3 (at 47 h). There was a decline in the total activity which was greater than twofold (Table 4). The specific activities of phospholipase C in the three serotypes declined about 10-fold during the stationary phase. The total and specific activities of phospholipase C did not differ among the *U. urealyticum* serotypes in the stationary phase. Therefore, these data show that enzyme assays must be done at the same stage of growth (e.g., exponential phase) for comparison among ureaplasma serotypes.

DISCUSSION

Microbial phospholipases may play an important role in mechanisms of infection. Thus, the presence of phospholipases in perinatal pathogens known to infect placentas or fetal lungs, in which phospholipases may interact, has taken on new importance. Phospholipases have been detected in *M. hominis* (21) and *Acholeplasma laidlawii* (27). In these studies the absolute values for phospholipases A and C were not given but were expressed as percentages of radioactivity in the free fatty acid fraction of the total radioactivity in membrane lipids. The phospholipase A activity in *M. hominis* membranes, expressed as the percentage of the radioactivity in fatty acids released, was 21.5% of the total membrane lipid activity (21). In *A. laidlawii* lysophospholipase A activity was detected in intact cells and membranes, but phospholipases C and A were not detected directly. The specific activity observed was 15.2 nmol hydrolyzed/min per mg of protein for intact cells and 48.0 nmol/min per mg of protein for membranes. In this study we demonstrated the presence and measured the specific activities of phospholipases A_1, A_2, and C in *U. urealyticum*. Our observations may be important since this mycoplasma is frequently found in placentas and fetal lungs in cases of spontaneous abortion, stillbirth, and early neonatal death (7, 13, 18).

Specifically labeled substrates of lecithin were used to directly assay for the hydrolytic action of phospholipases A and C in ureaplasma lysates. It is generally accepted that the hydrolysis of phospholipids by phospholipases is influenced by the physiochemical state of the substrate, i.e., interfacial characteristics (3, 25), and the transition temperature of the lipid (16). In order to meet the conditions described above, all of our assays were performed with unsonicated liposomes (substrate) and metal ions (Ca^{2+}) at a near-transition temperature (37°C). Liposomal substrates were considered to be more physiological than detergent-treated or sonicated substrates (3, 12). Further studies are in progress to explore this hypothesis.

The correct use of a specifically labeled substrate for determinations of phospholipases A_1 and A_2 is corroborated by the following facts. The labeling of the substrate at the *sn*-2 position of the glycerol backbone provided an approach for determining phospholipase A_1 and A_2 activities simultaneously by monitoring the formation of lysophospholipids and fatty acids, respectively. Although there is evidence that lysophospholipids formed might be reacylated back into the parent phospholipid (28), such reactions are ATP dependent and therefore were probably insignificant under our assay conditions. Transacylation reactions of two molecules of lysophosphatidylcholine to form diacylphosphatidylcholine have not been demonstrated in mycoplasmas or ureaplasmas. Hence, it is likely that the lysophospholipids and fatty acids formed in our assay were true reaction products of phospholipase hydrolysis of the substrate phosphatidylcholine. The possibility of hydrolysis of the lysophospholipids by lysophospholipase action, which could affect the determination of phospholipase A_1 and A_2 activities in cell lysates, must be considered. Although lysophospholipase activity was not assayed in cell lysates, the addition of unlabeled lysophosphatidylcholine to the assay mixture would inhibit any lysophospholipases present in the cell lysates (12). Assays for lysophospholipase activity in *U. urealyticum* are currently being studied. In light of the facts described above, our observations of low phospholipase A_1 activity compared with phospholipase A_2 activity in cell lysates of all three serotypes is a true reflection of the hydrolytic rate in *U. urealyticum*. These observations are in agreement with previous reports that most phospholipase A_1 activities are low, perhaps due to the fact that the enzymes are less abundant and rather labile (3).

Among the phospholipases, phospholipase A_2 is a more active enzyme, and its elevated specific activities in all three serotypes may be significant in the pathogenicity of *U. urealyticum*. Phospholipase A_2 activity either may be associated with a regulatory function of remodeling of the ureaplasma cell membrane phospholipids or may act as a component of the toxic agent in infectious processes or both. Also of significance to this study is the fact that the phospholipase A_2 activity in serotype 8 was higher than the activities in serotypes 3 and 4. Further studies will be required to confirm whether this higher level of activity occurs in primary isolates of serotype 8 or is associated with the high-passage status of the strain which we used. However, the strain used in this study was only in the fifth passage from the 1966 thrice-cloned strain of Purcell.

Phospholipase A is found in a wide variety of biological sources. In order to assess the significance of the ureaplasma enzyme activity, we compared its activity to previously reported values in other tissues and microorganisms (Table 5). In mammalian tissues, lysosomes seem to possess the highest phospholipase A activity, which may be related to their catabolic role in cell metabolism. The phospholipase A activities of microorganisms tend to be relatively low. However, free-living forms, such as *Dictyostelium discoideum* (a slime mold), have nearly one-half the specific activity of mammalian lysosomes because they have lysosomal activity. However, when 20 bacteria were examined for phospholipase A activity, the perinatal pathogenic bacteria, which were isolated from amniotic fluids in cases of premature labor, had the highest specific activities (2). These observations are of direct relevance to our study, which showed that the phospholipase A activity of all three *U. urealyticum* serotypes is significant. It is important to note that while the phospholipase A activities of serotypes 3 and 4 were comparable to the activities of the major perinatal pathogens (Table 5), the activity of serotype 8 was nearly threefold higher. The higher phospholipase A activity in *U. urealyticum*, as well as in the other perinatal pathogens, adds

TABLE 5. Comparison of phospholipase A activities from different sources

Source	Cell fraction	Phospholipase A activity (nmol/min per mg of protein)	Reference
Mammalian tissues			
Adrenal medulla (rat)	Lysosomes	6.10	22
Liver (rat)	Mitochondria	0.05	20
Placenta (sheep)	Homogenate	0.05	11
Lower eucaryote			
D. discoideum	Homogenate	3.8	8
Microorganisms			
Escherichia coli (01118)	Homogenate	0.06	9
Streptococcus group B	Sonic extract	0.04	3
Streptococcus epidermidis	Sonic extract	0.03	3
Perinatal pathogens			
Bacteroides fragilis	Sonic extract	0.16-0.42	3
Streptococcus viridans	Sonic extract	0.33	3
Fusobacterium	Sonic extract	0.30	3
U. urealyticum			
serotype 3	Lysate	0.3	This paper
serotype 4	Lysate	0.4	This paper
serotype 8	Lysate	1.1	This paper

credence to the hypothesis that microbial phospholipase A may play a role in the initiation of premature labor.

The specific labeling of the phospholipid substrate at the choline moiety (base) enabled us to determine phospholipase C activity by monitoring the formation of labeled glycerophosphorylcholine. The fact that the loss of radioactivity from L-α-dipalmitoylphosphatidylcholine-[cholinemethyl-^{3}H] could be quantitatively accounted for by [^{3}H]glycerophosphorylcholine indicated that the phospholipase C of ureaplasma cell lysates was specific for the phosphodiester bond. In addition, the very low level of radioactivity observed in the diacylglycerol, monacylglycerol, or fatty acid fractions not only was consistent with the specificity described above, but also indicated that there was limited diacylglycerol lipase activity in cell lysates. The detection of diacylglycerol as a reaction product (mass) by thin-layer chromatography methods indicated the presence of an active phospholipase C in *U. urealyticum*.

In contrast to the differences in phospholipase A_2 activity observed among the serotypes, the phospholipase C activities were comparable in the three serotypes tested. The reduction in phospholipase C activity in stationary-phase cells of the serotypes may primarily indicate enzyme inactivity in nonreplicating cells rather than physiochemical differences in the substrate. It is well known that distribution and packing of phospholipids in micelles and liposomes may affect the availability of substrate to the phospholipases. Since assays for exponential- and stationary-phase cells were carried out under standard physiological conditions, the phospholipase activity in *U. urealyticum* is more likely a property of rapidly growing exponential cells than of stationary-phase cells.

It is not yet known whether *U. urealyticum* phospholipases A_2 and C play a role as components of the toxic factor in production of disease. These enzymes may also be involved in the rapid turnover and remodelling of phospholipids. Therefore, studies are needed to characterize these enzymes in terms of their substrate specificities and to determine their possible role in the phospholipid metabolism of *U. urealyticum*, which may relate to the role of this organism as a perinatal pathogen in human disease.

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