The action of human and rabbit serum phospholipase A$_2$ on *Escherichia coli* phospholipids

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Abstract We have compared the properties of phospholipase A (E.C. 3.1.1.4) activity in whole human and rabbit serum toward the phospholipids of *Escherichia coli*. Using as substrate *E. coli* labeled during growth with either [1-^{14}C]-palmitic acid or [1-^{14}C]oleic acid, and then autoclaved to inactivate *E. coli* phospholipases and to render the labeled phospholipids accessible to exogenous phospholipases, we show that the deacylating activity in both human and rabbit serum is almost exclusively of the A$_2$ type. Rabbit serum is at least 20-fold more active than human serum. Activity in both sera is maximal at physiological Ca^{2+} concentrations (2 mM) and is abolished by ethylenediaminetetraacetic acid. To examine hydrolysis of intact (unautoclaved) *E. coli* treated with 25% serum, use was made of a phospholipase A-deficient *E. coli* strain (*E. coli* S17), thereby eliminating the possible contribution of bacterial phospholipases to degradation. Human and rabbit serum are about equally bactericidal toward *E. coli* and cause comparable structural damage. However, only rabbit serum produces substantial hydrolysis of the phospholipids of intact *E. coli* S17. Heated (56°C, 30 min) rabbit serum is non-bactericidal and retains phospholipase A$_2$ activity toward autoclaved, but not intact *E. coli*. The ability of heated serum to degrade phospholipids of intact *E. coli* S17 is restored, however, by adding 25% normal human serum, which is bactericidal. In this combination, doses of heated rabbit serum containing as much phospholipase A$_2$ activity (toward autoclaved *E. coli*) as is present in 25% unheated rabbit serum, produce roughly the same extent of hydrolysis of intact *E. coli* as does normal rabbit serum alone. Low doses with a phospholipase A$_2$ activity comparable to that of normal human serum elicit little or no hydrolysis. These findings indicate that hydrolysis of the phospholipids of intact *E. coli* S17 by serum occurs when: 1) the serum is bactericidal, and 2) when sufficient phospholipase A$_2$ is present. The difference in phospholipid hydrolysis that accompanies killing of *E. coli* by human or rabbit serum appears to reflect, therefore, the different amounts of phospholipase A$_2$ activity in the two sera. Phospholipid degradation is not required for the bactericidal action of serum. Bacterial phospholipid breakdown may be important, however, in the overall destruction and digestion of invading bacteria by the host.—**Kaplan-Harris, L., J. Weiss, C. Mooney, S. Beckerdite-Quagliata, and P. Elsbach.** The action of human and rabbit serum phospholipase A$_2$ on *Escherichia coli* phospholipids. *J. Lipid Res.* 1980. **21:** 617–624.

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Normal serum or plasma from man and several animals contains phospholipid-deacylating activity (E.C. 3.1.1.4) (1–7). While some of this activity has been attributed to heparin-releasable lipoprotein lipase acting on the 1-acyl position of a number of phospholipid substrates (phospholipase A$_1$ activity (1–3)), serum and plasma also contain phospholipase A$_2$ activity (4–6). The origin and function of this phospholipase A$_2$ have not been established. Thus, it is not known whether or not circulating phospholipase A$_2$ plays a role in the turnover of plasma phospholipids, or in immunologically mediated membrane damage. It is known, however, that the bactericidal effect of serum on gram-negative bacteria is accompanied by net hydrolysis of envelope phospholipids (8, 9). In the case of killing by human or rabbit serum of *Serratia marcescens*, this hydrolysis appears entirely due to activation of bacterial phospholipases (8). In contrast, serum phospholipase(s) A may participate in the degradation of phospholipids of *Escherichia coli* killed by serum (9).

In the present study we extended earlier observations (4–6, 8) on the properties of the serum phospholipase A$_2$ activity and we examined further its participation in the bacterial phospholipid degradation that occurs during killing of *E. coli* by human and rabbit serum.

To clarify the role of serum phospholipase A$_2$ in the antibacterial effects of serum, we compared the effects of serum from two sources, one relatively low in phospholipase A$_2$ activity (human serum) and one high in phospholipase A$_2$ activity (rabbit serum), on two serum-sensitive *E. coli* strains, *a*) *E. coli* S15, a K12 strain with a phospholipid-degradative apparatus

typical of wild type *E. coli* and *b*) *E. coli* S17, a mutant strain devoid of detectable phospholipase A activity, derived from *E. coli* S15 (10, 11).

MATERIALS AND METHODS

Materials

[1-^{14}C]Oleic acid and [1-^{14}C]palmitic acid (each 60 Ci/mol) were obtained from New England Nuclear Corp. Fatty acid-poor bovine serum albumin, Fraction V, was obtained from Pentex, Miles Research Products. Casamino acids were bought from Difco Laboratories and Hanks' balanced salt solution from Microbiological Associates. Thin-layer chromatography was performed on Silica Gel F-254 plates supplied by Brinkmann Instruments. All other chemicals were of reagent grade and were obtained from the usual sources.

Collection of serum

Venous blood from normal human volunteers and from New Zealand White rabbits was collected in sterile glass tubes. After clotting at 37°C for 45 min and clot retraction at 0°C for 30–60 min, the serum was taken and used the same day.

Bacteria

E. coli strains S15 and S17 (F$^-$, thi$^-$, thr$^-$, lac$^-$, mel$^-$) were kindly donated by Professor S. Nojima (Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan). The absence of both detergent-resistant and detergent-sensitive phospholipase A activities in S17 (10, 11) was confirmed by procedures previously described (12, 13). Both strains were grown in minimal medium buffered with triethanolamine at pH 7.75–7.9 (14). Bacterial cultures grown overnight to stationary phase were transferred to fresh medium (diluted 1:10) and the subcultures were grown to mid-late logarithmic phase by incubation for 3–4 hr at 37°C. Bacterial concentrations were determined by measuring absorbance at 550 nm. The bacteria were sedimented by centrifugation at 6000 *g* for 10 min and resuspended in sterile physiological saline in the desired concentration.

Labeling of *E. coli* phospholipids

Bacterial phospholipids were labeled during growth in subculture. Aliquots of an overnight culture of *E. coli* (S15 or S17) grown as described above were diluted 1:10 in fresh triethanolamine medium containing 0.2 μCi/ml of [1-^{14}C]palmitic acid or [1-^{14}C]oleic acid complexed with 0.02% bovine serum albumin (fatty acid-poor). After incubation for 2–3 hr at 37°C, the bac-

teria were sedimented by centrifugation at 6000 *g* for 10 min, resuspended in fresh triethanolamine medium and reincubated for 30 min to permit incorporation of the remaining unincorporated labeled precursor. The labeled bacteria were washed with 1% albumin, resuspended in saline, and used in one of two ways: 1) as live organisms, to examine the effect of serum on the envelope phospholipids of intact *E. coli*, caused either by serum phospholipase activity and/or by activation of bacterial phospholipases; or 2) after autoclaving for 15 min at 121°C and 2.7 kg/cm². This procedure inactivates heat-stable bacterial phospholipases (13) and renders the envelope phospholipids readily accessible to added phospholipase A.

The position of the incorporated labeled fatty acids was determined as previously described (12). More than 90% of the esterified [1-^{14}C]palmitic and [1-^{14}C]-oleic acids were located in the 1-acyl and 2-acyl positions, respectively, of *E. coli*'s diacylphospholipids. The distribution of the incorporated label closely corresponds to the chemical composition of *E. coli* phospholipids: diacylglycerophosphoethanolamine, 50–65%; diacylglycerophosphoglycerol, 15–20%; and cardiolipin, 10–15% (12).

Incubation procedures

Effects of serum toward live *E. coli* were determined in incubation mixtures containing 10 μmol Tris-HCl buffer (pH 7.5), 25 μl of Hanks' solution, 250 μg of vitamin-free casamino acids, 0.5 μmol CaCl$_2$, 60 μl serum (unless otherwise indicated), 2.5 × 10⁸ [^{14}C]-fatty acid-labeled *E. coli* and sterile physiological saline in a total volume of 0.24 ml. Typical incubation mixtures with autoclaved bacteria contained 80 μmol Tris-HCl (pH 7.5), 1.0 μmol CaCl$_2$, 2.5 × 10⁸ autoclaved [1-^{14}C]oleic acid-labeled *E. coli* (approximately 5 nmol of phospholipid; 10,000 cpm), serum (as indicated) and sterile physiological saline in a total volume of 0.5 ml. All incubations were carried out at 37°C.

Assay for bactericidal activity

In all experiments with live *E. coli* the bactericidal activity of serum was tested after incubation for 60 min. Samples of 10 μl were taken from the incubation mixtures, serially diluted in sterile isotonic saline and plated on nutrient agar. The number colony-forming units was determined after incubation at 37°C overnight.

Lipid extraction and fractionation

Lipids were extracted according to the procedure of Bligh and Dyer (15). The aqueous methanolic up-

per phase was removed and washed once with 0.5 volume of CHCl₃ to optimize recovery of monoacyl-phospholipids. The combined $CHCl_3$ extracts were dried under a nitrogen stream, redissolved in 0.05 ml $CHCl_3–CH_3OH$ 2:1 and transferred to Silica Gel F254 plates. Monoacylphospholipids, diacylphospholipids, and fatty acids were separated in a solvent system consisting of chloroform–methanol–distilled water–glacial acetic acid 65:25:4:1 (v/v). Under experimental conditions giving rise to ^{14}C-labeled free fatty acids as the only labeled product of hydrolysis (i.e., serum phospholipase A_2 action toward [1-^{14}C]oleic acid-labeled autoclaved *E. coli* or intact *E. coli* S17), chromatography was carried out in a solvent system consisting of petroleum ether–diethyl ether–glacial acetic acid 80:20:1 (v/v) which separates phospholipids and free fatty acids. Lipid species were identified by comparison of R_f with that of authentic standards visualized by exposure of the plates to iodine vapors. Liquid scintillation counting of thin-layer fractions scraped off the plates into counting vials was carried out as described previously (16).

Other procedures

Phospholipase A_2 activity was removed from human serum by adsorption onto autoclaved *Micrococcus lysodeikticus*, as previously described (8). Release of the periplasmic enzyme alkaline phosphatase from *E. coli* was measured as previously described (8, 17).

RESULTS

Phospholipase A_2 activity, measured against the phospholipids of autoclaved *E. coli* (6, 8) is at least 20-fold higher in rabbit serum than in human serum (8). It was necessary, therefore, to establish first that
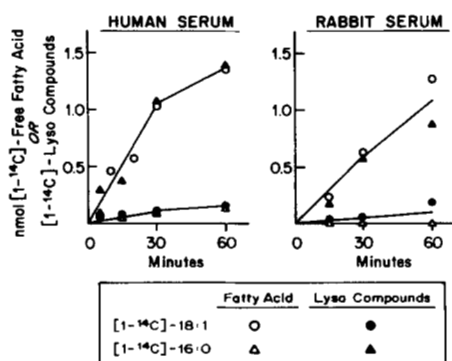
Fig. 1. Positional specificity of human and rabbit serum phospholipase A_2. Incubations up to 60 min with autoclaved *E. coli*, labeled either with [1-^{14}C]palmitic acid or with [1-^{14}C]oleic acid were carried out as described in Materials and Methods, except that 50 μl of fresh normal human serum and 1 μl of fresh normal rabbit serum per reaction mixture of 0.5 ml were used.
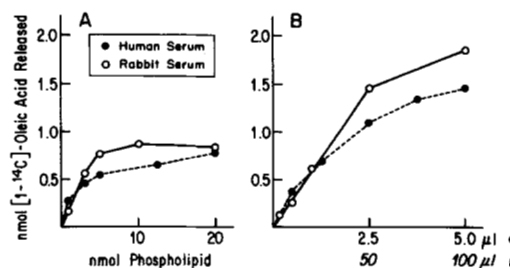
Fig. 2. Effect of increasing phospholipid (A) or serum concentration (B) on phospholipase A_2 activity of human and rabbit serum. Incubations were carried out as described in Materials and Methods. Incubation times and amounts of serum per reaction mixture were selected to yield convenient amounts of hydrolysis. Fig. 2A, normal human serum 20 min and 50 μl; normal rabbit serum 15 min and 2 μl. Substrate concentration was varied by varying the number of unlabeled autoclaved *E. coli* (2.5 × 10⁸ *E. coli* contain approx. 5 nmol of phospholipid). Fig. 2B, incubation times were 30 and 15 min for reaction mixtures containing human and rabbit serum, respectively. Each symbol represents the mean of the results of three independent experiments.

the catalytic properties of the two activities were generally similar, in order to be able to compare the phospholipase A_2 activity in human and rabbit serum towards intact *E. coli*.

Comparison of phospholipase A_2 activity of human and rabbit serum

The positional specificity of the serum phospholipase A activity was determined using *E. coli* autoclaved after labeling during growth either with [1-^{14}C]palmitic acid or with [1-^{14}C]oleic acid (labeling respectively the 1- and 2-ester position of the bacterial phospholipids (see Materials and Methods)). The radioactive products formed during hydrolysis of the labeled bacterial phospholipids by human or rabbit serum are almost solely ^{14}C-labeled free fatty acids from [^{14}C]oleic acid-labeled *E. coli* and ^{14}C-labeled lysophospholipids from [^{14}C]palmitic acid-labeled *E. coli* (**Fig. 1**). This indicates that degradation by both sera is almost exclusively the result of phospholipase A_2 activity.

During maximal phospholipid hydrolysis no radioactivity appears in association with cholesteryl esters, suggesting that lecithin-cholesterol-acyl transferase activity does not contribute to the phospholipase A_2 activity observed under our experimental conditions.

It has previously been shown by others (4, 5) that human and rat serum phospholipase A activity toward ^{32}P-labeled phospholipid substrates is maximal between pH 7.0 and 8.0. The phospholipase A_2 activity of both human and rabbit serum in our assay system is also maximal at physiological pH (not shown). **Fig. 2** shows the effect of increasing substrate and serum concentrations on hydrolysis of phospholipids of autoclaved *E. coli*. Hydrolysis reaches roughly the
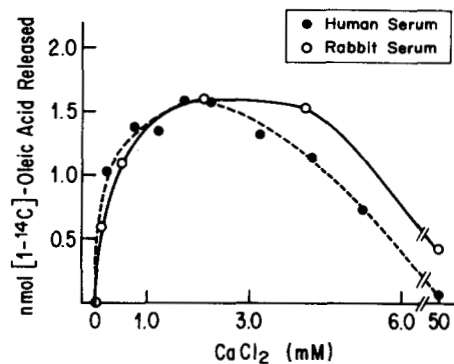
Fig. 3. Effect of Ca²⁺ concentration on phospholipase A_2 activity of human and rabbit serum on autoclaved [¹⁴C]oleic acid-labeled *E. coli*. For experimental procedure see Materials and Methods. Incubation time for 50 µl of human serum was 30 min, for 5 µl of rabbit serum 7.5 min. Results are of one of several similar experiments.

same plateau at a bacterial phospholipid concentration of 5–10 nmol per reaction mixture of 500 µl (Fig. 2A). This plateau is reached in 30 min at 50 µl of human serum and in 15 min at 2 µl of rabbit serum (Fig. 2B), reflecting the difference in activity of the two sera. Trypsin treatment of human serum increases its phospholipase A_2 activity up to four-fold. However, under our experimental conditions we have been unable to stimulate the activity in human serum with trypsin as dramatically as described by Etienne et al. (4).

As is typical for all phospholipases A_2 studied so far, activity in serum exhibits an absolute requirement for Ca²⁺ (4, 5) and is abolished by EDTA (2 mM). The optimal Ca²⁺ concentration is approximately 2 mM (**Fig. 3**), i.e., the Ca²⁺ concentration in normal serum. The phospholipase A_2 activity in human serum is slightly more sensitive than that in rabbit serum to the inhibitory effect of higher Ca²⁺ concentrations. Neither human nor rabbit serum phospholipase A_2 activities are markedly affected by varying Na⁺ or K⁺ concentrations over a range of 0 to 180 mM (not shown).

Degradation of bacterial phospholipids during the bactericidal action of human and rabbit serum on *E. coli* S15 (parent) and *E. coli* S17 (phospholipase A-deficient mutant)

The rate and extent of killing of *E. coli* S15 and *E. coli* S17 by 25% human or rabbit serum are roughly similar (not shown). After incubation for 60 min killing is nearly complete (>95% < 100%).

Killing of the parent strain *E. coli* S15 by either human or rabbit serum is accompanied by degradation of bacterial lipid (**Table 1**). Both ¹⁴C-labeled free fatty acids and ¹⁴C-labeled lysophospholipids accumulate whether the *E. coli* S15 were labeled with [1-¹⁴C]oleic acid or [1-¹⁴C]palmitic acid. This indicates that the action of deacylase activities is directed at both the 1- and 2-ester positions of the bacterial phospholipids. In contrast, the phospholipid hydrolysis that accompanies the killing by rabbit serum of *E. coli* S17 (the phospholipase A-deficient mutant) yields almost exclusively ¹⁴C-labeled free fatty acid when the label is [¹⁴C]oleic acid, but chiefly ¹⁴C-labeled lysocompounds when the label is [¹⁴C]palmitic acid. These findings are consistent with the conclusion that the rabbit serum phospholipase A_2 is primarily responsible for degradation of the phospholipids of *E. coli* S17, whereas bacterial phospholipase(s) (predominantly phospholipase A_1 (18–20)), activated in the parent strain during killing, contribute to degradation in *E. coli* S15. The release of some ¹⁴C-labeled free fatty acid in [¹⁴C]palmitic acid-labeled *E. coli* S17

TABLE 1. Hydrolysis of [¹⁴C]oleic acid- or [¹⁴C]palmitic acid-labeled *E. coli* S15 and *E. coli* S17 treated with human or rabbit serum

	E. coli S15				E. coli S17			
	¹⁴C-18:1		¹⁴C-16:0		¹⁴C-18:1		¹⁴C-16:0	
	% of Total Bacterial Lipid Radioactivity[a]							
Additions	Lyso[b]	FFA[c]	Lyso	FFA	Lyso	FFA	Lyso	FFA
Normal human serum	10.5	16.2	9.8	21.0	<1	<1	<1	<1
Adsorbed human serum	9.9	8.0	6.0	22.0	<1	<1	<1	<1
Normal rabbit serum	13.5	50.8	21.7	34.1	2.7	64.0	45.0	13.8

[a] Values given are corrected for radioactivity in lysocompounds and free fatty acids at 0-time: <2% and <2%, respectively, for [¹⁴C]oleic acid (18:1)-labeled *E. coli* S15 and *E. coli* S17, and [¹⁴C]palmitic acid (16:0)-labeled *E. coli* S15; <2% and <5%, respectively, for [¹⁴C]palmitic acid-labeled *E. coli* S17. *E. coli* incubated alone undergo no detectable net hydrolysis.

[b] Lyso, monoacylphospholipids (predominantly monoacylglycerophosphoethanolamine (13)).

[c] FFA, free fatty acids.

Experiments were carried out as described in Materials and Methods. Incubation time at 37°C was 60 min. In each instance viability at 60 min was <5%. Results are the mean of at least two closely similar experiments.

treated with rabbit serum suggests that lysophospholipase(s), known to be present in the phospholipase A-deficient mutant (21), is activated and further deacylates ^{14}C-labeled lysophospholipids generated by the serum phospholipase A_2.

In contrast to the effects of rabbit serum, most human sera tested do not cause appreciable net phospholipid hydrolysis during killing of the phospholipase-A-deficient mutant *E. coli* S17. The hydrolysis generated by human serum in *E. coli* S15 (Table 1) is therefore most readily explained by activation of bacterial phospholipases. That human serum phospholipase A_2 does not substantially contribute to this hydrolysis is further indicated by the observation that human serum, preadsorbed onto autoclaved *Micrococcus lysodeikticus*, a procedure that removes almost all phospholipase A_2 activity from human serum without altering its bactericidal activity (8), causes nearly as much hydrolysis as untreated serum (Table 1).

The phospholipase A-deficient *E. coli* mutant offers a useful tool for further evaluation of the differences in phospholipase A_2 activity of human and rabbit serum towards the bacterial phospholipids during serum-induced killing. Such differences may merely reflect the apparent differences in phospholipase A_2 activity of the two sera, but may also represent differences in other serum constituents, including antibacterial factors. For example, rabbit serum might be more destructive to the bacterial envelope than human serum and hence more effective in promoting access of serum phospholipase A_2 to the envelope phospholipids. The following experiments suggest that this is not the case. The release of the periplasmic
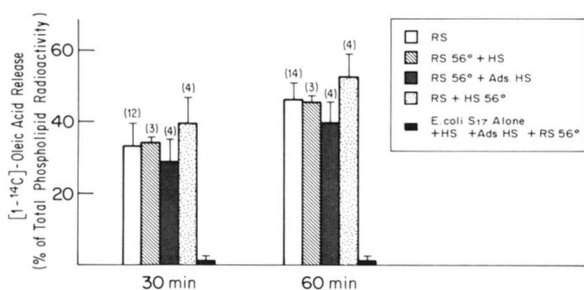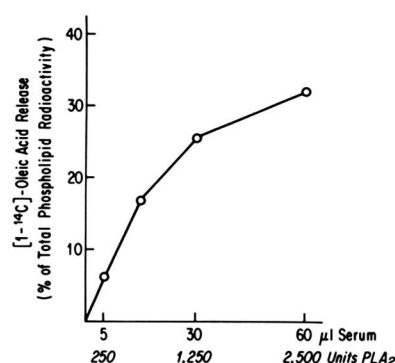

Fig. 5. Effect of increasing amounts of heated rabbit serum on hydrolysis of *E. coli* S17 phospholipids during bacterial killing by human serum. Standard incubation mixtures contained 2.5×10^8 *E. coli* S17 labeled with [^{14}C]oleic acid, 60 μl of normal human serum, and the indicated amount of heated rabbit serum. Incubations were carried out for 30 min at 37°C. Bacterial killing by human serum was >95%, with or without added heated rabbit serum.

enzyme alkaline phosphatase into the medium, a measure of the envelope damage that accompanies bacterial killing by serum (8), is in fact less pronounced during killing of *E. coli* S17 by rabbit serum than by human serum (not shown). Further, as shown in **Fig. 4,** human serum is capable of facilitating the action of rabbit serum phospholipase A_2 on *E. coli* phospholipids as effectively as rabbit serum itself. This was shown by preincubating rabbit serum at 56°C, which abolished its bactericidal activity without markedly affecting its phospholipase A_2 activity (toward autoclaved *E. coli*), and then combining the heated rabbit serum with 25% normal human serum.

As shown in Fig. 4, neither 25% human serum (normal or adsorbed) nor 56°C-heated rabbit serum alone produce appreciable net phospholipid hydrolysis in *E. coli* S17. By contrast, normal rabbit serum produces extensive hydrolysis during a one-hour incubation period. Hydrolysis is nearly the same when heated (non-bactericidal) rabbit serum is added to 25% normal or adsorbed (phospholipase A_2-depleted) human serum, or when heated human serum is added to 25% normal rabbit serum. These findings suggest that the differences in bacterial phospholipid degradation produced by human and rabbit serum indeed reflect the differences in level of phospholipase A_2 activity in the two sera. The results shown in **Fig. 5** support this conclusion. Phospholipid hydrolysis in *E. coli* S17, killed by adsorbed human serum, increases with increasing amounts of added 56°C-heated rabbit serum as a source of phospholipase A_2. At low doses of heated rabbit serum (<5 μl), containing as little phospholipase A_2 activity (toward autoclaved *E. coli*) as 25% normal human serum, little or no hydrolysis is produced.

Fig. 4. Phospholipid hydrolysis during killing of *E. coli* S17 by human or rabbit serum. Relative phospholipase A_2 activity of human and rabbit serum. *E. coli* S17 (2.5×10^8), labeled with [^{14}C]oleic acid, were incubated at 37°C for 30 or 60 min with normal or heated (56°C for 30 min) rabbit serum, added either alone or in combination with one of the following human sera: fresh normal serum, 56°C-heated serum or adsorbed serum. Each serum fraction was added in amounts of 60 μl in a total volume of 240 μl of the standard incubation mixture. The black bar represents *E. coli* S17 incubated alone or with either normal human serum, adsorbed human serum, or heated rabbit serum. Results are shown as mean ± SEM of the indicated number of experiments (n).

DISCUSSION

The phospholipase A_2 activity of human and rabbit serum appears to account for nearly all of the phospholipid deacylating activity in serum toward the phospholipids of both autoclaved and intact E. coli. We have found no evidence of appreciable phospholipase A_1 activity (1–3). Moreover, heparin administration to man or rabbit has no detectable effect on the amount or positional specificity of serum phospholipase A activity toward this bacterial substrate.[1] This is remarkable because numerous studies indicate that post-heparin serum lipoprotein lipase activity is associated with phospholipid-deacylating activity of A_1 specificity (1, 23), manifest in the hydrolysis of serum lipoprotein phospholipid (2, 3). These apparently discrepant observations possibly relate to the manner in which the phospholipids are presented for the demonstration of serum phospholipase A_1 or A_2 activities and hence to the physiological function of these two deacylating activities. This would imply that post-heparin serum phospholipase A_1 acts preferentially on the phospholipids of circulating lipoproteins, but that the preferred substrates for serum phospholipase A_2 are the bacterial envelope phospholipids, rendered accessible either by surface-active bactericidal constituents of serum (complement) or by autoclaving of the bacteria. We recognize that this view may well be too simple and that multiple factors determine the action of phospholipases A on one or another substrate (24, 25). Whatever the complexities of the system, our results firmly establish that under our experimental conditions the positional specificity of the serum deacylating activity is almost exclusively that of a phospholipase A_2.

Etienne and collaborators (4, 5) have shown that storage of serum or addition of very high concentrations of trypsin to whole human and rat serum increase phospholipid-splitting activity many-fold, and they have concluded that phospholipase A in plasma or serum exists in the form of a proenzyme. Under the conditions of our experiments high trypsin concentrations (50 μg/500 μl of serum) also increased activity as much as 4-fold (not shown). However, such an increase in activity after trypsin treatment of whole serum might be caused by mechanisms other than cleavage of a proenzyme. Elucidation of the activation phenomenon must await purification of the serum phospholipase(s) A_2.

The phospholipids of most strains of intact E. coli, including E. coli S15 and E. coli S17, are not degraded

by added phospholipases (25–27). This is also true for serum phospholipase A_2 in the absence of bactericidal serum factors. Thus, in contrast to normal rabbit serum, the addition of heated rabbit serum (non-bactericidal, phospholipase A_2-rich) to E. coli S17 does not cause phospholipid degradation. However, when bactericidal human serum (phospholipase A_2-poor) is supplied as well, prompt phospholipid degradation is again evident (Figs. 4 and 5). Hence the bactericidal events render previously inaccessible phospholipids susceptible to hydrolysis by both bacterial (E. coli S15) and exogenous phospholipases A (E. coli S15 and E. coli S17).

The participation of serum phospholipid-splitting activity in a degradative attack on live E. coli during exposure to bactericidal concentrations of serum has previously been reported (9). Unequivocal demonstration of the action of exogenous phospholipase A activity on bacterial phospholipids has been made possible by the availability of phospholipase A-deficient mutants (10, 11). The use of such mutants has been particularly helpful in studies on the biological role of phospholipid degradation in the antimicrobial action of polymorphonuclear leukocytes and serum (9, 12, 25, 27). It has been established in our laboratory that, while killing of E. coli by granulocytes or granulocyte fractions is accompanied by very early net degradation of bacterial phospholipid, the bactericidal event does not require phospholipid breakdown (25, 27, 28).

The observations of Kreutzer et al. (9) and the data presented here suggest that the effectiveness of killing of E. coli by serum is also independent of bacterial phospholipid degradation. Thus, these two studies have shown that the bactericidal activities of human, guinea pig, and rabbit sera toward E. coli S17 are not markedly different, even though human and guinea pig serum cause little or no phospholipid degradation while rabbit serum causes extensive hydrolysis in the phospholipase-deficient mutant. Moreover, the killing efficiency of the three sera toward E. coli S15, in which activation of bacterial phospholipase A coincides with the rapid killing phase, is essentially the same as toward E. coli S17.

The envelope changes that accompany killing of gram-negative bacteria by serum and also by polymorphonuclear leukocytes, include an increase in envelope permeability (8, 25, 27–32). The permeability alterations caused by potent purified bactericidal leukocyte proteins, that appear mainly responsible for the leukocyte's bactericidal action toward gram negative bacteria, are quite discrete and appear restricted to the outer membrane (25, 27–32). The envelope damage caused by serum is much more

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[1] Kaplan-Harris, L., J. Weiss, C. Mooney, S. Beckerdite-Quagliata, and P. Elsbach. Unpublished observations.

generalized and includes the release of large molecules such as alkaline phosphatase from the periplasmic space (8). Both the subtle alterations in the outer membrane produced by purified bactericidal leukocyte proteins and the cruder envelope lesions inflicted by serum are the same in the absence and presence of phospholipid hydrolysis. Evidence is lacking, therefore, that the fate of gram-negative bacteria vis à vis humoral or cellular host defense forces is much influenced by the action of either exogenous or endogenous phospholipases A. It is possible, however, that the early enzymatic attack on the gram-negative bacterial envelope phospholipids coincident with the bactericidal action of serum and granulocytes determines the rate and extent of the digestive process that is part of microbial destruction by the host (33). Whatever the role of phospholipid hydrolysis in the ultimate fate of microorganisms, it should be noted that its occurrence and onset are closely linked to the bactericidal event, whether caused by serum or by polymorphonuclear leukocytes (8, 9, 25, 27–31).⬛

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