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INFLUENCE OF SOME PATHOGENIC BACTERIA ON KININ FORMATION AND DESTRUCTION

BY

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The plasma kinins produce the four cardinal signs of inflammation (Elliott, Horton & Lewis, 1960), and it has been suggested that they may act as chemical mediators in the inflammatory reaction.

The amount of free kinin in an area at any one time depends on the available amount of kinin precursor as well as the amounts of kinin-forming and kinin-inactivating enzymes present. Plasma itself contains an inactive precursor of a kinin-forming enzyme. This precursor can be activated by dilution or acidification. Plasma also contains kinin precursor as well as kinin-inactivating enzyme. As kinin formation may be important in the inflammatory response, the effect of micro-organisms on kinin formation and inactivation is obviously of great interest.

In this investigation several bacteria have been examined for kinin-forming and kinin-inactivating activity. Microbes from the genera *Pseudomonas* (*Ps. aeruginosa*), *Escherichia* (*E. coli*) and *Streptococcus* (a β-haemolytic streptococcus) have been most extensively studied. They were chosen as microbes which were different in respect to Gram-staining, pathogenicity and biochemical characteristics.

METHODS

Bacterial cultures. These were obtained from Kapt. W. Wilhelmsen og frues bakteriologiske institutt, University of Oslo, and from the Bacteriological Laboratory, Oslo City Hospital.

Culturing of microbes. The different microbes were allowed to grow under constant shaking for 18 hr at 37° C in a medium consisting of 5 g glucose, 5 g peptone and 5 g meat extract per 1,000 ml. of water. The cultures were checked for contamination. The cultures were then centrifuged at 1,900 *g* for 30 min. The supernatant fluid and the sediment were investigated separately. After having been washed three times in 0.9% saline, the sediment was suspended in 0.1 M-phosphate buffer of *p*H 6.5 and the density of the suspension was examined in a Beckmann photometer at 680 mμ. One portion of each microbial suspension was diluted with 0.1 M-phosphate buffer of *p*H 6.5 until the optical density was 0.30 at 680 mμ. A standard suspension of microbes five times more concentrated than the diluted portion with this defined optical density was finally used for the tests.

Ultrasonic treatment of microbial suspensions. This was carried out with an MSE ultrasonic disintegrator at 20,000 cycles/sec for 5 min.

Kinin-forming activity. This was evaluated by incubating the test material with a stable substrate plasma preparation (Amundsen, Nustad & Waaler, 1963). Washed sediments (standard suspension), ultrasonically treated sediments, as well as supernatant fluids from the glucose broth cultures of the three types of microbes, were tested for such activity by incubating 0.2 ml. of the specimen to be tested with 0.8 ml. of the stable

substrate plasma at 37° C. Aliquots were removed from the incubates after 1, 6, 11, 16 and 21 min and tested for kinin activity.

In some experiments microbes were cultured in the stable substrate plasma itself, and at intervals samples were removed and tested for kinin activity.

RESULTS

Kininase activity of microbes

It may be difficult to reveal kinin-forming enzymes in a specimen which also possesses kininase activity. We have therefore first tested our samples for kininase activity. When such activity was present, kininase inhibitors were added during subsequent tests for kinin-forming enzymatic activity.

Three fractions of each microbial culture were examined for kininase activity, namely the supernatant fluid, the washed sediment and the ultrasonically disintegrated sediment (Table 1). The fresh glucose-peptone-meat extract medium itself showed no kininase activity.

TABLE 1

KININASE ACTIVITY OF MATERIAL DERIVED FROM MICROBES

Suspensions of microbes were cultured in glucose broth, centrifuged, washed, resuspended and treated ultrasonically as described in Methods. Supernatant refers to the centrifuged 18-hr culture. Kininase activity of specimens: none=0; 0.5 μg synthetic bradykinin eliminated by 0.9 ml. of test material in the course of 15 to 30 min=+; in the course of 5 to 15 min=++; and in less than 1 min=++++

	Kininase activity in		
		Sediment	
Microbe	Supernatant	No ultrasonic treatment	Ultrasonic treatment
Ps. aeruginosa	++++	+	++
E. coli	0	+	++
β-Haemolytic streptococci	0	0	++

The supernatant fluid of *Ps. aeruginosa* cultures possessed pronounced kininase activity (Fig. 1), whereas the supernatant fluids of *E. coli* and the β-haemolytic streptococci showed no such activity (Figs. 2 and 3). In a 1 : 200 dilution the supernatant fluid of *Ps. aeruginosa* cultures eliminated 0.5 μg of synthetic bradykinin within 10 min (Fig. 1).

The washed sediments from cultures of *Ps. aeruginosa* and *E. coli* possessed slight and almost equally pronounced kininase activities when tested in suspensions of standard optical density. A similar suspension of washed β-haemolytic streptococci showed no kininase activity.

A marked increase in kininase activity of all three microbial suspensions was observed after ultrasonic treatment at 20,000 cycles/sec for 5 min (Figs. 1, 2 and 3).

Kinins may be broken down by various types of enzymes. The plasma kininase is probably a carboxypeptidase, whereas the kininase activity present in erythrocytes is apparently due to another enzyme (Erdös, Renfrew, Sloane & Wohler, 1963). The plasma kininase is inhibited by disodium edetate whereas that present in erythrocytes is not (Erdös *et al.*, 1963). Disodium edetate in a concentration of 0.1% inhibited completely the kininase activity of ultrasonically treated suspensions of *E. coli* and *Ps. aeruginosa* but not that of

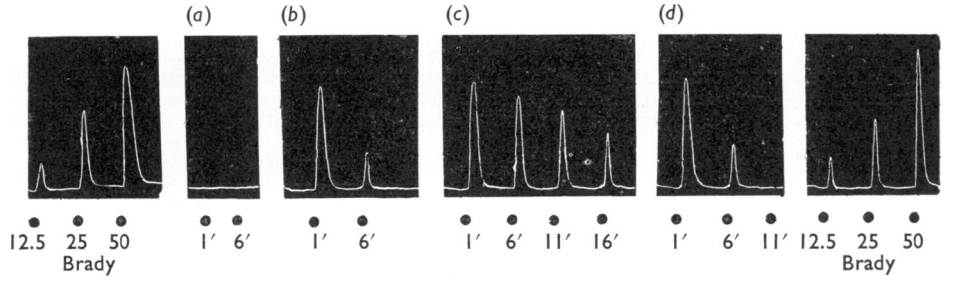

Fig. 1. Kininase activity of materials derived from *Ps. aeruginosa* culture. Incubation at 37° C of 0.9 ml. of test material with 0.1 ml. of saline containing 0.5 μg of synthetic bradykinin. Contractions of rat isolated uterus suspended in 10 ml. of de Jalon solution on addition of 0.1 ml. of incubates. Times of incubation are given below in min. Standard doses (ng) of synthetic bradykinin (Brady) were added to the 10-ml. organ-bath before and after tests. Test materials: (*a*) supernatant fluid after centrifugation at 1,900 *g* for 30 min of bacterial culture in glucose broth; (*b*) same supernatant fluid diluted 1 : 200 with saline; (*c*) resuspended, washed sediment after the same centrifugation for (*a*), standard concentration of sediment suspension being used (see Methods); and (*d*) same sediment suspension as used for (*c*) after ultrasonic treatment at 20,000 cycles/sec for 5 min.

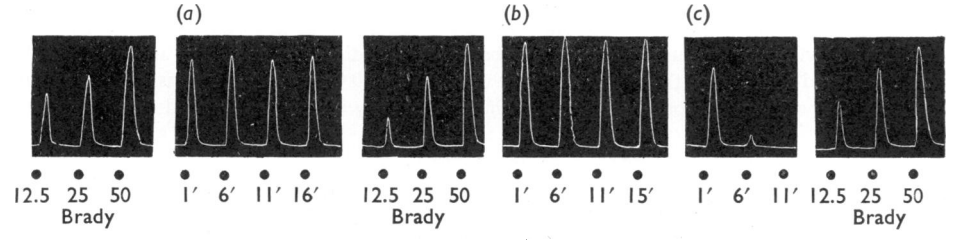

Fig. 2. Kininase activity of materials derived from culture of β-haemolytic streptococci. Assays carried out as described in Fig. 1. Test materials: (*a*) supernatant fluid after centrifugation at 1,900 *g* for 30 min of bacterial culture in glucose broth; (*b*) resuspended, washed sediment after centrifugation in (*a*), with standard concentration of sediment suspension (see Methods); and (*c*) same sediment suspension as for (*b*) after ultrasonic treatment at 20,000 cycles/sec for 5 min.

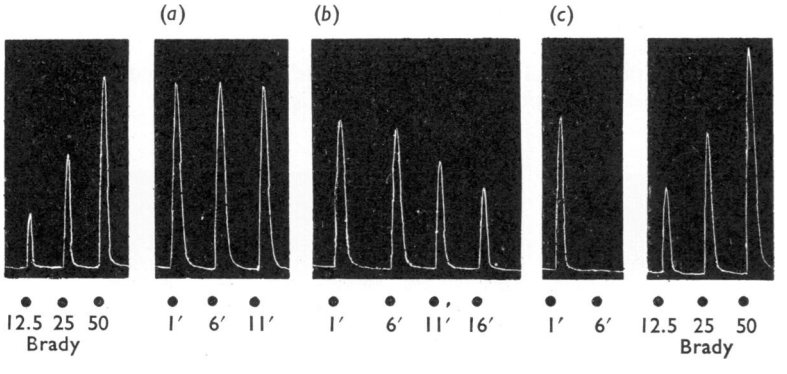

Fig. 3. Kininase activity of materials derived from *E. coli* culture. Assays carried out as described in Fig. 1. Test materials: (*a*) supernatant fluid after centrifugation at 1,900 *g* for 30 min of bacterial culture in glucose broth; (*b*) resuspended, washed sediment after centrifugation in (*a*), with standard concentration of sediment suspension (see Methods); and (*c*) same sediment suspension as for (*b*) after ultrasonic treatment at 20,000 cycles/sec for 5 min.

TABLE 2

EFFECT OF DISODIUM EDETATE ON KININASE ACTIVITY

Supernatant and sediment are used as in Table 1, the latter being ultrasonically treated. 0=No effect of disodium edetate; +=complete inhibition of kininase activity by disodium edetate at a concentration of 0.1% in incubate; and −=no kininase activity

Microbe	Kininase activity in	
	Supernatant	Sediment
Ps. aeruginosa	0	+
E. coli	−	+
β-Haemolytic streptococci	−	0

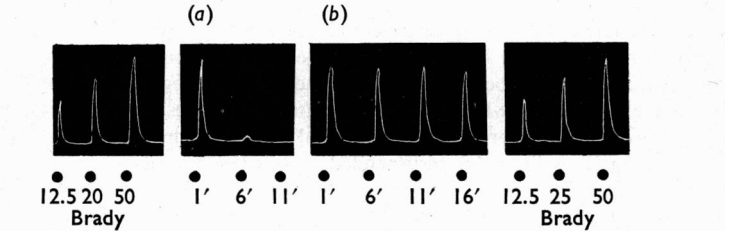

Fig. 4. Effect of disodium edetate on kininase activity of ultrasonically treated suspension of washed sediment of *E. coli* culture. The suspension was obtained and treated as described in Fig. 3. Test materials: (*a*) without disodium edetate in incubate; (*b*) with disodium edetate in incubate. 0.1 ml. of a 1% buffered solution of the chelating agent was added to 0.9 ml. of the suspension. Incubation was at 37° C for 10 min before the assay for kininase activity which was carried out as described in Fig. 1.

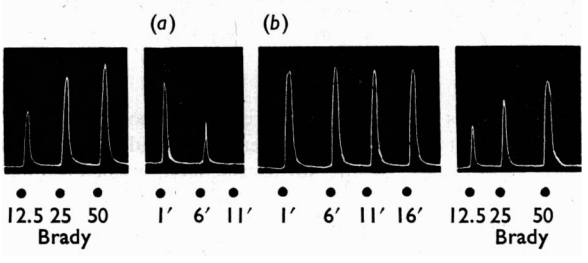

Fig. 5. Effect of disodium edetate on kininase activity of ultrasonically treated suspension of washed sediment of *Ps. aeruginosa* culture. The suspension was obtained and treated as described in Fig. 1. Test materials: (*a*) without disodium edetate in incubate; (*b*) with disodium edetate in incubate. Incubation with the chelating agent was as described in Fig. 4.

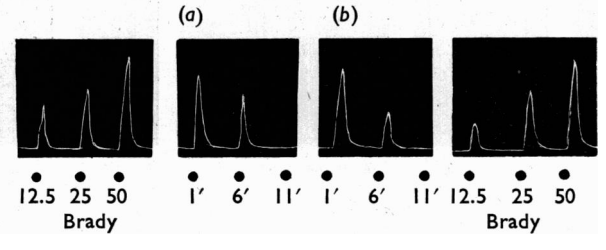

Fig. 6. Effect of disodium edetate on kininase activity of ultrasonically treated suspension of washed sediment of culture of β-haemolytic streptococci. The suspension was obtained and treated as described under Fig. 2. Test materials: (*a*) without disodium edetate in incubate; (*b*) with disodium edetate in incubate. Incubation with the chelating agent was as described in Fig. 4. Assays for kininase activity were as described in Fig. 1.

the β-haemolytic streptococci (Table 2, Figs. 4, 5 and 6). The kininase activity of the supernatant fluid after centrifugation of the *Ps. aeruginosa* culture was not inhibited by disodium edetate.

In addition to the experiments mentioned above, a few tests were also carried out with two more genera of microbes, namely *Proteus* and *Sarcina*. Ultrasonically treated suspensions of these microbes showed marked kininase activity as well.

Kinin-forming capacities of microbes

Cultures and suspensions of microbes were examined in various ways for kinin-forming capacity.

(1) Microbes were cultured in a stable substrate plasma (Amundsen *et al.*, 1963) at 37° C for 24 hr. During the first 5 hr, aliquots were removed every 30 min and tested for plasma kinin activity. A final test was also carried out after 24 hr. No kinin activity was ever detected in these cultures. During some experiments the substrate plasma became

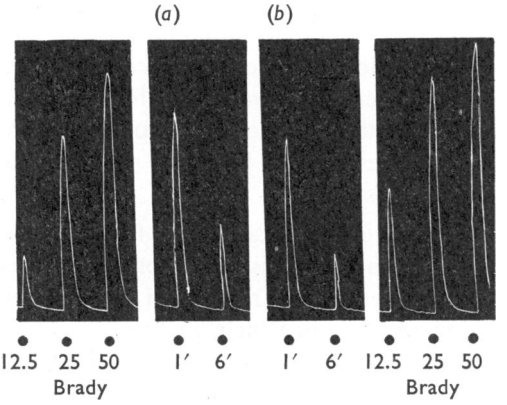

Fig. 7. Effect of disodium edetate on kininase activity of diluted supernatant fluid after centrifugation of *Ps. aeruginosa* culture in glucose broth. Centrifugation was at 1,900 *g* for 30 min. Supernatant fluid was diluted 1 : 200 in saline. Assays for kininase activity were carried out as described in Fig. 1. Test materials: (*a*) without disodium edetate in incubate; (*b*) with disodium edetate in incubate. Incubation with the chelating agent was as described in Fig. 4. Assays for kininase activity were as described in Fig. 1.

exhausted of its content of kinin precursor. It was thought that a continuous kinin formation could have occurred in the incubates, but that it might have been masked by the kininase activity of the microbes themselves. In some experiments disodium edetate was therefore added to the substrate plasma to a concentration of 0.1%. This did not stop the growth of the microbes being used in these experiments. Nor did it unveil any kinin activity in the incubates.

(2) It was considered possible that the procedure followed during the preparation of the substrate plasma could have removed from the plasma factors necessary for the production of plasma kinins by bacteria. All three species of microbes were therefore cultured for 24 hr in fresh citrated human plasma (3.1% sodium citrate dihydrate as 1 in 10 of the plasma volume). Plasma kinin activity was not detected in these incubates either.

(3) Washed sediments (standard suspension) as well as supernatant fluids from the ordinary glucose broth cultures of the three types of microbes were also tested for kinin-forming activity. No such activity was detected in any of the incubates. Similarly no kinin-forming activity was found in suspensions of ultrasonically treated microbes.

(4) In another set of experiments the procedure described under (3) was repeated, but with addition of disodium edetate to the test material. The specimen to be tested was first incubated with disodium edetate in a concentration of 0.1% for 10 min before being incubated with the substrate plasma. Neither was kinin activity revealed, however, in any of these incubates.

DISCUSSION

The possible role of plasma kinins in the development of inflammation prompted the present investigations on kinin formation and inactivation by microbes.

Kinin-forming enzyme activity has previously been demonstrated in products derived from *Clostridium histolyticum* (Prado, Monnier, Prado & Fromageot, 1956; Prado & Prado, 1962). Cysteine, which is a well-known inhibitor of kininase activity (Erdös *et al.*, 1963) was present in their incubation mixtures. Prado *et al.* (1956, 1962) do not, however, discuss the tendency to spontaneous plasma kinin formation in their bovine globulin substrate. It is therefore difficult to know how much of the plasma kinin activity detected by them had been due to enzymes derived from the micro-organisms and how much to spontaneous formation in the substrate.

We have been unable to detect enzymes capable of forming plasma kinins in any of the microbes used during this investigation. This was surprising in the case of the β-haemolytic streptococci, since some of the haemolytic streptococci are known to produce streptokinase which activates plasminogen to plasmin which, in turn, is capable of producing plasma kinins from a suitable substrate (Lewis, 1958). The streptokinase-forming ability of the β-haemolytic streptococcus used in these experiments has not been investigated, however. The kininase activity of the streptococci themselves could also possibly have masked a relatively weak kinin formation by the microbes.

It is well established that plasma contains inactive precursors to kinin-forming enzymes (Lewis, 1960). Treatments such as dilution, contact with a foreign surface or acidification activate these precursors to kinin-forming enzymes (Lewis, 1960). It seems possible that micro-organisms could induce plasma kinin formation through such a procedure. It is also possible that inhibition of plasma kininase activity due to lowering of *p*H (Edery & Lewis, 1962) may play some role in increasing the plasma kinin activity at a site of inflammation. It is well known that inflammatory exudates usually have a *p*H on the acid side of 7.

The most significant finding in the present investigation is the presence of kininase activity in all microbes tested. In *Ps. aeruginosa* a high kininase content was developed also in the surrounding medium. It is tempting to speculate whether this ability of microbes to eliminate kinins may be related to their pathogenicity. Here not only the amount but also the type of kininase activity may be significant. The kininase activities attached to the bodies of the different microbes were apparently not caused by one and the same enzyme. Thus the kininases derived from the β-haemolytic streptococci were not inhibited by

disodium edetate, whereas those obtained by ultrasonic treatment of *E. coli* or *Ps. aeruginosa* were. This finding may possibly be of pathophysiological importance.

Interestingly enough, *Ps. aeruginosa* seems to develop two different kininases, one being inhibited by disodium edetate, the other not. The first type of kininase is bound to the microbes themselves, the other is found in the supernatant fluid after centrifugation of the culture.

SUMMARY

1. Intact or ultrasonically treated bacteria as well as the media from cultures of *E. coli*, *Ps. aeruginosa* and β-haemolytic streptococci have been examined for presence of kinin-forming and destroying activities.

2. Kinin-forming activity could not be revealed in any of the bacterial species investigated.

3. Washed sediments of *E. coli* and *Ps. aeruginosa* cultures possessed weak kininase activities whereas sediments of cultures of β-haemolytic streptococci showed no kininase activity. Marked kininase activity was seen after ultrasonic treatment of all three types of microbial sediments. The kininase of *Ps. aeruginosa* and *E. coli*, but not that of β-haemolytic streptococci, was inhibited by disodium edetate.

4. In cultures of *Ps. aeruginosa* a marked kininase activity was found also in the medium. This activity was not inhibited by disodium edetate.

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