

FORMYL-MET-LEU-PHE INDUCES CHEMOTAXIS AND ACROSOMAL ENZYME RELEASE IN BULL SPERM

S. VIJAYASARATHY, S. SHIVAJI, M. IQBAL and P. BALARAM
Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

Received 27 March 1980

1. Introduction

The chemical nature of the factors that induce the selective fusion and vesiculation of the outer acrosomal and plasma membranes in sperm cells is yet to be established [1,2]. This process, known as the acrosome reaction, precedes fertilisation of the egg and is accompanied by the release of the degradative enzymes sequestered in the acrosomal vesicle of sperm [1]. The synthetic tripeptide, formyl-Met-Leu-Phe has been shown to induce chemotaxis in rabbit neutrophils [3]. It also causes the release of lysosomal enzymes, a process which must necessarily involve a fusion of the lysosomal and plasma membranes [4]. Functional analogies between lysosomes and the acrosome in sperm have been drawn [5,6]. Here we describe the selective release of bull sperm acrosomal enzymes, induced by formyl-Met-Leu-Phe. The specificity of this process is established by the inability of related di- and tripeptide analogs to cause enzyme release. Further, formyl-Met-Leu-Phe is also shown to induce chemotaxis in bull sperm. This observation demonstrates for the first time that low molecular weight peptides may indeed be capable of stimulating release of acrosomal contents, a process closely identified with the acrosome reaction.

2. Experimental

All peptides listed in table 1 were synthesised by solution phase procedures and checked for homogeneity by thin-layer chromatography (TLC). Their structures were established by 60 and 270 MHz ^1H

NMR. The extent of acrosomal enzyme release was monitored by incubating the sperm cells with the peptide followed by centrifugation and assay of the enzymatic activities in the supernatant. Typically, 4 ml fresh bull semen was diluted with 12 ml Krebs-Ringer bicarbonate buffer (pH 7.4) containing fructose (1 mg/ml) [7]. About 5 ml of the diluted semen was layered over 5 ml 1.3 M sucrose/0.9% NaCl and centrifuged at 6000 rev./min for 10 min. The pellet was resuspended in 20 ml buffer and spun down at 6000 rev./min $^{-1}$ /15 min $^{-1}$. The resultant sperm pellets were resuspended in a minimum volume of buffer. Peptides (100 μM) and BSA $^+$ (2 mg/ml) were added to 1 ml sperm suspension ($\sim 5 \times 10^6$ cells). The controls lacked the peptide. The mixtures were incubated at 37°C for 1 h and the cells, pelleted. The supernatants were collected and assayed for hyaluronidase [8], acrosin [9], lactate dehydrogenase (LDH) [10], aryl sulfatase [11], *N*-acetyl- β -D-glucosaminidase [12] and acid phosphatase [13] by standard procedures. The chemotactic response of bull sperm to the peptides was measured by the inverted capillary assay [14]. Freshly collected semen was suspended into yolk-citrate buffer containing 4 mM CaCl_2 . Experiments were carried out at 37°C.

3. Results and discussion

Table 1 summarises the results obtained using 8 peptides. There is a marked enhancement in the activities of hyaluronidase, aryl sulfatase, acid phosphatase and *N*-acetyl- β -D-glucosaminidase, in the supernatants of cell suspensions treated with formyl-Met-Leu-Phe. These enzymes have been shown to be present in the acrosome and their release accom-

Address correspondence to P. B.

Table 1
Average level of acrosomal enzymes released following incubation of sperm with peptides^a

Enzyme	f-Met-Leu-Phe	f-Pro-Leu-Phe	f-Leu-Met-Phe	f-Leu-Phe-Met	Boc-Phe-Leu	f-Met-Phe	Boc-Leu-Phe	Boc-Met-Phe
Hyaluronidase	+51.80(4)	+2.35(2)	+2.85(2)	-2.36(1)	-2.16(2)	-(1)	+0.04(1)	+0.05(1)
Aryl sulfatase	+70.70(7)	-(2)	-(2)	-11.36(1)	-30.34(2)	-2.53(1)	+0.02(1)	+3.68(1)
<i>N</i> -Acetyl- β -D-glucosaminidase	+89.90(9)	-(2)	-(2)	-4.80(1)	-24.39(2)	+5.71(1)	-7.23(1)	+12.19(1)
Acid phosphatase	+33.30(9)	-4.76(2)	-(2)	-2.25(1)	-5.50(2)	-51.20(1)	+3.47(1)	+3.53(1)
Acrosin	-(8)	-(2)	-(2)	-(1)	-(2)	-(1)	-(1)	-(1)
Lactate dehydrogenase	-(8)	-(2)	-(2)	-(1)	-(2)	-(1)	-(1)	-(1)

^a + and - signs indicate the percentage increase and decrease, respectively, of enzyme levels over the control. Figures in parentheses indicate the number of experiments averaged

panies in vitro acrosome reaction [15]. On the other hand there is no detectable activity of LDH, a cytoplasmic enzyme or acrosin, which is presumably tightly bound to the inner acrosomal membrane [1]. Acrosin activity could, however, be detected in the supernatants after exposure of sperm to low pH. The lack of acrosin release has been noted in studies of in vitro acrosome reaction of rabbit sperm, induced by rabbit serum [15]. Addition of the tripeptide sequence analogs, formyl-Leu-Phe-Met or formyl-Leu-Met-Phe did not result in any increase in the acrosomal enzyme levels. The tripeptide formyl-Pro-Leu-Phe and the dipeptides formyl-Met-Phe, Boc-Leu-Phe, Boc-Phe-Leu and Boc-Met-Phe were also largely without effect. This suggests a definite specificity of interaction between formyl-Met-Leu-Phe and bull sperm. The values in table 1 are expressed as % increase over control samples, without peptide. Moderately high levels of the acrosomal enzymes were obtained in the control samples, presumably due to the medium employed, which is known to facilitate acrosome reaction. Indeed, the addition of BSA is essential to detect peptide induced enhancements of acrosomal enzyme release. BSA has been used to promote in vitro acrosome reaction of sperm [16]. The levels of enzyme release in sperm induced by the tripeptide are lower than the corresponding values reported for lysosomal enzyme release in neutrophils [3]. This may, in part, reflect the low percentage of sperm cells capable of undergoing acrosome reaction, an observation generally reported in studies of in vitro fertilisation [15]. In studies on neutrophils [3], cytochalasin B has been used to enhance enzyme release, in contrast to the

experiments reported here. Addition of ATP (60 μ M) to the medium results in enhancements of 49%, 69% and 4% of aryl sulfatase, acid phosphatase and *N*-acetyl- β -D-glucosaminidase, respectively, over that observed in the presence of tripeptide alone. Addition of ascorbic acid (5 mM) leads to enhancements of 56%, 80% and 25% of these 3 enzymes over the peptide value. Fig.1 shows the chemotactic effect of the peptide on bull sperm as examined by the inverted capillary assay. It is interesting to note that the response increases linearly with concentration up to ~ 80 μ M. At higher peptide concentration there is a sharp fall in the chemotactic response. Prelimi-

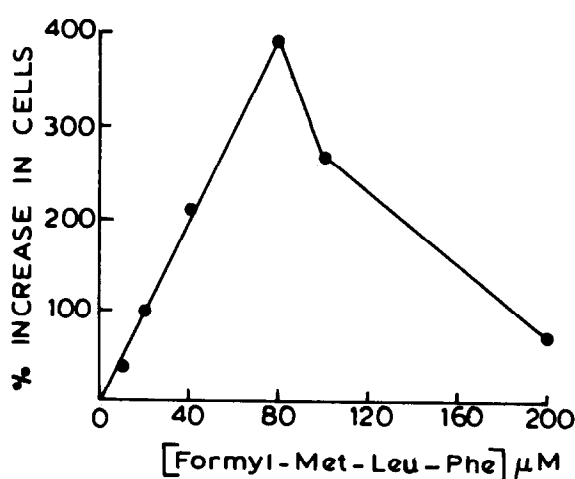


Fig.1. Chemotactic response of bull sperm. The ordinate represents the percentage increase in the number of cells entering a capillary containing the peptide solution, over that of a control capillary. Assay conditions were as in [14].

nary studies with formyl-Leu-Phe-Met and Boc-Leu-Phe suggest that these peptides are without effect, again emphasising the specificity of the phenomenon.

The acrosome reaction has been considered as an example of stimulus-secretory coupling, mediated by Ca^{2+} influx into the cell [17]. Interestingly, the transmembrane movement of Ca^{2+} accompanies interaction of neutrophils with chemotactic and lysosomal enzyme releasing factors [18,19]. We have demonstrated a high Ca^{2+} -ATPase activity in a subcellular fraction from bull sperm, that is enriched in plasma membranes (unpublished). The enhancement of peptide induced acrosomal enzyme release by ATP suggests a possible role for membrane bound Ca^{2+} -ATPase in altering intracellular Ca^{2+} levels. The effect of ascorbic acid is of particular relevance, in view of its role in the enhancement of chemotactic response and microtubule assembly in polymorphonuclear leukocytes [20]. Further, the involvement of microtubule assembly in the acrosome reaction of boar sperm has been established [21]. In vitro acrosome reaction has thus far been extensively studied in guinea pig, mouse, hamster and rabbit sperm, but studies on sperm with large acrosomes like bull and ram have been limited, because of a lack of systems for inducing capacitation and acrosome reaction [1,7]. The use of synthetic peptides merits further investigation in this direction, as does the possibility that low molecular weight factors [22,23], capable of inducing acrosome reaction, found in sperm extracts, blood serum and adrenal glands may be peptide in nature. It is also interesting to speculate that chemical factors may be involved in the directional movement of sperm, prior to fertilisation.

Acknowledgements

We are grateful to the staff of the Centralised Semen Collection Centre, Hebbal, for their generous gifts of bull semen. This research was supported by the Department of Science and Technology, Government of India.

References

- [1] Meizel, S. (1978) Development in mammals (Johnson, M. H. ed) vol. 3, pp. 1-64, North-Holland, Amsterdam.
- [2] McRorie, R. A. and Williams, W. L. (1974) *Ann. Rev. Biochem.* 43, 777-803.
- [3] Showell, H. J., Freer, R. J., Zigmond, H. S., Schiffmann, E., Aswanikumar, S., Corcoran, B. and Becker, E. L. (1976) *J. Exp. Med.* 143, 1154-1169.
- [4] Becker, E. L., Showell, H. J., Henson, P. M. and Hsu, L. S. (1974) *J. Immunol.* 112, 2047-2062.
- [5] Allison, A. C. and Hartree, E. F. (1970) *J. Rep. Fert.* 21, 501-515.
- [6] Morton, D. B. (1976) in: *Lysosomes in biology and pathology*, (Dingle, J. T. and Dean, R. T. eds) vol. 5, pp. 203-255, Elsevier/North-Holland, Amsterdam, New York.
- [7] Rogers, B. J. (1978) *Gamete Res.* 1, 165-223.
- [8] Linker, A. (1974) in: *Methods in enzymatic analysis* (Bergmeyer, H. U. ed) vol. 2, pp. 944-948, Academic Press, New York.
- [9] Scheuning, D. and Fritz, H. (1976) *Methods Enzymol.* 45, 330-342.
- [10] Bergmeyer, H. U., Bernt, E. and Hess, B. (1963) *Methods in enzymatic analysis* (Bergmeyer, H. U. ed) pp. 736-743, Academic Press, New York.
- [11] Robinson, D., Smith, J. N. and Williams, R. T. (1951) *Biochem. J.* 49, 74.
- [12] Findlay, J., Levy, G. A. and Marsch, C. A. (1958) *Biochem. J.* 69, 469-476.
- [13] Lowry, O. H., Roberts, N. R., Mei-Ling, W., Hixson, W. S. and Crawford, E. J. (1954) *J. Biol. Chem.* 207, 19-37.
- [14] Tso, W. W., Lee, W. M. and Wong, M. Y. (1979) *Contraception* 19, 207-212.
- [15] Akruk, S. R., Farooqui, A. A., Williams, W. L. and Srivastava, P. N. (1979) *Gamete Res.* 2, 1-13.
- [16] Lui, C. W. and Meizel, S. (1977) *Differentiation* 9, 59-66.
- [17] Green, D. P. L. (1978) in: *Development in mammals* (Johnson, M. H. ed) vol. 3, pp. 65-81, North-Holland, Amsterdam.
- [18] Boucek, M. M. and Snyderman, R. (1976) *Science* 193, 905-907.
- [19] Naccache, P. H., Volpi, M., Showell, H. J., Becker, E. L. and Shaafi, R. I. (1979) *Science* 203, 461-463.
- [20] Boxer, L. A., Vanderbilt, B., Bonsib, S., Jersild, R., Yand, H. H. and Baehner, R. L. (1979) *J. Cell Physiol.* 100, 119-126.
- [21] Peterson, R., Russell, L., Bundman, D. and Freud, H. (1978) *Biol. Reprod.* 19, 459-466.
- [22] Bavister, B. D. and Yanagimachi, R. (1977) *Biol. Reprod.* 16, 228-237.
- [23] Lui, W., Cornett, E. and Meizel, S. (1977) *Biol. Reprod.* 17, 34-41.