ASSOCIATION OF SPERMINE AND DIAMINE OXIDASE ACTIVITY WITH HUMAN SPERMATOZOA

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Summary. Human spermatozoa, separated from the seminal plasma, contained substantial amounts of spermine, and also a distinct diamine oxidase (EC 1.4.3.6) activity. Spermine, but not diamine oxidase activity, could be removed from the spermatozoa by washing the cells with buffers containing high concentrations of salts. Incubation of human spermatozoa in the presence of labelled spermine and partly purified amine oxidase from bovine blood gave rise to the formation of radioactive products that could not be removed from the spermatozoa by increasing the ionic strength. Similarly, partly purified diamine oxidase from human seminal plasma apparently catalysed the formation of labelled products from radioactive spermine that were more tightly bound to the spermatozoa than spermine itself.

INTRODUCTION

The occurrence of spermine in human semen has been known since 1677 (see Mann, 1964; Cohen, 1971). Although its concentration in human semen is much higher than in other tissues and body fluids, its function in semen remains obscure. It has no marked effects upon the motility or metabolism of spermatozoa of different mammals (Tabor & Rosenthal, 1956; Williams-Ashman & Lockwood, 1971). Fair, Clark & Wehner (1972) found a correlation between the concentration of spermine in the seminal plasma and the concentration of spermatozoa in the semen but Jänne, Hölttä, Haaranen & Elfving (1973) could not confirm this.

There is a growing body of evidence that products arising from the action of amine oxidases upon spermine and spermidine are toxic for a variety of cells (Tabor & Rosenthal, 1956; Bachrach, 1970) including spermatozoa. It is a matter of controversy whether it is the primary oxidation products (iminoaldehydes) or their transformation products that are the toxic agents (Bachrach 1970; Kimes & Morris, 1971a, b; Israel, Zoll, Muhammed & Modest, 1973) but it is known that iminoaldehydes are very labile, undergoing β -elimination and polymerization (Tabor, Tabor & Bachrach, 1964; Bachrach, 1970; Kimes & Morris, 1971a, b).

Zeller (1941) and Zeller & Joel (1941) reported that human seminal plasma contains diamine oxidase and they supposed that it would also oxidize higher

polyamines. We confirmed this observation (Jänne *et al.*, 1973) and found great differences in oxidase activity between individuals. We also detected a complex type of correlation between oxidase activity and sperm count.

In the present communication, we have explored some interactions of spermine and diamine oxidase with the spermatozoa in human semen.

MATERIALS AND METHODS

Materials

The semen samples were obtained from a local laboratory performing semen analyses. After separation of the seminal plasma by low-speed centrifugation, the spermatozoa were suspended in 25 mM-tris-HCl buffer, pH 7.4, containing 0.1 mM-EDTA. The sperm cell suspensions and seminal plasma samples were stored at -20° .

The [1,4- 14 C]spermine.4HCl (sp. act. 18.75 mCi/mmol) and [1,4- 14 C]putrescine (sp. act. 17.5 mCi/mmol) were purchased from the New England Nuclear Corporation. When subjected to paper electrophoresis (Raina, 1963), the radioactive spermine was found to be free of any radioactive contaminants.

Partial purification of amine oxidase and diamine oxidase

Amine oxidase was isolated from bovine blood plasma (Yamada & Yasunobo, 1962).

To isolate diamine oxidase, 171 ml human seminal plasma were fractionated with ammonium sulphate at 0°C . Proteins precipitated between 0.45 and 0.70 saturation were dissolved in 31 ml of 25 mM-tris-HCl buffer, pH 7.4, containing 0.1 mM-EDTA and were dialysed overnight against the same buffer. The product was chromatographed on a column of DEAE-cellulose (3×40 cm, Whatman DE-52) previously equilibrated against the tris-EDTA buffer. The column was washed with 100 ml of the equilibration buffer, and diamine oxidase was eluted with a linear gradient of NaCl from 0.1 to 0.4 M-NaCl. The most active fractions were pooled and freeze-dried.

Preparation of oxidized spermine

Radioactive spermine (15 μCi , sp. act. 18.75 mCi/mmol) was incubated with amine oxidase (19 ml dialysed ammonium sulphate fraction of bovine blood) for 30 min at 37°C . The solution was cooled and treated with trichloroacetic acid at a final concentration of 5%. Precipitated protein was centrifuged down and the clear solution was applied to a column (1×5 cm) of Dowex 50- H^{+} . The column was washed with 100 ml water and oxidized spermine was eluted with 2 N-HCl. The radioactive fractions were pooled, evaporated to dryness at 30 to 35°C under reduced pressure and the residues were dissolved in a small volume of distilled water. On paper electrophoresis, only one major radioactive fraction was found. At pH 4.3, this fraction migrated slightly more slowly than did spermine. A sample of the reaction mixture was adjusted to pH 8 and kept at 100°C for 30 min. Paper electrophoresis revealed that all radioactivity migrated at the same rate as labelled putrescine. This indicates degradation of an iminoaldehyde by β -elimination (Tabor *et al.*, 1964).

Diamine oxidase assay

Diamine oxidase activity was assayed using radioactive putrescine as the substrate and counting the radioactive pyrroline formed (Okuyama & Kobayashi, 1961; Tryding & Willert, 1968) or by measuring colorimetrically the formation of hydrogen peroxide in the presence of different amine substrates (Aarsen & Kemp, 1964). The activity of amine oxidase (spermine oxidase) was assayed by measuring the formation of hydrogen peroxide in the presence of spermine (Aarsen & Kemp, 1964).

Subcellular fractionation of spermatozoa

The sperm cell suspension was subjected to sonication (three times for 5 to 10 sec/time at full power) in a Branson Sonifer (model No. S125) using an ice-ethanol bath for cooling. The sonicates were layered over a linear sucrose gradient from 20 to 70% with 5.5 ml of 80% sucrose as a cushion at the bottom of the tube. The gradients were centrifuged in a SW 25 rotor for 45 min at 2500 rev/min (about 600 *g*) in a Spinco ultracentrifuge. Fractions (1 ml) were collected from the bottom of the tube. The heads and tails were well separated by this method (see the turbidity pattern in Text-fig. 2).

Other methods

Polyamines were isolated by a slight modification of the Dowex 50-H⁺ slurry method described earlier (Jänne & Williams-Ashman, 1971) and measured after paper electrophoresis (Raina, 1963) by staining the paper strips with ninhydrin as described earlier (Raina & Cohen, 1966). The DNA was measured by the method of Burton (1956) as modified by Giles & Myers (1965) and protein by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Association of spermine and diamine oxidase activity with spermatozoa

Human spermatozoa, separated from the seminal plasma and washed extensively with buffers of low ionic strength, contained appreciable amounts of spermine, but there was no obvious correlation between the concentration of spermine in the seminal plasma and the spermine content per spermatozoon. About 5% of the total diamine oxidase activity of human semen was also associated with the washed spermatozoa.

As shown in Table 1, spermine was removed from spermatozoa when they were washed with solutions of sodium chloride or magnesium chloride, the latter being more effective, but diamine oxidase was incompletely removed. While up to 1% of Triton X-100 with or without the addition of 0.2 M-dithiothreitol, failed to remove either spermine or amine oxidase, 1% sodium dodecyl sulphate removed spermine completely. No lipid-bound spermine was removed by repeated washings with acetone.

Oxidized spermine and its association with spermatozoa

When spermatozoa were incubated with labelled spermine, part of the label became associated with the cells and could not be extracted with standard

Table 1. The release of spermine and diamine oxidase activity from human spermatozoa

Treatment	% Remaining in spermatozoa	
	Spermine	Diamine oxidase activity
NaCl (M)		
0	100	100
0.05	73	78
0.1	60	82
0.2	25	50
0.3	14	57
0.5	6	56
MgCl ₂ (M)		
0	100	100
0.02	28	98
0.04	18	86
0.06	6	82
0.1	2	78

The spermatozoa were washed twice with tris-EDTA buffer and once with the buffer containing the concentrations of the salts indicated in Column 1.

TEXT-FIG. 1. Effect of the amine oxidase and diamine oxidase on the incorporation of radioactivity from labelled spermine into human spermatozoa. The spermatozoa were washed twice with cold tris-EDTA buffer, once with the buffer containing 0.1 M-MgCl₂ and once again with the standard buffer. The spermatozoa were suspended in a small volume of the buffer and incubated for 240 min at 37°C in the presence of 1 μCi [1,4-¹⁴C]spermine (0.053 μmol/ml) in the absence or presence of amine oxidase (spermine oxidase) (12 mg protein) or diamine oxidase (90 mg protein). After the incubation, the tubes were cooled and washed at 0°C with standard buffer containing MgCl₂ at the concentrations indicated. □, [1,4-¹⁴C]spermine + amine oxidase; △, [1,4-¹⁴C]spermine + diamine oxidase; ●, [1,4-¹⁴C]spermine.

Table 2. The incorporation of radioactivity from labelled spermine and oxidized spermine into human spermatozoa and its release by magnesium chloride

Concentration of $MgCl_2$ (M)	Radioactivity remaining in the spermatozoa			
	Spermine μmol	(%)	Oxidized spermine μmol	(%)
0	75.5	(100)	37.7	(100)
0.02	12.5	(16)	41.2	(109)
0.05	10.7	(14)	34.7	(92)
0.1	7.8	(10)	27.3	(72)

The spermatozoa were washed three times with tris-EDTA buffer and suspended in a small volume of the same buffer. Portions were incubated at 37°C for 120 min in the presence of 0.1 μCi (0.0053 $\mu\text{mol/ml}$) radioactive spermine or radioactive oxidized spermine. The cells were collected, washed four times with buffer, once with buffer containing $MgCl_2$ (as indicated), and were counted for radioactivity.

TEXT-FIG. 2. Subcellular distribution of radioactivity derived from labelled spermine in the absence or presence of amine oxidase from bovine blood plasma. Human spermatozoa were washed as described in the legend for Text-fig. 1. Cell suspensions were incubated in the presence of 0.5 μCi (0.024 $\mu\text{mol/ml}$) of [1,4- ^{14}C]spermine in the absence or presence of amine oxidase (6 mg protein) at 37°C for 60 min. The cells were centrifuged, washed twice with tris-EDTA buffer containing 0.1 M- $MgCl_2$. The spermatozoa were sonicated and centrifuged in a sucrose density gradient as described in the text. Turbidity ($A_{600\text{ nm}}$) and radioactivity were measured from each fraction. $\circ$, Turbidity; $\square$, [1,4- ^{14}C]spermine; $\blacktriangle$, [1,4- ^{14}C]spermine + amine oxidase.

buffer. Inclusion of low concentrations of MgCl_2 in the buffer, however, led to almost complete extraction of the label (Text-fig. 1). If either amine oxidase or diamine oxidase was present during the incubation with spermine, the labelled metabolic products were no longer extractable with buffered MgCl_2 .

When oxidized spermine was incubated with spermatozoa, it became attached to the cells more tightly than did spermine under the same conditions (Table 2).

In a further series of experiments, human spermatozoa were incubated in the presence of radioactive spermine, with or without amine oxidase. The cells were disintegrated after the incubation and the head and tail fractions were separated by a centrifugation of the sonicates through a sucrose density gradient. As shown in Text-fig. 2, most of the radioactivity derived from radioactive spermine in the absence of amine oxidase appeared on the top of the gradient whereas the radioactivity derived from labelled spermine in the presence of amine oxidase was clearly associated with the particulate fractions of the spermatozoa. The radioactivity appeared to be distributed roughly equally between the head fraction (on the left side) and the tail fraction (on the right side). Whether this kind of distribution also occurs in intact spermatozoa is not known because a secondary redistribution of the oxidized polyamine might take place after the disintegration of the sperm cell.

DISCUSSION

Although a major part of spermine in human semen is localized in the seminal plasma, the spermatozoa contain appreciable amounts of this polyamine under conditions that can be considered physiological. Whether the spermine in the spermatozoa is attached to the outer membrane of the cell, or binds more specifically to some intracellular component, is not known. The swift redistribution of polyamines after the disintegration of any type of cells makes it difficult to solve this point.

Oxidation products formed from spermine and seminal diamine oxidase, like those from spermine and amine oxidase, have a high affinity for human spermatozoa. The nature of the oxidation products is uncertain but the results are consistent with the known high chemical reactivity of oxidized spermine (Bachrach, 1970). It seems unlikely that appreciable amounts of such products would be formed during the short period that ejaculated spermatozoa remain in contact with the seminal plasma but, even in very low concentrations, oxidized polyamines will completely inactivate human spermatozoa (Tabor & Rosenthal, 1956). Both spermine and diamine oxidase in human semen appear to be derived from the prostate gland (Mann, 1964; Jänne *et al.*, 1973). It is thus possible that ejaculated spermatozoa make contact with preformed oxidation products and that such products may influence the motility and fertilizing capacity of spermatozoa.

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