THE HYDROLYSIS OF ADENOSINE TRIPHOSPHATE BY HUMAN SPERMATOZOA

I. F. DURR, A. ABLA AND A. MROUEH

*Department of Biochemistry, American University of Beirut,
Beirut, Lebanon*

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Summary. Human spermatozoa contain an ATPase which is stimulated by sodium and potassium ions in the presence of magnesium ions. The hydrolysis of ATP is inhibited by ouabain, sodium fluoride and Triton. The presence of an active 5'-nucleotidase in human spermatozoa is indicated by the accumulation of adenosine as the ultimate product of ATP hydrolysis.

The hydrolysis of ATP has been extensively studied in the spermatozoa of various animals (Nelson, 1954; Bishop, 1958; Tibbs, 1959; Uesugi & Yamazoe, 1966), but not so far in man. This paper provides evidence for an ATPase in spermatozoa, which is stimulated by sodium and potassium ions ($\text{Na}^+ + \text{K}^+$) in the presence of magnesium ions.

Semen was collected from healthy individuals and pooled. After liquefaction, sperm counts were determined by use of a Neubauer counting chamber as in the routine counting of red blood corpuscles. The semen was centrifuged at 900 *g* for 10 min, and the pellet was washed twice with a phosphate-based buffer, pH 7.4 (Peterson & Freund, 1968). The pellet was suspended in this buffer which contains the cations—sodium, potassium and magnesium. The spermatozoa maintained good motility (50 to 60%) for 3 hr. Such spermatozoa metabolized glucose or fructose at two to four times the rate of those of the same batch kept in their own plasma (I. F. Durr, unpublished data).

The content of each incubation mixture is indicated in Tables 1 and 2. The incubation was at 37° C and uniform shaking was maintained throughout the incubation. Labelled [$\text{U-}^{14}\text{C}$]ATP or [$\text{U-}^{14}\text{C}$]AMP were used as substrate. The reaction was terminated by immersing the tube in a boiling water bath for 1 min. After centrifugation, 20 μl of the supernatant solution was 'spotted' onto a Whatman No. 3 paper and subjected to ascending chromatography for 23 hr at 21° C. Solvent A consisted of n-butanol:formic acid:water, 77:13:10 by vol. (Markham & Smith, 1949), and was used to separate adenosine and adenine. Authentic samples gave R_F values of 0.14 and 0.3, respectively, the R_F value being the ratio of the distance between the starting line and the centre of the spot to the distance between the starting line and the solvent front. The other nucleotides of ATP did not move significantly. Solvent B consisted of n-butanol:glacial acetic acid:acetone:ammonia:water, 14:16:10:1:9 by vol. (Hollinger, 1971) and was used to separate ATP, ADP and AMP. Authentic

samples gave R_F values of 0.11, 0.28 and 0.52, respectively. Adenosine and adenine had an almost identical R_F value, 0.67, in this system. Authentic ATP, ADP, AMP, adenosine and adenine were 'spotted' onto the same paper on which the aliquot of the incubation mixture was 'spotted'. The UV-absorbing spots corresponding to those of the authentic samples were cut out, and placed in a vial for counting in a Packard scintillation counter having 85% efficiency for radioactive carbon compounds.

Text-figure 1 shows the rate of ATP hydrolysis as well as the formation of its

TABLE 1
CATION STIMULATION OF ATP HYDROLYSIS BY HUMAN SPERMATOZOA

Time of incubation (min)	Adenosine formed (nmol)		
	Mg ⁺⁺	(Na ⁺ + K ⁺)	Mg ⁺⁺ + (Na ⁺ + K ⁺)
60	21	35.8	57.7
90	33.6	54.2	93.7

The incubation mixture contained 2.7×10^8 spermatozoa, 200 nmol [$U\text{-}^{14}C$]ATP (4.8×10^5 counts/min), 180 μ mol of tris-HCl buffer, pH 7, supplemented with cations as indicated. The final volume was 2.2 ml. Spermatozoa used in this experiment were washed and suspended in 0.1 M-tris-HCl, pH 7, and supplemented with cations where indicated.

TABLE 2
INHIBITION OF ATP HYDROLYSIS BY SODIUM FLUORIDE OR OUABAIN-TREATED HUMAN SPERMATOZOA

Time of incubation (min)	Adenosine formed (nmol)		
	Control	Ouabain	NaF
30	9.87	2.98	—
60	16.03	4.31	2.67
90	27.58	5.08	4.59

The incubation mixture contained 1×10^8 spermatozoa, 200 nmol [$U\text{-}^{14}C$]ATP (1.9×10^5 counts/min), 1.8 ml Peterson & Freund buffer, pH 7.4, in a final vol. of 2.1 ml. The concentrations of ouabain and sodium fluoride were 0.1 and 1.0 mM, respectively.

A 1.8-ml vol. Peterson & Freund buffer contain 252 μ mol NaCl, 5.4 μ mol of each of KCl and $MgSO_4$, 7.6 μ mol Na_2HPO_4 and 15.3 μ mol NaH_2PO_4 .

metabolites. About 80% of ATP was hydrolysed in the first 20 min. Analysis of radioactivity in the various metabolites showed that adenosine was the major product and that human spermatozoa apparently contain an active 5'-nucleotidase. The presence of 3'-AMP did not affect the rate of adenosine formation from 5'-AMP. On the other hand, 5'-GMP inhibited the rate of 5'-AMP hydrolysis by 25%, thereby indicating that the nucleotidase is specific to 5'-nucleotides but not specific to adenine nucleotides. Levin & Bodansky (1966)

TEXT-FIG. 1. Rate of ATP hydrolysis and formation of various products (●, ATP; ○, ADP; △, AMP; ■, adenosine; ▲, adenine). The incubation mixture contained 3.7×10^8 spermatozoa, 1.8 nmol $[U-^{14}C]ATP$ (1.16×10^6 counts/min) in a final vol. of 3 ml. At the indicated time intervals, aliquots were assayed for ^{14}C as described in a section of the text. A blank contained the same ingredients except that the spermatozoa were boiled for 3 min.

TABLE 3
INHIBITION OF ATP HYDROLYSIS BY TRITON-
TREATED HUMAN SPERMATOZOA

Time of incubation (min)	Adenosine formed (nmol)	
	Control	Triton
30	49.5	19.2
60	80.6	39.7
90	117.2	58.6

The incubation mixture contained 4.5×10^8 spermatozoa, 200 nmol $[U-^{14}C]ATP$ (4.8×10^5 counts/min), 1.8 ml Peterson & Freund buffer, pH 7.4, in a final vol. of 2.2 ml. The sperm suspension (2 ml) was mixed with 0.2 ml of 1% w/v Triton-X-100 in H_2O for 5 min at room temperature. Subsequently, the spermatozoa were collected by centrifugation and suspended in Peterson & Freund's buffer.

described the properties of a 5'-nucleotidase isolated from bull seminal plasma. Further hydrolysis to adenine is a slow reaction. About 80% of the added ATP was recovered in adenosine. The remaining products, together with adenosine, quantitatively account for the added ATP. Addition of glucose, fructose, glucose-6-phosphate, fructose-6-phosphate, ribose or pyruvate did not significantly alter the pattern or rate of ATP metabolism. This is expected since the hexokinase and phosphofructokinase are rate-limiting, as shown by Peterson & Freund (1970). In other systems, the hydrolysis of ATP produced equal amounts of ADP and AMP (Hollinger, 1971), or mainly ADP with some AMP (Quinn & White, 1968). The rate of hydrolysis of [U-¹⁴C]AMP to adenosine was equal to the overall rate of hydrolysis of [U-¹⁴C]ATP to adenosine.

Since the major metabolite of ATP was adenosine, all subsequent assays involved measurement of [¹⁴C]adenosine. Table 1 shows that ATP hydrolysis is stimulated by ($\text{Na}^+ + \text{K}^+$) in the presence of magnesium ions, which activate the enzyme involved in the transport of sodium and potassium ions. The observed hydrolysis of ATP may not be solely due to the ($\text{Na}^+ + \text{K}^+$)-activated enzyme, since other ATPases may be present by analogy with other systems which also contain a magnesium-dependent ATPase (Bishop, 1958; Tibbs, 1959; Young & Nelson, 1969).

O'Donnell & Ellory (1970) showed that ouabain bound to bull spermatozoa, and that metabolic inhibitors such as sodium fluoride or detergents such as Triton decreased this binding. Consequently, the effect of such compounds on the hydrolysis of ATPase was investigated. The production of adenosine was determined at various time intervals ranging from 30 to 90 min. Tables 2 and 3 show that the production of adenosine was severely inhibited. It should be noted that the release of adenosine is the result of more than one enzyme. Hence, the observed inhibition does not imply an inhibition of ATPase. Further, there was no correlation between changes in the numbers of motile spermatozoa with magnitudes of inhibition by ouabain, sodium fluoride and Triton.

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