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REGULATORY PROPERTIES OF PRIMATE SPERM PHOSPHOFRUCTOKINASE*

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SUMMARY

1. The enzyme phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) has been solubilized from spermatozoa of the rhesus monkey (*Macaca mulatta*) by sonic irradiation and purified approx. 18-fold by a combination of $(NH_4)_2SO_4$ fractionation and gel filtration on Sephadex G-200. Sperm phosphofruktokinase is inactivated by molecular sieving but may be stabilized by addition of Fru-6-*P*, ATP, and $MgSO_4$ to the column elution medium.

2. The partially purified enzyme, free of ATPase activity, is insensitive to ATP inhibition in the presence of $(NH_4)_2SO_4$ but markedly inhibited in its absence. Orthophosphate functions both as a deinhibitor at high ATP levels and as a pure enhancer at low ATP levels.

3. ATP inhibition is also modulated by 3'-AMP, 5'-AMP, ADP, 3',5'-AMP, and Fru-1,6-P_2. In all cases, except the latter, modulators owe their effects to alterations in the binding of Fru-6-*P* to phosphofructokinase. ADP and 3',5'-AMP are the most potent compounds tested. Orthophosphate *plus* adenine mono- and dinucleotides are synergistic in relieving ATP inhibition.

4. Sperm phosphofructokinase is inhibited by low levels of citrate, the inhibition being synergistic with that of ATP. Citrate inhibition may be overcome by Fru-6-*P*, P_i, and 5'-AMP but not by Fru-1,6-P_2. P_i and 5'-AMP are synergistic in relieving citrate inhibition. Partially purified sperm phosphofructokinase is rapidly desensitized to both ATP and citrate inhibition.

5. It is concluded that the kinetic properties of phosphofructokinase are consistent with a role for the enzyme in the regulation of sperm cell glycolysis.

INTRODUCTION

The mammalian sperm cell, including that of primates, is unusual in that it relies almost exclusively *in vitro* on aerobic fructolysis (or glycolysis) to provide energy for

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sustenance of motility[1,2]. Presented with the choice of either oxidizing endogenous substrates *via* the electron transport chain or of converting exogenous fructose (the principal sugar of semen[1]) to lactate aerobically, the sperm cell uses the latter, thermodynamically less efficient, pathway. It does so despite the presence in the cell mid-piece of a mitochondrial system fully capable, in the absence of exogenous carbohydrate, of utilizing endogenous lipid and supporting motility through oxidative phosphorylation[1–7]. A further distinguishing characteristic of human[8] and monkey[2] ejaculated spermatozoa is the almost complete lack of a demonstrable Pasteur effect, with glycolytic rates being only 10–15% greater in the absence of air than in its presence. While not enhanced by anaerobiosis, glycolysis is, however, considerably stimulated in the sperm cell of a number of species by inorganic ions[1,9–12], hormones[13,14] and female reproductive tract fluids (E. R. CASILLAS AND D. D. HOSKINS, unpublished observations; refs. 15–19).

The present study is part of an effort designed to provide a molecular level basis on which to explain these singular properties of the mammalian sperm cell. In this paper we have directed our attention to phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) since this enzyme has been shown to play a regulatory role in a wide variety of tissues (for reviews, see refs. 21–23 among others). The mechanism of this regulation has proven to be one of controlling the rate of the phosphofructokinase reaction by varying the binding of Fru-6-*P* to phosphofructokinase. ATP and citrate are known to increase the K_m for Fru-6-*P*, an effect which is reversed by a number of modulators including P_i and adenine mono- and dinucleotides.

We describe here the stabilization, partial purification, and regulatory properties of phosphofructokinase derived from the sperm cell of the rhesus monkey (*Macaca mulatta*). The partially purified enzyme has been shown to possess properties similar in many, but not all, respects to that of mammalian diploid cells. The kinetic results are considered to be consistent with a regulatory role for phosphofructokinase in sperm glycolysis.

MATERIALS AND METHODS

Semen was collected at 3-day intervals from 18 rhesus monkeys (*M. mulatta*) ranging in weight from 8 to 12 kg by the electroejaculation technique of MASTROIANNI AND MANSON[24]. Coagulated ejaculates, contained in 10-ml beakers, were allowed to stand at room temperature for 1 h after which the exuded sperm-rich solution was removed, diluted with 2 vol. of Ca^{2+}-free Krebs–Ringer phosphate buffer for spermatozoa[25], and centrifuged at $700 \times g$ for 5 min. Sedimented cells were washed twice with a volume of Ca^{2+}-free Krebs–Ringer phosphate buffer equivalent to 3 times the exudate volume. After final centrifugation the cells from each animal were suspended in 1 ml of 75% $(NH_4)_2SO_4$ solution adjusted to pH 7.5 with KOH and stored at 4°. Cells from approx. 40 ejaculates were collected over a 2–3-week period, centrifuged at $28\,000 \times g$ for 30 min, resuspended in 1 ml Ca^{2+}-free Krebs–Ringer phosphate buffer per ejaculate, and subjected to sonic irradiation at 0° for 2 min using a Bronwill Biosonik III sonicator at an intensity setting of 30–40. The supernatant solution obtained on subsequent centrifugation at $28\,000 \times g$ for 15 min was used as the starting material for phosphofructokinase purification.

All nucleotides, sugar phosphates, and auxiliary enzyme preparations were products of Sigma Chemical Co. Other chemicals were of the highest purity obtainable. Phosphofructokinase activity was measured fluorimetrically at 23° using a Perkin–Elmer Model 203 fluorescence spectrophotometer with attached recorder and was based on the method of RACKER[26]. Each assay cuvette contained, in order of addition, 33 mM Tris–HCl buffer (pH 7.5); 0.2 mM $MgSO_4$; 50 mM KCl; 0.1 mM ATP adjusted to approx. pH 7 with NaOH; 10 μM NADH; 2 mM dithiothreitol, 0.2 ml auxiliary enzyme solution (containing 0.2, 5.0, and 1.5 units of rabbit muscle aldolase, α-glycerophosphate dehydrogenase, and triosephosphate isomerase, respectively), 1 mM Fru-6-*P* (sodium salt), and water in an amount such that after initiating the reaction with phosphofructokinase, the total volume was 3.0 ml. Reaction rates were calculated from the linear portion of the plot, obtained following an initial lag phase of approx. 1 min, that represented loss in native fluorescence of NADH. Under these conditions phosphofructokinase activity was proportional to enzyme concentration in the range 2–12 nmoles of NADH oxidized per min at 23°.

The effects of Fru-1,6-P_2 on the modulation of ATP inhibition were studied by measuring the rates of ADP formation in the presence of a 10-fold excess of an auxiliary enzyme preparation consisting of crystalline pyruvate kinase, lactate dehydrogenase, and 2 mM phosphoenol pyruvate, as described by PASSONNEAU AND LOWRY[21].

The auxiliary enzyme used in both assays were suspended in 10 mM Tris–HCl buffer (pH 7.5) containing 2.1 mg/ml of bovine plasma albumin and dialyzed for 3 h at 4° against 300 vol. of this same buffer before addition to the assay system.

ATP was assayed fluorimetrically as described by GREENGARD[27]. Concentrations of all other nucleotides were determined spectrophotometrically using appropriate extinction coefficients. Protein was determined by the method of LOWRY *et al.*[28].

RESULTS

Solubilization and stabilization of sperm phosphofructokinase

Phosphofructokinase is easily solubilized from the monkey spermatozoon by sonic irradiation. Sperm cells that are washed twice with and suspended in Ca^{2+}-free Krebs–Ringer phosphate buffer (pH 7.4) at a concentration of $1 \cdot 10^8$–$3 \cdot 10^8$ cells per ml release over 95% of the total measurable cellular phosphofructokinase into solution on sonication for 1 min at 0° (see MATERIALS AND METHODS). Recovery of the enzyme is not improved by increasing sonication time to a total 5 min or by addition of reducing agents, stabilizers, or chelating agents to the suspension media. Thus, 10 mM dithiothreitol, 0.1 mM ATP[29], or 2 mM EDTA added singly or in combination fail to improve enzyme recovery.

Soluble sperm phosphofructokinase in extracts containing approx. 0.5 mg protein per ml is almost completely inactivated by freezing at −20°, by dialysis overnight against 50 mM phosphate buffer (pH 7.5) or by lyophilization. Higher protein concentrations partially protect phosphofructokinase against inactivation during dialysis. At a level of 1.3 mg protein per ml a 60% loss in activity occurs overnight which can be almost completely regained by incubation at 30° for 30 min with high levels (50 mM) of dithiothreitol. The enzyme loses activity on standing at temperatures ranging from 4 to 30° at rates of approx. 10–20% per day but retains 90% of its activity for periods of 1 week or longer if stored in the undisrupted cell at 4° in the presence of

TABLE I

REQUIREMENTS FOR STABILIZATION OF MONKEY SPERM PHOSPHOFRUCTOKINASE DURING SEPHADEX G-25 GEL FILTRATION

The basic elution contained 10 mM β-mercaptoethanol, 2 mM EDTA, and 50 mM phosphate buffer (pH 7.50). Fru-6-*P*, ATP, and $MgSO_4$ were added where indicated at levels of 1, 0.1, and 5 mM, respectively. Specific activity is μmoles Fru-1,6-P_2 formed per min per mg protein at 23°.

Addition to elution media	*Specific activity*		*Ratio phosphofructokinase activity gel eluate to incubated extract*
	Extract incubated with elution media	*Gel eluate*	
None	0.31	0.05	0.16
Fru-6-*P* + ATP + $MgSO_4$	0.31	0.40	1.28
ATP + $MgSO_4$	0.31	0.25	0.81

75% $(NH_4)_2SO_4$ (ref. 30) at pH 7.5. Lesser protection is afforded if whole sperm cells are stored for 1 week at −20 or −196° in the absence of salt.

The enzyme contained in sonic extracts is almost completely inactivated on rapid passage through molecular sieves such as Sephadex G-25. Since it was anticipated that filtration on appropriate gels would allow partial purification of small amounts of phosphofructokinase with a limited amount of manipulation the protection of the enzyme against inactivation on Sephadex G-25 columns was studied in some detail. As shown in Table I, stabilization can be achieved by addition of Fru-6-*P*, ATP, and $MgSO_4$ to the elution medium. Similar results have been reported for lens[31] and heart[29,33] phosphofructokinase. Fru-6-*P* was omitted from elution media subsequently used during purification of phosphofructokinase on Sephadex G-200 columns, despite its stabilizing effect, since small amounts of a compound, presumably Fru-1,6-P_2, were formed during filtration that interfered with fluorimetric assays. The action of Fru-6-*P*, ATP, and $MgSO_4$ is attributable to a stabilization of the enzyme rather than to reactivation since it is not possible to restore phosphofructokinase activity in Sephadex G-25 column eluates by addition of these compounds either singly or in combination with reducing agents such as β-mercaptoethanol (10 mM) or dithiothreitol (50 mM).

TABLE II

PARTIAL PURIFICATION OF MONKEY SPERM PHOSPHOFRUCTOKINASE

Specific activity is defined as μmoles Fru-1,6-P_2 formed per min per mg protein at 23°.

Enzyme source	*Volume (ml)*	*Protein (mg/ml)*	*Total protein (mg)*	*Protein recovery (%)*	*Specific activity*	*Total units*	*Enzyme recovery (%)*
Sonic extract	42	0.63	26.3	100	0.16	4.22	100
Salt fraction (35–50% $(NH_4)_2SO_4$)	1.0	6.82	6.8	26	0.52	3.64	84
Sephadex G-200 eluate No. 3	1.4	0.36	0.52	1.9	1.57	0.84	19
Sephadex G-200 eluate No. 4	1.4	0.23	0.29	1.1	2.80	0.82	19
Sephadex G-200 eluate No. 5	1.4	0.28	0.35	1.3	1.42	0.50	12

Partial purification and general properties of sperm phosphofructokinase

Phosphofructokinase was purified from sonic extracts of monkey spermatozoa by a combination of $(NH_4)_2SO_4$ fractionation and filtration on Sephadex G-200 (Table II). A total of 42 ml of extract was fractionated with solid $(NH_4)_2SO_4$ between the limits 0–35 and 35–50% saturation. This and all subsequent operations were carried out at 0–4°. Solutions were stirred for 15 min after the addition of each increment of salt. The fraction precipitating at 35–50% saturation was centrifuged at 28 000 × *g* for 30 min, dissolved in 1 ml of a column elution medium containing 50 mM of phosphate buffer (pH 7.5) 0.1 mM of ATP, and 5 mM of $MgSO_4$ and applied to a 1.1 cm × 26 cm Sephadex G-200 column previously equilibrated with this same solution. 1.4-ml fractions were collected at a flow rate of 10 ml/h. Phosphofructokinase was eluted from the column, beginning with the column void volume (10 ml), and attained maximum specific activity after passage of an additional 4–5 ml of elution medium. The peak activity eluates contained approx. 20% of the phosphofructokinase of crude extracts and represented an 18-fold purification of the enzyme.

The purified enzyme is completely inactivated by freezing at −20° and is unstable at 4°, losing over 80% of its activity in 5 days. Partial reactivation (60%) of the enzyme stored for several days at 4° may be obtained by incubation at 30° for 15 min with 50 mM (final concentration) dithiothreitol in 50 mM phosphate buffer (pH 7.5) Much greater stability may be obtained, however, if 0.1 ml of 0.2 M dithiothreitol in the elution medium is added to the contents (1.4 ml) of each Sephadex G-200 eluate tube and the solutions stored at 4° as 100-μl portions in 5 mm × 50 mm centrifuge tubes covered with parafilm. Since partial inactivation occurs during routine handling, each 100 μl portion is discarded after a single day's use. Under the above conditions the enzyme is stable for 1 week. It rapidly loses its sensitivity to ATP inhibition, however, (see below), being at least 50% desensitized within 1 week. Citrate desensitization occurs at least as rapidly. The studies on the inhibition of phosphofructokinase by ATP and citrate and on the modulations of these inhibitions by nucleotides and P_i reported in this paper were carried out within 1 week of purification and, where critical, on the day following elution from Sephadex G-200 columns.

That the partially purified enzyme was free of ATPase activity, a requirement for the quantitative assay of the regulatory role for ATP, is indicated by the fact that Sephadex G-200 eluates when used in amounts 5-fold greater than used for phosphofructokinase assay failed to hydrolyze significant amounts of added ATP (2.8 μM) over a 15 min period at 23°.

Sperm phosphofructokinase has a broad pH optimum between pH 7.0 and 9.0 with a maximum at 8.2 as measured in 33 mM Tris–HCl buffer. Activities at pH 7.0 and 9.0 were 59 and 56%, respectively, of that obtained at pH 8.2.

Following passage of the partially purified enzyme through a second Sephadex G-200 column equilibrated with 50 mM potassium phosphate buffer (pH 7.5) and 0.1 mM ATP, an absolute requirement for Mg^{2+} was noted, with optimum activity at the 50 μM (equimolar with ATP) level. In contrast to the results of PASSONNEAU AND LOWRY with sheep brain phosphofructokinase[21], however, assay of sperm phosphofructokinase in the presence of additional Mg^{2+} (up to 5 mM) resulted in only a slight (less than 30%) diminution in activity.

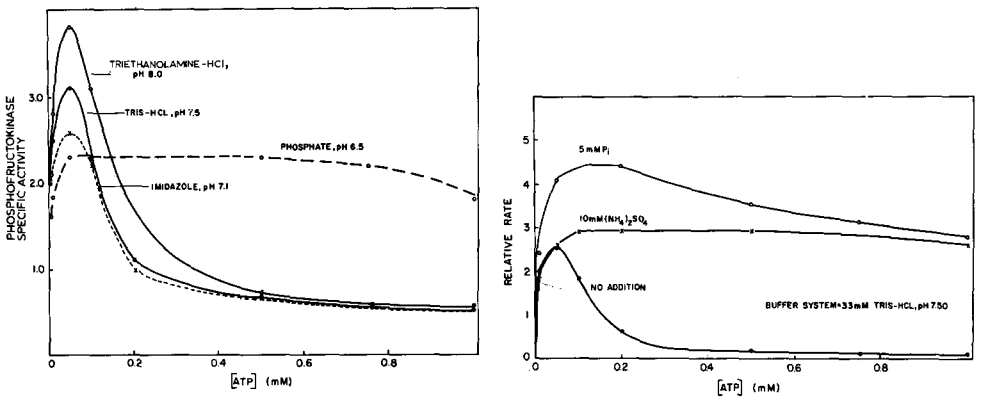


Fig. 1. Inhibition of sperm phosphofructokinase by ATP as a function of pH and buffer composition. Assay carried out using dialyzed auxiliary enzymes. Phosphofructokinase specific activity is μmoles Fru-1,6-P_2 formed per min per mg protein at 23°. Assay conditions for the experiments shown in Figs. 1 and 2 are identical except for use of dialyzed or undialyzed auxiliary enzymes, Each assay cuvette contained 2.4 μg protein and 83 μM Fru-6-*P*.

Fig. 2. Relief of ATP inhibition of sperm phosphofructokinase by P_i and $(NH_4)_2SO_4$. Each assay cuvette contained 2.4 μg protein and 83 μM Fru-6-*P*.

Inhibition by ATP

ATP is recognized as a potent inhibitor of phosphofructokinase derived from a variety of mammalian tissues, yeast, bacteria, and plants (for a review, see ref. 32). ATP is an equally potent inhibitor of sperm phosphofructokinase (Fig. 1). Marked inhibition, 60–70%, by ATP is apparent in all buffer systems tested except P_i on increasing the nucleotide concentration only 4-fold, *i.e.*, from 50 to 200 μM. Inhibition by ATP is abolished in all systems not only by P_i but also by $(NH_4)_2SO_4$ (10 mM) with levels of nucleotide greater than 3 mM being required to effect a 50% inhibition of activity. The effects of 10 mM $(NH_4)_2SO_4$ and P_i (5 mM) at pH 7.5 (Standard assay conditions) are shown in Fig. 2. P_i is seen to be both an enhancer of phosphofructo-

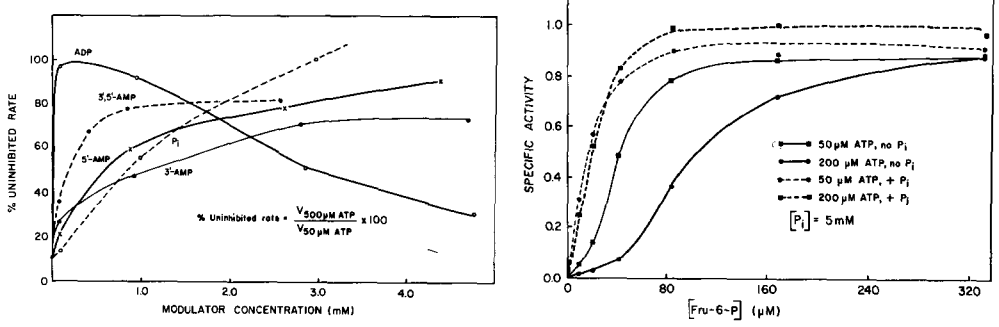


Fig. 3. Modulation of ATP inhibition by nucleotides and P_i. Each assay cuvette contained 2.5 μg protein and 83 μM Fru-6-*P*.

Fig. 4. Modulation of ATP inhibition of sperm phosphofructokinase by 3',5'-AMP .

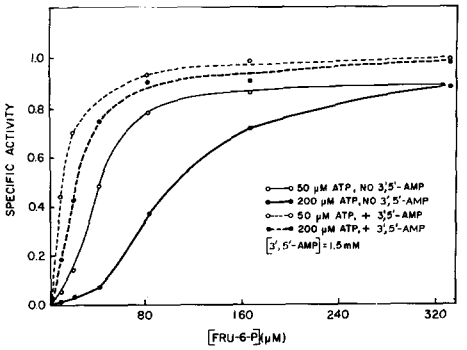


Fig. 5. Modulation of ATP inhibition of sperm phosphofructokinase by P_i.

kinase activity at optimum ATP substrate levels and a modulator of inhibition at inhibitory levels of nucleotide.

Modulation of ATP inhibition

In the presence of 500 μM ATP sperm phosphofructokinase activity is approx. 10% of that obtained at a level of 50 μM. As can be seen from Fig. 3, ADP, 3',5'-AMP, 3'-AMP, 5'-AMP, and P_i all effectively relieve ATP inhibition, with ADP and 3',5'-AMP exhibiting the most pronounced stimulatory effects at levels equimolar to ATP. The diphasic effect of the reaction product ADP is singular and most likely reflects competition with ATP at both a substrate and a regulatory site. That the action of all inhibitor modulators is due to relief of the inhibitor effects of ATP on Fru-6-*P* binding to phosphofructokinase is shown by the data of Figs. 4 and 5 and Table III. It is clearly seen from Figs. 4 and 5 that both 3',5'-AMP and P_i markedly alter the apparent K_m for Fru-6-*P* without significantly changing the reaction v_{max}. In all

TABLE III

THE EFFECT OF MODULATORS OF ATP INHIBITION OF SPERM PHOSPHOFRUCTOKINASE ON KINETIC CONSTANTS FOR Fru-6-*P*

Fru-6-*P* concn., 83 μM. Each cuvette contained 2.5 μg protein.

Expt. No.	*Modulator*	*ATP concn. (μM)*	K_m *(μM)*	v_{max}
1	None	50	40	0.84
		200	93	0.84
	5 mM P_i	50	14	0.90
		200	19	0.96
	1.5 mM 3',5'-AMP	50	10	0.98
		200	20	0.98
2	None	50	70	1.65
		200	178	1.68
	5 mM 5'-AMP	50	11	1.05
		200	24	1.37
	0.5 mM ADP	50	45	0.72
		200	11	1.65

cases Fru-6-*P* cooperative effects are noted in the absence of modulators but not in their presence. Whether the apparent absence of cooperative effects are real or perhaps illusory (due to compression of the scale of the abscissa) as shown by ATKINSON *et al.*[34] for yeast phosphofructokinase is not known. Table III, containing data derived from similar plots, shows the K_m and v_{max} values for Fru-6-*P* at low (50 mM) and high (200 mM) levels of ATP in the presence of all the modulators shown in Fig. 3 except 3'-AMP. Modulator levels were chosen on the basis of the least amount that gave maximum relief of 500 μM ATP-inhibited phosphofructokinase. In each case the addition of modulator lowered the apparent K_m for Fru-6-*P*. ADP, however, once again appeared to exert a singular effect, with v_{max} for Fru-6-*P* being greater with 200 than with 50 μM ATP.

In confirmation of earlier reports (see, for example, ref. 21) Fru-1,6-P_2 is a modulator of ATP inhibition of sperm phosphofructokinase with levels as low as 33 μM effecting a doubling of the rate of ATP-inhibited (500 μM) sperm phosphofructokinase. Results with Fru-1,6-P_2, however, have been less definitive than those with other modulators since little relief is noted below 33 μM Fru-1,6-P_2 and relief of ATP inhibition above this level appears to be concentration independent. The effect of Fru-1,6-P_2 on the binding of Fru-6-*P* to sperm phosphofructokinase has not been studied.

The modulating effects of P_i *plus* ADP and P_i *plus* 3',5'-AMP, studied under conditions of severe inhibition by 2 mM ATP, were found to be synergistic and yielded phosphofructokinase rates 7.1 and 3.5 times respectively, greater than the rates expected from the added effects of the individual modulators (Table IV). The data are in agreement with the results of LOWRY AND PASSONNEAU[35] using sheep brain phosphofructokinase. We have not, however, been able to confirm the internucleotide synergistic effects, *e.g.*, between ADP and 5'-AMP, reported by these authors.

Inhibition by citrate

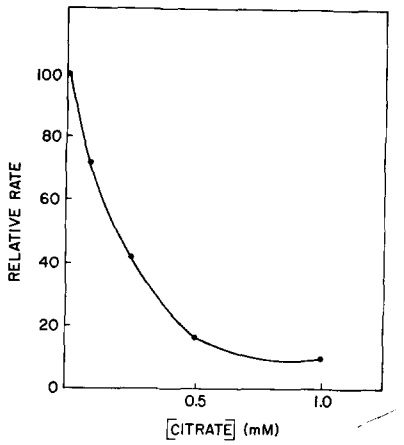
Sperm phosphofructokinase is inhibited by citrate with 50% inhibition of the enzyme being effected at 0.2 mM citrate and a substrate ATP level of 50 μM (Fig. 6).

TABLE IV

SYNERGISTIC STIMULATORY EFFECTS OF P_i *plus* ADENINE NUCLEOTIDES ON ATP-INHIBITED PHOSPHOFRUCTOKINASE

Each assay cuvette contained 83 μM Fru-6-*P*, 2.2 mM $MgSO_4$, 2 mM ATP, and 9.0 μg protein.

Expt. No.	*Modulator*	*Observed rate*	*Additive rate*	*Synergistic factor*
1	P_i	6	—	—
	ADP	15	—	—
	P_i + ADP	149	21	7.1
2	P_i	9	—	—
	5'-AMP	6	—	—
	3',5'-AMP	21	—	—
	ADP	28	—	—
	ADP + 3',5'-AMP	18	49	0
	5'-AMP + ADP	28	43	0
	P_i + 3',5'-AMP	104	30	3.5



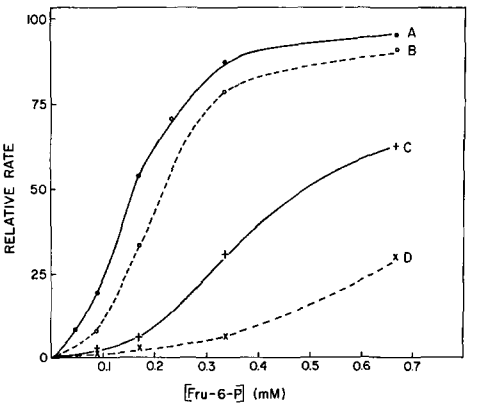


Fig. 6. Inhibition of sperm phosphofructokinase by citrate. Each assay cuvette contained 83 μM Fru-6-*P*, 50 μM ATP, 0.2 mM $MgSO_4$, and 4.8 μg protein.

Fig. 7. Modulation of citrate inhibition of sperm phosphofructokinase by Fru-6-*P* and synergism of citrate and ATP inhibition. Each assay cuvette contained 3.2 μg protein. Curve A, 30 μM ATP and 0.2 mM $MgSO_4$; Curve B, 50 μM ATP, 0.2 mM $MgSO_4$; Curve C, 30 μM ATP, 0.3 mM citrate ,0.5 mM $MgSO_4$; Curve D, 50 μM ATP, 0.3 mM citrate, 0.5 mM $MgSO_4$.

Similar results have been reported for the phosphofructokinases derived from skeletal muscle[36], adipose tissue[35], heart[38], liver[39], brain[21], plants[40], and yeast[41]. Citrate inhibition of sperm phosphofructokinase is at least partially overcome by increasing amounts of Fru-6-*P* (Fig. 7, Curves A and C). Also shown in Fig. 7 are the effects of slight alterations in the ATP level (from 30 to 50 μM) on the degree of inhibition by citrate and the ability of Fru-6-*P* to modulate the inhibition. By comparison of Curves A and C (30 μM ATP) with Curves B and D (50 μM ATP) it is apparent that ATP and citrate exert synergistic inhibitory effects. In agreement with the result of PARMEGGIANI AND BOWMAN[36] and others[21,37,40] both P_i and 5'-AMP are effective in relieving citrate inhibition. In an assay system (30 μM ATP, 167 μM Fru-6-*P*) inhibited with 0.3 mM citrate, 2.5 mM 5'-AMP and 5 mM P_i stimulated phosphofructokinase activity 2- and 3-fold, respectively. Detailed kinetic studies of the manner in which P_i and 5'-AMP relieve citrate inhibition have not been made. The action of P_i must be attributable to something other than its function as pure enhancer of phosphofructokinase activity, however, since the only marginal stimulation would be expected at an ATP level of 30 μM (See Fig. 2). To date we have been able to show only marginal synergistic effects of 5 mM P_i and 2.5 mM 5-AMP (less than 25% greater than additive rates) in relieving citrate inhibition, and have been completely unsuccessful in confirming earlier reports[21,39,42] that Fru-1,6-P_2 reverses citrate inhibition. This diphosphate at levels as high as 250 μM has no effect on citrate-inhibited (0.3 mM) phosphofructokinase. It is conceivable that these negative effects may be due to rapid desensitization of the enzyme to citrate inhibition and, hence, to modulators of citrate inhibition. With some preparations, partially purified sperm phosphofructokinase was found to have lost over 50% of its sensitivity to 0.3 mM citrate within less than 2 days after recovery from Sephadex G-200 columns.

DISCUSSION

The present paper represents an attempt at identification of those enzymes in the mammalian sperm cell which control the rate of aerobic glycolysis or fructolysis. Description of the properties of phosphofructokinase was given initial emphasis since a regulatory role for phosphofructokinase in a wide variety of tissues is now well established. The published evidence suggesting that phosphofructokinase plays a similar role in the sperm cell, however, is nonexistent with the possible exception of the statement by MORTON AND LARDY[20] that extracts of bull epididimal sperm catalyze the conversion of glycolytic intermediates beyond phosphofructokinase to lactate at rates faster than those intermediates that come before phosphofructokinase in the glycolytic sequence. Recent data obtained with monkey sperm in this laboratory has confirmed this report and has shown, in addition, that the levels of ATP and Fru-6-*P* found in monkey sperm extracts capable of converting fructose to lactate are of such a magnitude, 6 and 0.3 mM respectively, to indicate (based on the kinetic data of this paper) that fructolysis *in vivo* would be completely inhibited in the absence of modulators of ATP inhibition.

A number of compounds, including ADP, 3′-AMP, 5′-AMP, cyclic 3′,5′-AMP, Fru-6-*P*, and P_i, have all been shown capable of such a function. The modulating action of P_i and cyclic 3′,5′-AMP may be of particular significance in the economy of the sperm cell. For example, P_i acts both as a pure enhancer of phosphofructokinase at optimum ATP substrate levels and as a modulator of ATP inhibition. In addition, it has the added characteristic of acting synergistically with ADP and 3′,5′-AMP in relieving ATP inhibition. It may be well to consider these observations in light of the numerous reports that phosphate ion has pronounced stimulatory effects on glycolysis and fructolysis in the sperm cell of many species, including man (for discussion, see ref. 9). Of interest also is the observation that 3′,5′-AMP is a potent modulator of ATP inhibition. If current work confirms the suspicion that control of phosphofructokinase is tantamount to control of sperm glycolysis, then alterations in the intracellular level of modulators of ATP-inhibited phosphofructokinase, such as cyclic-AMP, would be of considerable importance. It is now known that the potential at least exists for such changes in the cyclic AMP levels in the sperm cell through regulation of adenylate cyclase. This enzyme has recently been shown in this laboratory to be present in the monkey sperm cell and to be sufficiently active to be of physiological importance.

The present paper is then considered to provide evidence which is consistent with a role for phosphofructokinase in regulation of primate sperm glycolysis. Confirmation of such a role is currently being sought using whole spermatozoa and extracts of physically disrupted spermatozoa.

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