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The Kinetics of Hydrolysis of ATP by Apyrase A from *Solanum tuberosum*

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Abstract—The kinetics of the hydrolysis of ATP by apyrase A from the *Solanum tuberosum* potato is studied at a pH of 6.5 and 25°C by the bioluminescent method. $K_M = 33$ μM and $V = 0.37$ μM/s are determined for ATP. It is shown that the hydrolysis of ATP proceeds in two stages. The fast and slow stages of the reaction are described by pseudo-first-order exponents. According to the hypothesis proposed, the intermediate complex of apyrase with ADP which is formed at the fast stage of the reaction possesses catalytic activity in the hydrolysis reaction of ATP but it is lower compared to free apyrase. This complex hydrolyzes ATP at the slow stage of the reaction.

Keywords: apyrase A, adenosine-5'-triphosphate, ATP, adenosine-5'-diphosphate, ADP, bioluminescent analysis

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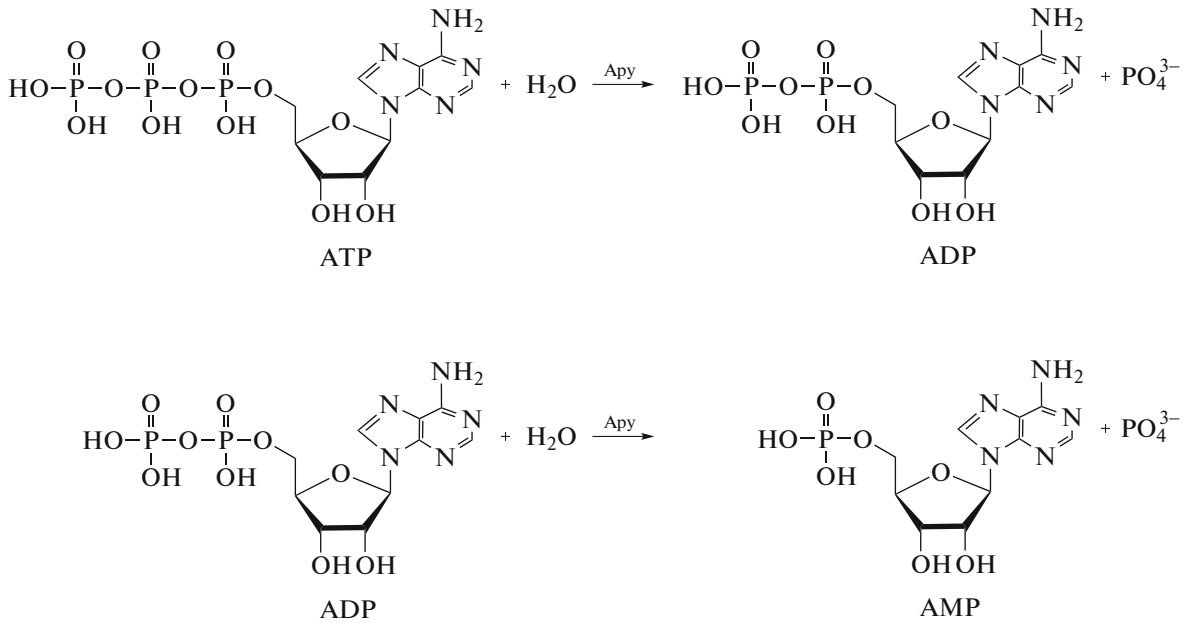
Apyrase (EC 3.6.1.5) is an enzyme that catalyzes the hydrolysis of adenosine-5'-triphosphate (ATP) into adenosine-5'-diphosphate (ADP) and the subsequent hydrolysis of ADP into adenosine monophosphate (AMP) with the formation of an inorganic phosphate ion at each stage [1, 2].

Apyrases (Apys) have been found in many eucaryotic systems. Their important role in the aggregation of platelets and biology of tumors has been shown [3–5]. Depending on the source, the kinetic properties of apyrases and their sensitivity to various effectors strongly differ due to the differences in the structure of the enzymes. One of the commercially available apyrases is apyrase A from the *Solanum tuberosum* potato which hydrolyzes ATP at a rate that is by an order of magnitude higher when compared to ADP [1, 2]. Due to this, the enzyme finds application in the removal of extracellular ATP from cell suspensions and in pyrosequencing [6]. This protein consists of one polypeptide chain (454 AAR) which forms a two-domain tertiary structure. The catalytic mechanism of the action of apyrase is still not completely clear. Two main models of hydrolysis of ATP by apyrase are discussed in the published sources. According to the first model, ATP and ADP are hydrolyzed over the same active site of the enzyme [1]. The second model is based on the assumption that the ADP produced at the first stage of the reaction moves to another site, over which its hydrolysis into AMP occurs. Both models cast doubt because they are not confirmed by the data of the kinetic studies [7]. Thus, no additivity of the rates of hydrolysis is observed in the case of the hydrolysis of equimolar amounts of ATP and ADP.

Various methods for the determination of the activity of apyrase and investigation of the kinetics of the reactions catalyzed by the enzyme are known. The colorimetric method of determining the reaction product, inorganic phosphate formed at both the first and second stages of the reaction, is the most common method. The method is based on the reaction of inorganic phosphate and ammonium molybdate with the formation of a complex, the intensity of coloration of which is proportionate to the amount of the phosphate [8]. The colorimetric method makes it possible to determine the total concentration of phosphate, the product of reactions (1) and (2) (Scheme 1), but does not allow correctly recording the kinetics of the hydrolysis of ATP. The high labor intensity and low sensitivity which do not allow monitoring the concentrations of the substrate and reaction products at the initial period of the hydrolysis reaction of ATP can also be regarded as the disadvantages of the colorimetric method. The well-known bioluminescent method of determining ATP, which is distinguished by a high level of sensitivity and specificity towards ATP [9], was applied in just two works but to screen various effectors of apyrase rather than investigate the kinetics of hydrolysis of ATP by apyrase [10, 11]. The kinetic parameters of the hydrolysis reaction of ATP were not correctly determined in these works.

The aim of this study consists of studying the kinetics of the hydrolysis of ATP by apyrase using the bioluminescent method of determining ATP, which makes it possible to correctly determine the kinetic parameters of this process, find some characteristic features of the catalysis by apyrase, and optimize the conditions of the removal of extracellular ATP from the samples under analysis using apyrase A.



Scheme 1. The scheme of the hydrolysis reactions of ATP by apyrase.

EXPERIMENTAL

Materials and Equipment

Potato apyrase (A6410, Sigma Aldrich, United States), ATP reagent (firefly luciferase, D-luciferin, magnesium ions) (Lumtek, Russia), adenosine-5'-triphosphate (ATP) (Sigma, United States), adenosine-5'-diphosphate (ADP) (Reanal, Hungary), sodium chloride NaCl (Khelikon Helicon, Russia), calcium chloride $CaCl_2$ (ICN, United States), distilled anhydrous dimethylsulfoxide (DMSO) (Lumtek, Russia), 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) (Sigma, United States), and mannitol (Aldrich, United States) were used in this study. The solutions were prepared using highly purified distilled deionized water obtained on a Labconco unit (United States). The reactions were carried out in an HB buffer containing 0.05 M HEPES, 0.15 M NaCl, and 8 mM $CaCl_2$ (pH of 6.5). The intensity of bioluminescence was recorded on FB 12 Femtomaster (Berthold, Germany) and LUM-1 (Lyumtek, Russia) luminometers. A GLP-21 pH meter (Crison, Spain) with the accuracy of up to 0.01 pH units was used for measuring the pH. The microbiological experiments were carried out in a GS laminar box (Babcock, Germany).

The Preparation and Reconstitution of the Lyophilized Apyrase Preparations

Two mL of a sterile normal saline was added to the manufacturer's vial containing 0.2 mg lyophilized apyrase. The obtained solution of apyrase (100 U/mL) was stored in aliquots at −70°C. To obtain lyophilized preparations, the solution of apyrase (0.1 mg/mL or 2 μM) obtained as described above was tenfold diluted with a normal saline containing 2% mannitol and 16 mM $CaCl_2$, frozen in aliquots of 50 μL each, and lyophilized. It was stored at −18°C. Prior to use, the lyophilized apyrase preparation was reconstituted in 100 μL of water, thus obtaining a solution of the enzyme with a concentration of 100 nM.

The Measurement of the Bioluminescent Signal for ATP

Twenty μL of a sample containing ATP was introduced into the microcuvette of the luminometer, 100 μL of the ATP reagent was added, and the bioluminescent signal RLU (number of pulses per s) was recorded.

The Study of the Kinetics of Hydrolysis of ATP in the Presence of Apyrase by the Bioluminescent Method

A solution of ATP in an HB buffer (pH of 6.5) and a solution of apyrase in a normal saline containing

8 mM $CaCl_2$ and 1% mannitol were prepared. Twenty-five μL of the solution of apyrase was added to 100 μL of the solution of ATP, and it was incubated at room temperature. Ten μL of the reaction mixture (in each instance) were collected at specific time intervals and placed in 50 μL of DMSO. The bioluminescent signal for the obtained sample (RLU_{smp}) was measured as described above. To calculate the concentration of ATP in the reaction mixture, the control solution with the known concentration of ATP in an HB buffer was used. Ten μL of the control solution of ATP was placed in 50 μL of DMSO, and the bioluminescent signal was measured for the solution of the control sample of ATP in DMSO (RLU_{contr}) as described above. The concentration of ATP in the reaction medium was calculated by the formula

$$[ATP]_{smp} = [ATP]_{contr}(RLU_{smp}/RLU_{contr}).$$

The concentration of ATP in the reaction mixture was varied in the range from 8 nM to 160 μM, and the concentration of apyrase, in the range from 0.4–4.0 nM. The diluted solutions were prepared by successive dilutions of the initial solution of ATP with an HB buffer, and the solutions of apyrase were prepared by successive dilutions of the initial solution of the enzyme with the normal saline containing 8 mM $CaCl_2$ and 1% mannitol.

The Study of the Effect of the Additions of Apyrase and ATP during the Reaction on the Kinetics of the Hydrolysis of ATP by Apyrase

The kinetics of hydrolysis of ATP was recorded as described above for a reaction mixture containing 80 μM ATP and 2 nM apyrase. After 200 s of the reaction when ~50% of the substrate had reacted, 2.5 μL of 100 nM apyrase or 5 μL of 1 mM ATP was added to 100 μL of the reaction mixture, and the recording of the kinetic curve of hydrolysis of ATP was continued.

The Study of the Effect of ADP on the Kinetics of Hydrolysis of ATP by Apyrase

Twenty-five μL of 10 nM apyrase was added to 100 μL of a 1 mM solution of ADP in an HB buffer (pH 6.5) containing 10 μM ATP, and the kinetics of hydrolysis of ATP was recorded as described above.

The Study of the Effect of Pyrophosphate on the Kinetics of Hydrolysis of ATP by Apyrase

Twenty-five μL of a 10 nM solution of apyrase was added to 100 μL of a 0.1 mM solution of ATP in an HB buffer (pH of 6.5) containing 0.09 mM sodium pyrophosphate, and the kinetics of hydrolysis of ATP was recorded as described above.

The Bioluminescent Analysis of Bacterial Cell Suspensions Using Apyrase

When determining the concentration of extracellular ATP, 20 μL of a cell suspension was introduced in 1 mL of 0.15 M NaCl and stirred. Twenty μL of the diluted cell suspension was placed in the microcuvette of the luminometer, 100 μL of the ATP reagent was added, and the bioluminescent signal was measured. The bioluminescent signal for the control sample of ATP in 0.15 M NaCl was measured in parallel. When determining the total concentration of ATP, 1 mL of DMSO was added to 120 μL of the cell suspension to destroy the cell walls and extract intracellular ATP. Twenty μL of the obtained extract was introduced in the cell of the luminometer, 100 μL of the ATP reagent was added, and the bioluminescent signal was measured. The bioluminescent signal for a solution of the control sample of ATP in DMSO was measured in parallel. When determining the concentration of intracellular ATP, 20 μL of 100 nM apyrase was added to 100 μL of the cell suspension to hydrolyze extracellular ATP, and it was incubated for 10 min. Then 1 mL of DMSO was added to inactivate apyrase and extract intracellular ATP. Twenty μL of the obtained extract was placed in the microcuvette of the luminometer, 100 μL of the ATP reagent was added, and the bioluminescent signal was measured. The bioluminescent signal for a solution of the control sample of ATP in DMSO was measured in parallel.

Each of the above-described experiments was repeated at least twice. The error of determining the kinetic quantities did not exceed ±5%.

The processing of the obtained experimental data and calculation of the kinetic constants were performed using the Microsoft Excel 2007 and 2010 and Origin Pro 8 programs.

RESULTS AND DISCUSSION

The Determination of the Kinetic Parameters of the Hydrolysis Reaction of ATP by Apyrase

To study the kinetics of hydrolysis of ATP by apyrase, we used the bioluminescent method of determining ATP based on the application of a luciferin–luciferase reagent [9, 12]:

$$ATP + LH_2 + O_2 \xrightarrow{Luc,\ Mg^{2+}} AMP + PP_i + LO + CO_2 + h\nu.$$

Due to the high level of sensitivity (the limit of detection of ATP is 10^{-13} M), specificity, and rapidity and simplicity, this method makes it possible to obtain the kinetic curves of the hydrolysis of ATP in a wide range of concentrations of ATP and the enzyme. Earlier, the bioluminescent method was applied to monitor the effectors of the activity of apyrase [10, 11]. The studies were performed at selected constant concentrations of ATP and apyrase; here, the concentrations

of the effectors were varied. In [10], the reaction mixture contained both apyrase and a luciferase reagent and had a pH of 7.75 during the incubation of apyrase with the effector, while the pH optimum of the activity of apyrase operating in a narrow range of pH is observed at a pH of 6.5. In [11], a special format of the HTS analysis of the activity of apyrase using a 384-well plate and commercial kits for the measurement of the activity of apyrase was also inapplicable for solving the problems set in this work. The results obtained by the authors [11] were certain effective values that made it possible to compare the effect of various effectors on apyrase but did not allow determining the kinetic parameters for the hydrolysis reaction of ATP by apyrase at the pH optimum of its potency.

We separated the stages of hydrolysis of ATP by apyrase and measurements of the residual concentration of ATP by applying the bioluminescent method for the measurement of ATP in biological systems developed by us earlier [9]. The principle of the method is as follows. Apyrase is added to a solution of ATP with pH of 6.5, and the reaction mixture is incubated at a constant temperature. Samples are collected at specific time intervals and placed in 100% DMSO. The final concentration of DMSO in the mixture being obtained is at least 80%. Immediate termination of the hydrolysis reaction occurs at such a high concentration of DMSO due to the inactivation of apyrase. Here, the concentration of ATP becomes fixed and remains unchanged for a long time, namely, for at least 8 h at room temperature and for several months at −18°C. The concentration of ATP is determined by the bioluminescent method as described in the *Experimental* section. Fast collection of the samples and their rapid processing made it possible to record the change in the concentration of ATP in the reaction mixture in the second and minute intervals of the reaction, as well as to perform the hydrolysis by apyrase and measurement of bioluminescence under the optimum conditions for each process.

To determine the kinetic parameters of the hydrolysis reaction of ATP by apyrase at 25°C, the kinetic curves were obtained at a fixed concentration of apyrase (4 nM) and a variable concentration of ATP (0.8 to 160 μM). The reaction mixture contained 0.05 M HEPES, 0.15 M NaCl, and 8 mM $CaCl_2$ (pH of 6.5). The Michaelis constant and maximum reaction rate were calculated from the obtained values of the initial reaction rates:

$$K_M = (33 \pm 6)\ \mu M \text{ and } V = (0.37 \pm 0.03)\ \mu M/s.$$

The value of K_M measured by us turned out to be lower by a factor of ~1.5 than the value of K_M determined by the colorimetric method [2]. This may be explained by the fact that we measured K_M for the first reaction (Scheme 1) rather than for the total reaction of the formation of inorganic phosphate. The smaller value of K_M acts as the indicator of the higher affinity of apyrase to ATP than to ADP. The kinetic curves of hydrolysis of ATP were obtained in a wide range of concentrations of ATP (8 nM to 80 μM) and apyrase (0.8 to 4.0 nM), the examples of which are shown in Fig. 1.

The shape of the obtained kinetic curves and depth of hydrolysis of ATP depend on the ratio of the concentrations of the substrate and enzyme. At low concentrations of apyrase (0.8–1.2 nM), no more than 50% ATP is hydrolyzed over 300 s of the reaction at all the used concentrations of ATP. At a concentration of apyrase of ≥2 nM, 80–90% of the hydrolysis of ATP takes place over the same time. As is shown in Fig. 1, two stages are observed in the kinetic curves of the hydrolysis of ATP by apyrase, namely, fast and slow, each of which proceeds by the pseudo-first order. Comparing the initial rates of the fast and slow stages of the hydrolysis of ATP (Table 1) shows that these quantities linearly depend on the concentration of the enzyme in the used range of concentrations of apyrase. An exception is the data obtained at a very low concentration of ATP (8 nM) when the fast stage proceeds over several seconds at all the used concentrations of apyrase, and it cannot be determined accurately. At nanomolar concentrations of ATP, the initial rate of the fast stage is orders of magnitude higher than the initial rate of the slow stage. At the saturating concentration of ATP (80 μM), these rates differ by a factor of about nine.

The two-stage kinetics of formation of phosphate was observed in the earlier works on the investigation of the kinetic properties of apyrase A [13], and the concept of fast and slow stages of hydrolysis of ATP by apyrase was introduced. It has been noted that the rate of formation of phosphate decreases and becomes equal to the rate of formation of phosphate in the case of the hydrolysis of ADP after the hydrolysis of ~50% of ATP. It was found in [1] using ^{32}P-labeled substrates that γ-phosphate (from the ATP molecule) is predominantly abstracted at the fast stage with a small admixture of β-phosphate (from the ADP molecule). When ATP labeled at the β-position was used, the formation of a small amount of the labeled phosphate (from ADP) was observed at the fast stage, and the main amount of the labeled phosphate was released at the slow stage of the process. The obtained results made it possible to assume that ATP acts as a competitive inhibitor in the hydrolysis reaction of ADP, and the inhibition constant is close to K_M for ATP [1].

The Effect of Additives on the Kinetics of the Hydrolysis of ATP by Apyrase

To obtain more detailed information about the nature of the fast and slow stages of the hydrolysis of ATP, we conducted a series of additional experiments. At a high initial concentration of ATP (80 μM), a solution of apyrase was added to the reaction mixture during the slow stage of the reaction (after 200 s when ~50% of the ATP had hydrolyzed) in such a way that

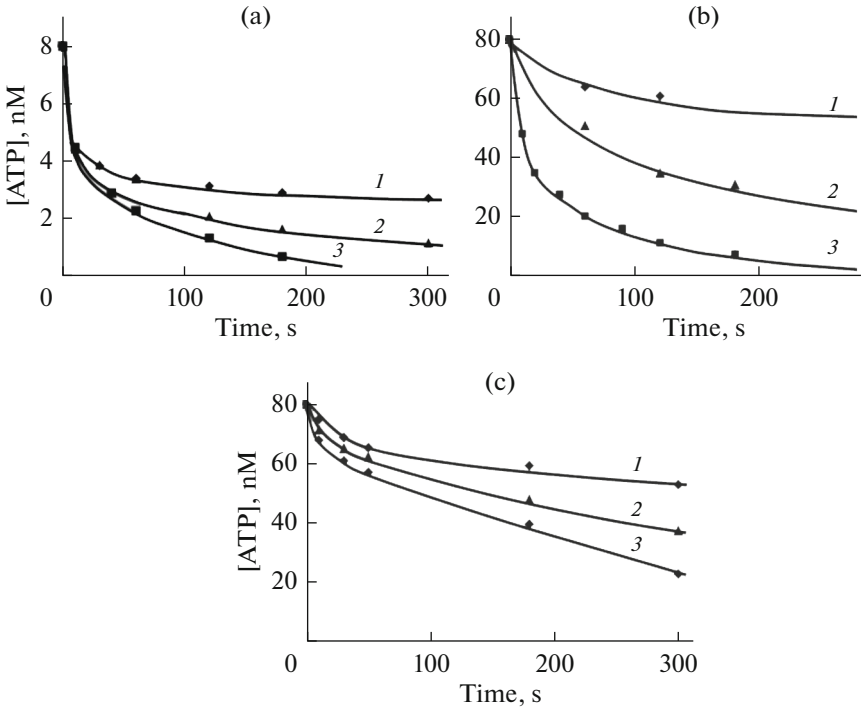


Fig. 1. Kinetics of hydrolysis of ATP by apyrase at 25°C. The composition of the reaction mixture is as follows: 0.05 M HEPES, 0.15 M NaCl, and 8 mM $CaCl_2$ (pH of 6.5). The concentration of ATP is (a) 8, (b) 80 nM, and (c) 80 μM. The concentration of apyrase is (a) (*1*) 0.8, (*2*) 2.0, and (*3*) 4.0 nM; (b) (*1*) 1.2, (*2*) 2.0, and (*3*) 4.0 nM; and (c) (*1*) 1.0, (*2*) 2.0, and (*3*) 4.0 nM.

the concentration of the enzyme doubled. Here, the reaction rate also doubled. Although the additions of apyrase increased the rate of the process proportionately to the increase in the concentration of the enzyme, they did not change the shape of the kinetic curve. Only the slow stage of the process was observed without the fast stage. In the next experiment, ATP was added to the reaction mixture during the slow stage of the reaction in such a way that the concentration of ATP became equal to the initial concentration of ATP. Here, the reaction rate did not change and remained equal to the rate of the slow stage. Therefore, the additions of ATP also did not affect the rate of the slow stage of the process.

To explain the obtained results, Scheme 2 was proposed.

$$E + ATP \leftrightarrow E \cdot ATP \rightarrow E \cdot ADP + P_i, \quad (1)$$

$$ATP + E \cdot ADP \rightarrow ATP \cdot E \cdot ADP \rightarrow ADP + E \cdot ADP + P_i, \quad (2)$$

$$E \cdot ADP \rightarrow AMP + E + P_i. \quad (3)$$

Scheme 2. The proposed scheme of hydrolysis of ATP by apyrase.

According to Scheme 2, apyrase interacts with ATP at the fast stage, and a complex E · ATP is formed, the transformation of which over the active site of apyrase

Table 1. Initial rates and rate constants of the fast and slow stages of the hydrolysis reaction of ATP in the presence of apyrase at various concentrations (the conditions are presented in the figure captions)

$[ATP]_0$	$[E]_0$, nM	$v_{0(fast)}$, μM/s	$v_{0(slow)}$, μM/s	k_{fast}, s^{-1}	k_{slow}, s^{-1}
	1.2	0.36	0.0028	0.06	0.0017
8 nM	2	0.36	0.0055	0.06	0.006
	4	0.35	0.008	0.058	0.010
	0.8	1.79	0.04	0.015	0.002
80 nM	2	3.24	0.07	0.032	0.006
	4	3.16	0.05	0.043	0.012
	1	0.50	0.06	0.0065	0.0009
80 μM	2	0.86	0.10	0.0114	0.002
	4	1.20	0.14	0.0162	0.0035

leads to the release of phosphate and formation of the complex E · ADP (reaction (1)). Further transformation of the complex E · ADP may lead to the formation of AMP and phosphate (reaction (3)). It is known that the catalytic constant of hydrolysis of ADP by apyrase A is smaller by a factor of about ten than the catalytic constant of hydrolysis of ATP [1, 2], because of which the E · ADP complex is accumulated in the reaction system as reaction (1) proceeds. This complex possesses enzymatic activity towards ATP but it is lower in comparison with the activity of a free enzyme (reaction (2)). The E · ADP complex in particular catalyzes the hydrolysis of ATP at the slow stage. At low concentrations of ATP and at the initial stage of hydrolysis of ATP, the fast stage of hydrolysis of ATP by apyrase is implemented. Under these conditions, the concentration of the E · ADP complex in the reaction medium is insignificant. At high concentrations of ATP and depth of hydrolysis of ATP ≥ 50%, the amount of the E · ADP complex sufficient for the implementation of the slow stage of hydrolysis of ATP is accumulated.

To verify this hypothesis, we obtained the kinetic curves of hydrolysis of 10 μM ATP in the presence of 2 nM apyrase in the initial reaction medium free from ADP and in a medium into which 0.8 mM ADP was preliminarily added. Therefore, we created conditions, under which the concentration of ADP is much higher than the initial concentration of ATP. The fast stage was absent in the presence of a large excess of ADP, while the rates of the slow stage of the reaction were the same in both cases. Therefore, the slow stage of the reaction is observed in the case of accumulation of a sufficiently high concentration of the E · ADP complex in the reaction medium. It cannot be ruled out that the structure of the E · ADP complex formed by reaction (1) (Scheme 2) is different from the structure of the E · ADP complex formed during the reaction of apyrase with ADP. This is indicated by the published data on the investigation of the inhibiting action of ADP on the hydrolysis of ATP by apyrase A. A ten-fold excess of ADP had just a slight inhibiting effect on the formation of phosphate from ATP labeled by the γ-phosphate group [1]. A 100-fold excess of ADP used by us made it possible to entirely eliminate the fast stage of hydrolysis of ATP even at a low concentration of the substrate.

It was important to find out which groups in the nucleotide molecule participate in the formation of the complex of ADP with apyrase. We studied the effect of pyrophosphate that is an analogue of the substrates of apyrase because its structure includes the same O–P bonds as nucleoside tri- and diphosphates that act as the target for apyrase on the hydrolysis of ATP by apyrase [14, 15]. The kinetic curves of the hydrolysis reaction of ATP by apyrase were obtained at 80 μM ATP and 2 nM apyrase in the absence and presence of 72 μM sodium pyrophosphate. As in the case of ADP, the rate of the hydrolysis reaction of ATP in the presence of pyrophosphate is equal to the rate of the slow stage of the reaction in the absence of pyrophosphate. Probably, the binding of the enzyme and ADP with the formation of the active E · ADP complex proceeds by the pyrophosphate bonds that exist in both ADP and pyrophosphate.

Therefore, apyrase A catalyzes a series of successive transformations of ATP into AMP, an important role in which belongs to the active E · ADP complex. Due to its participation, the kinetics of the hydrolysis of ATP is described by a biexponential dependence, namely, the reaction rate decreases with the accumulation of the primary reaction product (ADP). We should use the kinetic characteristics of the slow stage for the correct determination of the time of removal of ATP with an unknown concentration. This is especially important in the case of the hydrolysis of high concentrations of ATP. The calculation shows that the reaction time is not more than 10 min for the hydrolysis of ATP at a concentration of 80 μM in the presence of 4 nM of apyrase.

The Use of Apyrase for the Removal of Extracellular ATP

The determination of cells' viability plays an important role in biochemistry and biotechnology. There are many methods for determining cells' viability, one of which is ATP bioluminescence assay based on the determination of intracellular ATP as a universal energy source in living organisms [9]. The number of living cells, e.g., in the antituberculosis bacillus Calmette–Guérin (BCG) vaccine medicinal products, is determined by the concentration of intracellular ATP [16, 17]. During the preparation, lyophilization, and storage of a cell suspension, a certain number of cells are destroyed, and extracellular ATP is accumulated in the reaction medium, which interferes with the correct determination of the concentration of intracellular ATP. Apyrase is used to remove extracellular ATP from the cell suspension [16–18]. Table 2 presents the data on the measurement of intracellular and extracellular ATP in the experimental samples of a BCG vaccine which were obtained upon varying the conditions of sample preparation and lyophilization at the production plant of this medicinal product [17, 18]. The concentration of extracellular ATP was determined by the bioluminescent method without the preliminary destruction of the cell walls. To measure the concentration of intracellular ATP, the cell suspension was treated with apyrase and then placed in DMSO for the destruction of the cell walls, inactivation of apyrase, and release of intracellular ATP. The concentration of intracellular ATP was measured by the bioluminescent method as described in this study.

As is seen from Table 2, the concentration of extracellular ATP in the samples of the vaccine varies from 5 to 24 nM. An exception is the experimental liquid vaccine sample no. 2, in which the degree of cell destruction is quite high. The concentration of intra-

Table 2. Concentration of total, extracellular, and intracellular ATP in the cell suspension for different experimental samples of the liquid (samples 1, 2) and reconstituted after lyophilization (samples 3–14) BCG vaccine

Samples of the vaccine	Concentration of ATP in the vaccine cell suspension, nM			Concentration of extracellular ATP in total ATP, %
	total ATP	intracellular ATP	extracellular ATP	
1	341	330	11	3.2
2	516	386	130	25.2
3	24	16	8	33.3
4	27	22	5	18.5
5	35	28	7	20.0
6	37	30	7	18.9
7	40	27	13	32..5
8	42	18	24	57.1
9	43	30	13	30.2
10	43	37	6	13.9
11	44	21	23	52.3
12	44	38	6	13.6
13	51	38	13	25.5
14	55	34	21	38.2

cellular ATP for the reconstituted samples of the vaccine changes by a factor of two; and the concentration of extracellular ATP, by a factor of five. Therefore, the bioluminescent determination of intracellular ATP without the preliminary treatment of the sample under analysis with apyrase may lead to strongly overestimated results on the concentration of living microorganisms in the sample. The cell suspensions under analysis contained nanomolar concentrations of ATP which were substantially lower than K_M of apyrase for ATP. It can be concluded based on the results obtained in this study that the fast stage of the hydrolysis of ATP up to a deep degree of hydrolysis will mainly be observed under these conditions.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ADDITIONAL MATERIALS

There are no additional materials.

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