Tissue Based Enzymatic Determination of ATP in Human Erythrocyte Hemolysate Combined with Spectrophotometric Method

Makoto Kataoka, Norio Teshima, Makoto Kurihara, Katsuhiko Kuwa,† and Takuji Kawashima*
Laboratory of Analytical Chemistry, Department of Chemistry, University of Tsukuba, Tsukuba 305-8571
†*College of Medical Technology and Nursing, University of Tsukuba, Tsukuba 305-8577*

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A potato tissue based enzymatic determination of ATP combined with spectrophotometric detection is proposed. ATP can be hydrolyzed to give AMP via ADP and two moles of orthophosphate by apyrase (EC 3.6.1.5) contained in the potato tissue. The phosphate released is determined spectrophotometrically based on molybdenum blue formation (λ_{max}=712 nm). A linear calibration graph is obtained over the range 1.3×10^{-6} – 1.3×10^{-5} mol dm^{-3} ATP with a detection limit of 1.5×10^{-7} mol dm^{-3} (S/N = 3). The proposed method was successfully applied to the determination of ATP in human erythrocyte hemolysate.

Adenosine-5'-triphosphate (ATP) is the mediator of energy changes which occur in both catabolic, degradative processes and anabolic, biosynthesis processes. The hydrolysis of ATP is one of the most important reactions of metabolism because the energy released drives many various reactions.[1] Most commonly used methods for the determination of ATP include bioluminescent method,[2,3] chromatography[4] and biosensor using isolated enzyme.[5–7] Recently biocatalytic materials such as living bacterial cells[8,9] and tissue slices[8,10] have been used for the construction of biosensors in stead of isolated enzymes. Potato tissue (*Solanum tuberosom*) contains an ATP–diphosphohydrolase (apyrase, EC 3.6.1.5) which catalyzes the following reactions (1) and (2).

$$ATP + H_2O \rightarrow ADP + \text{orthophosphate} \quad (1)$$
$$ADP + H_2O \rightarrow AMP + \text{orthophosphate} \quad (2)$$

The paper describes a new method for ATP using these reactions combined with conventional molybdenum blue method. The recommended procedure is as follows: To a sample solution containing up to 2.6×10^{-5} mol dm^{-3} ATP in a 100-mL beaker, an aliquot of buffer solution (MOPS, pH 6.6) is added and diluted to *ca.* 25 mL with the same buffer solution. The solution is heated for 5 min at 37 ℃ and then *ca.* 2 g of sliced cubic potato tissues (5-8 mm^3) are added and allowed to proceed the enzyme reaction with stirring the solution in a thermostat for 8 min. Then, all of the solution is transferred into a 50-mL volumetric flask. To this solution 5 mL of ammonium molybdate (1.5 mol dm^{-3} H_2SO_4) and 2 mL of ascorbic acid solutions are added and diluted to 50 mL with water and vigoraously shaken. The molybdenum blue formation reaction is carried out at 50 ℃ in the thermostat for exactly 30 min after mixing the solution and then the absorbance at 712 nm is measured against a distilled water reference. The net absorbance is obtained by subtracting a blank absorbance.

A linear calibration graph was obtained over the range 1.3×10^{-6} – 1.3×10^{-5} mol dm^{-3} ATP with a correlation coefficient of 0.994 and a detection limit of 1.5×10^{-7} mol dm^{-3} (S/N = 3). The reproducibility of the method is satisfactory with the relative standard deviation of 0.51% for eight determinations of 1.0×10^{-5} mol dm^{-3} of ATP even if a different potato tissue was used. The effect of foreign substances on the determination of 1.0×10^{-5} mol dm^{-3} of ATP was examined. Equivalent amounts of iron (III), copper (II), inorganic phospate, glucose-6-phospate, adenosine-5'-monophosphate (AMP), adenosine-5'-diphosphate (ADP), inosine-5'-phosphates, guanosine-5'-phosphates, cytosine-5'-phosphates and xantosine-5'-phosphates showed a little interference. The interference of these nucleotides is attributable to the formation of phosphate by apyrase and acid phosphatase (EC 3.1.2.1) contained in the potato tissue. No interference of phosphate (8 μmol g^{-1})[11] contained in the tissue was observed by subtracting the blank absorbance. The proposed method was applied to the determination of ATP in a human erythrocyte hemolysate. To the heparinized human blood from healthy volunteers a 0.9% isotonic NaCl solution was added and the solution was centrifuged at 2000 rpm for 5 min. Red blood cells were collected and stored at –20 ℃ in a freezer. After the hemolysis of red blood cells, the erythrocyte hemolysate was centrifuged at 3000 rpm for 10 min and 1 mL of the supernatant solution was taken in the other centrifugal precipitation tube. To deproteinize, 1 mL of water and 6 mL of trichloroacetic acid solution (11.7%) were added to the supernatant solution and the solution was centrifuged at 3000 rpm for 10 min. Five mL of this solution was used for each measurement. To examine the recovery of ATP, known amounts of ATP were added to the sample. The results are shown in Table 1. The recovery of added ATP was found to be satisfactory. The concentrations of

Table 1. Determination of ATP in human erythrocyte hemolysate

Sample (age, sex)	ATP added /μmol l^{-1}	ATP found /μmol l^{-1}	ATP in sample[a] /mmol l^{-1}	Recovery /%
1	0	0.265	1.21	–
(24, male)	0.200	0.471	1.23	103
	0.399	0.669	1.22	101
	0.599	0.875	1.25	102
2	0	0.268	1.22	–
(22, male)	0.200	0.471	1.23	102
	0.399	0.673	1.24	102
	0.599	0.875	1.25	101
3	0	0.264	1.19	–
(24, female)	0.200	0.465	1.20	101
	0.399	0.667	1.21	101
	0.599	0.868	1.22	101
4	0	0.306	1.39	–
(23, female)	0.200	0.512	1.41	103
	0.399	0.713	1.42	102
	0.599	0.919	1.45	102

[a] Sample solutions were diluted 453 times for the analysis.

ATP by the present method are in good accord with those reported in the literatures.[6, 12, 13]

References and Notes

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