Communications to the Editor

[Chem. Pharm. Bull. 30(7)2651—2654(1982)]

dATP CONTENT IN COMMERCIAL ATP SAMPLES

Akiko Yoshioka, Kazumi Tanaka, Yusuke Wataya,
and Hikoya Hayatsu*
Faculty of Pharmaceutical Sciences, Okayama University,
Tsushima, Okayama 700, Japan

dATP content in commercial ATP samples was examined by use of periodate-mediated degradation of ATP and subsequent analysis on high-performance liquid chromatography. Out of ten samples obtained from eight different distributors, six contained dATP in amounts ranging from 0.1% to 0.9%. The significance of this contamination is discussed.

KEYWORDS —— ATP impurity; dATP HPLC; ATP HPLC; HPLC ATP dATP; Periodate oxidation ATP

In the course of our study on cellular nucleotide pools, in which we fractionated deoxyribo- and ribo-nucleoside triphosphates, we have become aware that commercial ATP samples contain dATP as impurities. By use of periodate-mediated selective degradation of ATP, coupled with high-performance liquid chromatography (HPLC), it is now possible to detect 1×10^{-12} mol of dATP present in 1×10^{-8} mol ATP as impurities. We report here that six out of ten ATP samples obtained from eight different reagent distributors contained dATP in significant amounts.

ATP samples were purchased from three Japanese, four American, and one German reagent companies. dATP as a standard sample was prepared by purification of a sample (P-L Biochemicals) on HPLC, using a column of Partisil 10 SAX (see below for conditions of the chromatography).

The periodate oxidation-HPLC procedure for determination of dATP in the presence of excess ATP was a modification of the one previously reported by Garrett and Santi.[1] A detailed study to establish the reaction conditions optimal for the determination will be described elsewhere. Briefly, the ATP in the sample was degraded with sodium periodate-methylamine, the excess periodate was removed by treatment with rhamnose, and the dATP was quantified by HPLC.

The HPLC was carried out using a Waters chromatography pump Model 6000A and an absorbance detector Model 440, attached to a Hewlett-Packard integrator 3390A. The chromatography on a column of Partisil 10 SAX (4.6 x 250 mm) was run using a 10-to-1 mixture of 0.4 M ammonium phosphate (pH 2.9) and acetonitrile as eluent. The flow rate was 2 ml/min, and the detection of peaks was by absorbance at 254 nm. The chromatography on a column of μBondapak C_{18} (4.0 x 300 mm) was run with 0.1 M triethylammonium phosphate (pH 6.0) containing 5% acetonitrile as eluent, at a flow rate of 1 ml/min. The amount of dATP was estimated from the peak height in HPLC, based on a calibration line obtained with a standard dATP sample. The calibration line was linear in the range 1×10^{-11} to 1×10^{-9} mol of dATP.

When commercial ATP samples were analyzed by HPLC following treatment with

periodate, elution profiles such as those shown in Fig. 1 were obtained. Figure 1-1a and 1b represent the profiles for a sample eluted through two different types of chromatographic columns, i.e. an anion exchange column (1a) and a reverse phase column (1b). As can be seen, this sample gave peaks corresponding to dATP. From Fig. 1-1a, it is also clear that the degradation of ATP was complete under the conditions employed. The dATP peaks were identified by the retention time, as well as by cochromatography on both the anion exchange and the reverse phase columns with a standard dATP sample. In this cochromatography, the dATP peak-heights of the test sample and the standard were strictly additive, indicating the

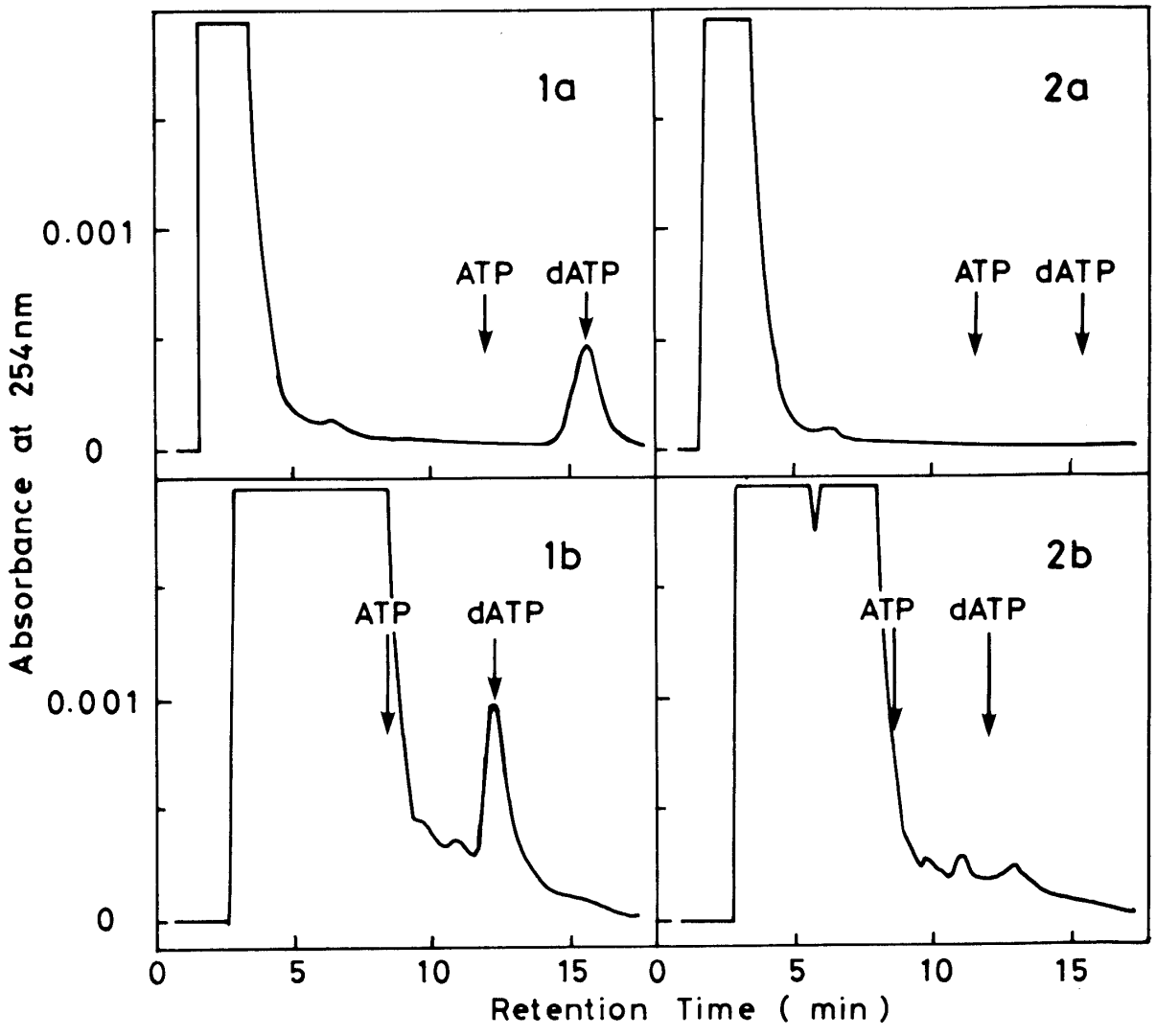

Fig. 1. Analysis of Periodate-treated ATP Samples by High-performance Liquid Chromatography
(1a) Sample 1 on Partisil 10 SAX, (1b) sample 1 on μBondapak C_{18}, (2a) sample 2 on Partisil 10 SAX, and (2b) sample 2 on μBondapak C_{18}. The "ATP" and "dATP" with arrows indicate the positions where standard samples were eluted.

exact coincidence of these two peaks. From the analysis, the sample was estimated to contain 0.93% dATP in total adenine nucleoside triphosphates. This experiment was repeated three more times and the dATP content found was 0.92%, 0.93% and 0.91%.

A sample of ATP did not give peaks corresponding to dATP (Fig. 1-2a and 2b). Since the limit of detection of dATP was 1×10^{-12} mol in this system, and the amount of ATP-sample injected was 1×10^{-8} mol, this result indicated that the dATP content in this sample was less than 0.01%. The experiment was repeated two more times and the dATP content found was less than 0.01% in both experiments. These observations also indicated that the analytical method employed can yield reproducible results.

Table I. dATP Content in Commercial ATP Samples

Distributor [a]	Sample number	dATP/(ATP + dATP) (mol %)
A	1	0.92
B	1	0.25
C	1	0.17
D	1	0.19 [c]
	2 [b]	0.15 [c]
E	1	0.11 [c]
F	1	< 0.01 [c]
G	1 [b]	< 0.01 [c]
H	1	< 0.01
	2	< 0.01

a) Distributors B, F and H are Japanese, C, D, E and G are American, and A is German.
b) Dipotassium salt. Others were disodium salts.
c) Results of single experiments. The others were averages of values in more than two experiments.

The dATP content as expressed by mol percentage in total adenine nucleoside triphosphates in ten commercial ATP samples are presented in Table I. Among these samples, six contained significant amounts of dATP, the content ranging from 0.1% to 0.9%.

The dATP as impurities in ATP samples can have its origin in DNA that contaminates the RNA being used as the starting material for its preparation. In a catalog of a manufacturer, it is stated that ATP is chemically synthesized from AMP, which, in turn, is prepared from RNA.

Although less-than 1% contamination by dATP will not pose problems in most ATP-requiring reactions, it can become a serious matter in some experiments. Thus, ATP has been shown to stimulate DNA synthesis both in cells (ref. 2 and references cited therein) and in cell-free systems.[3] In these studies, ATP was

used in a large excess (up to three orders of magnitude excess) of deoxyribonucleoside triphosphates. Under such circumstances, if the ATP sample is impure like those shown in Table I, the amount of dATP originating from the ATP can become comparable to that of dATP added to the reaction mixtures. As a result, the concentration of dATP in the system can become significantly higher than is desired.

For preparation of ATP free of dATP, it would be advisable to use DNA-free RNA as the starting material.

ACKNOWLEDGEMENT This work was supported by a grant for Scientific Research from the Ministry of Education, Science and Culture. Dr. F. Hanaoka of the University of Tokyo, Faculty of Pharmaceutical Sciences, and Dr. T. Oda of Okayama University Medical School are thanked for discussions.

REFERENCES

1) C. Garrett and D. V. Santi, Anal. Biochem., 99, 268 (1979).
2) C. Gärtner, E. A. Waldstein, and U. Hagen, Biochim. Biophys. Acta, 607, 247 (1980).
3) T. Spasokukotskaja, M. Staub, M. Sasvári-Székely, and F. Antoni, Biochim. Biophys. Acta, 656, 140 (1981).

(Received June 5,1982)