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Journal of Chromatography B, 784 (2003) 101–109
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Analysis of intracellular nucleoside triphosphate levels in normal and tumor cell lines by high-performance liquid chromatography

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Received 11 February 2002; received in revised form 25 September 2002; accepted 30 September 2002

Abstract

A reversed-phase ion-pair high-performance liquid chromatographic method for the direct and simultaneous determination of ribonucleoside triphosphates and their corresponding deoxyribonucleoside triphosphates in trichloroacetic acid cell extracts is presented. Using this system, high resolution of nine acid-soluble compounds, including ADP, CTP, dCTP, GTP, UTP, dGTP, dTTP, ATP and dATP in 16 normal or tumor cell lines, is obtained. The method is based on an extraction of nucleotides from cells with a solution of 6% trichloroacetic acid followed by neutralization with the addition of 5 *M* K_2CO_3 just prior to HPLC analysis. Chromatographic separations were performed using a Symmetry C_{18} 3.5 μm (150×4.6 mm) column (Waters) equipped with a NovaPak C_{18} Sentry guard column with UV detection at 254 nm. The HPLC columns were kept at 27 °C. The mobile phase was delivered at a flow-rate of 1.0 ml/min, with the following stepwise gradient elution program: A–B (60:40) at 0 min→(40:60) at 30 min→(40:60) at 60 min. Solvent A contained 10 m*M* tetrabutylammonium hydroxide, 10 m*M* KH_2PO_4 and 0.25% MeOH, and was adjusted to pH 6.9 with 1 *M* HCl. Solvent B consisted of 5.6 m*M* tetrabutylammonium hydroxide, 50 m*M* KH_2PO_4 and 30% MeOH, and was neutralized to pH 7.0 with 1 *M* NaOH. The calibration curves ($r>0.99$) of the components in cell extracts were established with their aqueous standards. The average within-day precision for the nine compounds was 0.9%, and the average day-to-day precision was 5.0%. The detection limits (pmol) of the nine reagents were 1.39 (ADP), 4.32 (CTP), 15.5 (dCTP), 2.38 (GTP), 4.42 (UTP), 9.45 (dGTP), 14.6 (dTTP), 2.44 (ATP) and 11.8 (dATP). The recovery of this method for the standards ranged from 82.4 to 120.5%. The results for the detection of nucleotide pools in 16 normal and tumor cell lines were presented. In conclusion, this simplified analytical method enables the simultaneous quantitation of NTP and dNTP in cell or tissue extracts and may represent a valuable tool for the detection of minute alterations of intracellular NTP/dNTP pools induced by anticancer/antiviral drugs and diseases.

Keywords: Tumor cells; Nucleoside triphosphates

1. Introduction

Carcinogenesis is a complicated process induced by the interaction of endogenous and exogenous factors. Unquestionably, a genetic change must have

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1570-0232/02/$ – see front matter
PII: S1570-0232(02)00780-8

occurred in this process. Nucleotides participate in most biochemical processes as activated precursors for DNA and RNA synthesis and as intermediate products in biosynthesis. The metabolic pathway for synthesis of nucleotides and for recycling bases and nucleosides to nucleotides is subject to alteration as a normal cell goes through the steps which ultimately yield a cancerous cell. Many anticancer and antiviral drugs interfere with the synthesis of DNA and RNA and/or their precursors, notably dNTP and NTP. Batiuk et al. [1] reported that guanosine and deoxyguanosine exhibited toxicity to Jurkat cells through two mechanisms: ATP deletion, causing necrosis, and the accumulation of dGTP, resulting in apoptosis. The determination of nucleotides and deoxynucleotides is of fundamental importance either to evaluate the cell energy state or to evaluate the degree of DNA and RNA biosynthesis. This latter aim is particular relevant in studies involving cell cycle regulation, viral infection of cell, molecular mechanism of tumorigenesis and metastasis and pharmacological activity of anticancer or antiviral drugs able to interfere with nucleic acid replication. An analytical method for the quantitative measurement of NTP and dNTP pools in cells is therefore required for studying the biological differences between normal and tumor cells and the influence of pharmacologically active agents on DNA and RNA synthesis and regulation.

Enzyme assay, bioluminescence, ^{31}P nuclear magnetic resonance spectroscopy (NMR), high-performance liquid chromatography (HPLC), high-performance capillary electrophoresis (HPCE) and gas chromatography (GC) can be applied to the determination of nucleotides [2]. The use of HPLC for determining cellular NTP and dNTP pool sizes has proven to be one of the most simple and rapid methods of measuring all of the NTPs and dNTPs at the same time. Since the concentration of dNTP present in mammalian cells is several orders of magnitude lower than the corresponding NTP, attempts have been made to remove NTP from cell extracts prior to dNTP analysis by periodate [3] and methylamine [4] oxidation procedure. Though this method is effective, the periodate oxidation procedure was reported to result in partial destruction of dGTP and some extra problematic peaks may coelute with the dNTP fractions with MeOH but not trichloroacetic acid (TCA) extraction [5]. Another similar approach implied removal of NTP by sequential boronate affinity chromatography of cell extracts prior to ion-exchange chromatography of dNTP eluted fractions [6]. Many attempts have been made in recent years to apply HPLC and capillary electrophoresis (CE) to separation and quantitation of NTP and dNTP in cell extracts. To the best of our knowledge however, only a few studies reporting the simultaneous determination of NTP and dNTP by HPLC have been published so far [5,7–9]. The present study reports the ion-pair reversed-phase HPLC method which, without any chemical manipulation of samples except for TCA treatment of cells, allow the direct simultaneous separation and quantitation of dNTP and NTP. This method was applied for detecting and comparing the nucleotide pool size of a series of normal and tumor cell lines, and we obtained the levels of NTP and dNTP in some common tumor cell lines in vitro. Our results indicate there are some differences associated with their biological properties between normal and tumor cell lines and between different tumor cell lines. To demonstrate the validation of the present HPLC method, specificity, linearity, recovery, precision, and detection limit of the standards were evaluated.

2. Experimental

2.1. Chemicals and reagents

All standards (ADP, 5′-CTPNa_2, 5′-GTPNa_2, UTPNa, ATPNa_2, dTTPNa_4, dCTPNa_2, dGTPNa_2, dATPNa_2) were purchased from Sigma (St. Louis, MO, USA). Tetrabutylammonium hydroxide was obtained from Sigma. KH_2PO_4 (analytical grade) was from Beijing Chemical Reagent Company. Methanol (MeOH, chromatographic grade) was from Nankai University. TCA was purchased from Beijing Nanshangle HuaGongChang. Sodium hydroxide, potassium carbonate and hydrochloric acid were obtained from Tianjin Chemical Reagent Factory, Beijing Liulidian HuaGongChang and Beijing Chemical Factory, respectively. All other chemicals were of analytical grade. Ultrapure water was obtained from a Milli-Q UF-Plus apparatus (Millipore).

2.2. Instrument and chromatographic conditions

The chromatographic system consisted of a Waters in-line degasser, a Waters-600 controller connected to a Waters-600 pump and a Waters 486 tunable absorbance detector and a Waters Millennium[32] workstation (Version 3.05) chromatography manager. Chromatographic separations were performed using a Waters Symmetry C_{18} 3.5 μm (150×4.6 mm) column, equipped with a NovaPak C_{18} Sentry guard column (Waters).The HPLC columns were kept at 27 °C in a column oven. The mobile phase was delivered at a flow-rate of 1.0 ml/min during the analysis using the following stepwise gradient elution program: A–B (60:40) at 0 min→(40:60) at 30 min→(40:60) at 60 min. Buffer A contained 10 m*M* tetrabutylammonium hydroxide, 10 m*M* KH_2PO_4 and 0.25% MeOH, and was adjusted to pH 6.9 with 1 *M* HCl and a pHS-2 detector (Shanghai). Buffer B consisted of 5.6 m*M* tetrabutylammonium hydroxide, 50 m*M* KH_2PO_4 and 30% MeOH, and was neutralized to pH 7.0 with 1 *M* NaOH and the pHS-2 detector. Solution A was at high risk of microbial contamination and was therefore passed through a 0.45-μm filter. Both solutions were prepared regularly and stored in the dark at 4 °C prior to use. Both solvents were degassed. The injection volume was 50 μl. Detection was UV absorption at 254 nm. The Millennium[32] workstation (version 3.05) chromatography manager was used to pilot the HPLC instrument and to process the data (area integration, calculation and plotting of chromatograms) throughout the method validation and sample analysis.

2.3. Cell culture

2.3.1. Cell culture condition

RPMI 1640 media and fetal calf serum (FCS) were purchased from Gibco BRL. Trypsin was obtained from Gibco BRL. The 16 normal and tumor cell lines were kept in our laboratory. The cell lines maintained in RPMI 1640 medium with 2 g/l $NaHCO_3$ supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml) at 37 °C in a humidified air atmosphere containing 5% CO_2, were used for in vitro experiments.

2.3.2. Cell lines

Balb/3T3 mouse fibroblast cell (B3T3), human embryonic lung fibroblast cell (HELF), human acute leukemic cell (HL-60), human myeloid leukemic cell (U937), human leukemic cell (K562), human mouth epidermal carcinoma cell (KB), vincristine-resistant human mouth epidermal carcinoma cell (KBr200, whose IC_{50} towards VCR is about 200-fold higher than KB's evaluated by MTT assay), human breast adenocarcinoma cell (MCF-7), adriamycin-resistant human breast adenocarcinoma cell (MCF/Adr), mouse melanoma cell (B16), mouse melanoma cell (B16-BL6, derived from B16), human salivary adenoid cystic cystocarcinoma cell (A2), human salivary adenoid cystic cystocarcinoma cell (AM), human colorectal adenocarcinoma cell (HCT-8), human transgenic colorectal adenocarcinoma cell (m_1HCT-8, derived from HCT-8 transfected with MEKK1 via lipofectAMINE), human colorectal adenocarcinoma cell transfected with pcDNA3.1 vector by lipofectAMINE (pHCT-8) were used. All of the above cells were cultured in RPMI 1640 medium under a humidified air atmosphere containing 5% CO_2 at 37 °C. The cells were harvested in their exponential growth phase.

2.4. NTP/dNTP extraction

All the extraction steps were performed on ice. Immediately before processing, cells were carefully counted and monitored for viability by the trypan blue exclusion method under the microscope, washed three times with ice-cold phosphate-buffered saline (PBS) and harvested. The cell pellets were deproteinized with the same volume of 6% TCA, vortex-mixed for 20 s, ice bathed for 10 min then vortex-mixed again for 20 s. Acid cell extracts were centrifuged at 13 000 rpm for 10 min at 4 °C. The resulting supernatants were supplemented with an equal volume of bidistilled water, vortex-mixed for 60 s, and either stored at −80 °C or neutralized by the addition of 5 *M* K_2CO_3 just prior to HPLC analysis.

2.5. Calibration standards

Aqueous standards were used for the calibration. The stocking solution of each standard was prepared

by individually dissolving the commercially available pure NTP and dNTP standards with ultrapure water. The concentration for each stocking solution was 2.83 (ADP), 2.28 (CTP), 2.42 (GTP), 2.37 (UTP), 3.66 (ATP), 0.78 (dCTP), 0.74 (dGTP), 0.70 (dTTP), 0.77 (dATP) m*M*. Then 1 ml of each stocking solution was mixed together and double diluted four times sequentially to prepare the working solution (comprising five data points for each standard, respectively).

2.6. Analytical method validation

2.6.1. System suitability

Under the conditions described, the following chromatographic parameters for the separation of NTPs and dNTPs were obtained from a standard mixture containing nine compounds. The values were obtained using the following expressions:

$$k' \text{ (capacity factor)} = \frac{(t_R - t_0)}{t_0}$$

$$R \text{ (resolution)} = \frac{(t_{R2} - t_{R1})}{(W_{1/2,1} + W_{1/2,2})}$$

$$N \text{ (efficiency)} = 5.54 \cdot \left(\frac{t_R}{W_{1/2}}\right)^2$$

$$T \text{ (tailing factor)} = \frac{B_{0.1}}{A_{0.1}}$$

where t_R is the retention time of each standard; t_0 is the retention time of water (unretained compound) which was determined as the time from injection to the first distortion of the baseline; $W_{1/2}$ is the peak width at halfpeak height of each standard; $B_{0.1}$ is the peak width at 10% peak height (to the right with respect to the perpendicular); $A_{0.1}$ is the peak width at 10% peak height (to the left with respect to the perpendicular).

2.6.2. Specificity and peak identification

All the compounds were identified by comparing their retention times with those of pure compounds by cochromatography with single pure NTP or dNTP.

2.6.3. Linearity

The linearity of the method was proven in standards at five known concentrations. Calibration curves were obtained by unweighted least-squares linear regression analysis of the peak area of the respective dNTP and NTP.

2.6.4. Precision

A series of standard solutions were analyzed and used for the determination of the variability associated with the HPLC analysis. The within-day precision of measurements was evaluated by analyses ($n=3$) of the standards at five different concentrations. The day-to-day repeatability was assessed on three separate days. The precision was calculated as the relative standard deviation (RSD).

2.6.5. Limit of detection

The limit of detection was determined as the concentration giving a signal-to-noise ratio (S/N) of 3:1.

2.6.6. Recovery

To assess the accuracy of the method, a selected cell extract pool was spiked with dNTPs (ds) and NTP (Ns) at known concentrations. The ratio of the average measured concentration and its nominal concentration was defined as the recovery.

2.7. Determination of samples

Using this method, the concentrations of ADP, CTP, dCTP, GTP, UTP, dGTP, dTTP, ATP and dATP in 16 tumor and normal cell extracts were analyzed. Quantitation was performed according to the external standard method by plotting the peak area against known concentrations of standards.

3. Results

3.1. System suitability

The separation of the standard solution of nine NTPs and dNTPs is shown in Fig. 1. Under the conditions described, the chromatographic parameters were obtained (data not shown).

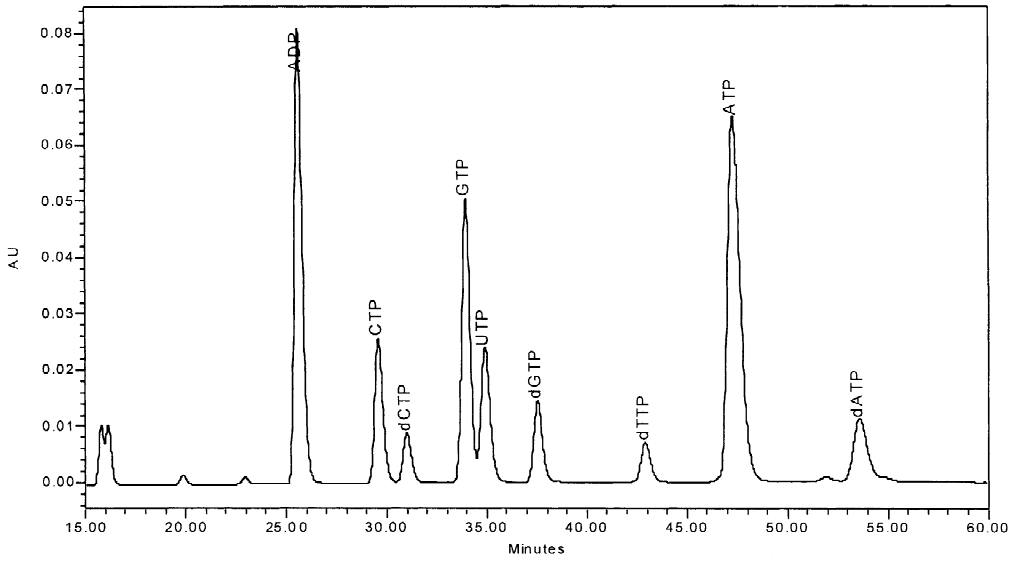

Fig. 1. HPLC separation of a standard mixture of ADP, CTP, dCTP, GTP, UTP, dGTP, dTTP, ATP and dATP (50 μl injection).

3.2. Linearity

The standard curves for ADP, CTP, dCTP, GTP, UTP, dGTP, dTTP, ATP and dATP were satisfactorily described by unweighted least-squares linear regression analysis. Over the range 0.5~20 nmol NTP and 50~4000 pmol dNTP, the regression coefficients (r) of the calibration curves for the nine NTPs and dNTPs were good and always >0.998.

3.3. Precision

The precision (RSD) levels were achieved by analyzing the standard solution at five concentrations. The mean within-day and day-to-day precisions of measurements are 0.9% ($n=5$) and 5.0% ($n=15$), respectively.

3.4. Detection limit

The limit of detection was determined as the amount giving an S/N of 3:1. The detection limits for each compound were 1.39 (ADP), 4.32 (CTP), 15.5 (dCTP), 2.38 (GTP), 4.42 (UTP), 9.45 (dGTP), 14.6 (dTTP), 2.44 (ATP) and 11.8 (dATP) pmol, respectively.

3.5. Recovery

To assess the accuracy of the method, a selected cell extract pool (with known values of NTP and dNTP concentrations) was spiked with NTPs (Ns) and dNTPs (ds). The determination of the amount of NTP or dNTP added and their theoretical levels are shown in Table 1.

Table 1
Recovery of NTPs and dNTPs in neutralized HCT-8 cell extract pools[a]

Component	ADP	CTP	GTP	UTP	ATP	dCTP	dGTP	dTTP	dATP
Theoretical amount (mM)[b]	0.0591	0.0188	0.0260	0.0432	0.1016	0.0130	0.0169	0.0124	0.01313
Experimental amount (mM)[c]	0.0585	0.0227	0.0245	0.0451	0.1040	0.0122	0.0139	0.0123	0.01314
Recovery (%)[d]	98.9	120.5	94.3	104.4	102.3	93.8	82.4	99.1	100.1

[a] Analysis was done in triplicate with neutralized samples.
[b] Theoretical = ds (Ns)·2/5 + HCT-8·3/5.
[c] Experimental = ds (Ns) (20 μl) + HCT-8 (30 μl) (50 μl injection volume).
[d] Recovery = experimental/theoretical × 100%.

3.6. Analysis of samples

Fig. 2 shows the chromatograms of a K562 cell extract either blank, with scale adjusted to the highest peak (ATP) (Fig. 2a) or spiked with a known amount of each dNTP (Fig. 2b). The identity of each NTP peak has also been assessed by coinjecting NTP standard substances (data not shown). The concentration of NTPs and dNTPs in the samples were calculated from the obtained calibration curve parameters. The intracellular concentration of each nucleotide was expressed as nmol/10^8cell (calcu-

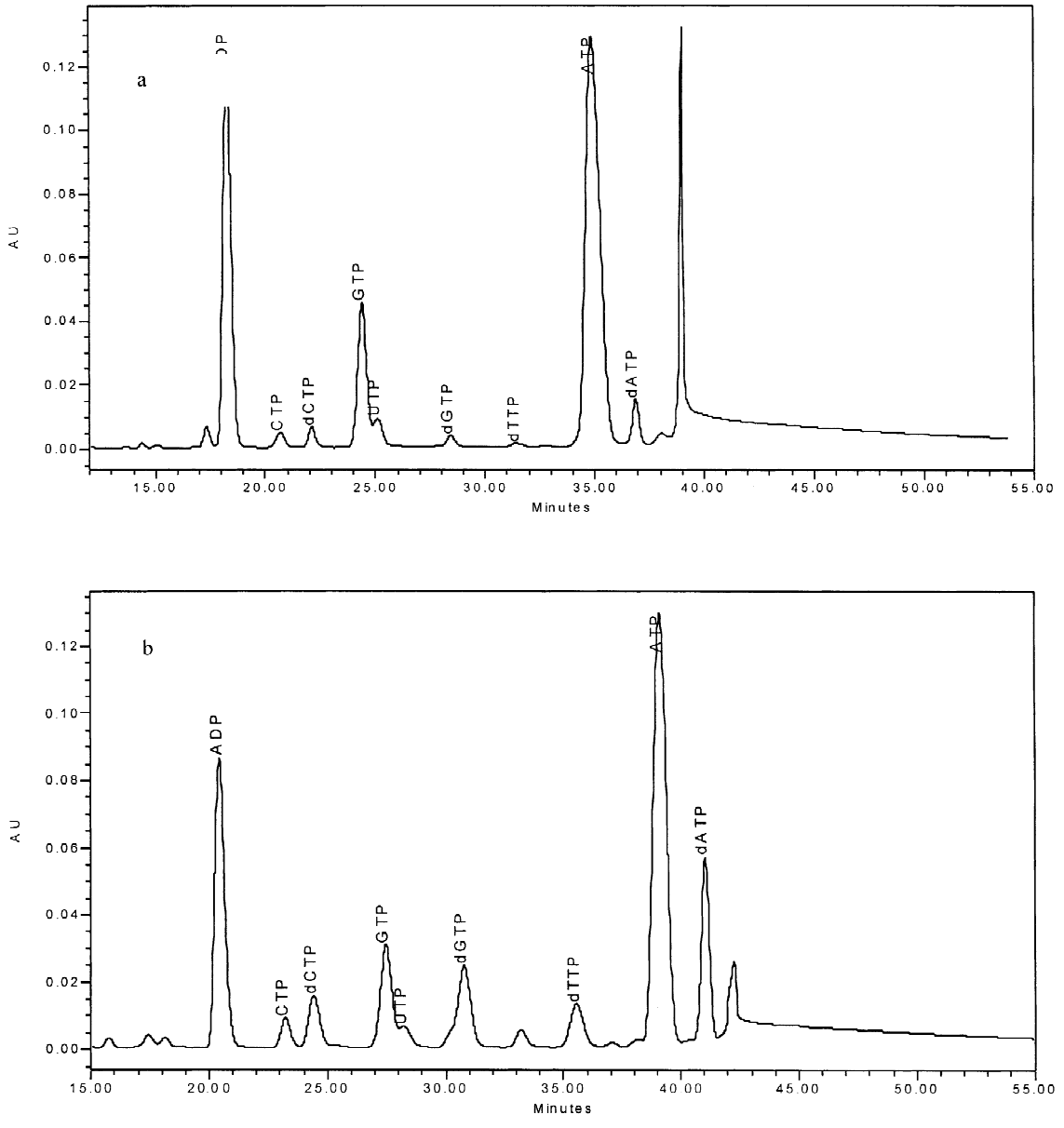

Fig. 2. Chromatograms of NTP/dNTP pools in K562 cell extracts (50 μl injection). (a) Untreated K562 cells with the scale adjusted to the highest peak (ATP). (b) Untreated K562 cells spiked with four dNTPs.

Table 2
Concentrations (SD) of nine nucleotides in cell lines with high metastasis potential and common tumor cell lines

Cell line	Concentration (SD) (nmol/10^8 cell)								
	ADP	CTP	dCTP	GTP	UTP	dGTP	dTTP	ATP	dATP
B16	93.2	34.5	–	33.7	49.7	10.5	8.2	78.5	7.5
	(3.1)	(1.1)		(3.1)	(1.8)	(1.3)	(0.7)	(5.6)	(0.05)
B16BL6	26.3	16.9	–	18.2	19.0	4.1	4.0	39.6	3.9
	(0.1)	(0.03)		(0.7)	(0.1)	(0.4)	(0.03)	(0.3)	(0)
Magnitude[b]	3.5**[a]	2.0**	–	1.9**	2.6**	2.5**	2.0**	2.0**	1.9**
A2	99.8	57.1	8.1	80.0	139.6	10.5	10.4	262.1	8.1
	(1.1)	(0.1)	–	(0.5)	(3.5)	(0.9)	(0.7)	(6.1)	–
AM	76.4	36.1	4.8	51.9	93.2	6.3	5.8	176.6	4.9
	(3.5)	(0.7)	(0.4)	(1.9)	(5.2)	(0.1)	(0.6)	(4.8)	(0.3)
Magnitude[b]	1.3**	1.6**	1.6	1.5**	1.5**	1.7**	1.8**	1.5**	1.5

[a] ** $P<0.01$ compared with common tumor cell lines.
[b] Magnitude = concentration (common tumor cell lines)/concentration (cell lines with high metastasis potential).

lated by dividing the total amount of each nucleotide in the sample by the number of cells and the mean cell volume of the sample) shown in Tables 2–4.

4. Discussion

Ion-pair reagents are widely used for the separation of polar compounds such as certain pharmaceuticals, amino acids, proteins and nucleic acids. Tetrabutylammonium [2,5,7,10] and triethylammonium [11,12] are the cationic ion species commonly used to form ion-pair complexes. Improvements in separations of nucleotides by ion-pair reversed-phase HPLC could be achieved by varying the amount of the organic modifier and the pH and gradient of the eluent within the limitations given by the use of silica-based columns (range of pH stability). Decosterd et al. [5] reported that the unneutralized control cell extracts stored at −80 °C were found to be stable for at least 2 months. The neutralized sample was stable at room temperature for at least 48 h. The choice of extraction procedure for the HPLC of NTPs and dNTPs from cell extracts is important for accurate quantification. Perchloric acid, TCA, trifluoroacetic acid and cold methanol are all the methods of choice [2,3,10]. We adopted 6% TCA as the cell extraction reagent, and the most

Table 3
Concentrations (SD) of nine nucleotides in drug-resistant tumor cell lines and non-drug-resistant tumor cell lines

Cell line	Concentration (SD) (nmol/10^8 cell)								
	ADP	CTP	dCTP	GTP	UTP	dGTP	dTTP	ATP	dATP
KB	127.8	37.3	3.9	41.4	63.8	4.3	8.4	150.5	8.0
	(0.04)	(0.02)	–	(0.05)	(0.08)	(0.01)	(0.03)	(0.2)	(0.05)
KBr200	152.7	52.9	5.0	75.5	172.6	7.3	12.1	262.7	7.7
	(11.4)	(4.2)	(0.3)	(10.5)	(6.0)	(2.2)	(0.8)	(21.6)	(0.7)
Magnitude[c]	1.2	1.4*[a]	1.2	1.9*	2.7**[b]	1.4	1.5**	1.8**	1.0
MCF-7	61.6	18.4	–	31.6	36.5	3.4	3.8	92.6	3.3
	(0.6)	(0.2)		(0.5)	(1.6)	(0.4)	(0.05)	(1.3)	(0.02)
MCF/Adr	138.1	52.2	–	60.9	97.8	9.5	10.5	183.0	8.1
	(1.1)	(0.3)		(0.8)	(1.2)	(0.3)	(0.5)	(3.5)	(0)
Magnitude[c]	2.2**	2.8**	–	1.9**	2.7**	2.8**	2.8**	2.0**	2.5**

[a] * $P<0.05$ with respect to the corresponding value of non-drug-resistant tumor cell lines.
[b] ** $P<0.01$ compared with non-drug-resistant tumor cell lines.
[c] Magnitude = concentration (drug-resistant tumor cell lines)/concentration (non-drug-resistant tumor cell lines).

Table 4
Concentrations (SD) of nine nucleotides in other tumor and normal cell lines[a]

Cell line	Concentration (SD) (nmol/10^8 cell)								
	ADP	CTP	dCTP	GTP	UTP	dGTP	dTTP	ATP	dATP
HCT-8	123.7	44.3	–	60.2	96.6	14.0	6.3	208.3	5.6
	(0.9)	(0.4)		(3.0)	(3.2)	(0.4)	(0.2)	(5.1)	(0.03)
m_1HCT-8	44.0	23.0	–	32.2	70.2	6.5	3.5	104.8	2.8
	(0.3)	(0.1)		(0.5)	(1.0)	(0.2)	(0.04)	(0.5)	(0.04)
Magnitude[c]	2.8**[b]	1.9**	–	1.9**	1.4**	2.2**	1.8**	2.0**	1.9**
pHCT-8	107.7	46.7	–	75.5	139.8	15.8	9.7	237.2	7.2
	(0.6)	(0.4)		(0.3)	(2.4)	(0.3)	(0.1)	(3.3)	(0.04)
Magnitude[c]	1.1**	1.0**	–	0.8**	0.7**	0.9**	0.7**	0.9**	0.8**
U937	84.4	44.5	6.1	62.8	113.8	12.6	8.8	209.1	7.6
	(4.3)	(2.2)	(0.05)	(2.4)	(9.4)	(3.5)	(0.6)	(12.7)	(0)
K562	128.1	24.9	18.6	73.6	32.1	10.4	8.1	227.3	15.4
	(0.7)	(0.4)	(0.6)	(0.8)	(0.7)	(1.8)	(1.1)	(3.9)	(4.8)
HL-60	111.7	51.4	7.1	69.9	118.1	5.4	8.0	261.3	7.6
	(1.1)	(0.6)	(0.3)	(1.2)	(1.3)	(0.3)	(0.1)	(5.1)	(1.0)
B3T3	49.4	26.4	–	33.5	44.6	8.0	6.1	94.8	5.4
	(0.2)	(0.5)		(0.1)	(0.7)	(2.0)	(0.1)	(4.1)	(0.1)
HELF	62.3	29.6	5.7	34.1	43.5	4.5	5.5	109.3	5.7
	(2.9)	(0.3)	(0.1)	(1.9)	(2.6)	(0.4)	(0.3)	(3.3)	(0.5)

[a] Preparation of cell extracts and chromatographic conditions are fully described under Experimental. Values are expressed as nmol/10^8 cells.

[b] ** $P<0.01$ compared with HCT-8.

[c] Magnitude = concentration (HCT-8)/concentration (m_1HCT-8 or pHCT-8).

important thing is to destroy the cells or organelles first and block the metabolism of nucleotides immediately by inactivation of the enzymes. We can evaluate the recovery of the extraction procedure by radio labeling of the nucleotide pool if necessary. Aqueous standards used for the calibration were reported by Schleyer et al. [13].

Our approach provides a simple procedure to simultaneously measure the nine NTPs and dNTPs in cell extracts in a single run. Based on the present experiment, we obtained the following results: the levels of NTP and dNTP in the tumor cell line with high metastasis potential were 1.5- to 3.5-fold lower than those in its corresponding common tumor cell line ($P<0.01$, see Table 2), such as B16-BL6 and B16, AM and A2. This may be associated with the cell differentiation state and malignant behavior. The amount of NTP and dNTP present in the drug-resistant tumor cell line was 1.4- to 2.8-fold higher than those in the corresponding non-drug-resistant tumor cell line ($P<0.05$ or $P<0.01$, see Table 3), such as KBr200 and KB, MCF/Adr and MCF-7. The highly active metabolic state of drug-resistant tumor cells may be consistent with these results. The concentrations of NTP and dNTP in m_1HCT-8 were 1.4- to 2.8-fold lower than those in HCT-8 and pHCT-8. Increased cell number and delayed peak time when the cells went into plateau phase was seen in the growth curve of m_1HCT-8 compared with HCT-8. A period of time delay, while an almost similar cell number in the plateau phase, was shown in the growth curve of pHCT-8 compared to HCT-8. The colony formation potency of m_1HCT-8 was significantly higher than that of HCT-8 ($P<0.01$) (data not shown). Based on these results, MEKK1 might play an important role in cell growth and proliferation. The level of NTP present in normal cell lines was lower than that in most tumor cell lines except for B16, B16-BL6 and MCF-7. B16-BL6 had lower amount of NTP than any other tumor or normal cell lines. The ATP/ADP ratio in most cell lines was >1, while it was <1 in the B16 cell line.

Not all of the nine compounds can be found in each cell type, e.g., Werner [2] reported that dGTP and dATP are not detectable in erythrocytes. The profile of purine nucleotides, nucleosides and bases

also shows large concentration changes during the maturation of red blood cells [14]. Moreover, modified nucleosides as biochemical markers in cancer detection was reported by Nakano et al. [15], for example, uracil was significantly elevated in the neoplastic colorectal mucosa of eight patients with colorectal cancer ($P<0.01$). The serum level of pseudouridine, primarily a degradation product of tRNA was investigated as a biochemical marker in small cell lung cancer. Serum pseudouridine levels changed considerably parallel with the changes in the clinical response [16]. Hesse et al. [17] reported quantitative determination of 2,8-dihydroxyadeninuria and xanthine for diagnostic and therapy-control of inborn metabolic disorders. Therefore, investigation of some modified nucleosides is also of great importance in the diagnosis or treatment of cancer.

In conclusion, this HPLC method is of great advantage in studies of the substances critical for evaluating physiological, pharmacological, or pathological phenomena affecting cell energy metabolism and nucleic acid biosynthesis.

Acknowledgements

This work was financially supported by the National Natural Science Foundation (China, 39870861). We thank the cooperation of central laboratory at Beijing Neurosurgical Institute and Mr. Liang Li for his concern and help, and Miss Shurong Zheng for her great support and help.

References

[1] T.D. Batiuk, C. Schnizlein-Bick, Z. Plotkin, P.C. Dagher, Am. J. Physiol. Cell. Physiol. 281 (2001) C1776.
[2] A. Werner, J. Chromatogr. 618 (1993) 3.
[3] S. Palmer, S. Cox, J. Chromatogr. A 667 (1994) 316.
[4] M.L. Mole, D.L. Hunter, P. Gao, C. Lau, Anal. Biochem. 259 (1998) 245.
[5] L.A. Decosterd, E. Cottin, X. Chen, F. Lejeune, R.O. Mirimanoff, J. Biollaz, P.A. Coucke, Anal. Biochem. 270 (1999) 59.
[6] D.S. Shewach, Anal. Biochem. 206 (1992) 178.
[7] D. Di Pierro, B. Tavazzi, C.F. Perno, M. Bartolini, E. Balestra, R. Calio, B. Giardina, G. Lazzarino, Anal. Biochem. 231 (1995) 407.
[8] D.R. Cross, B.J. Miller, S.J. James, Cell Prolif. 26 (1993) 327.
[9] F. Arezzo, Anal. Biochem. 160 (1987) 57.
[10] R.L. Claire III, Rapid Commun. Mass Spectrom. 14 (2000) 1625.
[11] T. Uesugi, K. Sano, Y. Uesawa, Y. Ikegami, K. Mohri, J. Chromatogr. B 703 (1997) 63.
[12] V. Reichelova, F. Albertioni, J. Liliemark, J. Chromatogr. B 682 (1996) 115.
[13] E. Schleyer, G. Ehninger, M. Zuhlsdorf, B. Proksch, W. Hiddemann, J. Chromatogr. 497 (1989) 109.
[14] A. Werner, W. Siems, G. Gerber, H. Schmidt, S. Gruner, H. Becker, Chromatographia 25 (1988) 237.
[15] K. Nakano, K. Shindo, T. Yasaka, H. Yamamoto, J. Chromatogr. 332 (1985) 127.
[16] S. Tamura, H. Fujioka, T. Nakano, T. Hada, K. Higashino, Cancer Res. 47 (1987) 6138.
[17] A. Hesse, A. Thon, A. Classen, H. Birwe, Chromatographia 25 (1988) 205.