Journal of Chromatography, 593 (1992) 157–163
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2426

Determination of inositol polyphosphates from human T-lymphocyte cell lines by anion-exchange high-performance liquid chromatography and post-column derivatization

Andreas H. Guse* and Frank Emmrich

Max-Planck-Society, Clinical Research Unit for Rheumatology/Immunology at the Institute for Clinical Immunology of the University, Schwabachanlage 10, D-8520 Erlangen (Germany)

ABSTRACT

The intracellular amounts of several inositol tris-, tetrakis- and pentakisphosphates and inositol hexakisphosphate were determined in resting and stimulated cells from human T-lymphocyte lines. The inositol polyphosphates were separated by anion-exchange high-performance liquid chromatography and were detected on-line by a recently developed post-column dye system. In the human T-lymphocyte cell line Jurkat, basal intracellular concentrations ranged between 25 ± 10 pmol per 10^9 cells for inositol 1,4,5-trisphosphate to 6380 ± 355 pmol per 10^9 cells for inositol hexakisphosphate. Similar basal concentrations were observed in the human T-lymphocyte cell line HPB.ALL, with the exception that inositol hexakisphosphate was approximately 665 ± 10 pmol per 10^9 cells. Stimulation of the human T-lymphocyte cell line Jurkat via the T-cell receptor by a monoclonal antibody directed against the T-cell receptor–CD3 complex induced time-dependent changes in the intracellular concentrations of multiple inositol polyphosphate isomers, including inositol 1,3,4-trisphosphate, inositol 1,3,4,5-tetrakisphosphate, inositol 1,3,4,6-tetrakisphosphate, an as yet unidentified inositol tetrakisphosphate isomer, inositol 1,3,4,5,6-pentakisphosphate, inositol 1,2,3,4,6-pentakisphosphate and DL-inositol 1,2,4,5,6-pentakisphosphate. Inositol 1,4,5-trisphosphate increased only transiently after 5 min, whereas DL-inositol 1,4,5,6-tetrakisphosphate (determined as the enantiomeric mixture) increased after 20 min.

INTRODUCTION

It is now widely accepted that inositol polyphosphates play an important role in the process of transmembrane signalling [1,2]. In particular, inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] causes the release of Ca^{2+} from intracellular stores and the increased cytosolic Ca^{2+} concentration initiates a number of cell-type specific physiological responses. In this paper, $InsP_2$, $InsP_3$, $InsP_4$, $InsP_5$, $InsP_6$ represent *myo*-inositol bis-, tris-, tetrakis-, pentakis- and hexakisphosphate with isomeric positioning of phosphate groups as indicated and assumed to be D-isomers.

Ins(1,4,5)P_3 has been described as playing a key part in the activation of T-lymphocytes [3]. These cells act as effector cells and are an important regulatory element directing the function of the immune system.

To measure changes in Ins(1,4,5)P_3 and other inositol polyphosphate, most workers have used conventional anion-exchange separation and radioactive detection in samples from cells previously labelled with *myo*-[^{3}H]inositol [4–6]. The disadvantages of these systems are: (i) the inability to measure real intracellular concentrations as labelling to isotopic equilibrium is not achieved in most systems; (ii) the discontinuous detection system (collecting of fractions, liquid scintillation counting) limits the separation efficiency; (iii) the lack of sensitivity for the on-line detection of *myo*-[^{3}H]inositol phosphates by radioactivity detectors.

Two post-column systems for the detection of non-radioactively labelled phosphorylated com-

0021-9673/92/$05.00

pounds have been published [7–10]. Although the first system was not sensitive enough for the determination of trace amounts of inositol phosphates (detection limit approximately 800 pmol $InsP_3$ per sample) [7,8], the second, the so-called "metal–dye detection" system [9,10] has already proved to be useful for the determination of the intracellular concentrations of inositol phosphates in HL-60 cells [11]. This post-column dye system, first described by Mayr [9], is based on the competition between anionic compounds eluting from the column and the dye 2-(4-pyridylazo)resorcinol for complex formation with Y^{3+}. Although the dye–Y^{3+} complex absorbs strongly at 520 nm, there is diminished absorption when the eluting anionic compounds compete for Y^{3+} binding. Inverting the analogue signal from the detector then leads to chromatogram with "positive" peaks.

This paper reports the determination of basal intracellular concentrations of inositol polyphosphates in the human T-cell lines Jurkat and HPB.ALL using the metal–dye detection system. In response to stimulation of the T-cell receptor–CD3 complex, increases were observed in the intracellular concentrations of Ins(1,4,5)P_3, Ins (1,3,4)P_3, Ins(1,3,4,5)P_4, Ins(1,3,4,6)P_4, Ins(1,4,5,6)P_4 and/or Ins(3,4,5,6)P_4 (determined as the enantiomeric mixture), an additional undefined $InsP_4$ isomer, Ins(1,2,3,4,6)P_5 and Ins(1,3,4,5,6)P_5. In contrast, the intracellular concentration of Ins(1,2,4,5,6)P_5 decreased with stimulation.

EXPERIMENTAL

Materials

Ins(1,3,4)P_3 was purchased from Calbiochem (Frankfurt, Germany). Ins(1)P_1, Ins(1,4)P_2, Ins(1,4,5)P_3, Ins(1,3,4,5)P_4, Ins(1,2,5,6)P_4, Ins(1,4,5,6)P_4 and Ins(1,3,4,5,6)P_5 were from Boehringer Mannheim (Mannheim, Germany). $InsP_6$ and alkaline phosphatase were from Sigma (Taufkirchen, Germany) $YCl_3 \cdot 6H_2O$ was purchased from Janssen (Nettetal, Germany). Hydrochloric acid and triethanolamine, both of analytical-reagent grade, were from Merck (Darmstadt, Germany). 2-(4-Pyridylazo)resorcinol was from Serva (Heidelberg, Germany). Doubly distilled water or Milli-Q water (Millipore-Waters, Eschborn, Germany) was used throughout the experiments. The monoclonal antibody to CD3, OKT3, was purified from hybridoma supernatant on protein A–Sepharose. Ins(1,3,4,6)P_4 was obtained by phosphorylation of Ins(1,3,4)P_3 in a rat liver homogenate [12].

Culture of the human T-lymphocyte cell lines Jurkat and HPB.ALL

The cells were cultured in RPMI-1640 medium (Gibco-BRL, Eggenstein, Germany) supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (50 μg/ml), L-glutamine (300 mg/ml) and 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) (20 m*M*) at 37°C with 5% carbon dioxide in air. The cell density in flasks containing 200 ml of medium was between $1.5 \cdot 10^5$ cells per ml of fresh culture and $1.5 \cdot 10^6$ cells per ml of culture ready to be harvested.

Stimulation protocol and extraction of inositol phosphates

The cells (approximately $2 \cdot 10^8$–$4 \cdot 10^8$ cells per 200 ml of medium) were harvested by centrifugation (6 min, 18°C, 450 *g*) and resuspended in 10 ml of fresh RPMI-1640 medium (as above) and were kept at 37°C for 10 min. Buffer (for control values) or OKT3 (final concentration 10 μg/ml) was then added for different periods of time. Two minutes before the end of the incubation period, the cells were pelleted (2 min, 450 *g*). The supernatant was decanted and the cells were lysed by the addition of 1 ml of ice-cold perchloric acid (10%, v/v). The perchloric acid extract was immediately vortex-mixed and freeze–thawed twice in liquid nitrogen. The perchloric acid extracts were left on ice for 30 min to extract the soluble inositol phosphates. Then the precipitated protein was removed by centrifugation (10 min, 8800 *g*) and the supernatant was titrated to pH 4–5 by the addition of potassium hydroxide solution (small volumes of the following concentrations were added successively: 7, 3.5, 1.75 and 0.875 *M*). The samples were left on ice for 30 min and then centrifuged to remove the potassium perchlorate precipitate (10 min, 8800 *g*).

Sample preparation for determination by high-performance liquid chromatography (HPLC)

Nucleotides were removed from the samples by extraction with charcoal as described previously [11]. Directly before injection into the HPLC sys-

tem, the samples were filtered through disposible filters (0.45 μm; Schleicher and Schüll, Dassel, Germany). The solid-phase extraction method that was originally introduced with the metal–dye detection system [9] was not used for two reasons: (i) the inositol phosphates were extracted from the cell pellet so that no interference from salts or buffers from the medium was expected; (ii) to avoid the acid-catalysed migration of phosphate groups on the inositol ring [11].

HPLC separation and determination by the metal–dye detection system

The determination of the inositol phosphates was carried out with a Kontron Instruments HPLC system consisting of two pumps (Model 420), two micro-mixing chambers (Model 494) and a UV–visible detector (Model 430) equipped with two MonoQ columns (5 × 0.5 cm; Pharmacia, Freiburg, Germany) in line. System control and data acquisition were performed with the computerized MT1-system/D450-software from Kontron Instruments (Munich, Germany).

The separation of inositol phosphates was achieved by an upward concave gradient as described previously [9]. The composition of the eluents was: A, 0.02 m*M* HCl–13.5 μ*M* YCl_3; B, 0.4 *M* HCl–21 μ*M* YCl_3. The dye solution, C [350 μ*M* 2-(4-pyridylazo)resorcinol, 1.6 *M* triethanolamine, pH 9.1], was pumped by a third HPLC pump (Beckman 110B, Munich, Germany) and mixed with the column eluate in a ratio of 1:2 by a micro-mixing chamber with a dead volume of 800 μl (Model 494, Kontron Instruments). This modification was introduced to reduce the baseline noise and resulted in a better sensitivity than with a T-junction and a knitted coil. The detector was auto-zeroed at the start of each chromatogram. The absorbance was measured at 520 nm and the analogue signal inverted, baseline-substracted and integrated by the MT1/D450-system.

Semi-preparative isolation of $InsP_5$ isomers

Phytic acid (0.2 g; Fluka, Buchs, Switzerland) was hydrolysed in 1.5 ml of sodium acetate solution (0.2 *M*, pH 4.0 with acetic acid) at 100°C for 6 h. Then a 50-μl portion was diluted with 1 ml of doubly distilled water and separated by anion-exchange HPLC as described above. Peak 7 (Fig. 1A) was collected, lyophilized and dissolved in 4.5 ml of doubly distilled water and a 100-μl aliquot was rechromatographed (Fig. 1B). A 1.5-ml portion was then separated as above. Peaks 4–8 were collected, lyophilized and dissolved in 300 μl of doubly distilled water. Aliquots (30 μl diluted with 1 ml of doubly distilled water) were rechromatographed (Fig. 1C–G). The yields of purified $InsP_5$ isomers were 1.2–2.4 μmol.

RESULTS

Evaluation of the analytical system

When carrying out the sample preparation procedure with a standard mixture of inositol pentakisphosphates and $InsP_6$, the recovery of all isomers was greater than 88% [mean 94 ± 5.2% (n = 8)]. These experiments also confirmed published data [11] that there is no acid-catalysed migration of phosphate groups under such conditions. In addition, some samples from cells were spiked with DL-Ins(1,2,3,4,5)P_5 as an internal standard. Recoveries of about 96% indicate no loss due to catabolism of the sample, *e.g.* by acid-stable phosphatase activities.

By monitoring the column eluate at 260 nm without using the post-column dye system, three charcoal extractions were necessary to remove the nucleotides from cell samples. To rule out that any of the peaks were due to elution of nucleotides or other polyanionic compounds (*e.g.* polysulphated substances), control samples were treated as follows. First, HPLC separation with a wavelength setting of 260 nm without using the post-column dye system was used to detect the remaining nucleotides. It was found that the nucleotides were eluted before Ins(1,3,4)P_3 and did not interfere with the detection of later eluting compounds. Secondly, control samples were treated with alkaline phosphatase. After adjusting the pH to 8 with triethanolamine, the samples were reacted with 12 U of alkaline phosphatase at 37°C for 24 h. Alkaline phosphatase treatment led to the disappearence of all peaks designated as inositol polyphosphates.

The linear regression analysis of the calibration graphs for several inositol polyphosphate isomers revealed r values > 0.99 in the range of 50 pmol to 1.5 nmol (Table I). When relating the slope of the calibration graphs to the number of phosphate

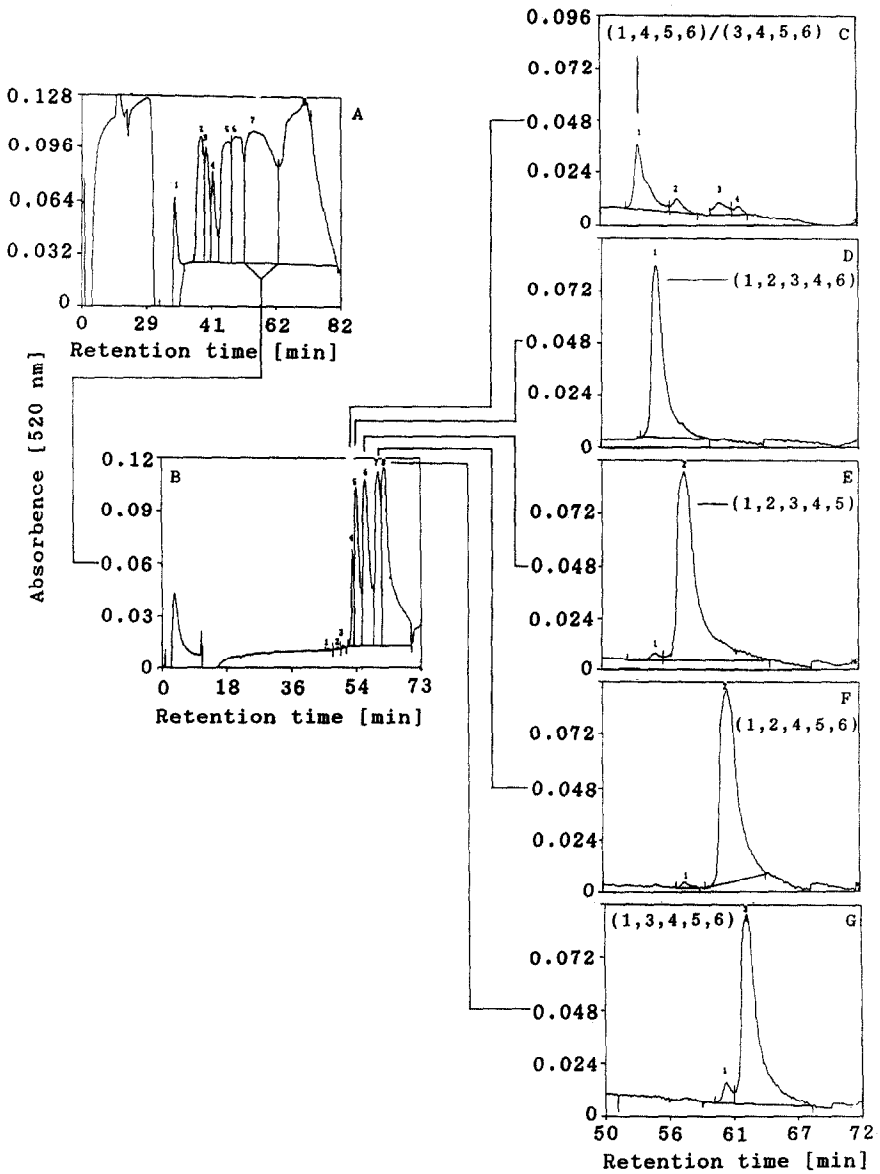

Fig. 1. Semi-preparative isolation of $InsP_5$ isomers. Samples were separated by anion-exchange HPLC on MonoQ columns (50 × 5 mm, two columns in-line; Pharmacia) by an upward concave gradient from 0.02 m*M* HCl–13.5 μM YCl_3 to 0.4 *M* HCl–21 μM YCl_3. The flow-rate through the columns was 1.2 ml/min whereas the dye solution [350 μM 2-(4-pyridylazo)resorcinol, 1.6 *M* triethanolamine, pH 9.1] was pumped at 0.6 ml/min. The absorbance of the mixture was measured on-line at 520 nm. A 50-μl aliquot of an $InsP_6$ hydrolysate containing $InsP_1$ to $InsP_6$ was separated (A), peak 7 was collected (A), lyophilized and dissolved in 4.5 ml of doubly distilled water and a 100-μl aliquot was rechromatographed (B), resulting in the resolution of DL-Ins(1,4,5,6)P_4 (B, peak 4), Ins-(1,2,3,4,6)P_5 (B, peak 5), DL-Ins(1,2,3,4,5)P_5 (B, peak 6), DL-Ins(1,2,4,5,6)P_5 (B, peak 7) and Ins(1,3,4,5,6)P_5 (B, peak 8). A 1.5-ml portion was then separated as above. Peaks 4–8 were collected, lyophilized and dissolved in 300-μl of doubly distilled water. Aliquots of 30 μl diluted with 1 ml of doubly distilled water were then rechromatographed (C–G).

TABLE I

CALIBRATION GRAPHS FOR INOSITOL POLYPHOSPHATES DETERMINED BY THE METAL–DYE DETECTION SYSTEM

Mixtures of standard Ins(1,4)P_2, Ins(1,4,5)P_3, Ins(1,3,4,5)P_4, Ins(1,4,5,6)P_4, Ins(1,3,4,5,6)P_5 and $InsP_6$ in the range 50–1500 pmol were determined on a MonoQ column (100 × 5 mm, Pharmacia) as described under Experimental. Linear regression analysis of the signal (mV min) as a function of the amount (pmol) gave the values in this table.

Inositol polyphosphate	Slope	Intercept	r
Ins(1,4)P_2	0.0319	0.1321	0.9995
Ins(1,4,5)P_3	0.0549	0.0893	0.9995
Ins(1,3,4,5)P_4	0.0670	−0.5710	0.9998
Ins(1,4,5,6)P_4	0.0687	−0.1460	0.9990
Ins(1,3,4,5,6)P_5	0.0819	−0.6000	0.9929
$InsP_6$	0.0943	−0.0850	0.9999

groups on the inositol ring, a linear correlation was found ($s_c = 0.01518n_P + 0.00573$, $r = 0.9909$, where s_c is the slope of the calibration graphs and n_P is the number of phosphate groups).

Determination of multiple inositol polyphosphates in human T-cell lines

In the human T-cell line Jurkat at least eleven inositol polyphosphates could be separated by HPLC and on-line metal–dye detection (Fig. 2). The isomeric identity of several compounds was determined by (i) comparing the retention times with commercially available standards and (ii) by the addition of the commercially available compounds to cell extracts before analysis. Ins(1,4)P_2, Ins(1,3,4)P_3, Ins(1,4,5)P_3, Ins(1,3,4,5)P_4, the enantiomeric pair Ins(1,4,5,6)P_4–Ins(3,4,5,6)P_4, Ins(1,3,4,5,6)P_5 and $InsP_6$ were clearly identified by this method. In addition, two further $InsP_4$ isomers and two $InsP_5$ isomers were found (Fig. 2, peaks 8, 10, 12, 13). One of the $InsP_4$ isomers (Fig. 2, peak 8) was identified as Ins(1,3,4,6)P_4 as it had an identical retention time to the standard compound that was prepared by phosphorylation of Ins(1,3,4)P_3 in rat liver extracts [12]. The second peak (Fig. 2, peak 10) eluted between Ins(1,3,4,5)P_4 and Ins(1,4,5,6)P_4–Ins(3,4,5,6)P_4. As no standard substance with the same retention time was available, the isomeric identity could not be determined.

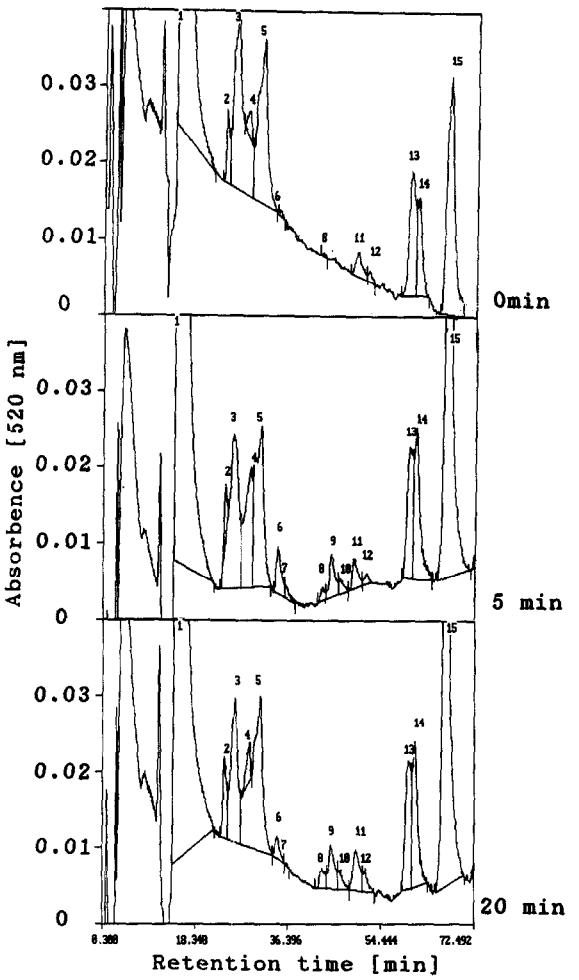

Fig. 2. HPLC determination of inositol polyphosphates from Jurkat T-lymphocytes. Samples from resting and stimulated cells (stimulation time is given on the right-hand side) were processed and analysed using the same conditions as in Fig. 1. Mixing of the column eluate and the dye solution was carried out in a micro-mixing chamber. The absorbance was measured at 520 nm and the inverted analogue signal was transferred to the computerized data system D450/MT1. Peaks were identified by co-chromatography with commercially available standards. Peaks: 1 = $InsP_1$ + P_i; 2 = Ins(1,4)P_2; 3–5 = ?; 6 = Ins(1,3,4)P_3; 7 = Ins(1,4,5)P_3; 8 = Ins(1,3,4,6)P_4; 9 = Ins(1,3,4,5)P_4; 10 = $InsP_4$ with unclear isomeric identity; 11 = Ins(1,4,5,6)P_4–Ins(3,4,5,6)P_4; 12 = Ins(1,2,3,4,6)P_5, 13 = Ins(1,2,4,5,6)P_5; 14 = Ins(1,3,4,5,6)P_5 and 15 = $InsP_6$.

Two additional peaks (Fig. 2, peaks 12 and 13) were detected between the standards Ins(1,4,5,6)P_4 and Ins(1,3,4,5,6)P_5. The first of these (Fig. 2, peak 12) eluted 1.4 min behind Ins(1,4,5,6)P_4, whereas the second (Fig. 2, peak 13) eluted 2 min before

$Ins(1,3,4,5,6)P_5$. The two compounds are probably $InsP_5$ isomers for several reasons: (i) in the original system [9,10] $Ins(1,4,5,6)P_4$ was shown to be the slowest eluting $InsP_4$ isomer followed by $Ins(1,2,3,4,6)P_5$; (ii) $InsP_5$ isomers prepared by the acid hydrolysis of phytic acid (see under Experimental) eluted between standard $Ins(1,4,5,6)P_5$ and standard $InsP_6$ and were identified according to their elution positions as $Ins(1,2,3,4,6)P_5$, DL-$Ins(1,2,3,4,5)P_5$, DL-$Ins(1,2,4,5,6)P_5$ and $Ins(1,3,4,5,6)P_5$ [10]; (iii) the first compound from Jurkat samples co-eluted with the $Ins(1,2,3,4,6)P_5$ standard while the second co-eluted with standard DL-$Ins(1,2,4,5,6)P_5$. According to these results the compounds found in Jurkat extracts are probably $Ins(1,2,3,4,6)P_5$ and DL-$Ins(1,2,4,5,6)P_5$.

The basal concentrations of individual inositol polyphosphates from Jurkat T-cells varied over more than 2 orders of magnitude from 25 ± 10 pmol per 10^9 cells ($n = 4$) for $Ins(1,4,5)P_3$ to 6380 ± 355 pmol per 10^9 cells ($n = 4$) for $InsP_6$ (Table I). In unstimulated cells $Ins(1,3,4,5)P_4$ was not detected under the conditions used. $Ins(1,4,5,6)P_4$–$Ins(3,4,5,6)P_4$ was predominant among the $InsP_3$ and $InsP_4$ isomers in resting cells at 525 ± 50 pmol per 10^9 cells ($n = 4$), whereas the concentration of each of the other $InsP_3$ and $InsP_4$ isomers was less than 100 pmol per 10^9 cells. Interestingly, of the $InsP_5$ isomers, the putative DL-$Ins(1,2,4,5,6)P_5$ and $Ins(1,3,4,5,6)P_5$ were approximately 24- and 16-fold higher than the amount of the putative $Ins(1,2,3,4,6)P_5$ isomer.

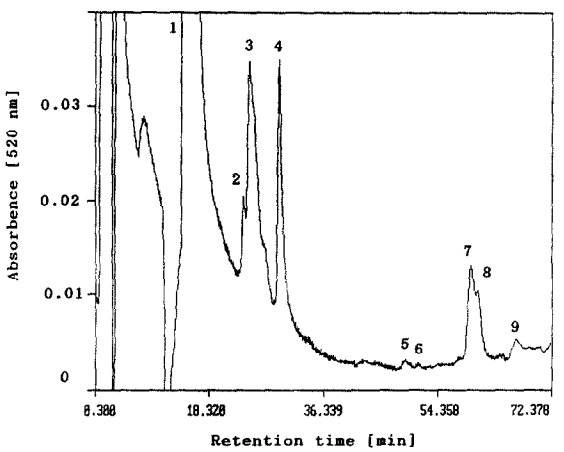

Fig. 3. HPLC determination of inositol polyphosphates from HPB.ALL T-lymphocytes. A sample from unstimulated HPB.ALL T-lymphocytes was analysed using the same conditions as in Fig. 2. Peaks: 1 = $InsP_1 + P_i$; 2 = $Ins(1,4)P_2$, 3,4 = ?; 5 = $Ins(1,4,5,6)P_4$–$Ins(3,4,5,6)P_4$; 6 = $Ins(1,2,3,4,6)P_5$; 7 = $Ins(1,2,4,5,6)P_5$; 8 = $Ins(1,3,4,5,6)P_5$; 9 = $InsP_6$.

TABLE II

TIME COURSE OF INOSITOL POLYPHOSPHATES IN JURKAT T-LYMPHOCYTES STIMULATED BY THE MONOCLONAL ANTIBODY OKT3 VIA THE T-CELL RECEPTOR–CD3 COMPLEX

The analysis of inositol polyphosphates was carried out as described in Fig. 2 and under Experimental.

Inositol polyphosphate	Concentration (mean ± S.D., $n = 4$) (pmol per 10^9 cells)		
	0 min	5 min	20 min
$Ins(1,3,4)P_3$	70 ± 10	460 ± 115[a]	340 ± 30[a]
$Ins(1,4,5)P_3$	25 ± 10	170 ± 15[a]	25 ± 5
$Ins(1,3,4,6)P_4$	35 ± 20	240 ± 30[a]	250 ± 20[a]
$Ins(1,3,4,5)P_4$	–	570 ± 105[a]	850 ± 15[a]
$InsP_4$ (?)	10 ± 5	185 ± 50[a]	190 ± 15[a]
$Ins(1,4,5,6)P_4$–$Ins(3,4,5,6)P_4$	525 ± 50	525 ± 70	850 ± 25[a]
$Ins(1,2,3,4,6)P_5$	115 ± 30	355 ± 30[a]	305 ± 20[a]
$Ins(1,2,4,5,6)P_5$	2740 ± 125	1790 ± 155[a]	1865 ± 85[a]
$Ins(1,3,4,5,6)P_5$	1855 ± 90	2190 ± 165[b]	2090 ± 115[b]
$InsP_6$	6380 ± 355	6860 ± 255	6680 ± 320

[a] $p \leq 0.01$ (Student's t-test).
[b] $p \leq 0.05$ (Student's t-test).

In the human T-lymphocyte cell line HPB.ALL similar basal concentrations of inositol phosphates were seen. The intracellular concentration of $InsP_6$ was about ten-fold lower in this cell line (Fig. 3).

Stimulation of Jurkat T-lymphocytes by the monoclonal antibody to CD3, OKT3 (10 μg/ml), revealed a complex pattern of mass changes in the different inositol polyphosphates (Fig. 2, Table II). The intracellular concentrations of Ins(1,3,4)P_3 and Ins(1,4,5)P_3 were increased transiently (Fig. 2), whereas the concentrations of the $InsP_4$ isomers [Ins(1,3,4,6)P_4, Ins(1,3,4,5)P_4 and the unidentified isomer] remained raised for 20 min. In contrast, Ins(1,4,5,6)P_4 and/or Ins(3,4,5,6)P_4 (identified as the enantiomeric mixture) remained at the control level for 5 min, but increased within 20 min (Fig. 2). Of the $InsP_5$ isomers, an increase in Ins(1,2,3,4,6)P_5 and Ins(1,3,4,5,6)P_5 within 5 min was noticed, which remained raised for 20 min also (Fig. 2). The very high level of $InsP_6$ also increased after stimulation. However, a significant effect could be observed only transiently after 3 min (data not shown). In contrast, Ins(1,2,4,5,6)P_5 did not increase, but decreased continuously for 20 min in response to stimulation by the antibody to CD3.

DISCUSSION

These results clearly show that the anion-exchange HPLC separation and post-column dye system [9,10] are suitable for the determination of intracellular concentrations of inositol polyphosphates in human T-lymphocyte cell lines. It could be shown that T-cell receptor–CD3 stimulation in intact Jurkat T-lymphocytes results in changes of the intracellular concentration not only of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 but of numerous inositol polyphosphates. Whereas the basal concentration or the receptor-mediated formation of some inositol polyphosphates was observed in other cell types [13–16], a novel finding is that an $InsP_5$ isomer, probably Ins(1,2,3,4,6)P_5, is formed in response to stimulation in the human T-cell line Jurkat. Also, the receptor-mediated decrease of Ins(1,2,4,5,6)P_5 in response to stimulation is a novel observation. Ins(1,2,4,5,6)P_5 probably serves as a source for $InsP_6$ as it has been observed that Ins(1,2,4,5,6)P_5 rather than the other $InsP_5$ isomers is phosphorylated to $InsP_6$ by a cytosolic extract from Jurkat T-lymphocytes [17].

A receptor-mediated increase of the intracellular concentration of Ins(1,3,4,5)P_4, Ins(1,3,4,6)P_4, Ins(3,4,5,6)P_4, Ins(1,3,4,5,6)P_5 and $InsP_6$ has been described in the human myeloid cell line HL-60 [11]. However, the time courses of the intracellular concentrations of these individual isomers in HL-60 cells on stimulation with formyl-Met-Leu-Phe were very different from those observed in Jurkat T-cells, indicating different regulatory mechanisms. In particular, in HL-60 cells there was a significantly faster synthesis of Ins(1,3,4,5)P_4, Ins(1,3,4,6)P_4 and Ins(3,4,5,6)P_4, with a peak value at 1–2 min and a rapid decrease to control values within 5 min [11].

ACKNOWLEDGEMENT

The Max-Planck Research Unit for Rheumatology/Immunology is funded by the German Ministry for Research and Technology (BMFT) by grant No. 01 VM 8702.

REFERENCES

1 M. J. Berridge, *Annu. Rev. Biochem.*, 56 (1987) 159.
2 M. J. Berridge and R. F. Irvine, *Nature (London)*, 341 (1989) 197.
3 A. Goldsmith and A. Weiss, *Science*, 240 (1988) 1029.
4 C. Grado and C. E. Ballou, *J. Biol. Chem.*, 236 (1961) 54.
5 M. J. Berridge, R. M. C. Dawson, C. P. Downes, J. P. Heslop and R. F. Irvine, *Biochem. J.*, 212 (1983) 473.
6 R. F. Irvine, A. J. Letcher, D. J. Lander and C. P. Downes, *Biochem. J.*, 223 (1984) 237.
7 J. L. Meek, *Proc. Natl. Acad. Sci., U.S.A.*, 83 (1986) 4162.
8 J. L. Meek and F. Nicoletti, *J. Chromatogr.*, 351 (1986) 303.
9 G. W. Mayr, *Biochem. J.*, 254 (1988) 585.
10 G. W. Mayr, in R. F. Irvine (Editor), *Methods in Inositide Research*, Raven Press, New York, 1990, p. 83.
11 D. Pittet, W. Schlegel, D. P. Lew, A. Monod and G. W. Mayr, *J. Biol. Chem.*, 264 (1989) 18489.
12 S. B. Shears, *J. Biol. Chem.*, 264 (1989) 19879.
13 S. B. Shears, J. B. Parry, E. K. Y. Tang, R. F. Irvine, R. H. Michell and C. J. Kirk, *Biochem. J.*, 246 (1987) 139.
14 C. A. Hansen, S. von Dahl, B. Huddel and J. R. Williamson, *FEBS Lett.*, 236 (1988) 53.
15 T. Balla, L. Hunyadi, A. C. Baukal and K. J. Catt, *J. Biol. Chem.*, 264 (1989) 9386.
16 F. S. Menniti, K. G. Oliver, K. Nogimori, J. F. Obie, S. B. Shears and J. W. Putney, Jr., *J. Biol. Chem.*, 265 (1990) 11167.
17 A. H. Guse and F. Emmrich, unpublished results.