Polarized subcellular distribution of the 1-, 4- and 5-phosphatase activities that metabolize inositol 1,4,5-trisphosphate in intestinal epithelial cells

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In intestinal epithelial cells, $\text{Ins}(1,4,5)\text{P}_3$ is metabolized both by an intracellular 5-phosphatase and by less specific extracellular phosphatases [Rubiera, Velasco, Michell, Lazo & Shears (1988) *Biochem. J.* **255**, 131–137]. A total of 91 % of intracellular $\text{Ins}(1,4,5)\text{P}_3$ 5-phosphatase was particulate, and was preferentially associated with plasma membranes rather than with other subcellular organelles. A soluble $\text{Ins}(1,4,5)\text{P}_3$ 3-kinase activity was also characterized, further supporting the idea that inositol phosphates are important in enterocyte function. We have studied the distribution of $\text{Ins}(1,4,5)\text{P}_3$ phosphatase activities in basolateral and brush-border domains of the plasma membrane. Compared with homogenates, the extracellular phosphatases were 13–17-fold enriched in brush-border membranes, but only 2-fold enriched in basolateral membranes. The 1- and 4-phosphates of $\text{Ins}(1,4,5)\text{P}_3$ were hydrolysed at equal rates by the extracellular phosphatases; these enzymes are proposed to have digestive functions. The intracellular particulate 5-phosphatase was 2-fold enriched in brush-border membranes and 13-fold enriched in basolateral membranes, at the same pole of the cell where $\text{Ins}(1,4,5)\text{P}_3$ is believed to be generated. This is opposite to the polarized distribution of particulate 5-phosphatase in hepatocytes [Shears, Evans, Kirk & Michell (1988) *Biochem. J.* **256**, 363–369]; these differences in subcellular distribution may be important in determining cell-specific metabolism of $\text{Ins}(1,4,5)\text{P}_3$.

INTRODUCTION

$\text{Ins}(1,4,5)\text{P}_3$ is a widespread receptor-induced intracellular signal that mobilizes cellular Ca^{2+} stores (see [1] for a review). $\text{Ins}(1,4,5)\text{P}_3$ is usually metabolized by one of two routes (see [2] for a review); it is dephosphorylated, and thereby deactivated, by a specific 5-phosphatase. Alternatively $\text{Ins}(1,4,5)\text{P}_3$ can be converted to $\text{Ins}(1,3,4,5)\text{P}_4$, which appears to co-operate in the regulation of cellular Ca^{2+} fluxes (see [3] for a review). As it has become possible to probe the spatial organisation of Ca^{2+} fluxes within single cells, it has become clear that these are frequently highly localized in origin. A factor that may contribute to this asymmetry is the intracellular distribution of the $\text{Ins}(1,4,5)\text{P}_3$ -mobilizable Ca^{2+} stores, which in some cases may be close to the site of $\text{Ins}(1,4,5)\text{P}_3$ production [1,3]. Also relevant is the spatial relationship between the sites where $\text{Ins}(1,4,5)\text{P}_3$ is generated and metabolized [2,3]. These sites appear to be polarized in hepatocytes, which have functionally distinct domains of plasma membrane; the $\text{Ins}(1,4,5)\text{P}_3$ 5-phosphatase is largely restricted to the bile canalicular region of the cell, whereas receptor-activated production of inositol phosphates probably occurs at the opposite, sinusoidal, pole of the cell [4]. This asymmetry could generate intracellular concentration gradients of $\text{Ins}(1,4,5)\text{P}_3$, locally high in the region of the $\text{Ins}(1,4,5)\text{P}_3$ -mobilizable Ca^{2+} store [4]. Since the spatial asymmetry of the inositol lipid signalling system may be of functional significance, we have now investigated the subcellular distribution of the $\text{Ins}(1,4,5)\text{P}_3$ 5-phosphatase in a different polarized cell type, the intestinal epithelial cell. These cells are amongst those shown to contain $\text{Ins}(1,4,5)\text{P}_3$ 5-phosphatase activity [5] and a $\text{Ins}(1,4,5)\text{P}_3$ -releasable Ca^{2+} store [6–8].

A complication in these studies is that intestinal epithelial cells

hydrolyse $\text{Ins}(1,4,5)\text{P}_3$ with predominantly extracellular phosphatase(s), as well as with the specific intracellular 5-phosphatase [5]. The function of the extracellular enzyme(s) is unknown, since it would presumably not encounter intracellularly generated $\text{Ins}(1,4,5)\text{P}_3$. We [5] have speculated that such enzyme activities could serve to digest dietary inositol polyphosphates before inositol uptake. An alternative possibility arises from the suggestion that extracellular inositol phosphatase activity may in some cases reflect a relatively non-specific enzyme, the primary function of which is to deactivate those inositol polyphosphates proposed to act at the cell surface [9,10]. It is self-evident that if extracellular $\text{Ins}(1,4,5)\text{P}_3$ hydrolysis was found to predominate at the brush-border region of the cell, this would strongly support the idea that the responsible phosphatase has a digestive function. We have therefore assessed the distribution of the extracellular $\text{Ins}(1,4,5)\text{P}_3$ phosphatase activities in the plasma membrane domains of the enterocyte.

MATERIALS AND METHODS

Enzyme assays

$\text{Ins}(1,4,5)\text{P}_3$ phosphatase was assayed with ^3H - and ^{32}P -labelled substrates at 37 °C in 0.25 ml of the following medium: 120 mM-KCl, 20 mM-Hepes (pH 7.0 with KOH), 10 mM-NaCl, 5 mM-MgSO₄, 1 mM-dithiothreitol, 1 mM-EGTA, 50 μM non-radioactive $\text{Ins}(1,4,5)\text{P}_3$, 0.2 mg of saponin/ml and 0.5 mg of BSA/ml. Where indicated, incubations also contained 10 mM-glucose 6-phosphate (Glc6P) to inhibit extracellular phosphatase activity towards $\text{Ins}(1,4,5)\text{P}_3$ [5]. Incubations were initiated with appropriate amounts of tissue, chosen so that less than 10 % of the $\text{Ins}(1,4,5)\text{P}_3$ was dephosphorylated to InsP . Reactions were

Abbreviation used: Glc6P, glucose 6-phosphate.

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quenched after 10 min with 0.1 ml of 2 M-HClO₄. Acid-quenched samples were neutralized with > 85% recovery [5,11], and chromatographed either on gravity-fed columns [5] or by h.p.l.c. In the latter case, samples were applied to a Whatman Partisil 10 SAX h.p.l.c. column (4.6 mm × 250 mm; Krackeler Scientific Co., Durham, NC, U.S.A.), preceded by an Adsorbosphere 5 SAX guard column (Alltech Associates, Deerfield, IL, U.S.A.). The elution procedure was a slight modification of that described previously [12], using a gradient generated from water and 1.7 M-ammonium formate, pH 3.7, with phosphoric acid (solvent B): 0–5 min, B = 0%; 5–12 min, B increased linearly to 44%; 12–17 min, B = 44%; 17–24 min, B increased linearly to 59%; 24–32 min, B = 59%; 32–44 min, B increased linearly to 100%; 44–50 min, B = 100%. The flow rate was 1 ml/min. The radioactivity in the eluate from the column was measured with a Flo-1 on-line detector (Radiomatic Instruments, Tampa, FL, U.S.A.) using a 1:5 (v/v) mixture of eluate/Monoflow 4 (National Diagnostics, Manville, NJ, U.S.A.). The counting efficiency throughout the gradient was about 20% for ³H and 50% for ³²P; there was no significant crossover between the ³H and ³²P channels of the detector.

Ins(1,3,4,5)P₄ phosphatase was assayed in the medium used to measure Ins(1,4,5)P₃ phosphatase (see above), except that 9 μM-[4,5-³²P]Ins(1,3,4,5)P₄ (1500 d.p.m.) replaced the Ins(1,4,5)P₃. Incubations also contained [³H]InsP₄ (2500 d.p.m.) where indicated, and were initiated by addition of a hepatic 100000 g particulate fraction (0.24 mg of protein/ml), and were quenched after 70 min. Analysis was by gravity-fed columns [5,11].

Ins(1,4,5)P₃ kinase was assayed in the medium used to measure Ins(1,4,5)P₃ phosphatase (see above), with the following modifications: 5 mM-Na₂ATP was added, and the concentration of MgSO₄ was increased to 10 mM. Furthermore, 10 mM-Glc6P and 10 mM-2,3-bisphosphoglycerate were added, and the concentration of [³H]Ins(1,4,5)P₃ was 0.5 nM. In these incubations the dephosphorylation of Ins(1,4,5)P₃ and Ins(1,3,4,5)P₄ was inhibited by > 90% ([5], and results not shown). These conditions were chosen so as to improve our ability to detect Ins(1,4,5)P₃ kinase activity, rather than to determine precisely the activity of this enzyme *in vivo*. Reactions were quenched with HClO₄ and the products were analysed on gravity-fed columns [5,11]. For some experiments the [³H]InsP₄ product was purified by h.p.l.c. (see above) and desalted [4] for further analysis.

Sucrase and ouabain-sensitive K⁺-stimulated ATPase were assayed as described in [13]. Glutamate dehydrogenase, β-galactosidase and arylesterase were assayed as described in [11]. Protein was assayed by the method of Bradford [14], using gamma globulin as a standard.

Preparation of membrane fractions

Enterocytes were isolated from the intestines of two 250–300 g Sprague–Dawley rats [5]. Subcellular fractions were then prepared from enterocytes as follows. The cells were homogenized and the 2000 g 'mitochondrial' fraction was obtained according to Masola & Evered [15]. The supernatant from this spin was then further fractionated by centrifugation for 30 min at 10000 g. The pellet was saved, and the resultant supernatant was again centrifuged at 50000 g for 1 h to obtain the final pellet. Enriched fractions of plasma membranes were isolated from the basolateral and brush-border domains of the enterocyte by the method of Dominguez *et al.* [13], except that two Sprague–Dawley rats (250–300 g) were used for each preparation, and the initial washing buffer also contained 1 mM-dithiothreitol. In some experiments, 100000 g supernatant and particulate fractions were also prepared from homogenized enterocytes as in [5], or from liver as in [11].

Materials

[³H]Ins(1,4,5)P₃ and [4,5-³²P]Ins(1,4,5)P₃ were purchased from DuPont–New England Nuclear. The distribution of the ³²P between the 4- and 5-phosphates of Ins(1,4,5)P₃ (5:95, results not shown) was determined following its dephosphorylation by erythrocyte ghosts [5]. [4,5-³²P]Ins(1,3,4,5)P₄ was synthesized as described in [4]. Non-radioactive Ins(1,4,5)P₃ was purchased from Calbiochem. Other chemicals were as obtained from the sources previously described [5,11].

RESULTS AND DISCUSSION

Subcellular distribution of Ins(1,4,5)P₃ 5-phosphatase activities

Studies of Ins(1,4,5)P₃ metabolism in enterocytes are particularly complex; whilst these cells contain an intracellular Ins(1,4,5)P₃ 5-phosphatase, they also possess a different species of highly active phosphatase that shows less specificity towards Ins(1,4,5)P₃ [5]. The simplest procedure that we have used to distinguish between these two enzyme activities relies on our previous observation [5], which is confirmed in results described below, that 10 mM-Glc6P inhibits the relatively non-specific phosphatases without significantly affecting Ins(1,4,5)P₃ 5-phosphatase. Furthermore, our earlier studies [5] with intact and permeabilized cells demonstrated that the Glc6P-sensitive Ins(1,4,5)P₃ phosphatase was predominantly an extracellular enzyme. In contrast, Glc6P-insensitive Ins(1,4,5)P₃ 5-phosphatase was predominantly intracellular [5]. In the results described below we have obtained more detailed information on the individual Ins(1,4,5)P₃ phosphatase activities by using h.p.l.c. to analyse the separate InsP₂ products, namely Ins(1,4)P₂, Ins(1,5)P₂ and Ins(4,5)P₂. It should also be noted (results not shown) that the rate of Ins(1,4,5)P₃ dephosphorylation by enterocytes was not increased when cells were incubated in an 'extracellular' medium (Krebs–Henseleit buffer plus 1 mM-CaCl₂) instead of our 'intracellular' medium (see the Materials and methods section). Thus the experiments described below do not significantly underestimate the extent of extracellular Ins(1,4,5)P₃ phosphatase activity as a consequence of the incubation conditions that we have chosen to use.

The Ins(1,4,5)P₃ phosphatase activity that is predominantly extracellular, and Glc6P-sensitive, accounted for around 75% of total Ins(1,4,5)P₃ dephosphorylation by homogenates of enterocytes (Table 1), similar to our earlier results [5]. The distribution of the intracellular Glc6P-insensitive Ins(1,4,5)P₃ 5-phosphatase was studied in 100000 g supernatant and particulate fractions. The supernatants contained only 9 ± 1% (n = 4) of total cellular Glc6P-insensitive Ins(1,4,5)P₃ phosphatase activity. These data indicate that Ins(1,4,5)P₃ 5-phosphatase is predominantly a particulate enzyme.

The distribution of Ins(1,4,5)P₃ phosphatase activity between various subcellular fractions was also studied. Considerable amounts of this activity were found in particulate fractions sedimenting at 2000, 10000 and 50000 g (Table 1). However, the bulk of this activity was substantially inhibited by 10 mM-Glc6P (Table 1) and, as discussed above, was therefore predominantly due to extracellular Ins(1,4,5)P₃ phosphatases. The residual Glc6P-insensitive 5-phosphatase, and the K⁺-stimulated ATPase activity (a plasma membrane marker) were both enriched in a 10000g fraction of cell homogenates (Table 1). The 5-phosphatase was not associated with lysosomes or mitochondria, markers for which (β-galactosidase and glutamate dehydrogenase respectively) were concentrated in the 2000 g pellet (Table 1). Both arylesterase (the endoplasmic reticulum marker) and sucrase (the marker for brush-border membranes) were about evenly distributed between all three particulate fractions. Thus

Table 1. Distribution of total Ins(1,4,5) P_3 phosphatase activity, Ins(1,4,5) P_3 5-phosphatase and various subcellular markers in membrane fractions prepared from enterocytes

Particulate fractions sedimenting at 2000, 10000 and 50000 g were prepared as described in the Materials and methods section. Enzyme assays were performed as described in the Materials and methods section, using gravity-fed columns for the Ins(1,4,5) P_3 phosphatase assays, which each contained about 20000 d.p.m. [3H]Ins(1,4,5) P_3 . Total Ins(1,4,5) P_3 phosphatase activity was assayed in the absence of Glc6P. The 5-phosphatase activity was taken to be that insensitive to 10 mM-Glc6P (see text). Data are means $\pm$ S.E.M. from between 4 and 13 determinations.

Enzyme	Activity per fraction (nmol/min per mg of protein)			
	Homogenate	2000 g	10000 g	50000 g
K ⁺ -stimulated ATPase	3.4 $\pm$ 0.25	4.4 $\pm$ 0.5	22.3 $\pm$ 1.6	8.4 $\pm$ 0.5
Sucrase	23.4 $\pm$ 1	19.1 $\pm$ 1	29.1 $\pm$ 2	36.1 $\pm$ 1.7
Glutamate dehydrogenase	133 $\pm$ 25	408 $\pm$ 53	97 $\pm$ 13	27 $\pm$ 8
Arylesterase	1.5 $\pm$ 0.2	2.2 $\pm$ 0.2	3.6 $\pm$ 0.6	3.1 $\pm$ 0.1
β -Galactosidase	1.4 $\pm$ 0.1	4.3 $\pm$ 0.3	1.7 $\pm$ 0.3	0.5 $\pm$ 0.04
Total Ins(1,4,5) P_3 phosphatase	42 $\pm$ 5	125 $\pm$ 11	199 $\pm$ 16	104 $\pm$ 19
Ins(1,4,5) P_3 5-phosphatase	11 $\pm$ 1.5	17 $\pm$ 2	33 $\pm$ 5	17 $\pm$ 1.4

Ins(1,4,5) P_3 5-phosphatase appeared to predominate in plasma membranes, as in several other cell types (see [2]). Although the distribution of extracellular Ins(1,4,5) P_3 phosphatase is not resolved in Table 1, it is by definition associated with plasma membranes.

Identification and subcellular distribution of Ins(1,4,5) P_3 3-kinase

Homogenized enterocytes phosphorylated [3H]Ins(1,4,5) P_3 to an [3H]Ins P_4 in the presence of ATP; in the 10 min incubations described in the Materials and methods section, 31 $\pm$ 2 % of the substrate was phosphorylated and 4 $\pm$ 0.3 % was dephosphorylated (n = 3, 70–140 μ g of protein/ml). The proportion of cellular Ins(1,4,5) P_3 kinase activity that was recovered in 100000 g supernatants was 83 $\pm$ 9 % (n = 3). Thus, as in many other cells (see [2]), Ins(1,4,5) P_3 kinase and phosphatase reside in different regions of the cell. Based upon studies with other tissues, the [3H]Ins P_4 product was expected to be Ins(1,3,4,5) P_4 . In order to check this prediction, the [3H]Ins P_4 was incubated with 9 μ M-[^{32}P]Ins(1,3,4,5) P_4 and a hepatic 100000 g particulate fraction (see the Materials and methods section). Approx. 75 % of the genuine Ins(1,3,4,5) P_4 was metabolized, by both a 5-phosphatase and a 3-phosphatase, at a ratio of approx. 4:1 ([16], and results not shown). The rates of dephosphorylation of both the 3H - and ^{32}P -labelled Ins P_4 were identical (results not shown). Moreover, the 3H : ^{32}P ratio of the ensuing Ins P_3 was identical to the 3H : ^{32}P ratio of Ins P_3 arising from the dephosphorylation in parallel experiments of similar amounts of genuine 3H - and ^{32}P -labelled Ins(1,3,4,5) P_4 (results not shown). These data are entirely consistent with Ins(1,3,4,5) P_4 being the structure of the [3H]Ins P_4 formed by phosphorylation of Ins(1,4,5) P_3 in intestinal epithelial cells.

The distribution of Ins(1,4,5) P_3 phosphatases in plasma membrane domains of the intestinal epithelial cell

The distribution of Ins(1,4,5) P_3 phosphatases between basolateral and brush-border membranes was further studied in more purified preparations. Table 2 shows that our preparations of basolateral and brush-border membranes were, relative to homogenates, each about 20-fold enriched in their specific marker enzymes (K⁺-stimulated ATPase and sucrase respectively). In neither membrane was there any enrichment of arylesterase, glutamate dehydrogenase or β -galactosidase (results not shown). All of these results are in agreement with other workers' data on the purity of these preparations [17–19].

Ins(1,4,5) P_3 metabolism by basolateral and brush-border

Table 2. Distribution of Ins(1,4,5) P_3 1-, 4- and 5-phosphatases and subcellular markers in membranes derived from the basolateral and brush-border domains of the enterocyte

Enzyme assays were performed as described in the Materials and methods section, using h.p.l.c. for measuring Ins(1,4,5) P_3 phosphatases (see legends to Figs. 1 and 2 for details). The incubations were all performed in the absence of Glc6P, except for the 5-phosphatase in brush border membranes, which could only be accurately determined in the presence of 10 mM-Glc6P (see Fig. 2 and text). For Ins(1,4,5) P_3 5-phosphatase, which is the only one of the enzymes with significant cytosolic activity (9 % of total, see text), this soluble proportion was subtracted from the total activity in homogenates. Data are means $\pm$ S.E.M. from four membrane preparations, each assayed between two and four times.

Enzyme	Activity per fraction (nmol/min per mg of protein)		
	Homogenate	Basolateral	Brush border
K ⁺ -stimulated ATPase	4 $\pm$ 1	90 $\pm$ 17	4 $\pm$ 1
Sucrase	32 $\pm$ 3	21 $\pm$ 12	643 $\pm$ 60
Ins(1,4,5) P_3 1-phosphatase	8 $\pm$ 2	17 $\pm$ 2	101 $\pm$ 14
Ins(1,4,5) P_3 4-phosphatase	8 $\pm$ 2	15 $\pm$ 5	134 $\pm$ 20
Ins(1,4,5) P_3 5-phosphatase	9 $\pm$ 2	114 $\pm$ 10	18 $\pm$ 3

membranes was analysed using both 3H - and ^{32}P -labelled substrates, and the reaction products were separated by h.p.l.c. in order to obtain detailed information on the products of dephosphorylation. A representative experiment with basolateral membranes is shown in Fig. 1. In the absence of Glc6P, two Ins P_2 peaks were resolved (Fig. 1a). The earlier eluting of these (peak 3) represented 90 % of total Ins P_2 , and contained both Ins(1,4) P_2 and Ins(1,5) P_2 [5]. The proportion of each isomer in this peak was estimated by comparing its ^{32}P : 3H ratio (0.054) with the corresponding ratio for Ins(1,4,5) P_3 (0.24), and taking into account the fact that the [4,5- ^{32}P]Ins(1,4,5) P_3 had 95 % of its label in the 5-phosphate and 5 % in the 4-phosphate position (see the Materials and methods section). Thus peak 3 in Fig. 1(a) was 80 % Ins(1,4) P_2 and 20 % Ins(1,5) P_2 . Peak 4 in Fig. 1(a) (10 % of total Ins P_2) was Ins(4,5) P_2 [5]. In other words, in the absence of Glc6P (Fig. 1a), the three Ins P_2 isomers, i.e. Ins(1,4) P_2 , Ins(1,5) P_2 and Ins(4,5) P_2 , accumulated at the following relative rates: 72:18:10 respectively. The addition of 10 mM-Glc6P eliminated the formation of Ins(1,5) P_2 and Ins(4,5) P_2 by inhibiting

Fig. 1. H.p.l.c. analysis of the metabolism of [^3H]- and [4,5- ^{32}P]-Ins(1,4,5) P_3 by basolateral membranes of intestinal epithelial cells

Approx. 0.04 μCi of [^3H]Ins(1,4,5) P_3 and 0.007 μCi of [4,5- ^{32}P]Ins(1,4,5) P_3 were incubated for 10 min with 12.5 nmol of non-radioactive Ins(1,4,5) P_3 and 4.3 μg (a) or 3 μg (b) of protein of a basolateral membrane preparation, in the absence (a) or the presence (b) of 10 mM-Glc6P, as described in the Materials and methods section. The reaction products were separated by h.p.l.c. Peak 1, InsP; peak 2, P_1 ; peak 3, Ins(1,4) P_2 + Ins(1,5) P_2 ; peak 4, Ins(4,5) P_2 ; peak 5, Ins(1,4,5) P_3 [5]. The radioactivity (c.p.m.) for each inositol phosphate was computed from the area under each peak, which for (a) were: InsP, ^{32}P = 0, ^3H = 1681; Ins(1,4) P_2 /Ins(1,5) P_2 , ^{32}P = 442, ^3H = 8119; Ins(4,5) P_2 , ^{32}P = 271, ^3H = 936; Ins(1,4,5) P_3 , ^{32}P = 1819, ^3H = 7473. For (b): Ins(1,4) P_2 /Ins(1,5) P_2 , ^3H = 5031, ^{32}P = 0; Ins(1,4,5) P_3 , ^{32}P = 5878, ^3H = 11 125. All data are corrected for background (50 c.p.m.). The rates of 1-, 4- and 5-phosphatase were estimated from the rate of appearance of the appropriate Ins P_2 product, as nmol/min per mg of protein (see the text); -Glc6P: 1-phosphatase, 17; 4-phosphatase, 31; 5-phosphatase, 123; +Glc6P: 1-phosphatase, 0; 4-phosphatase, 0; 5-phosphatase, 130. These results are representative of 12 h.p.l.c. runs. Note that different h.p.l.c. columns were used in (a) and (b), so elution times are different.

Ins(1,4,5) P_3 1- and 4-phosphatases; there was no significant effect of Glc6P on the rate of formation of Ins(1,4) P_2 by the Ins(1,4,5) P_3 5-phosphatase (Figs. 1a and 1b). Thus basolateral membranes predominantly hydrolysed Ins(1,4,5) P_3 with a 5-phosphatase that was insensitive to 10 mM-Glc6P.

A representative assay of Ins(1,4,5) P_3 dephosphorylation by brush-border membranes is described by Fig. 2. The rates of 1-, 4- and 5-phosphatase were evaluated as described above. In the absence of Glc6P (Fig. 2a), the earlier-eluting Ins P_2 peak (number 3) had a ^{32}P : ^3H ratio of 0.35, similar to the ratio for Ins(1,4,5) P_3 (0.36), demonstrating that the Ins P_2 had retained most of the highly labelled [5- ^{32}P]phosphate, and was predominantly Ins(1,5) P_2 . This isomer and Ins(4,5) P_2 (peak 4) were present in about equal proportions, indicating that brush-border membranes primarily attacked Ins(1,4,5) P_3 with 1- and 4-phosphatases at about equal rates.

The brush-border membranes were also incubated with Ins(1,4,5) P_3 in the presence of 10 mM-Glc6P, which inhibited 1- and 4-phosphatase activities by around 90% (Fig. 2). Note that when Glc6P was present, in order to increase the amounts of products formed, the protein concentration was increased by 7-fold. The ^{32}P : ^3H ratio of peak 2 (0.17) was now less than the corresponding ratio for Ins(1,4,5) P_3 (0.33), indicating that about half of peak 3 was composed of Ins(1,4) P_2 (see Fig. 2b); the rate of Ins(1,4) P_2 production was too low to be detected in the absence of Glc6P (Fig. 2a). The relative amounts of each Ins P_2 isomer formed from Ins(1,4,5) P_3 by brush-border membranes ($\pm$ Glc6P, Figs. 2a and 2b) indicate that the Ins(1,4,5) P_3 5-phosphatase activity was substantially less than the 1- and 4-

phosphatase activities. A second conclusion is that the 5-phosphatase was relatively insensitive to Glc6P. Thus it seems likely that the 5-phosphatase activity of brush-border membranes is due to the specific intracellular enzyme.

The Ins(1,4,5) P_3 phosphatase activities of brush-border and basolateral membranes (Figs. 1 and 2) were compared with phosphatase activities in cell homogenates. For the 5-phosphatase, an accurate determination of the enrichment of the particulate enzyme in the purified membrane fractions necessitated the subtraction of soluble activity (9% of total, see above) from that determined in the homogenates. The activity in the membrane fractions of the predominantly extracellular 1- and 4-phosphatases (which by definition are particulate) were compared directly with that in the homogenates. The 5-phosphatase was enriched 2-fold in brush-border and 13-fold in basolateral membranes (Table 2). In contrast, the 1- and 4-phosphatases were more enriched in the brush-border region of the cell (13–17-fold) than in the basolateral membranes (2-fold).

The data in Table 2 demonstrate that the predominantly extracellular Ins(1,4,5) P_3 phosphatase activities are preferentially expressed on the luminal surface of the intestinal cell. This raises the question of the function of these activities *in vivo*, since Ins(1,4,5) P_3 is normally considered to be restricted to the cell interior [20]. Probably this Ins(1,4,5) P_3 hydrolysis reflects part of the process by which a family of phosphatases [21] sequentially dephosphorylate dietary inositol phosphates, which include Ins P_6 [22], ultimately to liberate inositol. It is also possible that Ins(1,4,5) P_3 is not formed during this process, and so the naturally occurring substrates for the 1- and 4-phosphatases may be the

Fig. 2. H.p.l.c. analysis of the metabolism of [^3H]- and [4,5- ^{32}P]Ins(1,4,5) P_3 by brush-border membranes of intestinal epithelial cells

Approx. 0.04 μCi of [^3H]Ins(1,4,5) P_3 and 0.007 μCi of [4,5- ^{32}P]Ins(1,4,5) P_3 were incubated for 10 min with 12.5 nmol of non-radioactive Ins(1,4,5) P_3 and 1.2 μg (a) or 8.1 μg (b) of protein of brush-border membranes, in the absence (a) or presence (b) of 10 mM-Glc6P, as described in the Materials and methods section. The reaction products were separated by h.p.l.c. Peak 1, InsP; peak 2, P_1 ; peak 3, Ins(1,4) P_2 + Ins(1,5) P_2 ; peak 4, Ins(4,5) P_2 ; peak 5, Ins(1,4,5) P_3 [5]. The c.p.m. for each inositol phosphate was computed from the area under each peak, which for (a) were: InsP, ^{32}P = 0, ^3H = 273; Ins(1,4) P_2 /Ins(1,5) P_2 , ^{32}P = 497, ^3H = 1427; Ins(4,5) P_2 , ^{32}P = 491, ^3H = 1283; Ins(1,4,5) P_3 , ^{32}P = 2994, ^3H = 8353. For (b): InsP, ^{32}P = 0, ^3H = 178; Ins(1,4) P_2 /Ins(1,5) P_2 , ^{32}P = 372, ^3H = 2249; Ins(4,5) P_2 , ^{32}P = 305, ^3H = 881; Ins(1,4,5) P_3 , ^{32}P = 3367, ^3H = 10226. All data are corrected for background (50 c.p.m.). The rates of 1-, 4- and 5-phosphatase (nmol/min per mg of protein) were calculated as in the legend to Fig. 1: -Glc6P: 1-phosphatase, 115; 4-phosphatase, 135; 5-phosphatase, not detected; +Glc6P: 1-phosphatase, 10; 4-phosphatase, 12; 5-phosphatase, 13. The results shown are representative of 12 h.p.l.c. runs.

alternative Ins P_3 species that are produced. Our data also indicate that these phosphatase activities are unlikely to be solely due to alkaline phosphatase (a constituent of brush-border membranes [17]), since that enzyme preferentially removes the 1-phosphate of Ins(1,4,5) P_3 (see [23]), whereas the extracellular phosphatases in intestinal cells removed both 1- and 4-phosphates with equal affinity (Table 2). In any case, our assays were performed at pH 7, at which alkaline phosphatase activity should be relatively low. Our data do not prove whether a single enzyme is responsible for both 1- and 4-phosphatase activities towards Ins(1,4,5) P_3 , although this is a strong possibility in view of their similar subcellular distribution and reaction velocities, and quantitatively similar inhibition by 10 mM-Glc6P (Table 2, Fig. 2). If a single enzyme were responsible, it is then intriguing that it has little, if any, affinity for the 5-phosphate of Ins(1,4,5) P_3 ; such a reaction has apparently evolved as a specifically intracellular activity.

General discussion

Enterocyte basolateral membranes possess receptors for epidermal growth factor [24], and enterocyte function is regulated by several neurohumoral substances [25] which stimulate inositol phosphate production in other cell types, although as yet such activity has not been demonstrated in enterocytes [5]. Thus our demonstration that these cells contain a Ins(1,4,5) P_3 3-kinase is particularly relevant because it indicates that Ins(1,3,4,5) P_4 is produced by these cells. Enterocytes also contain several other components of the inositol lipid signalling system, including protein kinase C [26], Ins(1,4,5) P_3 -mobilizable Ca^{2+} pools (see

the Introduction) and Ins(1,4,5) P_3 5-phosphatase (see above). Intestinal mucosa also contains a phosphatidylinositol-specific phospholipase C [27].

The activity of Ins(1,4,5) P_3 5-phosphatase was much greater in the basolateral region of the cell than in the region of the brush border (Table 1). It therefore seems likely that the sites of Ins(1,4,5) P_3 generation and inactivation are in close proximity. This is completely opposite to the situation in hepatocytes, where the Ins(1,4,5) P_3 5-phosphatase is concentrated in the bile canalicular region, away from the basolateral pole [4]. Thus in polarized cells the Ins(1,4,5) P_3 5-phosphatase can be asymmetrically distributed, and our data now lead to the novel conclusion that the pole of the cell at which the enzyme is concentrated can vary between cell types. It is possible that these differences establish cell-specific concentration gradients of Ins(1,4,5) P_3 across the cell interior, which may also contribute to regulating the flux of Ins(1,4,5) P_3 between its alternative metabolic routes. As indicated in the Introduction, these factors may be relevant to the highly localized changes in cytosolic Ca^{2+} that have been detected in some cells.

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