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Extraction and recovery of inositol phosphates from tissues

The recent interest in inositol phosphates as second messengers (e.g. Berridge & Irvine, 1984) has led to many experiments being performed using *myo*-[³H]inositol to label cells, followed by stimulation and subsequent quantification of radiolabelled inositol phosphates (e.g. Berridge *et al.*, 1982, 1983). We have recently become aware of two instances in which considerable loss of radiolabelled inositol phosphates can occur during extraction and analysis, and have found a simple remedy which we suggest could be used routinely to circumvent any future problems.

When extracting inositol phosphates from concanavalin A-treated murine thymocytes labelled with *myo*-[³H]inositol (Moore *et al.*, 1984) we found that inositol tetrakisphosphate (InsP₄) levels were very much reduced if instead of a chloroform/methanol extraction we used trichloroacetic acid. This was caused by absorbance of [³H]InsP₄ onto plastic surfaces and cellulose acetate filters used in our procedure; [³H]Ins(1,3,4,5)P₄ was lost in this way, but [³H]Ins(1,4,5)P₃ was not. Thus a specific loss of InsP₄ occurred which distorted the relative amounts detectable in the tissue. In view of the probable second messenger role of InsP₄ (Irvine & Moor, 1986) this loss is undesirable.

Another extraction method which under some circumstances could lead to loss of radiolabelled inositol phosphates was that of Sharpes & McCarl (1982) using Freon and tri-*n*-octylamine. When [³H]Ins(1,4,5)P₃ (Amersham) was extracted from water using this method, < 10% remained in the aqueous upper phase. Maleic acid in the original InsP₃ solution (i.e. having the InsP₃ either in a Tris/maleate buffer rather than a Tris/HCl buffer, or in the presence of 75 mM-maleate, pH 7) prevented this loss, which we ascribe to the trapping of InsP₃ as the octylamine salt, and its displacement by the maleate anion (Wreggett & Irvine, 1987).

We believe both these losses during extraction are caused by the well-known phenomenon of trace amounts of compounds being bound to non-specific sites, this problem typically being alleviated by increasing the mass of the compound concerned. Thus, in this particular case, the problems may only be confined to very small amounts of tissue, and only to particular pieces of apparatus (type of filter etc.); for example, we know (C. P. Downes & P. T. Hawkins, personal communication) that no loss of any inositol phosphates occurs during extraction with trichloroacetic acid of carbachol-stimulated parotid slices (Hawkins *et al.*, 1986) and this may be perhaps because of the greater mass of tissue, or slightly different techniques. Rather than investigate this in detail, we preferred to find a simple solution: if this

Table 1. Effect of the addition of phytate hydrolysate on Ins(1,3,4,5)P₄ recovery from thymocyte extracts

[³H]Ins(1,3,4,5)P₄ (Irvine *et al.*, 1986) was added to thymocytes or to the buffer described by Zilberman *et al.* (1987) in which they were bathed, and then extracted either with chloroform/methanol (Zieberman *et al.*, 1987) or by trichloroacetic acid followed by ether washes (Batty *et al.*, 1985). Recovery was assessed by comparison with a freeze-dried sample of InsP₄. All samples were analysed by h.p.l.c. (Batty *et al.*, 1985) and the InsP₄ region collected and counted. Phytate hydrolysate, when added, contained 25 µg of phosphorus.

Extraction method	Recovery of added [³ H]Ins(1,3,4,5)P ₄ (%)
Neutral chloroform/methanol	79 ± 7
Neutral chloroform/methanol + thymocytes	88 ± 0.3
Trichloroacetic acid	8 ± 7
Trichloroacetic acid + thymocytes	10 ± 1
Trichloroacetic acid + phytate hydrolysate	95 ± 9
Trichloroacetic acid + thymocytes + phytate hydrolysate	90

were routinely adopted, such potential artefacts would not occur.

Table 1 shows that inclusion of a phytic acid hydrolysate containing InsP₁₋₆ (total 25 µg of phosphorus) completely overcomes the extraction loss of InsP₄ from thymocytes described above. We prepared this hydrolysate as in Desjobert & Petek (1956), i.e. 1 g of phytate (Sigma) was dissolved in 10 ml of a pH 4.0 acetate/acetic acid (0.1 M) buffer, stoppered and incubated in a boiling-water bath for 8 h. It was then passed down a Amberlite 1R-120 column (BDH Chemicals) in the H⁺ form, dried, redissolved in water and its phosphorus content determined (Rouser *et al.*, 1970). This gave sufficient phytate hydrolysate to last the lifetime of an average scientist (if added to all samples in the dose given above). If used routinely, phytate hydrolysate should prevent any future problems with losses of radiolabelled inositol phosphates. The phytate hydrolysate also increases the reproducibility of ion-exchange columns (Wreggett & Irvine, 1987) for the same reason (i.e. increasing the mass to above trace quantities) which is another reason to advocate its routine use.

The only potential disadvantage would be if mass measurements of inositol phosphates (e.g. Rittenhouse & Sasson, 1985) are required, or if purification of inositol phosphates is desired. Here, however, the masses are

greater anyway, and so losses are less likely. If measurement of *myo*-inositol after dephosphorylation remains a method of choice for mass detection and analysis of inositol phosphates (Rittenhouse & Sasson, 1985), then a mixture of inositol phosphates prepared by chemical phosphorylation of an inositol other than *myo*-inositol would be a useful alternative. As long as radiolabelling is the predominant method used for inositol phosphate analysis, we recommend routine addition of a phytate hydrolysate.

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Batty, I. R., Nahorski, S. R. & Irvine, R. F. (1985) *Biochem. J.* **232**, 211–215

Berridge, M. J. & Irvine, R. F. (1984) *Nature (London)* **312**, 315–321

Berridge, M. J., Downes, C. P. & Hanley, M. R. (1982) *Biochem. J.* **206**, 587–595

Berridge, M. J., Dawson, R. M. C., Downes, C. P., Heslop, J. P. & Irvine, R. F. (1983) *Biochem. J.* **212**, 473–482

Desjobert, G. R. & Petek, F. (1956) *Bull. Soc. Chim. Biol.* **38**, 871–883

Hawkins, P. T., Stephens, L. & Downes, C. P. (1986) *Biochem. J.* **238**, 507–516

Irvine, R. F. & Moor, R. M. (1986) *Biochem. J.* **240**, 917–920

Irvine, R. F., Letcher, A. J., Heslop, J. P. & Berridge, M. J. (1986) *Nature (London)* **320**, 631–634

Moore, J. P., Johannsson, A., Hesketh, T. R., Smith, G. A. & Metcalfe, J. C. (1984) *Biochem. J.* **221**, 675–684

Rittenhouse, S. E. & Sasson, J. P. (1985) *J. Biol. Chem.* **260**, 8657–8660

Rouser, G., Fleischer, S. & Yamamoto, A. (1970) *Lipids* **5**, 494–496

Sharpes, E. S. & McCarl, R. L. (1982) *Anal. Biochem.* **124**, 421–424

Wreggett, K. A. & Irvine, R. F. (1987) *Biochem. J.* **245**, 655–660

Zilberman, Y., Howe, L. R., Moore, J. P., Hesketh, T. R. & Metcalfe, J. C. (1987) *EMBO J.* **6**, 957–962

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