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# cDNA cloning of human and rat brain *myo*-inositol monophosphatase

## Expression and characterization of the human recombinant enzyme

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Inositol monophosphatase (EC 3.1.3.25) is a key enzyme in the phosphoinositide cell-signalling system. Its role is to provide inositol required for the resynthesis of phosphatidylinositol and polyphosphoinositides. It is the probable pharmacological target for lithium action in brain. Using probes derived from the bovine inositol monophosphatase cDNA we have isolated cDNA clones encoding the human and rat brain enzymes. The enzyme is highly conserved in all three species (79 % identical). The coding region of the human cDNA was inserted into a bacterial expression vector. The expressed recombinant enzyme was purified and its biochemical properties examined. The human enzyme is very similar to the bovine enzyme.

## INTRODUCTION

*myo*-Inositol monophosphatase (EC 3.1.3.25) is a key enzyme in the phosphoinositide cell-signalling system [reviewed in 1–3]. It hydrolyses Ins(1)*P*, Ins(3)*P* and Ins(4)*P* to provide the inositol required for the resynthesis of phosphatidylinositol and polyphosphoinositides. The enzyme has been purified from a variety of sources including rat and bovine brain [4,5], as well as lily pollen [6]. In each case the enzyme appears to be composed of two similar or identical 30 kDa subunits. The enzyme has an absolute requirement for $Mg^{2+}$ [7], and $Li^{+}$ has been reported to trap a phosphoryl enzyme intermediate preventing subsequent nucleophilic attack by water [8]. It has been suggested that blockade of inositol monophosphate hydrolysis and consequent depletion of inositol for phosphatidylinositol synthesis underlies the anti-manic and anti-depressant actions of $Li^{+}$ [9]. Recently a cDNA encoding the bovine brain enzyme has been described [10]. In this paper, we describe the isolation of cDNA clones encoding the human and rat brain inositol monophosphatases. We have expressed the human enzyme in bacteria and purified it to homogeneity. Its properties were found to be very similar to those of the bovine enzyme.

## MATERIALS AND METHODS

### Enzyme assays

Enzyme activity was determined by measuring release of [$^{14}$C]inositol from DL-Ins(1)*P* containing L-[U-$^{14}$C]Ins(1)*P* as label as described previously [11]. One unit of enzyme activity represents 1 $\mu$mol of substrate hydrolysed/min, at 37 °C. Protein concentrations were determined by the method of Bradford [12]. Kinetic analyses were performed as described previously [5].

### cDNA cloning

Human cDNAs were isolated from a commercially available hippocampal cDNA library constructed in $\lambda$ZAP (Stratagene Ltd., Cambridge, U.K.) by standard recombinant techniques [13]. A $^{32}$P-radiolabelled oligonucleotide-primed fragment of the bovine inositol monophosphatase cDNA was used to probe the library [14]. Rat cDNAs were isolated from a rat brain cDNA library constructed in $\lambda$gt11 (Clonetech Laboratories Inc., Palo Alto, CA, U.S.A.) in a similar way. The coding regions of these clones were sequenced in both strands by the dideoxy chain termination method [15]. Nucleic acid and protein sequences were analysed and compared using the Intelligenetics software package (Intelligenetics Inc., Mountain View, CA, U.S.A.).

### RNA blot hybridization

Total RNA was extracted from various rat tissues by the method of Chirgwin *et al.* [16]. Poly(A)$^{+}$RNA was prepared by oligo(dT)-cellulose chromatography [17], separated on a denaturing agarose gel [18], and transferred to Hybond-N membranes (Amersham) as recommended by the manufacturer. A $^{32}$P-radiolabelled oligonucleotide-primed rat cDNA insert [14] was used to probe the blot.

### Expression of recombinant human inositol monophosphatase in bacteria

The T7 polymerase bacterial expression system (pRSET5a) was used as described previously [10]. The coding region of the human inositol monophosphatase cDNA was reconstructed to contain an *Nde*I site at the start codon and a *Pst*I site just downstream of the stop codon using PCR methodology [19]. Oligonucleotides 5′-AATATTTTCAGCATATGGCTGATCCTTG3′ and 5′-ATGACTATGAGCTGCAGTAATTAATCTTC-3′ were synthesized on an Applied Biosystems 380B instrument. Inositol monophosphatase cDNA (100 ng) in PBluescript II SK (Stratagene Ltd., U.K.) was subjected to PCR under standard conditions [19], denaturation at 94 °C for 2 min, annealing at 55 °C for 2 min and polymerization at 72 °C for 6 min. Twenty cycles were performed with the last polymerization step lasting 12 min. The *Nde*I/*Pst*I-digested PCR product was cloned into *Nde*I/*Pst*I-digested pRSET5a and transformed into *Escherichia coli* strain DH5$\alpha$ competent cells. Positive clones were identified

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                                                           -1  +1
-36   CTC CGA CTC AAG ATA TTT GTC AAA TAT TTT CAG AAG ATG GCT GAT CCT TGG CAG
                                                       M   A   D   P   W   Q      6

 19   GAA TGC ATG GAT TAT GCA GTA ACT CTA GCA AGA CAA GCT GGA GAG GTA GTT TGT
      E   C   M   D   Y   A   V   T   L   A   R   Q   A   G   E   V   V   C      24

 73   GAA GCT ATA AAA AAT GAA ATG AAT GTT ATG CTG AAA AGT TCT CCA GTT GAT TTG
      E   A   I   K   N   E   M   N   V   M   L   K   S   S   P   V   D   L      42

127   GTA ACT GCT ACG GAC CAA AAA GTT GAA AAA ATG CTT ATC TCT TCC ATA AAG GAA
      V   T   A   T   D   Q   K   V   E   K   M   L   I   S   S   I   K   E      60

181   AAG TAT CCA TCT CAC AGT TTC ATT GGT GAA GAA TCT GTG GCA GCT GGG GAA AAA
      K   Y   P   S   H   S   F   I   G   E   E   S   V   A   A   G   E   K      78

235   AGT ATC TTA ACC GAC AAC CCC ACA TGG ATC ATT GAC CCT ATT GAT GGA ACA ACT
      S   I   L   T   D   N   P   T   W   I   I   D   P   I   D   G   T   T      96

289   AAC TTT GTA CAT AGA TTT CCT TTT GTA GCT GTT TCA ATT GGC TTT GCT GTA AAT
      N   F   V   H   R   F   P   F   V   A   V   S   I   G   F   A   V   N     114

343   AAA AAG ATA GAA TTT GGA GTT GTG TAC AGT TGT GTG GAA GGC AAG ATG TAC ACT
      K   K   I   E   F   G   V   V   Y   S   C   V   E   G   K   M   Y   T     132

397   GCC AGA AAA GGA AAA GGG GCC TTT TGT AAT GGT CAA AAA CTA CAA GTT TCA CAA
      A   R   K   G   K   G   A   F   C   N   G   Q   K   L   Q   V   S   Q     150

451   CAA GAA GAT ATT ACC AAA TCT CTC TTG GTG ACT GAG TTG GGC TCT TCT AGA ACA
      Q   E   D   I   T   K   S   L   L   V   T   E   L   G   S   S   R   T     168

505   CCA GAG ACT GTG AGA ATG GTT CTT TCT AAT ATG GAA AAG CTT TTT TGC ATT CCT
      P   E   T   V   R   M   V   L   S   N   M   E   K   L   F   C   I   P     186

559   GTT CAT GGG ATC CGG AGT GTT GGA ACA GCA GCT GTT AAT ATG TGC CTT GTG GCA
      V   H   G   I   R   S   V   G   T   A   A   V   N   M   C   L   V   A     204

613   ACT GGC GGA GCA GAT GCA TAT TAT GAA ATG GGA ATT CAC TGC TGG GAT GTT GCA
      T   G   G   A   D   A   Y   Y   E   M   G   I   H   C   W   D   V   A     222

667   GGA GCT GGC ATT ATT GTT ACT GAA GCT GGT GGC GTG CTA ATG GAT GTT ACA GGT
      G   A   G   I   I   V   T   E   A   G   G   V   L   M   D   V   T   G     240

721   GGA CCA TTT GAT TTG ATG TCA CGA AGA GTA ATT GCT GCA AAT AAT AGA ATA TTA
      G   P   F   D   L   M   S   R   R   V   I   A   A   N   N   R   I   L     258

775   GCA GAA AGG ATA GCT AAA GAA ATT CAG GTT ATA CCT TTG CAA CGA GAC GAC GAA
      A   E   R   I   A   K   E   I   Q   V   I   P   L   Q   R   D   D   E     276

829   GAT TAA TTA AGG CAG CTC ATA GTC ATC CAG TTG
      D   END
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**Fig. 1. Sequence of human brain inositol monophosphatase**

The nucleotide sequence surrounding the open reading frame of the cDNA is shown. The amino acid sequence is given using the single-letter code. The amino acid sequence is numbered to the right of the sequence line and the nucleotide sequence is numbered to the left.

by restriction analysis and DNA sequencing. For subsequent expression studies, the expression vector was transformed into competent BL21-DE3 cells.

### Purification of human recombinant inositol monophosphatase

*E. coli* bacteria (strain BL21-DE3) were grown and induced as described previously [10]. After induction, cells were pelleted and frozen until required for purification. The bacterial pellets (1–2 g/litre of fermentation mixture) were thawed, resuspended in 10 vol. of 20 mM-Tris/HCl/1 mM-EGTA buffer, pH 7.8 (buffer A) and sonicated on ice (3 × 1 min). The homogenate was then centrifuged at 100000 *g* for 20 min and the resultant supernatant heated for 1 h at 68 °C. The heat-treated supernatant was then centrifuged as before and 3 ml of the resultant supernatant was loaded, at a flow rate of 1 ml/min, on to an HR5/5 Mono Q column (LKB Pharmacia), previously equilibrated with buffer A. The column was eluted at 1 ml/min with a gradient of 0–300 mM-NaCl in buffer A and 1 ml fractions were collected. Portions (5 μl) of each fraction were subjected to SDS/PAGE on 12% gels according to the method of Laemmli [20]. Gels were stained with Coomassie Blue and the appropriate inositol monophosphatase-containing fractions were pooled for subsequent analysis.

## RESULTS AND DISCUSSION

### Cloning of human brain inositol monophosphatase

A human hippocampal cDNA library constructed in λZAP was screened using the bovine inositol monophosphatase cDNA as a probe [10]. Phage (100000) were plated and three independent cDNA clones were isolated. All three contained approx. 2 kb inserts subsequently shown to have identical sequences (results not shown). One of these clones was characterized further and found to contain a 277-amino acid open reading frame (Fig. 1). The predicted protein sequence is very similar to that of the bovine enzyme (Fig. 2) and has an estimated subunit $M_r$ of approx. 30000, as does the bovine enzyme [5].

### Cloning of rat brain inositol monophosphatase

A rat brain cDNA library constructed in λgt11 was screened using the bovine cDNA as a probe. Plaques were plated and two

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Bovine      1   MADPWGECMDYAVTLAgQAGEVVrEAlKNEMNiMvKSSPaDLVTATDQKVEKMLItSIKEKYPSHSFI
Human       1   MADPWGECMDYAVTLARQAGEVVcEAiKNEMNVMlKSSPvDLVTATDQKVEKMLISSIKEKYPSHSFI
Rat         1         CMDYAViLARQAGEmirvAlKNkMdVMiKSSPaDLVTvTDQKVEKMLmSSIKEKYPyHSFI
Consensus       madpwqeCMDYAVtLArQAGEvvreAlKNeMnvM-KSSPaDLVTaTDQKVEKMLisSIKEKYPsHSFI

Bovine     69   GEESVAAGEKSILTDNPTWIIDPIDGTTNFVHgFPFVAVSIGFvVNKKmEFGiVYSClEdKMYTgRKG
Human      69   GEESVAAGEKSILTDNPTWIIDPIDGTTNFVHRFPFVAVSIGFaVNKKiEFGVVYSCVEgKMYTaRKG
Rat        62   GEESVAAGEKtvfTeqPTWIIDPIDGTTNFVHRFPFVAVSIGFvVNKemEFGVVYSCVEdKMYTgRKG
Consensus       GEESVAAGEKsilTdnPTWIIDPIDGTTNFVHrFPFVAVSIGFvVNKkmEFGvVYSCvEdKMYTgRKG

Bovine    137   KGAFCNGQKLQVShQEDITKSLLVTELGSSRTPETVRiiLSNiErLlClPiHGIRgVGTAAlNMCLVA
Human     137   KGAFCNGQKLQVSQQEDITKSLLVTELGSSRTPETVRmVLSNMEkLfCIPvHGIRSVGTAAVNMCLVA
Rat       130   KGAFCNGQKLrVSQQEDITKSLLVTELGSSRkPETlRiVLSNMErLcsIPiHGIRSVGTAAVNMCLVA
Consensus       KGAFCNGQKLqVSqQEDITKSLLVTELGSSRtPETvRivLSNmErL-ciPiHGIRsVGTAAvNMCLVA

Bovine    205   aGaADAYYEMGIHCWDVAGAGIIVTEAGGVLlDVTGGPFDLMSRRVIAssNktLAERIAKEIQiIPLQ
Human     205   TGGADAYYEMGIHCWDVAGAGIIVTEAGGVLmDVTGGPFDLMSRRVIAAnNriLAERIAKEIQvIPLQ
Rat       198   TGGADAYYEMGIHCWDmAGAGIIViEAGGVLlDVTGGPFDLMSRRiIAAsNiaLAERIAKEleiIPLQ
Consensus       tGgADAYYEMGIHCWDvAGAGIIVtEAGGVLlDVTGGPFDLMSRRvIAasN--LAERIAKEiqiIPLQ

Bovine    273   RDDED
Human     273   RDDED
Rat       266   RDDEs
Consensus       RDDEd
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**Fig. 2. Comparison of the amino acid sequence of bovine, human and rat inositol monophosphatase**

Identity between sequences is represented by a vertical line. A consensus sequence is shown below the three species. Upper-case letters represent residues conserved in all three species. Lower-case letters represent residues conserved in two out of three species. A (-) represents residues that differ in all three species.

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QAX    1  MtsrtttatEldeiYtfAVqLgkdAGnllmEAarlrfsnNnaN hdkesttqefteKdSaVDiVTqTD
IMP    1  M   adPwqE cmdY  AVtLARqAGevvcEA      ikNemN            V mlKsSpVDlVTaTD
SUH    1  M    hP      mlniAVraARkAG                nliaknyetpdaVeasqkgsnDfVTnvD
Con       M-----p--e----y--AV-lar-AG----ea-------nn-n----e----v---k-s-vD-VT-tD

QAX   68  edVEafikSaIntrYPSHdFIGEEtyAkssqStrpyLvThttPTWvvDPlDGTvNytHlFPmfcVSIa
IMP   48  qkVEkmlISsIkekYPSHsFIGEESvAagEkS    iL T DnPTWiIDPiDGTTNFvHRFPfvAVSIg
SUH   45  kaaEaviIdtIrksYPqHtiItEES gelE      gtdqD vqWvIDPlDGTTNFikRlPhfAVSIa
Con       --vEa-iis-I---YPsH-fIgEEs-a--e-s----l-t-d-ptWviDPlDGTtNf-hrfP-faVSIa

QAX  136  FlVdgtpviGVicapmlGqlfTAcKGrGAwlNetQrLplvrQ pmpKSapggcVfscEwGkdRkdrPE
IMP  111  FaVnkkiEfGVVYscveGkmyTArKGkGAfcN GQkLqvSqQeDitKS   lLV  TElGssR  tPE
SUH  105  vrikgrtEvaVVYdpmrnelfTAtrGqGAqlN GyrLlgStarD   ldgtiL  aT
Con       f-v-g--e-gVvy-pm-g-lfTA-kG-GA-lN-gqrL--s-q-d--ks----lv--te-g--r---pe

QAX  203  gnlyRkVeSfvNMaaEvggrggkggmVHGvRSlGsAtldlaytAmGsfDiwwEgGcweWD VA AGIa
IMP  171    tvRmVlS   NM  E    KLFcipVHGiRSvGtAAvnmclVAtGgaDayyEmGihcWD VAGAGI
SUH  156    gfpfkakqyattyinivgKLF necadfRrtGsAAldlayVAaGrvDgffEiGlrpWDfaAG  el
Con       ----r-v-s--nm--e----klf---vhg-Rs-GsAaldlayvA-G--D---E-G---WD-vAgagi-

QAX  269  IlqEAGGlitsanppeDwaTaeipDvkLgSRlylvvRpagpsegetaRegqERtirEvwrrVraLdyt
IMP  227  IVtEAGG       vlmD vTGGpfD  LMSR     R    vIaAnNRilaERiakE  iqVipLqrd
SUH  219  lVrEAGG       ivsD fTGG   hnyM        ltgnIvAgN   prvvkamlanmrdeLsda
Con       iv-EAGG--------D--Tgg--d--lmsr-----r-----i-a-nr---er---e----v--L---

QAX  337  rpga
IMP  275  ded
SUH  265  lkr
Con       ---- -
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**Fig. 3. Comparison of the amino acid sequence of human inositol monophosphatase (IMP) with QAX and SUH-B homologues**

Identity between sequences is represented by a vertical line. In the consensus sequence (Con), completely conserved residues are shown in upper case, residues conserved in two out of three sequences are shown in lower case, and a (-) represents unconserved residues. The alignment was carried out using the Genalign program of the Intelligenetics Inc. software package (solution parameters were residue length = 1; deletion weight = 1; length factor = 0; matching weight = 1). *Qa-x* is a gene (*x*) found in a quinic acid (qa)-inducible gene cluster (i.e. *qa-x*) of *Neurospora crassa*. *Qa-x* encodes a presumptive protein of unknown function [21]. SUH-B is an *E. coli* gene product thought to be involved in the regulation of protein translation [26].

apparently identical cDNA clones containing 2.1 kb inserts were isolated and sequenced. They both contained an open reading frame of 270 amino acids beginning from residue eight of the bovine and human clones (Fig. 2). Therefore the rat cDNA clones are not quite full length.

### Sequence comparison of human, rat and bovine inositol monophosphatases

Aligning the protein sequences from the bovine, human and rat enzymes demonstrates that all three sequences are highly conserved (Fig. 2). The human enzyme is 85% identical with both the rat and bovine enzymes. In fact, 79% of residues are identical in all three species. The high degree of similarity between species is striking, but it does not make the identification of key residues easier.

Inositol monophosphatase showed no significant sequence similarity to bovine Ins(1,4,5)$P_3$ 3-kinase [21] or to human Ins (c 1:2)$P$ 2-phosphohydrolase [22], two other enzymes involved in the phosphatidylinositol signalling pathway. Limited sequence similarity between the bovine enzyme and bovine Ins(1,3,4)$P_3$/Ins(1,4)$P_2$ 1-phosphatase was reported recently [23]. However, comparison with the rat enzyme decreases the identity between the enzymes from 9 of 20 residues to 7 of 20. As both enzymes hydrolyse similar substrates and are inhibited by $Li^+$, it is perhaps surprising that they do not share more extensive sequence similarity. A comparison of the inositol monophosphatase sequence with the Genbank and EMBL databases revealed no significant similarity to any other enzymes. However, there is similarity to three proteins with apparent regulatory functions. They are the QAX protein of *Neurospora crassa* [24], the QUT-G protein of *Aspergillus nidulans* [25] and the SUH-B protein of *E. coli* [26]. All three proteins show approx. 35% identity with inositol monophosphatase. *Qa-x* and *Qut-G* are both part of a gene cluster induced when quinic acid/shikimic acid is provided as the sole carbon source for the respective organisms. No enzymic function has been ascribed to these gene products. *Suh-B* encodes a gene product involved in the regulation of a transcription factor. Aligning these sequences with inositol monophosphatase (Fig. 3) demonstrates considerable similarity, suggesting a similar overall structure of these proteins despite their apparently unlinked functions. The significance of this is unclear, but it seems unlikely that key functional residues are conserved in these homologues and this may be useful in future studies to identify catalytic and binding residues.

### Northern blot analysis of inositol monophosphatase

A Northern blot containing poly(A)$^+$RNA from various rat tissues was probed with a radiolabelled rat cDNA insert. The probe hybridized to a 2.2 kb mRNA species present in all tissues examined (Fig. 4). The human cDNA also hybridized to a 2.2 kb mRNA in IMR-32 human neuroblastoma cells and human placenta (results not shown). Interestingly, the rat probe also hybridized to some other tissue-specific mRNA species, notably, a 1.4 kb mRNA present only in testes, a 4.2 kb mRNA in cerebellum and a very large 8 kb mRNA present in brain, liver, kidney and adrenal gland. It is unclear whether these other mRNA species represent alternatively spliced transcripts of the inositol monophosphatase gene, or are related gene products whose function is unknown.

### Expression and purification of human inositol monophosphatase

To confirm that the human cDNA encodes a functional enzyme, the open reading frame was inserted into a T7 polymerase bacterial expression vector, pRSET5a. Bacterial cells containing the expression vector were induced by isopropylthio-

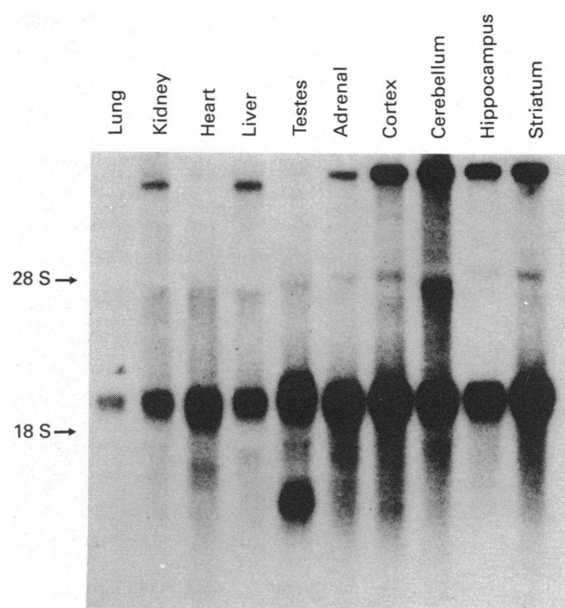


**Fig. 4. Northern blot analysis of inositol monophosphatase mRNA**

A Northern blot containing 3 $\mu$g of poly(A)$^+$RNA from various rat tissues was hybridized with a $^{32}$P-oligolabelled rat cDNA insert and subsequently washed twice with 5 × SSPE/0.1%-SDS at 65 °C, then with 0.3 × SSPE/0.1%-SDS at 65 °C. The position of the 18 S and 28 S ribosomal RNAs are indicated.

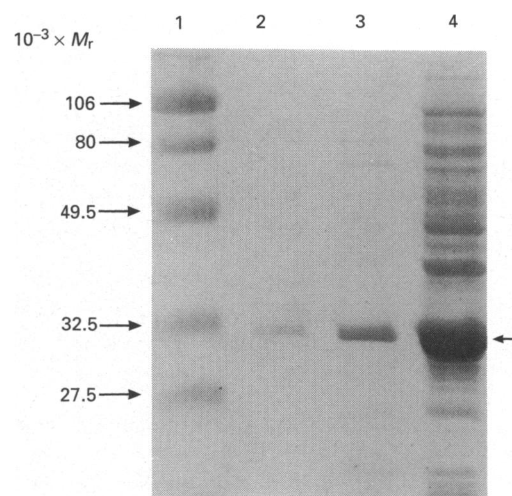


**Fig. 5. Enzyme purification**

SDS/PAGE of induced bacterial extract expressing inositol monophosphatase. The lanes contain portions of bacterial homogenate after centrifugation (lane 4), heat-treated supernatant (lane 3), pooled Mono Q column fractions containing enzyme (lane 2) and $M_r$ standards (lane 1). Sizes of marker proteins are given on the left, and the position of inositol monophosphatase is arrowed.

$\beta$-D-galactoside and analysed by both SDS/PAGE and enzyme assay (Fig. 5 and Table 1).

Recombinant bacteria expressed a prominent polypeptide, of $M_r$ 30000 (Fig. 5), which was absent from bacteria not containing the expression vector (results not shown). Bacterial lysates contained significant amounts of enzyme activity, which was further purified by heat treatment, centrifugation and ion-

**Table 1. Purification of recombinant human enzyme**

Purification was as described in the Materials and methods section.

| Step | Total activity (munits) | Total protein (mg) | Specific activity (munits/mg) | Yield (%) |
|---|---|---|---|---|
| Bacterial homogenate | 751100 | 214 | 3500 | 100 |
| Bacterial supernatant | 481000 | 90 | 5300 | 64 |
| Heat-treated supernatant | 264000 | 22 | 12000 | 35 |
| Mono Q | 177000 | 9 | 19700 | 24 |

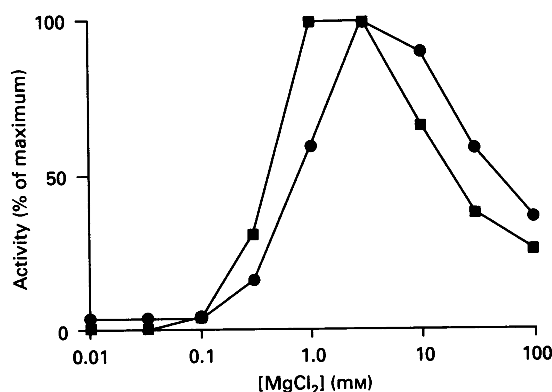


**Fig. 6. $Mg^{2+}$-dependence**

A typical example of an experiment demonstrating the $Mg^{2+}$-dependence of human (■) and bovine (●) inositol monophosphatase is shown.

exchange chromatography. SDS/PAGE of a typical purification is shown in Fig. 5. A single band of $M_r$ 30000 was obtained. Table 1 demonstrates a 5.4-fold purification of the enzyme, suggesting that recombinant inositol monophosphatase constituted nearly 20 % of the original bacterial protein. This level of expression will enable the isolation of large amounts of pure enzyme for X-ray crystallography and other studies. The biochemical properties of the human enzyme were further examined.

### Kinetic properties of recombinant human inositol monophosphatase

In agreement with previous work on the rat and bovine enzyme [4,5] we found that activity was totally dependent on $Mg^{2+}$. Under standard assay conditions, the apparent affinity of the human enzyme for $Mg^{2+}$ was approximately 2-fold greater than that of the bovine enzyme, and the human enzyme was also rather more sensitive to inhibition by high $Mg^{2+}$ concentrations (Fig. 6). In the absence of KCl, activation by $Mg^{2+}$ occurred at approximately 2-fold lower concentrations, but maximum enzyme activity was independent of KCl (results not shown). As can be seen from Table 2, the recombinant human brain enzyme has similar kinetic properties to the native human platelet enzyme as well as both the native and recombinant bovine enzymes [5,10]. The $K_m$ for DL-Ins(1)$P$ was slightly lower for the human enzyme, suggesting that it may have a slightly higher affinity for substrate than the bovine enzyme. These data also suggest that there is no significant post-translational modification of the native human enzyme that might change its behaviour compared with the recombinant enzyme.

**Table 2. Kinetic properties of inositol monophosphatases**

Initial rates determined with several substrate concentrations were fitted to a Michaelis–Menten expression by non-linear least-squares regression analysis. Values for $K_m$ and $V_{max.}$ were determined in the present study and are given as means ± S.E.M. ($n$ = 3). Bovine brain values are as reported in ref. [5] but confirmed by us. The human platelet value (*) was determined separately (C. Brazell, A. Prior, A. Heald & T. H. Corn, unpublished work).

| Enzyme | $K_m$ for DL-Ins(1)$P$ (mM) | $V_{max.}$ (μmol/min per mg of protein) |
|---|---|---|
| Human recombinant | 0.075 ± 0.003 | 36.8 ± 1 |
| Human platelet | 0.108 ± 0.003* | ND |
| Bovine recombinant | 0.12 ± 0.007 | ND |
| Bovine brain | 0.16 ± 0.02 | 13.3 ± 0.9 |

### Inhibition of recombinant human inositol monophosphatase

$P_i$ inhibited competitively with a $K_i$ value of 0.14 mM (cf. bovine enzyme, 0.5 mM), and $Li^+$ is an uncompetitive inhibitor with an apparent $K_i$ value of 0.3 mM (cf. bovine enzyme, 0.26 mM). The competitive inhibitor 1$S$-phosphoryloxy-2$R$,4$S$-dihydroxycyclohexane [27] inhibited with an apparent $K_i$ value of 2.7 μM (cf. bovine enzyme, 1.1 μM). All comparisons are from data obtained in parallel experiments carried out in this study. Overall, these data confirm that the human enzyme is very similar to, but not identical with, the bovine enzyme. The availability of multiple protein sequences and recombinant human enzyme will assist future studies using site-directed mutagenesis and chemical-modification techniques to characterize important residues for the structure and function of this enzyme.

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