Inositol monophosphatase is a highly conserved enzyme having localized structural similarity to both glycerol 3-phosphate dehydrogenase and haemoglobin

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The cDNA coding for an inositol monophosphatase in the oocytes of the African clawed frog, *Xenopus laevis*, has been isolated and sequenced. The predicted primary structure of this enzyme is markedly conserved when it is compared with its mammalian functional homologues; up to 84 % of the amino acid residues are identical, and conservative substitutions increase the similarity to 95 %, suggesting that this sequence represents the most parsimonious primary structure for the protein to maintain not only catalytic activity but also perhaps the facility to interact with other macromolecules. Two regions of the protein, each of about 11 residues and separated by about 90 residues, have been identified as a consensus found also in glycerol 3-phosphate dehydrogenase (EC 1.1.1.8). One of these regions is also found to be particularly conserved in the α -globin of birds and reptiles; birds and some turtles are known to modulate the oxygen affinity of their haemoglobin with inositol polyphosphate in the same way as with 2,3-bisphosphoglycerate in other species. This region is also conserved in the β -globin of most species, beginning with lysine-82, which is known to participate in the binding of organic phosphates. These regions of the inositol monophosphatase may represent motifs for the binding of its substrate.

INTRODUCTION

In the absence of an extracellular supply of the nutrient *myo*-inositol, the enzyme inositol monophosphatase (EC 3.1.3.25) has an important role in providing inositol to the cell through its recycling of the inositol phosphates that are generated by the inositol-linked second messenger system and by *de novo* synthesis from glucose 6-phosphate (see Berridge *et al.*, 1989; Sherman, 1989, for reviews). This enzyme is uncompetitively inhibited by millimolar concentrations of Li⁺ (Hallcher & Sherman, 1980), an observation which has not only provided a practical tool to study receptor-stimulated inositol phosphate metabolism in intact cells (Berridge *et al.*, 1982), but has also been the basis for an hypothesis concerning the effect of lithium in neuropharmacology and in developmental biology (Berridge *et al.*, 1982, 1989; Busa, 1988). It is important to understand the molecular basis for this rare example of uncompetitive inhibition, and thus information is required to identify the active site of this enzyme.

An inositol monophosphatase activity, with a broad substrate specificity, has been purified from bovine brain and the cDNA subsequently isolated to reveal that the protein is a homodimer comprising 30 kDa subunits (Gee *et al.*, 1988; Diehl *et al.*, 1990). More recently, cDNAs coding for the functional homologues from human and rat have been reported, and noted to be practically all identical in structure and function (McAllister *et al.*, 1992).

In an attempt to increase our understanding of the structure of inositol monophosphatase, I decided to isolate the cDNA for the functional homologue from an organism which is phylogenetically diverse from the mammals. To this end the African clawed frog, *Xenopus laevis*, was an attractive candidate, not only from an evolutionary point of view but also because the embryonic

development of this amphibian is remarkably affected either by treatment of early embryos with lithium salts or by micro-injection of lithium into specific blastomeres in a fashion which can be neutralized by co-injection with *myo*-inositol (Kao *et al.*, 1986; Busa & Gimlich, 1989), leading to the proposal that the teratogenic effect of this ion was by depletion of endogenous *myo*-inositol through inhibition of an inositol monophosphatase (Busa, 1988; Busa & Gimlich, 1989; Berridge *et al.*, 1989). This proposal can be tested experimentally using information on the structure of this enzyme in *Xenopus*.

MATERIALS AND METHODS

Screening of a cDNA library

An amplified cDNA library constructed in λ -ZAPII (ZAP-MO3X) was provided by Dr. John Shuttleworth, University of Birmingham, Birmingham, U.K. This was prepared from progesterone-matured *Xenopus* oocyte poly(A)⁺ mRNA ligated into the *Eco*RI site of the polylinker. The library was plated using *Escherichia coli* XL1 BLUE (Stratagene), and plaque lifts on to Duralon membranes (Stratagene) were taken using standard techniques (Sambrook *et al.*, 1989). The library was screened with a 1 kb cDNA fragment essentially consisting of the open-reading frame (ORF) for bovine inositol monophosphatase, prepared in a *Dde*I/*Eco*RI digestion of the original full-length cDNA in pBLUESCRIPT (Diehl *et al.*, 1990), purified after agarose electrophoresis using GeneClean (BIO 101, Inc.), and labelled by random priming (Prime-It, Stratagene) incorporating [³²P]dCTP (Amersham).

The membranes were pre-hybridized for about 2 h at 60 °C in 0.9 M-NaCl/1 % SDS/10 % poly(ethylene glycol) (PEG) 8000, and then the probe was added together with *E. coli* tRNA at a final concentration of 100 μ g/ml and left overnight at 60 °C. The

Abbreviations used: 2,3-BPG, 2,3-bisphosphoglycerate; ORF, open reading frame; CONS, consensus sequence; PEG, poly(ethylene glycol); FIMP, *Xenopus* inositol monophosphatase; SSC, 0.9 % (g/u) NaCl/0.44 % sodium citrate.

The nucleotide sequence data reported in this paper appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under accession no. X65513.

membranes were washed twice for 5 min at room temperature in $2 \times \text{SSC}/1\%$ SDS and once for 30 min at 60°C in $1 \times \text{SSC}/0.1\%$ SDS, and then put down for autoradiography at -80°C with Kodak XAR film in cassettes with intensifying screens for 48–96 h. Duplicated plaques were isolated in a tertiary level screening by standard methods (Sambrook *et al.*, 1989).

Preparation of plasmids and DNA sequencing

The cDNAs were isolated from the phage as recombinants in pBLUESCRIPT(SK) by using the *in vivo* excision protocol outlined by the manufacturers (Stratagene). Plasmids were cloned in XL1 BLUE, selected on LB plates with $20\ \mu\text{g}$ of ampicillin/ml, and the DNA was obtained in mini-preparations by a minor modification of the method of Saunders & Burke (1990). The first pellet was resuspended in $10\ \mu\text{l}$ of TE (for 1.5 ml of original bacterial culture) to which was added $100\ \mu\text{l}$ of TE containing 111.1 mg of CsCl and 0.1% (w/v) ethidium bromide, and then processed as described (Saunders & Burke, 1990); TE was 10 mM-Tris and 1 mM-EDTA, pH 8. For the purposes of both sequencing and genetic engineering, the final pellet from this method was resuspended in water and the plasmid was precipitated with $0.4\ \text{M}$ -NaCl/ 7% (w/v) PEG-8000.

Nested deletions of cDNAs in pBLUESCRIPT were generated from either side of the insert using Exonuclease III (New England Biolabs) and S1 nuclease (Amersham) by essentially standard techniques (Sambrook *et al.*, 1989).

Double-stranded DNA sequencing was performed on both strands with Sequenase Version 2.0 (USB Corporation) according to the manufacturer's instructions using either the M13⁻²⁰ or M13^{REV} primers synthesized by an in-house facility.

The assembly of nucleotide sequences, their subsequent analysis and the characterization of the predicted protein of the ORF were performed using software (Devereux *et al.*, 1984; Staden, 1986; Pearson & Lipman, 1988) running on a microVAX 3900 with VMS V5.4-1. Comparison of protein sequences for local sequence similarities with statistical considerations was also conducted using PROSCRCH (Collins *et al.*, 1988) on the Distributed Array Processor at the University of Edinburgh.

RESULTS AND DISCUSSION

Predicted size of *Xenopus* inositol monophosphatase by Western analysis

Extracts of *Xenopus* oocytes subjected to PAGE and blotted on to nitrocellulose were screened with a polyclonal antibody that had been raised against pure bovine inositol monophosphatase; a more complete description of this antibody and its properties is to be published elsewhere, and is available on application to the author. The cross-reactivity of the anti-bovine antibody with a protein of similar size in *Xenopus* oocytes (about 30 kDa) suggested that the *Xenopus* enzyme shares some structural identity with its bovine homologue (Fig. 1).

Analysis of the cDNA

Scores of positive plaques were obtained from the low-stringency primary screen of about 5×10^5 recombinants, of which six were isolated as plasmids and shown by *Eco*RI digestion and by initial sequencing to be identical (1.4 kb); one of these was then completely sequenced in both directions (Fig. 2). The 1475 bp fragment (*Xenopus* inositol monophosphatase; FIMP) had an ORF of 852 bp which contained two codons for methionine at positions +1 and +25. The former was chosen as the translational start site based on comparison with the bovine

sequence (Diehl *et al.*, 1990); neither position was associated with an obvious Kozak consensus (Kozak, 1989). The 5'-untranslated region was 163 bp long and the 3'-untranslated region of 442 bp [excluding the poly(A)⁺ tail] contained a polyadenylation signal 32 bp upstream from the start of the poly(A)⁺. This sequence has an overall 71% identity with the bovine homologue which covers the ORFs; a similar identity is found for a 39 bp stretch, located at the same position with respect to the ORF, in the 3'-untranslated region (bases +997 to +1035 in FIMP).

Comparison of the predicted product with mammalian inositol monophosphatase

The ORF coded for a protein of 284 amino acids having a molecular mass of 30 701 Da, agreeing with the results from the Western analysis. The predicted primary structure for FIMP has 87–89% similarity with the sequence for the inositol monophosphatase from bovine, human and rat brain, with 75% of the residues being identical; the three mammalian enzymes share an even greater degree of conservation (McAllister *et al.*, 1992). A matrix analysis (results not shown) comparing the *Xenopus* and bovine sequences at high stringency (11 matches in a window of 12 residues) revealed that these sequences are highly conserved from FIMP residues 42 to 281 (90–95% similar/75–84% identical), in which lies one short stretch of poor conservation (FIMP residues 170–178; 66% similar/33% identical); this suggests the existence of two separate domains in the primary structure. The predicted FIMP is longer than either the bovine or the human inositol monophosphatase by seven amino acids, four of which are interspersed throughout the sequence with the remainder comprising the C-terminal triplet. The FIMP product also contains a serine which is not present in the mammalian sequences, but lacks a residue which is present in the latter group within this same region of the C-terminus (these differences are indicated in Fig. 2). Taken together, these data support the

Fig. 1. Anti-(bovine inositol monophosphatase) immunoreactivity in extracts of *Xenopus* oocytes

Approx. $50\ \mu\text{g}$ of protein was loaded on to a 12.5% polyacrylamide gel and then transferred to a nitrocellulose membrane after electrophoresis. The blocked membrane was incubated with a polyclonal antibody raised against bovine inositol monophosphatase, and bound complexes were visualized by standard methods using a horseradish peroxidase P-conjugated anti-IgG antibody. A unique band at about 30 kDa was recognized in the oocyte extracts (lane 1); the reactivity in an equivalent amount of the supernatant of a bovine brain homogenate is shown for comparison (lane 2).

-163 CGCTTAGAAACTGTGGATCCCCGGGCTCGAGAAATTCGGGCTGCACACGCCGGGAAGCGCCTTTCATTGCTG
-90 AGGGTTCTGCTGCGATCTTATTGCAAGTGTGGGCGCAACCTGTAGTACAGAGGGTCTTTCATCATTACACACAGCGGCACAGGTGACA
1 ATGGAGGACCGGTGGCAAGAGTCATGATTCTTACCTGTTTCAATCCGAAGGACGGCTCAGTGGTGTGTGCAGCACTAAAGAA
M E D R W Q E G C M D F L A V S I A R K A G S V V C A A L K E 30

91 GATGTTTCAATAATGGTTAAAACTAGCCTAGCCCCAGCAGATCTGGTGACAGCCACAGATCAAAAAGTAGAAGAAATGATTATCTCATCC
D V S I M V K T S L A P A D L V T A T D Q K V E E M I I S S 60

181 ATTAAGGAAAAATATCCATCGCAGCTTTTATTGGCGAGGAGTCTGTAGCTCGAGGAGCTGGAAGCAGCGCTAACAGATAATCCAACCTGG
I K E K Y P S H S F I G E E S V A A G A G S T L T D N P T W 90

271 ATCATAGATCCCATAGATGGCACAACTAACTCGTACACAGGTTTCATTGTTGGCTGTGTCTATTGGATTGCTGTGCACAAGCAGGTT
I I D P I D G T T N F V H R F P F V A V S I G F A V H K Q V 120

361 GAATTTGGAGTTGTCTACAGCTGTGTAGAAGCAAAAATGTACTCGGAGAAAGGAAAAGGTTTCACTTCTGTAATGGTCAGAAATTACAA
E F G V V Y S C V E E D K M Y T G R X G K G S F C N G Q K L Q 150

451 GTCTCGGACAGAAAGATATTACAAATCCATGATAATAACGGAGCTAGGGTCAAATCCGGAATCCAGAGTTTATTAACCGGTGTCACGT
V S G Q K L I T K S M I A T E L G S N R N P E F I K T V S L 180

541 TCTAACATGGAGAGACTGCTGTGTATTCCCATCCCGGATTAGAGCAGTTGGTACAGCAGCAGTAANACTGTGCTCTCGTGGCTACTGGA
S N M E R L L C I P I H G I R A V G T A A V N M C L V A T G 210

631 GGTGCAGATGCTTATTATGAGATGGGTCTTCATTGCTGGGATATGGCAGCTCGTCCGTCATCGTTACAGAAGCAGGCGGAACAATCTGT
G A D A Y Y E M G L H C W D M A A A S V I V T E A G G T I L 240

721 GATGCAACAGGAGGCGCTGTTGACCTGATGTCTCGAGGATCATATCGGCTAGCAGCCGAGAAATAGCGGAGCGAATCGCTAAAGAGCTA
D A T G G G L F D L M S C R I I S A S S R E I A E I A K E L 270

811 CAGATTATACCCCTGGAAACGGGATGATGGCAAAAGTACCAACTGAAATGCCAGTTTAACTAAAGGTGTTTTTGAATCTATGAATATTAT
Q I I P L E R D D G K S T N * 284

901 GGAATTATGTACATAAGTTTATGnATTGTCTGTAATAGGCTTTATTTTCATGCAACnATTTTITAGCnAACATGTACATTTTITAG
991 TCTGTTATATTCAAATTTAAATAGTTGTTCTTTGTTTACTAATAAGTTTACCTATTTCATACCTTCCTGCCCATCTTCTACAGAGTTGG
1081 AATAAAGTAGTACCATACTGCTGACACCATCGATTCTATAGTATCCGAGTTCTCTGTGATCTACGCTGACCCCTACTCGCCGACTGGAAGCC
1171 AATnGAATGAATGCTGnAGGTTCAAGTCTCAAGCTACAAATTAATGTGCGTAGATGAANAAGTATGAGTAAGGAAAAATTACGATGCTCTAAA
1261 ACATnAAATATAGAGATGTTTTTTTTTCCCTGCCAAAAAATAAAAAA

The numbers on the left-hand side represent the positions of the nucleotides with reference to the position of the first ATG which starts at position 1; those on the right-hand side refer to the position of the amino acid residues. The amino acids not found in the mammalian functional homologues are boxed, and the relative position of an absent residue which is found in the mammalian homologues is indicated by $\wedge$. The underlined amino acids indicate the region of poor intra-species similarity, and underlined bases indicate remarkable regions in the non-coding sequence (see the text).

Protein sequences shown are *Xenopus laevis* inositol monophosphatase (FIMP), human alkaline phosphatase (AlkP), α -globin of aspic viper (AVHb), the product of *L. biflexa* *trpE* gene (TrpE) and human inosine monophosphate dehydrogenase (IMPD). Matches are indicated as identical (!) and either very (:) or reasonably (.) conserved.

Enzyme	First amino acid	Sequence
AlkP	92	RFPYVALSKTYSVDKHV
FIMP	104	RFPFVAVSIGFAVHKQV
AVHb	82	KLSDLHAQKLRVDPANFKILSQCLLST-LANHRNPEFGPAV-LASVDKFLG
FIMP	140	KGSFCNGQKLQV-SQG-KDITKSMIITELGSNRNPEFIKTVSLSNMERLLG
TrpE	49	WDVSAISVILTEAGGKLTDLNG
FIMP	223	WDMAAASVIIVTEAGGTILDATG
IMPD	286	IISSIKEKYPSHSFIGEESVAAGAGSTLTD : :
FIMP	57	MIKYIKEKYPSLQVIGGNVVTAQAQKNLID

One major disadvantage of this high degree of conservative structure is that there is no obvious information concerning the important functional residues in the primary sequence. It was recently reported that the mammalian inositol monophosphatase appeared to have a substantial similarity to a small group of proteins (McAllister *et al.*, 1992), although very little is known

about their function. Statistical analysis using PROSRCH (with a PAM of 100; see Collins *et al.*, 1988) indicates that with FIMP these similarities are highly significant [*suhB* suppressor protein in *E. coli*, SD = 244.7; the quinate-metabolizing proteins of *Neurospora crassa* and *Aspergillus nidulans*, SD = 149.1, where the parameter SD indicates the number of standard deviations from the mean of a distribution of random alignments by the chi-square statistic (Collins *et al.*, 1988). Some limited but significant (SD = 9.60) local similarity is also found in an hypothetical 22 kDa protein from the *trpE* gene of *Leptospira biflexa* (Yelton & Peng, 1989) and with the α -globin of the aspici viper (Duguet

Fig. 3. DOTPLOT presentation of a high-stringency matrix analysis comparing *Xenopus* inositol monophosphatase and *Drosophila* sp. glycerol 3-phosphate dehydrogenase (G3PD)

The protein sequences for both enzymes were analysed by the Wisconsin COMPARE software, which looked for regions of 10 residues containing 9 matches. The two short sequences which were recognized are indicated as CONS-1 and CONS-2 in Table 2.

et al., 1974) (Table 1); this similarity to haemoglobin is discussed further below. A search of the EMBL/Genbank database using TFASTA (which translates DNA sequences into all six reading frames before making a comparison) also noted some limited similarity over the entire sequence of the FIMP protein (47% similar/26% identical; results not shown) with the ammonium transport protein of *E. coli* (Fabiny *et al.*, 1991). Limited local similarity was also found with human alkaline phosphatase (Watanabe *et al.*, 1989) (76% similar/53% identical over 17 residues) and with human inosine monophosphate dehydrogenase (Natsumeda *et al.*, 1990) (60% similar/40% identical over 30 residues); these alignments are shown in Table 1. No outright functional correlates from these comparisons, however, can be concluded.

Consensus with glycerol 3-phosphate dehydrogenase

One group of proteins which provided an interesting local similarity to FIMP was the cytosolic glycerol 3-phosphate dehydrogenases (EC 1.1.1.8) from all eukaryotes. A matrix analysis comparing FIMP to *Drosophila* sp. glycerol phosphate dehydrogenase at high stringency (9 matches in a 10-residue window) revealed two short regions of structural similarity (Fig.

3). which are highly conserved across a wide range of species, and which are separated by a dissimilar stretch of about 85–110 residues in either protein (Table 2). A search of the databases for other proteins which might contain these consensus sequences did not reveal any matches across the entire sequence, even for proteins known to interact with (poly)ol phosphate, apart from some limited similarity to *Schizosaccharomyces pombe* cyclic AMP phosphodiesterase, Ca^{2+} -transporting ATPase and a group of proteins referred to as the Line-1 reverse transcriptases (Table 2). Some conservative similarity was also displayed by the proteases associated with RNA-directed RNA polymerases (results not shown).

Similarity to haemoglobin

The significant similarity of inositol monophosphatase to viper α -globin described earlier (Table 1) begins with a conserved match of consensus sequence 2 (CONS-2) which is maintained with conservative substitutions in all of the globins; those which have lysine at position 82 or 87 (position 1 in CONS-2) consist of the β -globins of most species and the α -globins of all birds and a few reptiles (Table 3). Sequences shown in Table 3 are those (not an exhaustive list) which maintain a conservative substitution at this position; not included are the vast majority of α -globins (including those of the non-baboon primates), which have an alanine at this position.

Conclusions

The high degree of conservation of the primary sequence for inositol monophosphatase has been noted for the mammalian homologues (McAllister *et al.*, 1992), and is rendered even more striking when the *Xenopus* sequence is studied, considering the phylogenetic separation of amphibians and mammals. A random screen of sequences from *Xenopus* compared with their human counterparts provided a range from about 63–80% similar/49–66% identical, for proteins such as the cyclins or superoxide dismutase, to 88–99% identical (e.g. enolase, actin). This suggests that the inositol monophosphatase occupies an important place in the evolutionary development of eukaryotes, such that this primary sequence may represent the most parsimonious one required to maintain either activity as a phosphatase or else the ability of the protein to interact with other macromolecules (perhaps to some degree in the ability of the 30 kDa monomers to aggregate as the active homodimer).

Inositol monophosphatase has a rather broad range of mono-

Table 2. Alignment of the available sequences for inositol monophosphatase and a sampling of those for glycerol phosphate dehydrogenase

Regions referred to as CONS-1 and CONS-2 are consensus sequences revealed by matrix analysis as shown in Fig. 3. The numbers refer to the position of the preceding amino acid residue in the primary sequence. Residues which are not identical matches are indicated; lower case letters in the consensus are residues conserved in the majority of sequences, whereas an upper case letter indicates a residue found in all sequences. Also shown are some other sequences in the databases which resemble CONS-2.

Sequence		Enzyme	
CONS-1		CONS-2	
20	AGSV-VCAALK	140	KGSFCNGQKLQV
19	..E.-..E...	137	..A.....
19	..E.-..RE...	137	..A.....
12	..EM-IRK...	130	..A.....R.
111	..DA.E..G...	214	..EML-.....G
194	..DT.EI.G...	289	..EML-.....G
222	..-..ALGG...	315	..ELL-G..L..G
	AgevxvcxALK		KxxxxnGQkLqx
Miscellaneous		KGETSG--NGQKLLQI	
		KFTVIR--NGQL-LQV	
		KATANIIVNGQK-LEA	
		<i>Xenopus</i>	
		Human	
		Bovine	
		Rat	
		Inositol monophosphatase	
		<i>Drosophila</i>	
		Mouse	
		Yeast	
		Consensus	
		Glycerol phosphate dehydrogenase	
		Yeast cyclic AMP phosphodiesterase	
		Ca^{2+} -ATPase	
		Line-1 reverse transcriptase	

Table 3. Comparison of CONS-2 sequence of inositol monophosphatase and glycerol phosphate dehydrogenase (see Table 2) with a similar region found in the sequences of all known globins

Most α globins have an alanine in position 82 except for the groups shown here. The first group of α -globins is comprised of those which maintain a positively charged residue at position 82, which is also found in most β -globins except for those of the ostrich and painted turtle. The second group of α -globins maintain a conservative polar/charged residue at this position; not included are the three groups of α -globins having a polar/uncharged residue: either a threonine (rat, rabbit, guinea pig and armadillo), a serine (bullfrog) or an asparagine (horse, donkey, zebra). Residues which are not identical to the top α -globin sequence are indicated.

Protein	Sequence	Source
	KxxxxnGQkLqx	CONS-2
α -Globins	82 KLSDLHAQKLRV	Aspic viper, most birds
	H....	Baboons, kangaroo, chicken, pheasant
	Q....	Cape monitor
	YN...	Snake-necked turtle
	YD...	<i>Xenopus</i>
	I...T...	Painted turtle
	...E..FD..H	Cobra
	...E..CD..H	Iguana
	R.....HN...	Caiman
	R..E...HS...	Crocodile, alligator
β -Globins	87 ...E..CD..H	Many species
	Q..E..CD..H	Ostrich
	T..K..CE..H	Painted turtle
α -Globins	82 E.....H....	Bison, cow, dolphin, manatee, yak
	D.....H....	Echidna, elk, goat, hyrax, sloth, whale
	D.....Y....	Jaguar, leopard, lion, tiger
α -D-Globins	E..N...YN...	Most birds, turtle

phosphorylated substrates and will hydrolyse 2'-AMP/2'-GMP and glycerol 3-phosphate at about 50% and 33% of the rate observed with InsP_1 at 2 mM (Gee *et al.*, 1988). The presence of the CONS-1 and -2 region in the comparison with glycerol 3-phosphate dehydrogenase does suggest that these two regions may represent a motif which is responsible for the binding of a glycerol 3-phosphate moiety (maintained in the structure of all of the substrates for the inositol monophosphatase).

The similarity of CONS-2 with the globins is interesting, considering that it is lysine-82 of β -globin which participates in the binding of either 2,3-bisphosphoglycerate (2,3-BPG) or inositol polyphosphate in crystallized human haemoglobin (Johnson & Tate, 1969; Arnone, 1972; Arnone & Perutz, 1974). Variant forms of haemoglobin which have this lysine replaced by either a negatively charged or a neutral show a marked decrease in the ability of polyanions to modulate oxygen affinity (Bonaventura & Bonaventura, 1980). Most species (including human) have an α -globin which contains an alanine at position 82, and thus would probably not interact with organic polyphosphates (as discussed in Bonaventura & Bonaventura, 1980). Avian haemoglobin has its oxygen affinity modulated by either 2-hydroxy- InsP_5 or an InsP_4 (Issacks *et al.*, 1977; Issacks & Harkness, 1980; Bartlett, 1982), and while the haemoglobin of some turtles contains high concentrations of InsP_5 , this compound may only have functional significance during embryonic development (Issacks & Harkness, 1980). Avian and (at least some) reptilian α -globins maintain a lysine at this position, as does the α -globin of baboons and kangaroo (Table 3), and the preferred binding site for pure InsP_5 (see Bartlett, 1982) or 2,3-BPG in either of the globin chains of these haemoglobins has not been demonstrated. The fact that the remainder of the consensus with CONS-2 involves residues in β -globin which interact with the haem moiety does suggest that this similarity is likely to be coincidental. It seems probable that this sequence has been maintained in enzymes such as inositol monophosphatase and glycerol phosphate dehydrogenase, and possibly even in some α -globins (note in Table 3 that ostrich and turtle β -globins do not

contain a lysine-82), as a general motif for the binding of organic phosphates. The substrates for inositol monophosphatase differ from 2,3-BPG and InsP_5 in that they contain a β -hydroxy group which may then bind to CONS-1.

Future work directed towards an examination of these sequence comparisons may lead to the understanding of the biochemical mechanism of this very important enzyme, and perhaps target key residues for the design of specific pharmacological compounds to regulate its activity *in vivo*.

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