at pH 3.5 revealed a single spot with an Rf of 0.80 compared to lysine.

Amino acid composition of the hexadecapeptide following acid hydrolysis was Asp(1.1), Thr(0.9), Ser(1.0), Glu(2.0), Val(1.0), Ile(1.0), Leu(4.2), Tyr(0.9), Lys(1.0), His(0.9), Arg(1.9). Complete removal of protecting groups was assessed by total enzymatic hydrolysis[8] revealing no unnatural amino acids and peptide extinction coefficient of 1690 with maximal absorbance at 277 nm.

The quantitative amount of truncated or failure sequences in the purified hexadecapeptide was determined by complete sequence analysis, a stringent technique of assessing purity[11,12]. The presence of deletion peptides for truncated sequences never exceeded 6% (cycle 8 of the degradation) on a molar basis, as evaluated by gas-liquid chromatography[13].

The dose-response relationships of the synthetic hexadecapeptide and ubiquitin are presented for prothymocyte-to-thymocyte differentiation (figure 1), CR^- B cell-to-CR^+ B cell differentiation (figure 1) and determination of intracellular cyclic AMP levels in sarcoma 180 cells (figure 2). The activity of the synthetic hexadecapeptide paralleled that of ubiquitin in each assay, showing approximately 40% activity by comparison with ubiquitin in each assay. For each assay the synthetic hexadecapeptide showed, like ubiquitin, a concentration range with maximal activity and, at higher concentrations, inhibition. The activity of the synthetic hexadecapeptide fragment of ubiquitin paralleled that of the parent molecule in all 3 assays. By contrast thymopoietin induces selective T cell differentiation in vitro[4] and a synthetic tridecapeptide fragment of thymopoientin (residues 24–41) also induced selective T cell differentiation in vitro, with no induction of CR^- to CR^+ B cell differentiation[15,16]. Additionally, the synthetic tridecapeptide segment of thymopoietin, like thymopoietin itself, produced impaired neuromuscular transmission in mice[17], an effect not caused by ubiquitin or the synthetic hexadecapeptide fragment of ubiquitin (data not shown).

Thus the synthetic peptide fragments of ubiquitin and thymopoietin showed contrasting biological activities, each having the biological characteristics of the parent molecules. We infer that each contains the amino acid sequence of the parent molecule involved in biological activity and that the putative epinephrin-mimetic active site of ubiquitin is present within the COOH-terminal 16 amino acids of the 74 amino acid sequence of ubiquitin and does not require the full tertiary structure of ubiquitin for biological activity in the assays studied. Yet there is rigorous evolutionary conservation of the entire amino acid sequence of ubiquitin, with complete identity between cattle and man[18] and close similarity of the NH_2-terminal sequence between these mammalian ubiquitins and ubiquitin isolated from the higher plant celery[4]. This suggests that there are constraints on amino acid substitutions in ubiquitin that are unrelated to the formation of an active site comprising an amino acid sequence capable of stimulating adenylate cyclase through β-adrenergic receptors. Thus, ubiquitin almost certainly has other vital function(s) (possibly nuclear[5,6]) which have contributed to its extraordinary evolutionary conservation in living cells, but which have not yet been detected in our biological assays.

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Inhibition of liver fructose 1,6-bisphosphatase activity by Zn^{2+}: Reversal by imidazole pyruvate[1]

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Summary. Imidazole pyruvate was found to be a very potent natural chelating agent in reversing the inhibition of liver fructose 1,6-bisphosphatase activity by Zn^{2+}. This metabolite may play a physiological role in gluconeogenesis.

The activity of fructose 1,6-bisphosphatase (Fru-P_2ase, EC 3.1.3.11) is markedly activated by a variety of chelating agents[3]. The basis of this activation is attributed to the chelation of the endogenous Zn^{2+}, a specific and very potent metal inhibitor of Fru-P_2ase[3,4]. Based on the observations that Zn^{2+} inhibition of Fru-P_2ase activity can be reversed by histidine at concentrations found in rabbit liver under gluconeogenic conditions[3,5], it was recently proposed that 'Zn^{2+} and histidine together may act to modulate the levels of Fru-P_2ase activity'[6]. We report here that imidazole pyruvate, which may exist in about the same concentration as histidine in liver cells[7], is significantly more potent than histidine in reversing the inhibitory effect of Zn^{2+}.

Materials and methods. Chelex 100 was obtained from Bio-Rad. Imidazole pyruvate, 1-methylhistidine, 3-methylhistidine were purchased from Calbiochem. Other chemicals were obtained from Sigma. Fru-P_2ase was purified from rabbit liver, and assayed at pH 7.5 in the presence of

2.5 mM $MgSO_4$ as previously described[8]. Whenever chelating agents were added, they were incubated with the enzyme in the assay mixture for 5 min prior to the addition of substrate. Protein concentration was determined spectrophotometrically at 280 nm. A solution containing 1 mg of purified enzyme/ml in 1-cm light path has an absorbance of 0.735.

Results and discussion. As shown in the table, the specific activity (μmoles/min/mg) of the purified enzyme was 3.1 as measured in the routine untreated assay system. It increased to 15.4 when the enzyme was assayed in a system in which all the components of the assay mixture (except $MgSO_4$) were treated with Chelex 100 according to the procedure previously described[4]. In the presence of 0.1 mM EDTA, the specific activity further increased to about 19, regardless of whether the assay system was treated or untreated with Chelex 100. This indicates that either the treatment with Chelex 100 failed to completely remove the endogenous Zn^{2+} or the $MgSO_4$ used was contaminated with a trace amount of Zn^{2+}. The table also shows that in the presence of 1.5 μM Zn^{2+}, the specific activity decreased to less than 1, and this inhibition by Zn^{2+} was completely reversed by 0.1 mM EDTA.

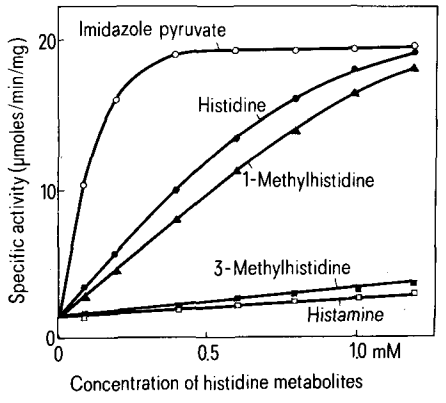

Fig. 1. Effect of various histidine metabolites on the inhibition of Fru-P_2ase activity by Zn^{2+} in a Chelex 100-treated system. Zn^{2+} (1.5 μM) and various histidine metabolites at the indicated concentrations were incubated with the enzyme in the assay mixture for 5 min prior to the addition of substrate. The nomenclature of 1-methylhistidine or 3-methylhistidine is based on IUPAC system.

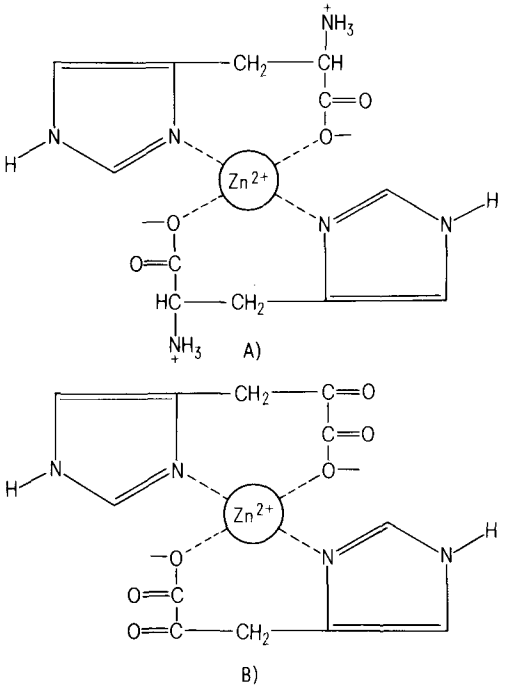

Fig. 2. A proposed structure of the chelate complex between Zn^{2+} and histidine (A) or imidazole pyruvate (B). Since Zn^{2+} has a coordination number of 4, 1 molecule of Zn^{2+} may potentially be chelated with 2 molecules of histidine or imidazole pyruvate. The imidazole ring of histidine illustrated in this figure represents the 1-H tautomer which was reported to be more stable than the 3-H tautomer[9].

The concentration of histidine in liver cells under fed and fasting conditions were reported to range from 0.2 to 1.0 mM[5]. Within this range we found that imidazole pyruvate was significantly more potent than histidine in reversing the inhibition by Zn^{2+} (figure 1). The minimum concentrations of these 2 compounds required to completely reverse the inhibitory effect of 1.5 μM Zn^{2+} were approximately 0.4 mM for imidazole pyruvate and 1.2 mM for histidine. This indicates that imidazole pyruvate is superior to histidine in chelating this metal inhibitor. According to White et al.[7], a specific aminotransferase is functioning at all times in liver cells for each α-amino acid (with the possible exception of lysine and threonine) and the equilibrium constant for all these transamination reactions is close to 1. This indicates that histidine and imidazole pyruvate are present in about the same amounts in liver cells. Thus, if histidine should be involved in the regulation of gluconeogenesis, as has been suggested[3,5,6], imidazole pyruvate might play even a more important role. We found that 1-methylhistidine was nearly as effective as histidine in reversing the inhibitory effect of Zn^{2+}, while 3-methylhistidine and histamine (the decarboxylated product of histidine) were nearly ineffective (figure 1). These data suggest that the chelation of Zn^{2+} by histidine or imidazole pyruvate involves the carboxylate group and the N-3 of the imidazole ring, as proposed in figure 2.

Specific activity of the purified liver Fru-P_2ase under various assay conditions

Additions**	Treatment of assay system with Chelex 100*	
	Untreated	Treated
None	3.1	15.4
0.1 mM EDTA	19.1	19.2
0.3 μM Zn^{2+}	1.8	4.1
1.0 μM Zn^{2+}	0.9	1.3
1.5 μM Zn^{2+}	0.8	0.8
1.5 μM Zn^{2+} + 0.1 mM EDTA	19.2	19.1

* All the components of the assay mixture (except $MgSO_4$) were treated with Chelex 100 according to the procedure previously described[4]. ** EDTA or Zn^{2+} was incubated with Fru-P_2ase in the assay mixture for 5 min prior to the addition of substrate.

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