The avian *MINPP* gene can help to alleviate the planet's "phosphate crisis"

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The chicken intestinal tract lacks phytase, so dietary phytate (IP_6) is not digested, yet this contains 80% of organic phosphate in poultry feed. Inorganic phosphate is added to the feed, which consumes the planet's reserves of a major fertilizer ingredient. Some of this phosphate, together with undigested phytate, is excreted; runoff from the manure imperils adjacent aquatic systems with phosphate-driven eutrophication (Abelson, 1999). Efforts to ameliorate this "phosphate crisis" (Abelson, 1999), by adding microbial phytase to animal feed, are costly (Ward, 2001), and represent a high-risk factor for occupational asthma and rhinitis in farm workers (Baur *et al*., 2002). A novel solution arises from our discovery that chickens already have an active, intracellular phytase, previously named MINPP (Multiple INositol Polyphosphate Phosphatase) that resides in the endoplasmic reticulum (ER).

Mammalian MINPP has not previously been considered a member of the phytase family because of its very weak activity (6 nmol/mg protein/min; microbial phytases are 10^3- and 10^4-fold more active; Mullaney *et al*., 2002). MINPP from *Gallus gallus* (Romano *et al*., 1998; Chi *et al*., 1999), with an added C-terminal poly[His] epitope tag, was expressed in *Pichia pastoris*, and purified by Ni-agarose affinity chromatography. At pH 5.0, recombinant avian MINPP hydrolyzed IP_6 with a K_m of 0.14 mM and an unexpectedly high V_{max} of 715 nmoles/mg protein/min (>100-fold greater than the phytase activity of mammalian MINPP; Nogimori *et al*., 1991). Glycosylation did not affect activity. A pH profile of MINPP showed a peak at pH 5.0, although the enzyme retained near-maximal activity over a broad range of pH 4.5 to 7.5, which also spans the pH of much of the chicken's intestinal tract. An end-point assay indicated that 80% of IP_6 phosphate was released by MINPP. Knowledge of the three-dimensional structure of microbial phytases has permitted the rational design of mutants with improved capacity to digest IP_6 (Tomschy *et al*., 2000). The structure of MINPP has not previously been determined, but we now predict a model structure that has some similarities to microbial phytases. This model attributes function to two amino-acid residues (T27; Q78) not previously thought part of MINPP's catalytic site. We verified this model by site-directed mutagenesis: A T27A mutant was 96% less active, and a Q78A mutant had 2-fold higher catalytic efficiency (i.e., K_m / V_{max}).

While MINPP has a number of useful properties as a phytase, its most practical attribute is that it is already endogenous to the chicken. Thus, we next considered what strategies might be used to deliver MINPP to its required site of action *in vivo*. Avian MINPP, like its mammalian counterparts, is normally an ER luminal resident (Romano *et al*., 1998; Chi *et al*.,

1999). We transiently over-expressed full-length MINPP in the LMH immortalized chicken liver cell line under the control of the CMV promoter. Much of the MINPP accumulated inside cells in an inactive form, possibly because it was misfolded. To avoid this problem, we next transiently expressed a truncated mutant [)ADEL-MINPP] from which the C-terminal ER-retention tetrapeptide was removed. This construct retained full activity, escaped the ER, and entered the secretory pathway; in cell cultures, 92% of activity was extracellular. While in transit through the membrane-delimited secretory pathway, the enzyme was insulated from cytosolic inositol phosphates. Thus, cellular levels of the MINPP substrates in control liver cells (IP_5 = 12.6 ± 2.4, IP_6 =12.2 ± 1.8) were similar to those in cells expressing)ADEL-MINPP (IP_5 = 11.8 ± 2.7. IP_6 = 13.9 ± 3.3). The)ADEL-MINPP expression did not affect either cell viability or proliferation.

We propose that a transgenic chicken expressing)ADEL-MINPP in intestinal epithelial cells would constitutively secrete the enzyme into the digestive tract, improve hydrolysis of dietary IP_6, and reduce the need for inorganic phosphate feed supplementation, reduce pollution, and thereby ease the planet's phosphate crisis. Public perception that it might be hazardous to introduce "foreign" proteins into food has contributed to the delay in the commercial exploitation of other transgenic animals expressing exogenous, microbial phytase (Forsberg *et al*., 2003; Hollingworth *et al*., 2003). We anticipate that the consumer can more readily accommodate genetic modifications that alter expression of an endogenous chicken gene, because it is conceptually akin to the acceptable practice of breeding.

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