Bioavailability of three homopoly amino acids in growing rats and chicks
by Nancy Creviston Dew

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Home Economics
Montana State University

Abstract:
A basic application of genetic engineering to improve protein quality of a substance is the incorporation of homopoly amino acids (HPAAs) into the original protein. Growth trials were conducted with growing rats and chicks to determine if three HPAAs, poly-L-lysine (PLL), poly-L-methionine (PLM), and poly-L-trypotophan (PLT), were nutritionally available sources of the respective amino acids. The control diet provided all amino acids in the free L-form. Other diets deviated from the control by providing lysine as PLL, methionine as PLM, tryptophan as PLT, or by being void of lysine, methionine, or tryptophan. Chick trials lasted two weeks. Rat trials lasted nine days and included a nitrogen balance study. The rats and chicks fed the control and PLL diets grew, while those fed PLM, PLT, and diets void of an amino acid demonstrated depressed or no growth. These trials present evidence that PLL is an available source of lysine and PLM is not an available source of methionine. Due to difficulties in quantitative analysis, no conclusions can be drawn regarding PLT.

It is suggested from this data that protein quality could be improved by the incorporation of PLL into the protein. The incorporation of PLM would be of no value.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date 12-2-82

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[1] Apples of Gold, compiled by Jo Petty. C. R. Gibson, Co., Norwalk, CN.

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ABSTRACT

A basic application of genetic engineering to improve protein quality of a substance is the incorporation of homopoly amino acids (HPAAs) into the original protein. Growth trials were conducted with growing rats and chicks to determine if three HPAAs, poly-L-lysine (PLL), poly-L-methionine (PLM), and poly-L-trypotophan (PLT), were nutritionally available sources of the respective amino acids. The control diet provided all amino acids in the free L-form. Other diets deviated from the control by providing lysine as PLL, methionine as PLM, tryptophan as PLT, or by being void of lysine, methionine, or tryptophan. Chick trials lasted two weeks. Rat trials lasted nine days and included a nitrogen balance study. The rats and chicks fed the control and PLL diets grew, while those fed PLM, PLT, and diets void of an amino acid demonstrated depressed or no growth. These trials present evidence that PLL is an available source of lysine and PLM is not an available source of methionine. Due to difficulties in quantitative analysis, no conclusions can be drawn regarding PLT. It is suggested from this data that protein quality could be improved by the incorporation of PLL into the protein. The incorporation of PLM would be of no value.

Chapter 1

INTRODUCTION

The shortage of food proteins that contain balanced quantities of all the essential amino acids increases with expanding world population. This fact forces a growing number of people to become dependent on plant protein sources, all of which are deficient in one or more essential amino acids. Three of the most common limiting amino acids in plant proteins are lysine, methionine, and tryptophan. Populations whose diets depends on plants as the major protein source would benefit from supplementing the diet with amino acids that are limiting in plants. An obvious method of supplementation for the purpose of improving protein quality is simply adding the chemically synthesized L-amino acid to a food product. However, this is costly and the nutritional value of the amino acid may be lost in processing (Carpenter, 1973). Other methods for accomplishing this improvement in protein quality include: 1) fermented food products produced with an organism excreting increased quantities of the limiting amino acids; 2) addition of another substance, whose protein contains complimentary amounts of the limiting amino acids, into the diet; and 3) genetic modification of the protein itself. Recombinant DNA procedures and genetic engineering techniques are methods that are capable of producing a multitude of novel protein products.

Before such protein products can be introduced to a food supply market, their biological availability and possible toxicity must be determined. As some novel proteins do not occur naturally, their

biological value may be questionable and the possibility exists that normal gastrointestinal processes may not be effective in digesting and absorbing them. One elementary example of a genetically engineered protein now available for assay is the homopoly amino acid.

This thesis was designed to assess the biological availability of three homopoly amino acids, poly-L-lysine, poly-L-methionine, and poly-L-tryptophan. This was accomplished by analysis of growth data of weanling rats and chicks fed these peptide polymers as the sole source of the respective amino acid.

Chapter 2

LITERATURE REVIEW

Introduction

The search for economical sources of high quality protein is vigorous and includes exploration of conventional and unconventional protein sources and methods of modification for the purpose of improving their quality. One such method is the genetic alteration of a protein source which would result in a protein specifically engineered for high biological value for a specific animal species. The most basic alteration for improvement of an existing protein source would be the incorporation of polymers of the limiting amino acid, or a homopoly amino acid. The nutritional value of homopoly amino acids will be determined by their physiological availability. Physiological availability is partially determined by protein quality and by the fate of the protein in digestion and absorption.

Protein Quality

Several methods for determining protein quality have been developed. Biological value for an organism is based on the proportion of absorbed nitrogen retained, or the protein's ability to support growth and maintenance (Mitchell, 1923). Net protein utilization is a measurement combining biological value with the digestibility of the food protein (Bender and Miller, 1953). Protein efficiency ratio is simply determined by dividing an animal's weight gain by its protein intake (Osborne, et. al. 1919). Although these methods have

been extensively used, they do meet with criticism and different methods for measuring protein quality have been developed and evaluated (Pellett, 1978; Hegsted and Chang, 1965).

The method of the amino acid score involves comparison of the amino acid composition of food proteins to an amino acid reference pattern (FAO/WHO, 1973). A protein deficient in one or more limiting amino acids will produce a lower amino acid score than a protein with an adequate amino acid pattern. Amino acid imbalances, or amino acids in disproportionate amounts, can also have quality lowering effects on a food's protein value.

The most basic form of measuring the biological availability of a protein is the measurement of growth of an animal fed the protein in question versus a protein known to be of good value. The purpose of the measurement is not to determine the protein's quality per se, but to determine if the protein source can be digested and utilized by the animal.

Protein Digestion

Protein digestion is a product of a variety of factors, including pH, hormones, and enzymes. The pH of the environment is important for activation of a zymogen or for providing the optimal environment an enzyme needs to be functional. Hormone secretions serve as a stimulus for organ function and the release of enzyme rich secretions. The enzymes act as catalysts in the actual degradation of the consumed proteins to the end products of free amino acids and peptides. A more detailed discussion of these functions follows.

pH

The major function pH plays in the digestion of proteins is in its effect on the enzymes necessary to cleave the peptide bonds. In the stomach, a pH 5 or below is necessary for initial activation of pepsinogen to pepsin (Tang, 1976), which itself is then capable of catalyzing the reaction of pepsinogen to pepsin. When the stomach contents are emptied into the duodenum, the chyme must be neutralized or its acidity will render pancreatic enzymes inactive. Controlled by secretin and pancreozymin released from the duodenal wall, a mechanism exists which stimulates alkaline secretions from the pancreas that effectively neutralize the acid chyme from approximately pH 2 to 6.5 (Florey, et al. 1941; Harper, 1959) within the first fifteen inches of the duodenum (Iber, 1980).

Hormones

The presence of protein in the antral portion of the stomach stimulates the production of gastrin in the gastric mucosa (Woodward, 1960). Gastrin is absorbed into mucosal capillaries and causes the fundic portion of the stomach to secrete the highly acidic gastric juice (Jacob and Francone, 1974). As chyme enters the duodenum, the presence of polypeptides and acid stimulate the release of secretin and cholecystokinin-pancreozymin (CCK-PZ) by duodenal mucosa. Secretin stimulates the release of an enzyme deficient alkaline pancreatic juice which serves to dilute the acidity of the chyme. CCK-PZ stimulates the production and secretion of the enzyme rich pancreatic juices (Jacob and Francone, 1974). CCK-PZ may also be responsible for

stimulating the release of enterokinase by the intestinal mucosal epithelial cells (Kim and Freeman, 1977). The pancreatic juices reach the duodenum via the major pancreatic duct (Jacob and Francone, 1974).

Enzymes

The first enzyme involved in protein digestion is pepsin, whose activation of the low pH environment is the result of the liberation of a 44 residue peptide from the amino-terminal end of its zymogen (Tang, 1976). Pepsin, an acid (or carboxyl) protease, has broad specificity but does exhibit some partiality toward peptide bonds adjacent to the aromatic amino acids (phenylalanine, tyrosine, and tryptophan), methionine, and leucine (Sanger and Tuppy, 1951). The proteolytic activity of pepsin initiates protein digestion, producing shorter peptides, but few free amino acids.

After the stomach, the remainder of protein degradation occurs in the small intestine. The enzymes released from the pancreas are in the zymogen forms of trypsinogen, chymotrypsinogen, proelastase, and procarboxypeptidases (Gitler, 1964). Intestinal glands lining the crypts of Lieberkuhn produce enterokinase and aminopeptidase; the former is secreted from the gland and the latter is liberated from sloughed mucosal cells (Jacob and Francone, 1974).

Activation of Pancreatic Enzymes

The first and therefore most critical activation is that of trypsinogen by enterokinase. As the pancreatic juices enter the duodenum, enterokinase is secreted and hydrolyzes a lysine-

isoleucine peptide bond in trypsinogen, thus activiting it to trypsin (Yamashina, 1956). Trypsin is then capable of activating chymotrypsinogen and proelastase to the active enzymes of chymotrypsin and elastase. Trypsin also activates carboxypeptidase A and carboxypeptidase B from their zymogens.

Substrate Specificity

Trypsin generally hydrolyzes the peptide bonds adjacent to lysine or arginine. Chymotrypsin is preferential to those bonds involving the aromatic amino acids, phenylalanine, tyrosine, and tryptophan (Ryle and Porter, 1959). Elastase exhibits partiality to the neutral aliphatic amino acids (glycine, alanine, valine, leucine, isoleucine, serine, threonine, cysteine, and methionine) (Naughton and Sanger, 1961).

Made obvious by the name, carboxypeptidases cleave one amino acid at a time from the peptide starting from the terminal carboxyl group. Carboxypeptidase A will hydrolyze any carboxyl-terminal peptide bond except those involving lysine or arginine. Carboxypeptidase B specificaly cleaves only lysine and arginine residues from this site (Neurath, 1960). The aminopeptidases attack the peptide from the amino terminal end and hydrolyze peptide bonds removing one amino acid at a time (Smith, 1960).

Completion of Protein Digestion

The digestion of proteins continues as the luminal contents pass through the small intestine. Some peptidase activity has been

documented in the rat ileal luminal contents, probably due to the enzymes liberated from sloughed mucosa, and not to secreted enzymes from the pancreas or intestine itself (Silk et al., 1976). As the digestive enzymes break the appropriate peptide bonds and the protein sources become oligo-, tetra-, tri-, and di- peptides and more free amino acids, these products make their way to the mucosa lining the small intestine. The majority of protein digestion and absorption, which occurs prior to and in the upper jejunum, is rapid (Kim and Freeman, 1977). Nixon and Mawer (1970a), using human subjects, showed that some absorption from a milk protein meal occurred in the duodenum and that 80 percent of the dietary protein was absorbed in the first fifty to one hundred centimeters of the jejunum. However, some products of a protein meal were found in the human jejunal and ileal fluids four or more hours after ingestion (Adibi and Mercer, 1973).

Transport

Nixon and Mawer (1970b) found that after a milk protein meal, some amino acids (lysine, valine, arginine, tyrosine, phenylalanine, methionine, and leucine) were absorbed rapidly in the free form. However, proline, glycine and the dicarboxylic acids which remained peptide linked much longer, were shown in a different study (Nixon and Mawer, 1970a) to be absorbed. This finding led these authors to believe that transport, and subsequent absorption of these amino acids occurred in a peptide form. The following discussion will outline current knowledge and theories of transport mechanisms

concerning free amino acids and peptides.

Transport of Free Amino Acids-Specific Carrier Systems

Transport of free amino acids across the intestinal membrane is thought to occur with the use of specific carriers. There is some agreement that four specific transport systems (Table 1) exist, (Gray and Cooper, 1971; Saunders and Isselbacher, 1966). These systems are outlined in Table 1.

Adibi and Gray (1967) demonstrated that affinity for the neutral amino acid system is greatest for methionine, followed in decreasing order by isoleucine, leucine, valine, phenylalanine, tryptophan, and threonine.

Some structural aspects are important for all the transport systems to work, as illustrated in Figure 1 (Saunders and Isselbacher, 1966). The correct stereoisomerism is required; that is the L-form for all amino acids except methionine, in which case the D-form is also acceptable. The carboxyl group attached to the alpha carbon atom must remain free and can not be substituted. The alpha amino group must also remain free, however substitution of the hydrogen of the amino group is acceptable resulting in reduced transport. Amino acids with the amino group in the beta rather than alpha position will be accepted for proper transport (Randall and Evered, 1964).

Table 1. Intestinal Amino Acid Transport Mechanisms

Type	Amino acid transported	Type of transport	Relative rate
Neutral (Monoamino-monocarboxylic)	Aromatic (tyrosine, tryptophan, phenylalanine Aliphatic (glycine,[a] alanine, serine, threonine, valine, leucine, isoleucine) Methionine, histidine, glutamine, asparagine, cysteine	Active Na+-dependent	Very rapid
Dibasic (diamino)	Lysine, arginine, ornithine, cystine	Active, partially Na+-dependent	Rapid (10% of neutral)
Dicarboxylic	Glutamic acid, aspartic acid	Carrier-mediated, ?active, partially Na+-dependent	Rapid
Imino acids and glycine	Proline, hydroxyproline glycine[a]	Active, ?Na+-dependent	Slow

[a]Shares both the neutral and imino mechanism with low affinity for the neutral (Gray and Cooper, 1971).

Competition exists among the amino acids sharing each transport system (Christensen, 1963). The amino acid with the highest affinity for the carrier would have the highest absorption rate and would inhibit the transport of the amino acids with lower affinity. Other