

Relationship between relative protein value and some *in vitro* indices of protein quality

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Abstract. Besides amino acid composition of a protein, their bioavailability is an important determinant of the protein quality. In view of the observations over the last decade or two, implicating the small peptide uptake by the mammalian intestine as a major route of protein absorption, a few animal and plant proteins were subjected to sequential enzymatic digestion *in vitro* with pepsin, pancreatin + trypsin and erepsin and the release of amino acids as small (including dipeptides) and large peptides and free amino acids, was determined. The relative protein values of α -lactalbumin, egg whites, casein, gluten, zein and protein isolates of soybeans and groundnuts was determined using rat growth method. It was observed that relative protein value were positively correlated with the essential amino acid index of protein, quantity of essential amino acids released as small peptides and the dipeptide content of enzymatic digests, while there was a negative correlation between relative protein value and essential amino acid content of large peptide fraction.

Keywords. Protein quality; relative protein value; *in vitro* indices; small peptides; dipeptide release.

Introduction

It is well recognized that amino acid (AA) composition of a protein [at least of the essential amino acids (EAAs)] determines the protein quality. However, AA composition by itself cannot predict the quality fully, because of variations in their bioavailability. Bioavailability of AAs can be influenced by several factors like protein conformation, intra and intermolecular bondings (Fukushima, 1968; Shotton and Hartley, 1970; Green *et al.*, 1973), modification of the AAs as well as the matrix of the protein in the food-stuff such as the presence of inhibitors, toxic factors, its containment in indigestible cell wall etc. (Kakade *et al.*, 1973; Pope *et al.*, 1975; Ramachandra *et al.*, 1977; Marable and Sanzone, 1981). It has been demonstrated that mammalian intestine can take up small peptides in addition to free AAs. Several kinetic advantages associated with the small peptide absorption as against the free AA absorption (Adibi, 1971; Matthews *et al.*, 1969), make the quality and quantity of digestion products of a protein, more relevant to the bioavailability of protein AAs.

Modern concepts of protein synthesis require that all AAs be available at the same time. This is determined by the rate of release of AAs from proteins during their

Abbreviations used: AA, Amino acid; EAA, essential AA; EAAI, EAA index; PDR, pepsin digest residue; PPDD, pepsin pancreatin digest dialysate; PER, protein efficiency ratio; NPU, net protein utilization; NPR, net protein retention; SPI, soya protein isolate; GNPI, groundnut protein isolate, AN, amino nitrogen.

digestion *in vivo* by enzymes of the gastro intestinal tract. The measurement of release of at least essential amino acids (EAAs) during protein digestion *in vivo* or *in vitro* should therefore be a reasonable approach to determine the bioavailability of the AAs and hence the protein quality (Stahman and Woldegiorgis, 1975). *In vivo* studies in experimental animals (Chen *et al.*, 1962) as well as in humans (Chung *et al.*, 1976) have shown that following a protein meal, the contents of the mammalian intestine are rich in small peptides of 2–6 AA residues.

Methods based on enzymatic digestion of protein *in vitro* have long been in use to determine AA bioavailability and to predict protein quality (Melnick *et al.*, 1946). The pepsin digest residue (PDR) AA index of Sheffner *et al.* (1956), pepsin pancreatin digest dialysate (PPDD) index of Mauron (1973), Ford and Salter's (1966) enzyme digestion on gel filtration column and the multienzyme (four proteolytic enzymes) digestion method (Satterlee *et al.*, 1979) for estimating a digestibility factor to determine the quality of protein are a few of the well known *in vitro* indices of protein quality. Various *in vitro* indices have been shown to be well correlated with the quality of protein as assessed by the bioassay methods in rat or human, indicating their usefulness in predicting the quality of proteins (Sheffner *et al.*, 1967).

Several methods are available to evaluate the quality of a protein using rat growth response, such as protein efficiency ratio (PER), net protein utilization (NPU), net protein retention (NPR), relative protein value (RPV) etc. However it has been shown by Samonds and Hegsted (1976), in a collaborative study between eight laboratories, that, RPVs (Hegsted *et al.*, 1968) unlike PER or NPR were more consistent with no significant interlaboratory variations.

In view of the scanty information available on the relevance of the release of AAs as peptides during digestion of proteins, to the protein quality, an attempt was made to correlate the RPVs of a few dietary proteins with the quantity and quality of peptides released during their digestion *in vitro*, sequentially with the enzymes of the mammalian gastro intestinal tract.

Materials and methods

Bovine serum albumin, hen egg white, casein (bovine milk), wheat gluten, corn zein and α -lactalbumin (bovine milk) were obtained from Sigma Chemical Co., St. Louis, Missouri, USA. Soya protein isolate (SPI) and groundnut protein isolate (GNPI) were gifted by the Central Food Technological Research Institute, Mysore. Protein isolates of rice and redgram dhal were prepared by isoelectric precipitation, washed with acetone and dried.

Pepsin (hog stomach mucosa), trypsin (porcine) and pancreatin (hog pancreas) were purchased from Sigma Chemical Co., St. Louis, Missouri, USA, while erepsin (hog duodenum) was obtained from Nutrition Biochemical Co., Cleveland, Ohio, USA. All other chemicals used, of analytical grade were obtained locally.

Relative protein value determination

RPVs of seven proteins viz. casein, α -lactalbumin, egg white, gluten, zein, SPI and GNPI were determined. After the protein content ($N \times 6.25$) of the protein sources was

Table 1. Protein content of isolates and diets and the relative protein values of some protein isolates.

Protein source	Protein* (%)	Protein in diet* (%)	Slope†	RPV
Egg white	74.9	3.99, 5.66, 9.88	4.41	110.5
α -Lactalbumin	75.4	3.88, 6.18, 9.12	3.99	100.0
Casein	81.5	4.43, 7.56, 10.26	2.70	67.6
Soya protein isolate	88.2	8.62, 11.84, 14.79	2.26	56.7
Groundnut protein isolate	87.7	8.17, 12.93, 15.38	1.68	42.0
Wheat gluten	78.8	8.40, 12.90, 17.40	0.71	17.7
Corn zein	92.9	9.70, 13.72, 18.06	0.022	0.55

* Protein % = $N \times 6.25$ (as determined by Kjeldahl's method).

† Slope of the regression line between protein intake (G) and body weight gain (G).

determined by the micro kjeldahl method, three diets with different protein contents were prepared using each protein source. The protein content of the sources and the diets used are given in table 1. The composition of the basal diet is given below:

Composition of the basal diet

Vitamin mixture USP xix (Horowitz, 1975)	1 %
Mineral mixture (NAS-NRC, 1963)	4 %
Refined groundnut oil	10 %
Starch	85 %

Choline chloride (0.2 % w/w) and vitamins A and D (vanitin) were added to the diets in appropriate quantities. The protein sources were incorporated into the basal diet at the expense of starch, to give the desired percentage of protein in the diet. The actual method used for determining the RPVs was essentially the same as described by Pellet and Young (1980).

in vitro digestion of proteins

Total amino nitrogen (AN) content of the protein sources and their AA composition were determined in their acid hydrolysates (6N HCl for 20–22 h at 110°C). Integrated EAAI of these proteins were calculated using the EAA content of α -lactalbumin (instead of egg white) as the reference protein (because α -lactalbumin was used as reference protein in RPV determination), according to the method of Oser (1951).

The proteins, suspended in 0.05 N HCl and pH adjusted to 1.8 (1 g substance in 100 ml) were digested sequentially *in vitro* with pepsin, pancreatin + trypsin and erepsin according to the method of Ford and Salter (1966). The enzymatic digests, which contained negligible amounts of TCA precipitable material, were lyophilysed, re-dissolved in minimum quantities of glass distilled water and stored frozen at -20°C till analysed. Aliquots of the reconstituted enzymatic digests were then loaded on columns of copper Sephadex G-25 and eluted according to the method of Fazakarley and Best (1965). The peptide and free AA fractions were freed of copper by treatment with H_2S

and their AA compositions were determined in an automatic AA analyser. The percentage of each EAA of the protein, in different fractions was calculated and their average was determined. The dipeptide content of the peptide fractions was determined by a method developed for this purpose (Raghunath and Narasinga Rao 1983).

Simple correlation analyses were done between RPV of proteins, integrated EAAI of proteins, dipeptide content of the enzymatic digests, EAA content of large peptide fraction (P_1) and EAA content of small peptide fraction (P_2). Multiple correlation coefficient was determined between RPV, EAAI and dipeptide content of enzymatic digest, in an attempt to see, whether or not the addition of the *in vitro* digestion parameter *viz.* dipeptide content increases the correlation between RPV and EAAI. Differences between plant and animal proteins in the *in vitro* digestion indices were tested by students 't' test and modified 't' test.

Results and discussion

Relative protein values of the proteins studied are given in table 2. The values obtained in this study are in general, in good agreement with the reported values of RPV of these proteins (Staples *et al.*, 1979; Cossack and Weber 1983). The ranking of the proteins according to the observed RPV values and the integrated EAAI tallied well and these in turn, corresponded well with the rankings reported in literature based on other quality parameters such as PER, relative growth index etc. (Jewell *et al* 1980).

Table 2. Integrated essential amino acid indices, relative protein values and some *in vitro* digestion indices of some proteins.

Protein	RPV	Integrated‡ EAAI	Dipeptide† AN in enzyme digest	EAA in large* peptide (P_1) fraction	EAA in small* peptide (P_2) fraction
<i>Animal proteins</i>					
Bovine serum albumin	N.D.	79.05	25.9	15.1	27.1
Egg whites	110.5	114.30	46.7	—	—
α -Lactalbumin	100.0	100.00	34.0	15.5	25.2
Casein	67.6	78.97	28.9	23.2	36.8
Mean \pm SEM			33.88 \pm 4.590 ^a	17.9 \pm 2.64 ^b	29.7 \pm 3.59 ^a
<i>Plant proteins</i>					
Soya protein isolate	56.7	37.40	17.3	—	—
Groundnut protein isolate	42.0	27.40	25.9	—	—
Wheat gluten	17.7	31.30	20.1	—	—
Corn zein	0.55	30.60	15.5	41.2	8.5
Rice protein isolate	N.D.	81.40	17.9	30.7	10.4
Redgram protein isolate	N.D.	51.93	14.1	34.2	7.8
Mean \pm SEM			18.47 \pm 1.708	35.37 \pm 3.087	8.9 \pm 0.777

* Values given are: percentage of EAAs of the protein, released into the fraction under consideration.

† Values given are: percentage of AN in the acid hydrolysate of enzymatic digest, contributed by dipeptides.

‡ Integrated EAAI determined according to the method described in the text.

Differences between animal and plant proteins: ^a $P < 0.01$ by modified 't' test; ^b $P < 0.02$ by students 't' test. N.D. Not determined. —, Peptides were not separated into two fractions on copper-sephadex G 25 column

Significant positive correlations were observed between RPV, integrated EAAI of proteins and the dipeptide content of the *in vitro* enzymatic digests of these proteins (table 3). These observations are in keeping with the well known concept that EAA content of the protein is a major determinant of the quality of the protein (Block and Mitchell, 1946). There was a significant positive correlation between EAAI and the quantity of dipeptides in the enzymatic digest indicating that EAA composition of the proteins may determine to a certain extent the type of digestion products they yield. This observation corroborates the findings of Jewell *et al.* (1980) and Bodwell (1981) who showed that content of certain AAs of protein could be used to predict its digestibility, which in turn is significantly correlated with the digestibility of the protein *in vivo* and *in vitro*.

Table 3. Simple and multiple correlation between *in vivo* and *in vitro* indices of protein quality of some animal and plant proteins.

Parameter	Integrated EAAI	EAA in P_1	EAA in P_2	Dipeptide in enzyme digest
RPV	0.9073 (7) ^a	-0.9996 (3) ^b	0.7351 (3)	0.8749 (7) ^a
Integrated EAAI	—	-0.8685 (6) ^b	0.6255 (6)	0.9002 (7) ^a

^a $P < 0.01$; ^b $P < 0.05$.

0.9073 and 0.8749 not significantly different from each other. Multiple correlation coefficient between RPV, integrated EAAI and dipeptide content of enzyme digest $R = 0.9171$.

Figures in parenthesis indicate the number of proteins used for the correlation analysis.

The multiple correlation coefficient between RPV, EAAI and dipeptide content of enzymatic digest ($R = 0.9171$) was not significantly different from either the correlation between RPV and EAAI ($R = 0.9073$) or between RPV and dipeptide content of enzymatic digest ($r = 0.8749$). This observation indicates that addition of an *in vitro* enzyme digestibility parameter like the amount of dipeptides released during digestion may not in any significant way improve the correlation between RPV and EAA content of proteins, in agreement with the observation of Satterlee *et al.* (1977). However, there was no significant difference between the two simple correlation coefficients *viz.* those between RPV and EAAI and RPV and dipeptide content of enzymatic digest, indicating that an *in vitro* enzymatic digestion parameter such as dipeptide content of the enzyme digest could be at least as useful as the EAA composition of the protein in predicting protein quality. This finding suggests, that the quantity of dipeptides released during *in vitro* enzymatic digestion, may be useful to predict the protein quality in the same way as PDR index, PPD index, PPDD index or multienzyme digestibility index of Satterlee, etc.

In an attempt to find out whether the RPV of the protein is related to a more general *in vitro* digestion parameter like the quantity of small peptides (2.6-3.0 AA residues on an average per peptide) and large peptides (4.8-5.7 AA residues on an average per peptide) released during *in vitro* enzymatic digestion of proteins, RPV and EAAI of the

proteins were correlated with the percentage of the EAA of the protein released into large peptide (P_1) and small peptide (P_2) fractions. It was observed that both RPV determined experimentally and EAAI of the proteins, were negatively correlated with EAA content of large peptide fraction (P_1) and positively correlated with EAA content of small peptide fraction (P_2) of the enzymatic digests of proteins. These correlations, which agree well with those of Amiot *et al.* (1981), indicate that better quality proteins yield greater quantities of small peptides than poorer quality ones, while the latter class of proteins yield greater amounts of larger peptides than the former during sequential enzymatic digestion *in vitro*. Similar correlations were also observed between the EAAI and RPV of proteins and NEAA content of P_1 and P_2 peptide fractions (data not given here).

It was observed in this study that animal proteins which have better RPVs than the plant proteins, yield significantly greater amounts of small peptides (including dipeptides) than plant proteins which give greater quantities of large peptides (table 2). These observations which are on the expected lines, are in good agreement with the observations of Amiot *et al.* (1981) and Satterlee *et al.* (1981) who also showed that animal proteins yield higher amounts of small molecular weight peptides, while plant proteins yield more of large molecular weight peptides during their enzymatic digestion *in vitro*. In general., significantly higher quantities of all classes of amino acids (such as basic, acidic, aromatic and neutral) were released as large peptides in plant protein digests, while in case of animal protein digests though larger amounts of all classes of AAs were released as small peptides, statistical significance was observed only with basic, acidic and neutral AAs (data not given here).

The results obtained in the present study, in general agreement with earlier such reports (Rich *et al.*, 1980; Marshall *et al.*, 1979), would thus suggest that an *in vitro* digestion parameter like the release of dipeptides during enzymatic digestion of proteins may be as useful as the EAA index of the protein itself, in predicting protein quality. It also indicates that differences do exist between plant and animal proteins in the type, of enzymatic digestion products and that quantity of small peptides released, more so the dipeptides, may be an important contributory factor to protein quality.

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