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The Digestibility of Gelatin Complexed with Propylene Glycol Alginate

Suhaila Binti Mohamed* & George Stainsby

Procter Department of Food Science, University of Leeds, Leeds, Great Britain

ABSTRACT

The hydrolysis of gelatin and its thermostable complexes with propylene glycol alginate by trypsin and pepsin at 37°C was monitored using ninhydrin and trinitrobenzene sulphonic acid (TNBS). The rate of breakdown of the complexes was the same as that of gelatin alone under these conditions, even though almost a fifth of the ε-amino groups are covalently bonded to polysaccharide. The TNBS method was simpler and more reproducible than the ninhydrin method.

INTRODUCTION

The ability of various proteins to form thermostable gels with propylene glycol alginate (PGA) in relation to the shape, size and structure of protein molecules has been studied (Mohamed & Stainsby, 1984*a*). The underlying idea was to make unattractive, novel or waste protein into attractive, palatable, textured gels with the addition of flavour. Extensive evidence suggests that chemical cross-links are formed between the ε-amino groups on the proteins and ester groups on PGA (McKay *et al.*, 1985). A reduction of 12–18% of the available lysine has been detected for proteins with globular and disordered structures, respectively, due to the formation of cross-bonds in the gel (Mohamed & Stainsby, 1984*b*).

Alginates are neither digested nor absorbed by humans, and the

* Present address: Faculty of Food Science and Technology, University of Agriculture, Malaysia.

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formation of a three-dimensional covalent network may hinder the digestion of the protein in the gel. The digestibility of these protein–alginate gels would also interest immobilised enzyme technologists who use these gels as the immobilising matrix (Salleh & Mohamed, 1982). The topic therefore merits investigation.

Digestibility can be measured by determining the increase in α-amino groups, liberated by the cleavage of peptide bonds. Two methods for determining alpha amino groups were compared, based on the colour formation of amino groups with ninhydrin and trinitrobenzene sulphonic acid (TNBS). These reagents were selected because they produce more intense colours with α-amino than with ε-amino groups: in the early stages of digestion the ε-amino groups far outweigh α-amino groups.

MATERIALS AND METHODS

Twice crystallised and lyophilised pepsin powder from hog stomach mucosa was bought from Sigma, containing 2755 units per mg solid. Six times crystallised and salt free lyophilised powdered trypsin was obtained from Armour Pharmaceutical Co. Ltd, Eastbourne, UK, containing 1 million units per g powder.

The alginate ester was Manucol E/RE, from Alginate Industries Ltd, and the protein used was air-dried gelatin.

Other chemicals were of reagent grade from BDH, Poole, Dorset, UK.

Treatment of gelatin for the digestibility study

To 20 ml of 3 % gelatin solution were added 10 ml 0·4 % enzyme solution (trypsin or pepsin). Seventy millilitres of the appropriate buffer was added and the mixture incubated at 37 °C. Samples were taken every 30 min, heated to inactivate the enzyme, and suitably diluted.

Gelatin–alginate gel preparation for the digestibility study

Twenty millilitres of gel were made by mixing 10 ml 6 % gelatin dissolved in pH 9·6 carbonate buffer and 10 ml 4 % PGA ester. The gels were homogenised in 70·0 ml of the appropriate buffer for the enzyme digestion. To the homogenate 10·0 ml of 0·4 % enzyme were added, followed by incubation at 37 °C. A sample from the liquid part of the

mixture (avoiding any gel suspension) was taken every 30 min. One millilitre of the sample was diluted 50 times for the ninhydrin assay, and 12·5 times for the TNBS assay. One per cent $NaHCO_3$ (pH 8·1) was used as buffer for trypsin digestion, and 0·05 M HCl containing 0·09 M KCl (pH 2·07) was used as buffer for pepsin digestion.

Ninhydrin method

Fresh ninhydrin reagent was made daily using 0·8 g ninhydrin and 0·12 g hydrindantin in 30 ml 2-methoxyethanol and 10 ml 4 M acetate buffer (pH 5·5). Twenty millilitres of the solution under test were added to 2·0 ml of ninhydrin reagent and the mixture heated in boiling water for 15 min. The tube was cooled and 3·0 ml of 50 % ethanol added. The tube was allowed to stand for 10 min before the absorbance at 570 nm was read. L-leucine (BDH) was used as standard to confirm that Beer's law was obeyed over a wide concentration range. The reaction product was stable for several hours and the molar extinction for leucine was found to be 22 300 cm^2/mol. Different amino acids produced slight differences in colour intensity, but in these experiments dealing with peptides the differences can be considered insignificant.

Trinitrobenzene sulphonic acid (TNBS) method

The method of Fields (1971) was used. Half a millilitre of the test solution was added to 1·0 ml of 0·1 M, pH 9·5, borate buffer and 0·5 ml 4 % aqueous TNBS solution. The mixture was shaken and allowed to react for 10–20 min. The reaction was stopped by adding 4 ml NaH_2PO_4 containing 1·5 mM sulphite. The extinction was read at 420 nm.

RESULTS AND DISCUSSION

The number of α-amino residues released per 10^5 g protein, during enzymatic attack, was calculated from the molar extinctions of glycine for the TNBS method ($E_{420}^{1cm} = 21\,600\ cm^2$/mol) and from the molar extinction of leucine for the ninhydrin method. No correction has been made for the molar extinction of leucine for the ninhydrin method. No correction has been made for the production of α-amino groups through autodigestion of the enzymes, as this has been shown to be almost

TABLE 1
Number of α-Amino Residues Released During Hydrolysis of Gelatin and Gelatin–PGA Complex

Time (*h*)	*Gelatin*		*Gelatin–PGA complex*	
	Trypsin	*Pepsin*	*Trypsin*	*Pepsin*
TNBS method (α-NH_2 *residues per* 10^5 *g protein*)				
0	30·3	34·0	25·5	8·50
0·5	68·2	42·5	62·1	19·25
1·0	71·2	45·0	64·5	27·25
1·5	73·3	46·5	65·2	29·25
2·0	72·1	49·3	62·1	33·25
2·5	71·8	51·3	58·7	39·25
Ninhydrin method (α-NH_2 *residues per* 10^5 *g protein*)				
0	27·4	34·1	36·4	12·5
0·5	73·8	44·3	69·4	25·9
1·0	72·6	48·1	72·9	28·0
1·5	74·1	52·2	74·0	26·8
2·0	72·9	54·5	76·1	30·6
2·5	72·9	46·6	75·2	33·5

negligible (Mohamed, 1981). The results are given in Table 1, from which it is seen that digestion by trypsin is almost complete in an hour. In this time, the number of α-amino groups released from gelatin was 40·9 residues as compared with 39·0 residues from the complex. Thus the covalent bonding in the complex did not hinder the rate of interaction with trypsin, even though the enzyme needs to diffuse through the gel to reach the sites for its hydrolytic action, and about one-fifth of the lysine groups are covalently bonded to the polysaccharide.

A similar situation prevails when pepsin is used, but the rate of attack is very much reduced. Though part of the reduced rate is due to the enzyme itself, as shown by the figures for gelatin, there is a further restriction in the extent of activity in the complex as this is de-swollen in acid, and penetration by enzyme should be more difficult than in neutral conditions.

The results presented in Table 1 do not represent quite all the amino groups that were released from the protein complexes, as the system is heterogeneous. Only those groups on fragments which have passed into solution have been determined. Others will occur on the residual gel network, and have to be excluded from the analysis as such particles, if

present in the spectrophotometer, would by their scattering produce spurious absorbance readings. When TNBS was used, the existence of orange-coloured species was particularly noticeable in the pepsin digests of the complex.

The results obtained when ninhydrin was used to monitor the digestion generally support those found using TNBS, but the ninhydrin method has disadvantages in its sensitivity to interference from traces of metal ions and from atmospheric ammonia. The TNBS is simpler, and has been found to be more reproducible for the present purpose (Mohamed, 1981).

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