STUDIES ON GRAFTING OF ACRYLONITRILE ONTO CASEIN

S. KULASEKARAN, Y. LAKSHMINARAYANA, S. RAJADURAI, K. T. JOSEPH & M. SANTAPPA

Central Leather Research Institute, Madras

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Acrylonitrile was grafted to casein using persulfate catalyst. This copolymer and pure casein were subjected to amino-acid analysis. A comparison of the compositions of the two substances indicated that about eleven amino-acids were affected. Of them, only seven—arginine, cystine, glycine, lysine, serine, threonine and valine could be interpreted as having served as grafting sites. The degree of polymerization (about 6) calculated from the degree of grafting (18.5) and the amount lost of the above amino-acid residues was very low. This may be attributed to the structural peculiarities of casein.

Introduction

In an earlier communication,[1] our preliminary findings on grafting acrylics onto casein using persulfate catalyst have been reported. In this paper, further evidence in support of the covalent binding of acrylics with casein is presented.

Experimental

a) Materials

1. Acrylonitrile (AN) from BDH was purified by standard method.
2. Casein, potassium persulfate and Tri-ethanolamine, AR, and Di-methylformamide (from E. Merck, India).
3. Sodium lauryl sulfate. (Ahura Chemicals, India).
4. Parachlorometacresol (from SISCO Chem. Industries, India).
5. Methanol (SD's Lab-Chem. Industries, India).
6. Acetic acid (Reechem Private Limited, India).

b). Methods

1. Preparation of 10% casein solution

Casein (20 g.) was soaked overnight in water (100 ml.). It was then treated gradually with a solution of triethanolamine (4 g.) in water (76 ml.) at 60°C and was kept at that temperature for 2 hrs., to obtain a clear solution of casein. pH 7.75.

2. Preparation of casein acrylonitrile copolymers

A three-necked R.B. flask (500 ml.) fitted with a mechanical stirrer, a condenser, a

thermometer, a nitrogen inlet tube and two dropping funnels was placed in a constant temperature water bath. 10% casein solution (200 g.) was placed in the reaction flask. Nitrogen was bubbled through the solution throughout the reaction. The acrylonitrile monomer (10 g.) was taken in one of the dropping funnels. A mixture of potassium persulfate (0.8 g.) and sodium lauryl sulphate (0.15 g.) was dissolved in water (10 ml.). The catalyst emulsifier mixture was taken in the other dropping funnel. The temperature of the casein solution was kept at 45°C at the start of the reaction. The monomer and the catalyst were added dropwise into the flask simultaneously over a period of 10 minutes. After the addition, the contents of the flask were kept at 45°C for 1 hour and then raised to 75°C and maintained at that temperature for 1 hour and cooled. The resultant polymer solution was obtained as pale yellow emulsion which was preserved with 0.2% parachloro meta-cresol dissolved in methanol (5 ml.).

3. Separation of homopolymer and graft copolymer from the polymer solution

The polymer solution obtained from the above experiment was treated with excess of 10% acetic acid thereby the whole polymer components got precipitated from the bath. It was filtered and repeatedly washed with distilled water. The residue after drying was taken in dimethyl formamide (300 ml.) and shaken intermittently in a shaker for a period of 72 hours. The solvent layer was separated from the undissolved residue. The solvent system on evaporation gave the homopolymer (5 g.). The undissolved residue was repeatedly washed with water and dried to give the graft copolymers (23 g.).

4. Nitrogen estimation

% Nitrogen of casein—acrylonitrile copolymer was estimated by the micro Kjeldahl method using selinium dioxide catalyst in the usual manner.[8] The nitrogen values for the casein graft and the pure casein were 16 and 14 respectively.

5. Moisture determination

This was carried out using 5 g. of the sample in the Advance Research Instruments Model M-3 Infra-red moisture balance.

6. Amino acid analysis

Caseins (100 mg. each) were hydrolysed with 6N HCl in evacuated and sealed tubes at 105°C for 22 hours. The amino-acid analysis was carried out on the hydrolyzate using Beckman Model 120C amino-acid analyser.

Results and discussion

The moisture contents of control casein and grafted casein were 8% and 7% respectively. Since the samples used for nitrogen estimation and amino-acid analyses were not predried, the figures obtained were recalculated using the corresponding moisture content to get the corresponding figures on dry basis.

The corrected percentage nitrogen values of pure and grafted caseins were 15.2 and 17.2 respectively. The percentage nitrogen in the grafted casein is the sum total of the contributions from both the polyacrylonitrile and casein and therefore it could be apportioned between these two components of the copolymer. Conversely, the

actual amount of grafted polymer could be obtained using the formula*

$$x = \frac{\%\ N \text{ of the copolymer} - \%\ N \text{ of casein}}{0.108}$$

where x is the amount of grafted polymer in the 100 g. of casein graft. Using this formula the value of x (amount of grafted polymer) for test sample was found to be 18.5185. This agreed well with the figure obtained from direct yield determination. The percentage grafting for this figure was found to be 22.7 or nearly 23%.

The amino-acid composition figures are given in Table 1. ASTM data[8] for the composition of casein are also given in the Table for comparison purposes to ascertain the degree of instrumental and other such errors. For tryptophan, ASTM value was taken as such. Since the phosphorous and carbohydrate contents in casein are very low (0.85 and 0.81 respectively) these were not estimated.[4,5]

In Table 2, the compositions of the control and grafted casein are presented in milli moles to give greater insight. Secondly, the data for the grafted casein have been recalculated for 122.72 to facilitate easy comparison between pure casein and casein graft on equal protein basis and are given in column 4. The amount of the amino-acids affected during polymerization was obtained by substracting column 4 total (798 milli moles) from column (3) total (883 milli moles). This is about 85 milli moles. To these amino acid residues, the amount of polyacrylonitrile grafted is 22.7. In colligative terms, this is equivalent to 429 milli moles. The average DP therefore is 5.0471 or say 5 approximately.

All the affected amounts of amino acids cannot certainly be taken as having served as grafting sites. For, persulfate is an oxidizing agent and many oxidizers are known to affect amino acids like tryptophan, methionine and tyrosine even where they are in reptide linkages.[6-9] Casein is already known to be amongst the simplest proteins susceptible to attack even by such large-sized reagents as enzymes.[10] As for histidine, though its imidazole ring is resistant to such strong oxidizing agents like nitric acid, chromic acid and alkaline permanganate, it has been found to be readily cleaved by peroxide type oxidizing agents such as hydrogen peroxide and per-benzoic acid.[11] Coming to serine, phosphates are linked to it in casein. Such ester linkages are known to undergo β-hydrogen elimination[12-16] and form dehydro-alanine. But, this linkage is affected even by acid hydrolysis.[17] So, an equivalent amount of serine may be assumed to have been lost in the control casein also. For that matter, all losses in acid hydrolysis in both control and grafted caseins are also equal when considered on equal protein basis as has been done above. Again, that threonine which is not linked to phosphate groups is also found missing lends credense to the idea that some of the serine residues with free

*When the polymer grafted in 100 g. of casein graft is x, the amount of casein is (100-x) g.

Nitrogen contributed (1) by the polymer is $\frac{14x}{53}$; and (2) by the casein is $\frac{100-x}{100} \times$ % N of pure casein

$$\therefore \frac{14x}{53} + \frac{100-x}{100} \times \%\ N \text{ of casein graft}$$

$$\text{On simplification } x = \frac{\%\ N \text{ of copolymer} - \%\ \text{of pure casein}}{0.108}$$

TABLE I

Amino acid	*Composition of the graft casein/100 g.*	*Composition of the control casein used*	*ASTM data*
Alanine	2.63	3.2	3.2
Arginine	2.48	3.93	4.1
Aspartic acid	6.3	7.8	7,1
Cystine	—	0.465	0.34
Glutamic acid	20.4	25.3	22.40
Glycine	0.81	1.87	2.00
Histidine	1.83	2.88	3.1
Isoleucine	5.14	6.26	6.1
Leucine	9.75	9.80	9.2
Lysine	5.66	9.84	8.2
Methionine	1.8	2.4	2.8
Phenyl alanine	4.43	5.37	5.00
Proline	9.4	11.6	10.6
Serine	3.9	5.9	6,3
Threonine	3.2	4.6	4.9
Tryptophan	—	—	1.2
Tyrosine	4.6	5.87	6.3
Valine	5.86	8.95	7.2
	88.19	116.035	110.04

TABLE 2

Amino acid	*Mol. weight*	*Test sample composition in milli moles/ 100 g. of dry casein*	*Unaffected amino acids in 122.7 g. of dry casein graft*	*Percentage affected during polymerization*
Alanine	89 . 10	35 . 9147	36 . 2267	%
Arginine	174 . 20	22 . 5603	17 . 4725	22 . 552%
Aspartic acid	133 . 10	58 . 6026	58 . 0916	0%
Cystine	240 . 30	01 . 9357	–	100%
Glutamic acid	147 . 10	171 . 9918	170 . 2034	0%
Glycine	75 . 10	24 . 9001	13 . 2372	46 . 8388%
Histidine	155 . 20	18 . 5561	14 . 4714	22 . 0127%
Isoleucine	131 . 20	47 . 7134	48 . 0817	0%
Leucine	131 . 20	74 . 6951	74 . 2056	0%
Lysine	146 . 20	67 . 3051	47 . 5138	29 . 4053%
Methionine	149 . 20	16 . 0858	14 . 8066	7 . 9524%
Phenyl alanine	165 . 20	32 . 5061	32 . 9113	0%
Proline	115 . 10	100 . 7819	100 . 2312	0%
Serine	105 . 10	56 . 1370	45 . 5421	18 . 8733%
Threonine	119 . 10	38 . 6230	32 . 9753	14 . 6226%
Tryptophan	204 . 20	5 . 876	–	–
Tyrosine	181 . 2	32 . 3951	31 . 1566	3 . 8230%
Valine	117 . 1	76 . 4304	61 . 474	19 . 6427%
		883 . 0102	798. 5444	

side chains have served as grafting sites. Free radicals from alcohols have also been obtained by oxidation with persulfate.[18] So, effectively, suffice it if tyrptophan, histidine, methionine and tyrosine are totally neglected for the calculation of DP. The data collected on these lines are presented in Table 3. The new DP works out to be 6.33 or nearly 6. Even this corrected value is quite low.

The above evidence is in favour of only the seven amino acids shown in Table 3 as the affected ones. The present observations are in line with the general theory of bond-strengths. The –SH and –NH groups are vulnerable and can be easily turned into free radicals. In the case of glycine, the NH is that of the peptide group –CONH–. Among the –CH groups, the tertiary –CH can be expected to be very weak.[19–20] (the bond strength being 90 Kcals as compared to 94 and 97 Kcals for secondary and primary CH's respectively)[21]. This is present in valine, leucine and isoleucine. But, strangely enough, only valine seems to have been affected and the reason for its odd behaviour is not clear. Still more strange is the fact that OH group also appears to have served as a grafting site. Normally, in respect of homolytic reaction. C–H (108 Kcals) is stronger than the –CH (@ 90 to 97 Kcals), group. But, this is so in respect of an isolated molecule. In a medium, the bond strength of a polar bond depends upon the dielectric constant of the medium. Since OH is 40% ionic according to Pauling and since water is a highly polar solvent, it is likely that OH has become weak enough for the hydrogen to be easily abstracted.[22]

Any grafting usually results by a chain transfer mechanism, even when the initiator used has a tendency to promote homopolymerization. Active centres created on the protein back bone by such mechanism remain immobilized in which condition, the termination/chain tranfer actions have been shown to be far less frequent. Conse-

TABLE 3

Amino acid	*Test composition in milli moles/ 100 g. of dry casein*	*Unaffected amino acid in 122.75 g. of dry casein graft*	*Affected amino acid amount*
Arginine	22.5603	17.4723	5.0878
Cystine	01.9357	01.9357	0.0
Glycine	24.9001	13.2372	11.6629
Lysine	67.3051	47.5138	19.7913
Serine	56.1370	45.5421	10.5949
Threonine	38.6230	32.9753	5.6477
Valine	76.1304	61.4174	15.013
			67.7859

quently, the rate and molecular weight (or DP for that matter) are higher.[24] But, in the present case, the grafted chains, have been found to be of low DP (5 or 6). This may be partly due to the alkaline medium, which has the effect of good solvent and partly, it may be caused by the structural peculiarities of the protein itself.

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