Degradation of Encephalitogenic Protein in Aerobic Ascorbic Acid Solutions[1]

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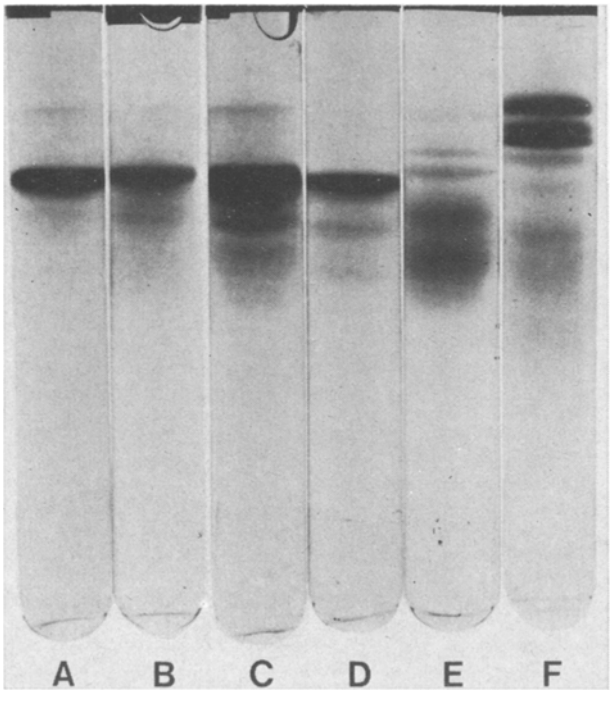
Summary. Aerobic ascorbic acid solutions are capable of extensively cleaving the peptide chain of the myelin basic protein. Cleavage occurred most readily with C-terminal to aspartic acid, serine, threonine, glutamic acid and leucine residues.

Thirty percent of the protein content of central nervous system myelin consists of a single basic protein[3]. This protein is responsible for the autoimmune disease allergic encephalomyelitis[4,5]. Its properties have been studied extensively because of its involvement not only in this disease, but also because of its potential involvement in human demyelinating diseases such as multiple sclerosis. It is reported to be a highly ordered single chain rod-shaped protein having a specific tertiary structure which is asymmetrical and relatively devoid of α helical or β-structure[6]. It is an excellent substrate for acid and neutral proteinases, and its tyrosines are all readily iodinated. The basic protein has been sequenced and contains 170 amino acids. Its basic charges are spread rather evenly throughout the molecule. The basic protein contains no cysteine and a single tryptophan.

ORR[7] has shown that catalase incubated with ascorbic acid results in degradative changes in the catalase molecule. Similarly, RICHHEIMER and ROBINSON[8] have shown that the ammonia liberated from transferrin incubated with ascorbic acid could not be completely accounted for by deamination of asparaginyl and glutaminyl residues within the molecule. Therefore, they suggested that the degradative effect of ascorbic acid on proteins results from breakage of the peptide chains and deamination of the resulting peptides. This paper presents data indicating that ascorbic acid degradation of basic protein also results in the cleavage of the peptide chain.



Polyacrylamide gel electrophoresis at 37 °C after 48 h of solutions which were initially a) 10^{-4} *M* basic protein; b) 10^{-4} *M* basic protein, 0.01 *M* H_2O_2, and 0.01 F $FeCl_3$; c) 5×10^{-4} *M* basic protein, 0.10 *M* H_2O_2, and 0.10 F $FeCl_3$; d) 10^{-4} *M* basic protein and 0.01 F ascorbic acid; e) 5×10^{-4} *M* basic protein and 0.10 F ascorbic acid (proteins of molecular weight greater than 12,000 molecular weight as determined by Sephadex chromatography); and f) 5×10^{-4} *M* basic protein and 0.10 F ascorbic acid (proteins of molecular weight less than 12,000 molecular weight as determined by Sephadex chromatography). All solutions were prepared in I = 0.2, pH 7.4 phosphate buffer.

Materials and methods. The bovine myelin basic protein was provided by Eli Lilly and Company. The protein was dissolved in 10 ml aliquots of pH 7.4 phosphate buffer which was 0.0146 F KH_2PO_4 and 0.0620 F Na_2HPO_4. 5 separate reactions were performed: 1. 10 ml buffer, 20 mg protein and 17.6 mg ascorbic acid (0.01 *M*). 2. 10 ml buffer, 20 mg protein, 10 µl H_2O_2 (0.01 *M*) and 10 µl ferric chloride solution (0.01 *M*). 3. 10 ml buffer and 20 mg protein. 4. 10 ml buffer, 100 mg protein and 176 mg ascorbic acid (0.1 *M*). 5. 10 ml buffer, 100 mg protein, 100 µl H_2O_2 (0.1 *M*) and 100 µl ferric chloride solution (0.1 *M*). After 48 h at 37 °C, the solutions were passed down separate Sephadex G-76 columns (4×100 cm). The effluent was monitored at both 280 nm and 238 nm. Gel electrophoresis was performed on each reaction mixture by the procedure of REISFELD, LEWIS and WILLIAMS[9].

In another set of experiments separate reactions were performed as follows: 1. 4 mg basic protein and 3.5 mg ascorbic acid were dissolved in 2.5 ml of either the phosphate buffer or 0.2 *M* triethylamine/carbonate buffer (pH 8.0). The reaction was for 48 h at 37 °C. 2. 4 mg basic protein and 35 mg ascorbic acid were dissolved in 5 ml buffer of either the phosphate buffer or 0.2 *M* triethylamine/carbonate buffer (pH 8.0). The reaction was for 48 h at 37 °C.

C-terminal analysis was performed[10] on each of the reaction mixtures before and after the small ions were removed by passing the reaction mixtures down a Sephadex G-10 column (1.8×55 cm). Each column was separately made using unused Sephadex to prevent any amino acid contamination of the samples.

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C-terminal analysis of degraded basic protein

Amino acids[a]	Relative amounts of aa's in protein	Relative amounts of C-terminal groups			
		0.1 F ascorbic acid		0.01 F ascorbic acid	
		Phosphate buffer	Triethylamine buffer	Phosphate buffer	Triethylamine buffer
Arg[b]	1.00	1.00	1.00	1.00	1.00
Ala	0.78	0.01	0.005	0.03	0
Asp	0.50	0.07	0.75	0	0.41
Flu	0.44	0.05	0.83	0	0.54
Gly	1.39	0.05	0.03	0.09	0
Leu	0.56	0.06	0.22	0	0
Ser	1.06	0.07	0.52	0.04	0.28
Thr	0.30	0.03	0.23	0.01	0.13

[a] All amino acids are listed which were observed on C-terminal analysis to be present in concentrations greater than 10^{-3} that of arginine in any one of the 4 experiments.
[b] Quantitative comparison of the C-terminal analysis of untreated basic protein with the analysis of treated basic protein indicated that only the C-terminal arginine of the protein contributed to the arginine value determined from the degraded protein. Therefore, we have assumed that the arginine concentration in the degraded and undegraded protein solutions are identical.

Results and discussion. The auto-oxidation of ascorbic acid is a complex process which produces many products including hydrogen peroxide and free radicals. The Figure shows the gel electrophoresis of the basic protein after treatment with either hydrogen peroxide or ascorbic acid. The greater the concentration of either the ascorbic acid or the peroxide, the more extensive the degradation of the protein. In these experiments the breakdown due to ascorbic acid appears to be greater than that due to the peroxide.

The gel electrophoresis of the degraded protein does not indicate the nature of the degradation. In order to study the peptide breakage, we performed C-terminal analysis on the degraded material. The basic protein contains a single C-terminal residue, arginine. The Table gives the relative amounts of amino acids recovered from C-terminal analysis of the degraded protein. Also given in the Table is the content of each amino acid contained within the protein relative to arginine, as determined by amino acid sequence of the protein[11].

By comparing the relative amounts of the different amino acids with the undegraded protein with those observed from C-terminal analysis of the degraded protein, one can determine whether or not the degradation by ascorbic acid possesses a sequence specificity. It would appear that there is some specificity of the reaction. Peptide chains with C-terminal aspartic acid, serine, threonine, glutamic acid and leucine were released most easily. The degradation was more extensive in the triethylamine buffer. It is known that in the presence of phosphate, the basic protein aggregates (E. H. EYLAR, personal communication). The aggregation is minimal in the triethylamine buffer; therefore, the aggregation of the basic protein may have an effect on ascorbic acid degradation.

We have shown that ascorbic acid is capable of extensively cleaving the peptide chain within the myelin basic protein. It has been suggested that the protein degradation which has now been demonstrated in aerobic ascorbic acid solutions of catalase[7], transferrin[8,12], and basic protein may be related to the biological function of ascorbic acid[13].

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Effect of Cholesterol Oxidation on (Na^+, K^+) ATPase Activity of Erythrocyte Membranes

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Summary. By incubation of human erythrocyte ghosts with cholesterol oxidase (EC 1.1.3.6) part of the cholesterol of the membrane is replaced by 4-cholesten-3-one. This alteration in the sterol composition is accompanied by an inhibition of the (Na^+, K^+) ATPase of the erythrocyte membrane.

In a previous paper[2] we were able to demonstrate that the alterations in (Na^+, K^+) ATPase activity of erythrocytes from rats fed 20,25-diazacholesterol is due to the partial replacement of cholesterol by its biological precurser, desmosterol. In order to get more insight into the role of cholesterol on (Na^+, K^+) ATPase activity, we tried to alter the cholesterol molecule in the intact membrane.

A very simple method to induce changes in the sterol fraction of erythrocyte ghosts is the oxidation with cholesterol oxidase (cholesterol-O_2 oxidoreductase, EC 1.1.3.6) from Norcardia erythropolis. This enzyme effects the oxidation of cholesterol to 4-cholesten-3-one in the

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