samples (Shaw and Coleman, 1974). The oils from all the chemically treated oranges contained less of the major terpene alcohol, linalool, and more of the aldehydes α-sinensal, citronellal, and dodecanal and of the sesquiterpene hydrocarbon valencene than the oil from control fruit. An increase in α-sinensal, which has an odor threshold in water of only 3.8 ppb (Ahmed and Dougherty, 1973), may be significant since its aroma resembles that of overripe citrus.

When control and treated oils from well-matured Valencia were compared, only minor quantitative differences in major constituents were observed. In quantitative composition the oils from control and treated, well-matured Valencia oranges were similar to the oil from the treated, barely-mature fruit. This finding suggests an acceleration of the maturing process when barely-mature oranges are sprayed with rind-injuring abscission agents, but not when well-matured fruit are similarly treated. Similar effects were observed in oils from early- and mid-season oranges.

The overripe flavor observed in oil from barely-mature oranges treated with rind-injuring abscission agents was probably not primarily due to an increase in α-sinensal content since this flavor was also present in oils from well-mature treated fruit with the same concentration of α-sinensal as the control oils. Other components must contribute to the overripe flavor, and their identities and flavor effects in juice and oil will have to be studied.

The flavor evaluations of this study were made only by a trained panel of expert tasters. One further important point that must be determined is whether the general consumer can detect a difference in juice flavor when oil from treated fruit is used, and whether any difference that might be detected is objectionable.

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Effects of Alkali on Glycoproteins. β-Elimination and Nucleophilic Addition Reactions of Substituted Threonyl Residues of Antifreeze Glycoprotein

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A small molecular weight antifreeze glycoprotein (AFGP) was used as a model protein to study the effects of alkali on a glycoprotein containing glycosyl groups O-linked exclusively to threonyl residues. Further, this AFGP contains no phosphate, sulfhydryl, or disulfide groups. Base-catalyzed β elimination is involved in removal of the carbohydrate moieties from the protein resulting in formation of olefinic amino acid residues (β-methyldehydroalanine). The rate and extent of β elimination under various conditions of pH and temperature were monitored by absorbance at 241 nm or by loss of threonine or galactosamine. Initial rate data indicate that the β-elimination reaction is dependent on hydroxide ion concentration but not on AFGP concentration. Nucleophilic additions of sodium sulfite and N^α-acetyl-L-lysine to the unsaturated residues were studied. The addition products of N^α-acetyl-L-lysine were identified by periodate treatment and by combined GC/MS to be the D and L isomers of N^ϵ-(1-methyl-2-amino-2-carboxyethyl)-L-lysine.

Glycoproteins serve a wide range of important biological functions and are widely distributed (Spiro, 1973). The two major types of bonds between the carbohydrate and the protein are easily distinguished by their different susceptibilities to alkaline and enzymatic cleavage, and a number of techniques for their identification have been developed (Downs and Pigman, 1976). When the carbohydrates are linked to the amide group of asparagine, the bond is relatively resistant to alkali. When the carbohydrates are attached by O-glycosidic linkages to seryl or threonyl residues, base-catalyzed β elimination is involved in the facile removal of carbohydrate chains from the protein core of the glycoprotein (Pigman and Moschera, 1973). Glycoproteins with base-labile O-glycosidic linkages occur mainly in animal protein sources (Spiro, 1973).

β elimination of substituted seryl and threonyl residues has been described for a wide variety of complex proteins (Tanaka et al., 1964; Adams, 1965; Carubelli et al., 1965; Gottschalk, 1972; Simpson et al., 1972; Plantner and

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Carlson, 1975). Dehydroalanine is formed from substituted serine and β-methyldehydroalanine from substituted threonine. Formation of these unsaturated components has been monitored by absorption at 240 nm (Carter and Greenstein, 1946; Price and Greenstein, 1947; Neiderhiser et al., 1971). Following β elimination, acid hydrolysis yields pyruvate from dehydroalanine and α-ketobutyrate from β-methyldehydroalanine; formation of the α-keto acids has been utilized to study the relative rates of β elimination from serine and threonine, and to determine the ratio of glycosylated serine to glycosylated threonine in proteins (Plantner and Carlson, 1975). The unsaturated β-elimination products can be reduced with $PdCl_2$ or aqueous sodium borohydride to alanine and α-aminobutyric acid (Tanaka and Pigman, 1965).

The nucleophilic addition products of dehydroalanine have been studied extensively because dehydroalanine can also be formed from O-phosphoserine (Sen et al., 1977) and cystine (Nashef et al., 1977) residues in proteins by the alkaline β-elimination reaction (Corfield et al., 1967; Ziegler et al., 1967; Asquith et al., 1969; Simpson et al., 1972; Spiro, 1972). Two cross-linked products of nutritional significance have been characterized as new amino acids, lanthionine (Horn et al., 1941) and lysinoalanine (Bohak, 1964; Patchornik and Sokolovsky, 1964). They are nucleophilic addition products of the sulfhydryl group of cysteine and the ϵ-amino group of lysine residues, respectively, to dehydroalanyl residues. DL-Lanthionine was shown to support growth of rats to the extent that the L component is cleaved to yield one molecule of L-cysteine (Jones et al., 1948). The nutritional implications and possible toxic effects of lysinoalanine are still being investigated (*Nutr. Rev.*, 1976).

Dehydrothreonyl (β-methyldehydroalanyl) residues, formed from O-glycosylthreonyl or O-phosphothreonyl residues under alkaline conditions, and their addition products, are less well characterized due to the comparative lack of suitable model proteins and the possibly lower reactivity of the dehydrothreonyl grouping (Spiro, 1972; Pigman and Moschera, 1973; Rando, 1974). Investigators in our laboratory had previously isolated and characterized an antifreeze glycoprotein (AFGP) from fish serum, which has particular advantages for such studies. AFGP contains carbohydrate substituted exclusively to threonyl residues through O-glycosidic linkages, but it contains no phosphate, sulfhydryl, or disulfide groups, and only one poorly reactive nucleophile, the α-amino group of an amino terminal alanine (DeVries et al., 1971; Feeney, 1974). The alkaline β-elimination reaction was utilized by earlier workers to establish the structure and role of carbohydrate in AFGP (Feeney, 1974). In this paper we report on studies on the β elimination of carbohydrate from AFGP under various experimental conditions, and on the identification of external nucleophilic addition products.

MATERIALS AND METHODS

Materials. Antifreeze glycoproteins from Antarctic fish were isolated and purified as previously described (DeVries et al., 1971). The smallest inactive antifreeze glycoprotein, AFGP-8 (2700 g/mol), was used for the following studies due to its relatively greater availability. AFGP-8 consists of four repeating tripeptides of Ala-Ala-Thr, with an additional one or two alanines at the C-terminal, in which each threonyl residue is glycosidically linked to the disaccharide galactosyl-N-acetylgalactosamine. AFGP-8 also differs from the larger active AFGP by having some proline following threonine in the sequence. A 1→3 glycosidic linkage between the two monosaccharides was reported by Vandenheede et al. (1972).

Sodium sulfite was purchased from J. T. Baker Chemical Company; N^α-acetyl-L-lysine, L-lysine monohydrochloride, α-glycyl-L-lysine hydrochloride, and N^ϵ-methyl-L-lysine hydrochloride were from Sigma Chemical Company; trifluoroacetic anhydride, 1.25 N HCl in methanol and 1.25 N HCl in butanol, were from Regis Chemical Company. All other reagents were of analytical grade.

Alkaline Treatment of AFGP-8. AFGP-8 was treated in most cases with 0.5 N NaOH at a concentration of 6 mg/mL (2.22×10^{-3} M) at various temperatures in a thermostatically controlled water bath. The reactions were monitored by absorbance at 241 nm after dilution with water to a concentration of 0.1 mg/mL and by analysis for amino acids and galactosamine. To study the effect of pH on β elimination, reactions were performed in 0.2 M phosphate–citrate or –NaOH buffers (pH 8.5, 9.4, 10.3, and 11.3 as measured at 50 °C) in a 50 °C water bath for 24 h. The effect of protein concentration on the initial rate of the alkaline β-elimination reaction was examined over a tenfold concentration range of AFGP-8 (0.02–0.2 mg/mL) in 0.5 N NaOH at 60 °C by following absorbance at 241 nm in a Cary 118C spectrophotometer equipped with a thermostated cuvette holder.

Amino Acid and Galactosamine Analyses. Aliquots of AFGP-8 subjected to alkaline treatment were taken at different time intervals and hydrolyzed first for galactosamine (3 N HCl, 95 °C, 15 h) and then for amino acids (6 N HCl, 110 °C, 22 h) in evacuated, sealed tubes; analyses were done on the Technicon automatic amino acid analyzer. Norleucine was added prior to acid hydrolysis to serve as an internal standard.

Sulfite Addition. Sulfite additions were done in 0.1 M Na_2SO_3–NaOH solutions (pH 9.3, 10.1, 11.0, and 11.9 as measured at 37 °C) on β-eliminated AFGP-8 preparations (0.5 N NaOH at 37 °C for 24 h) which had been neutralized, desalted on a Sephadex G-10 column, and then lyophilized. Three milligrams of the lyophilized β-eliminated AFGP-8 were incubated with 1 mL of one of the four Na_2SO_3 solutions at 37 °C for 24 h. The samples were then hydrolyzed in 6 N HCl for amino acid analysis. Cysteic acid was used as the standard.

N^α-Acetyl-L-lysine Addition. Fifty milligrams of AFGP-8 and 70 mg of N^α-acetyl-L-lysine were reacted in 5 mL of 0.5 N NaOH at 50 °C for 24 h. The sample was then neutralized with HCl and desalted on a Sephadex G-10 column. The protein peak was lyophilized and hydrolyzed in 6 N HCl. The hydrolyzed sample was run on the amino acid analyzer using the pH 5 buffer system of Williams and Woodhouse (1967). New peaks were collected separately from the analyzer, then passed through a Bio-Rad AG 11A8 ion retardation column to remove most of the salts. The isolated new peaks were oxidized with periodate according to the procedure of Bohak (1964) and then rerun on the amino acid analyzer. Combined gas chromatograph–mass spectrometry of the trifluoroacetyl-O-n-butyl ester derivatives of the new amino acids were performed on a Finnigan Model 3200 combination mass spectrometer–gas chromatograph (Finnigan Model 9500) equipped with a Finnigan Model 6000 MS data system. The gas chromatograph was equipped with a column (5 ft $\times$ 0.25 in.) of 3% OV-17, using a temperature program of 4 °C/min (from 75 to 250 °C) and N_2 carrier gas. Derivatives were prepared according to the procedure of Gehrke and Stalling (1967).

RESULTS AND DISCUSSION

Alkaline Treatment of AFGP-8. Initially, absorbance at 241 nm was used to follow the β-elimination reaction of AFGP-8, but interference, due to chromogen formation

Table I. Effect of Time on β Elimination of Antifreeze Glycoprotein on Losses of Threonine and Galactosamine[a]

Time, h	Threonine loss, %	Galactosamine loss, %
0	0	0
0.25	16	22
1	24	30
2	46	53
4	59	58
7	61	68
10	65	73
24	76	88
48	86	92

[a] Reaction was done at 6 mg of AFGP-8/mL of 0.5 N NaOH at 37 °C. Norleucine was added as internal standard in the amino acid analyses.

Table II. Effect of Temperature on Extent of β Elimination of Antifreeze Glycoprotein[a]

Temp, °C	Threonine loss, %	Galactosamine loss, %
3	7	11
23	59	60
37	76	83
50	88	100
60	90	nd[b]

[a] Reactions were done at 6 mg of AFGP-8/mL of 0.5 N NaOH for 24 h. Norleucine was added as internal standard in the amino acid analyses. [b] Not determined.

Table III. Effect of pH on Extent of β Elimination of Antifreeze Glycoprotein[a]

pH[b]	Threonine loss, %	Galactosamine loss, %
8.5	0.7	2.4
9.4	2.0	6.3
10.3	15	nd[c]
11.3	53	54

[a] Reactions were done at 6 mg of AFGP-8/mL in the appropriate 0.2 M phosphate-citrate or -sodium hydroxide buffer at 50 °C for 24 h. [b] Measured at 50 °C. [c] Not determined.

(Vandenheede et al., 1972), at this wavelength was present to various extents at different temperatures. Attempts to obtain an extinction coefficient for β-methyldehydroalanyl residues were not successful due to difficulties involved in separating the interfering materials from the small-sized AFGP-8. We therefore chose to monitor the reactions by following loss of threonine by amino acid analysis in most cases. Data obtained by absorbance at 241 nm were not used in subsequent thermodynamic calculations. A typical reaction course is shown in Table I. At 37 °C, 46% (1.84 residues/mol) of the threonine and 53% (2.12 residues/mol) of the amino sugar had been destroyed after 2 h. The figures were 76 and 88%, respectively, after 24 h. The losses of galactosamine were 8–10% higher than the losses of threonine at all times. This was also observed in other alkaline treatments with AFGP-8 at various temperatures and pH values. Plantner and Carlson (1975) found that the losses of β-hydroxy amino acids during β elimination and hydrolysis of mucin-type glycoproteins were always greater by 10–20% than the release or destruction of N-acetylgalactosamine. They attributed this observation to nonspecific losses of the hydroxy amino acids. In the case of AFGP-8, base hydrolysis of O-glycosyl side chains and peptide bonds resulting in a O-terminal substituted threonyl residue is possible since we were unable to obtain complete destruction of all threonine even at 60 °C for 24 h (Table II). Losses in galactosamine were greater since its destruction can occur in two ways: (1) N-acetylgalactosamine is rapidly converted to the Morgan-Elson chromogen when the sugar chain is released by base-catalyzed β elimination (Vandenheede et al., 1972; Plantner and Carlson, 1975), and (2) when the disaccharide is free, as released by base hydrolysis, alkali is responsible for the equivalent of a β elimination with the removal of galactose and the formation of a double bond in N-acetylgalactosamine if the glycosidic linkage is 1→3 (Mayo and Carlson, 1970; Pigman and Moschera, 1973).

Komatsu (1969) found that treatment of AFGP-8 with 0.5 N NaOH at 20 °C for 6 h resulted in the destruction of only 50% of the threonine, consistent with our current results. However, the same treatment of active AFGP (molecular weights >10 000 but with no proline) resulted in complete loss of threonine and the appearance of an equivalent number of reducing sugars (Komatsu et al., 1970). DeVries et al. (1971) incubated active AFGP with 0.2 N NaOH at 45 °C for 6 h and obtained practically sugar-free protein with no detectable splitting of peptide bonds.

A possible explanation for the more labile O-glycosides of active AFGP is the absence of prolyl residues. Heller and Raftery (1976) reported that prolyl residues are interspaced between two threonyl residues in vitelline envelopes of limpet eggs. Extensive alkaline incubation of vitelline envelopes (0.5 N NaOH at 37 °C for 144 h) could not destroy all threonines; an equality in molar ratio between threonine and galactosamine suggested that the sugar linkage to protein might be through an O-glycosidic bond. The possible role of neighboring prolines in the enhanced resistance of substituted threonines to β elimination is only speculative. The proximity of proline to substituted threonine was also reported for other glycoproteins such as bovine κ-casein (Fiat et al., 1972), IgG H chain (Smyth and Utsumi, 1967), and mucin derived from human colloid breast carcinoma (Adams, 1965).

Effect of Temperature. The relative degrees of β elimination of AFGP-8 in 0.5 N NaOH for 24 h at different temperatures are shown in Table II. No galactosamine could be detected after 24 h at 50 °C, while 10% of the threonyl residues still remained at 60 °C after 24 h. Again, the loss of galactosamine was greater than that of threonine. The extent of destruction of threonine, hence the extent of β elimination, increased almost linearly with the increase in temperature up to about 40 °C and then started to level off. This could suggest that the reactions at lower temperatures did not approach completion after 24 h.

Effect of pH. Table III shows the effect of pH on the relative extents of β elimination of AFGP-8. At pH 11.3, about 50% of the original threonine was lost after 24 h at 50 °C. Only slight losses occurred at pH values below 10 under the same conditions. Greater losses of galactosamine were again observed. The initial rate of β elimination of AFGP-8 was found to be directly dependent on the concentration of hydroxide ion used for the reaction (Table IV). The reason for the marked increase in initial rate at pH 8.5 (50 °C) is not known, although the result was readily reproducible.

Effect of Concentration of AFGP-8. At the dilute concentrations of proteins used, initial rates of β elimination as determined by absorbance at 241 nm were found to be independent of protein concentration over a tenfold range (0.02–0.2 mg/mL 0.5 N NaOH) at 60 °C.

Kinetic and Thermodynamic Parameters of β Elimination of AFGP-8. Initial rates of β elimination

Table IV. Effect of Hydroxide Ion Concentration on
Initial Rate of β Elimination of Antifreeze Glycoprotein[a]

[OH⁻], M × 10²	Initial rate,[b] min⁻¹	Initial rate/[OH⁻], M⁻¹ min⁻¹
0.001	4.13 × 10⁻⁵	4.13[c]
0.01	6.14 × 10⁻⁵	0.614
0.1	5.70 × 10⁻⁴	0.570
1.0	2.26 × 10⁻³	0.226
50[d]	2.04 × 10⁻¹	0.408
	Av	0.454

[a] Reactions were done at 3.70×10^{-5} M AFGP-8 in 0.2 M phosphate buffers (see Table III) at 50 °C and monitored by OD_{241} readings. [b] Expressed as [β-methyldehydroalanine]/[glycothreonine]min where β-methyldehydroalanine concentration was calculated by using a molar extinction coefficient of 4200 M⁻¹ cm⁻¹ (Carter and Greenstein, 1946). [c] Left out of average. [d] 0.5 N NaOH was used.
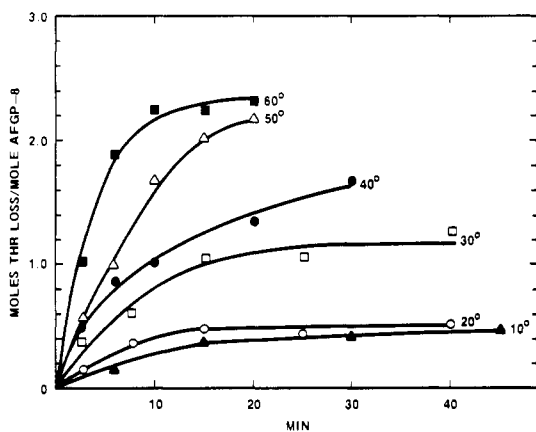
Figure 1. Time dependence of loss of threonine from antifreeze glycoprotein β eliminated in 0.5 N NaOH at several temperatures. Threonine loss was calculated on basis of four residues per mole of AFGP-8.

Figure 2. Arrhenius relationship for initial rates of β elimination of antifreeze glycoprotein. Rates were calculated on basis of threonine loss from Figure 1 using pseudo-first-order kinetics.

Table V. Sulfite Addition to β-Eliminated Antifreeze Glycoprotein at Different pH Values[a]

pH[b]	Sulfite addition, %
9.3	55
10.1	70
11.0	67
11.9	69

[a] AFGP-8 was alkaline treated for 24 h at 37 °C in 0.5 N NaOH to give 76% decrease of threonine. Three milligrams of the treated AFGP were incubated for 24 h at 37 °C in 1 mL of four solutions containing 0.1 M Na_2SO_3 adjusted with NaOH to the desired pH. Percent addition was calculated according to the actual available β-methyldehydroalanine. Cysteic acid was used as standard on the amino acid analyzer. [b] Measured at 37 °C.

(threonine loss) of AFGP-8 at different temperatures are shown in Figure 1. The plateau observed after an initial increase in the rates at the lower temperatures may suggest that some O-glycoside groups are more labile to alkaline removal than others. In Figure 2, the Arrhenius plot for the β elimination reaction gives an activation energy of 9.60 kcal/mol substituted threonine. This value is about half of those obtained for the β elimination of most disulfides (Nashef et al., 1977) and O-phosphoseryl residues (Sen et al., 1977). The free energy (ΔF^*) value of 22.4 kcal/mol is similar to that for β elimination of disulfide (20 kcal/mol) and O-phosphoserine (24.1 kcal/mol). Enthalpy (ΔH^*) and entropy (ΔS^*) for the β elimination of O-glycosides from threonines in AFGP-8 were calculated to be 8.94 kcal/mol and -39.9 cal deg⁻¹ mol⁻¹, respectively, at 60 °C. Donovan et al. (1972) found an activation energy of approximately 7 kcal/mol for the alkaline degradation of the glycoprotein ovomucin, changes in the carbohydrate composition of which have been suggested to be partly responsible for egg-white thinning occurring in eggs (Kato et al., 1970; Kato and Sato, 1972; Smith et al., 1974; Osuga and Feeney, 1977).

Sulfite Addition. Based on earlier reports (Simpson et al., 1972; Spiro, 1972), the sulfite addition reaction was investigated as a method for quantifying the β-elimination reaction. The results of sulfite additions to alkaline treated AFGP-8 in solutions of various pH values are shown in Table V. The β-eliminated AFGP-8 had a 76% loss in threonine by amino acid analysis. Above pH 10.1, about 70% addition to β-methyldehydroalanyl residues was achieved. At pH 9.3 only 55% addition was observed.
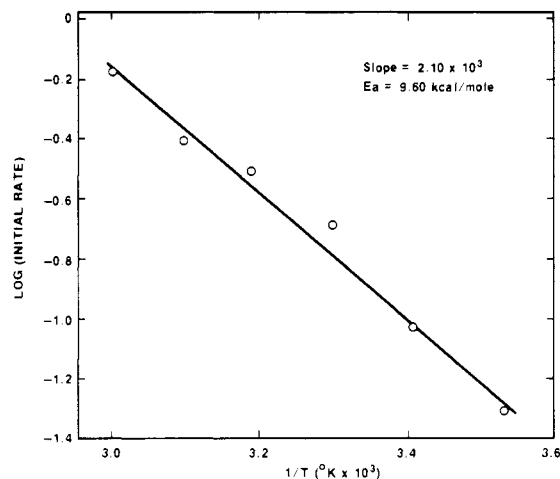
Komatsu et al. (1970) previously reported only 30% conversion at pH 9 with alkali-treated active AFGP. The sulfite addition product, 2-amino-3-sulfonylbutyric acid, has been isolated and characterized by Spiro (1972) who reported a 75% conversion while the conversion to cysteic acid through dehydroalanyl residues can be made quantitative. The extra methyl group of β-methyldehydroalanyl residues probably presents some steric hindrance to nucleophiles. Since we were also unable to obtain quantitative conversion under our experimental conditions, this alkaline–sulfite treatment was not utilized to follow the formation of β-methyldehydroalanyl residues.

Addition of N^{α}-Acetyl-L-lysine to β-Methyldehydroalanine. The addition product formed from reaction of N^{α}-acetyl-L-lysine and β-methyldehydroalanine is of special interest due to the reported nephrotoxicity of the homologue of this compound, lysinoalanine (*Nutr. Rev.*, 1976). β-Methyllanthionine, the addition product of the sulfhydryl group of cysteine to β-methyldehydroalanine, has been found to occur in bacterial antibiotics (Gross et al., 1975).

Treatment of AFGP-8 with excess N^{α}-acetyl-L-lysine in 0.5 N NaOH at 50 °C for 24 h yielded two new peaks, in approximately equal amounts, which eluted before lysine on the amino acid chromatogram after acid hydrolysis (Figure 3A). Upon periodate oxidation, both peaks disappeared with formation of lysine and a new unknown peak (Figure 3B). We suspect that the two N^{α}-acetyl-L-lysine addition products isolated after acid hydrolysis are the D and L isomers of N^{ϵ}-(1-methyl-2-amino-2-carboxyethyl)-L-lysine, since β elimination would destroy
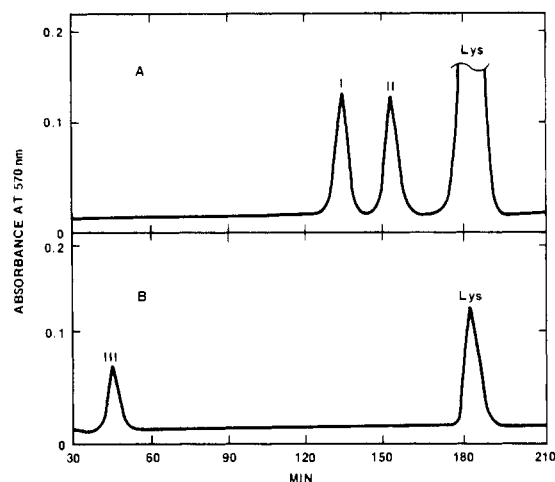
Figure 3. Ion-exchange chromatographic separation of basic residues from (A) acid hydrolysates of antifreeze glycoprotein β eliminated in presence of N^α-acetyl-L-lysine in 0.5 N NaOH at 50 °C for 24 h, and (B) isolated peak I or II after periodate oxidation. Peak III was not identified.

the native asymmetry at the α carbon and subsequent α-β addition of the nucleophile to the unsaturated derivative would result in formation of the DL isomers.

Since we could obtain only microgram amounts of the new compounds, it was not possible to perform elemental

analysis. They were derivatized for combined GC/MS studies. Their corresponding N-TFA-n-butyl esters were not separable on the gas chromatogram (Figure 4) and both give identical mass spectra (Figure 5). The mass spectra suggested a parent peak of mass 551 and contained characteristic fragments for lysyl and threonyl derivatives (Gelpi et al., 1969). The molecular weight of 551 corresponds to the N^ϵ-(1-methyl-2-amino-2-carboxyethyl)-L-lysine derivatives.

Since small amounts of N^α-acetyl-L-lysine were hydrolyzed to lysine under the treatment conditions, there is the possibility that the α-amino group might also add across the double bond. This is unlikely because base treatment of AFGP-8 in presence of the dipeptide α-glycyl-L-lysine or L-lysine followed by acid hydrolysis produced the same two peaks on the amino acid chromatogram. Also, N^ϵ-methyl-L-lysine gave no addition products under the same conditions.

The significance of this particular β-elimination reaction of O-glycosylthreonines and subsequent ε-amino addition reaction in the processing of food proteins is not known. The carbohydrate moieties are found to be released at a faster rate from serine than from threonine in mucin-type glycoproteins (Plantner and Carlson, 1975). Also the possible lower reactivity of β-methyldehydroalanine with nucleophiles has been suggested (Spiro, 1972; Pigman and Moschera, 1973; Rando, 1974). Therefore, it is unlikely that nucleophilic addition products with β-methyl-
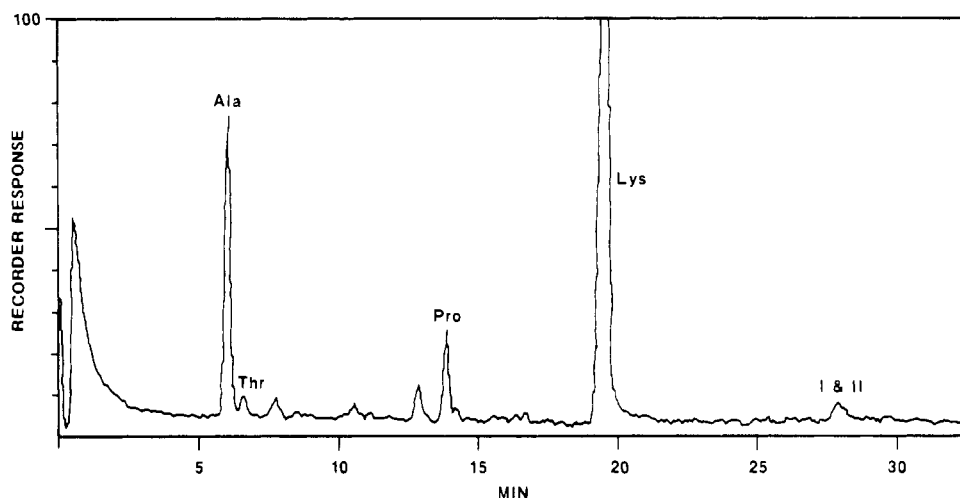

Figure 4. Gas chromatographic separation of derivatized residues from acid hydrolysates of antifreeze glycoprotein β eliminated in presence of N^α-acetyl-L-lysine in 0.5 N NaOH at 50 °C for 24 h. Identification of alanine, threonine, proline, and lysine derivatives was made by comparison of their elution times to those of derivatized standard amino acids, and of their mass spectra to those reported by Gelpi et al. (1969). The elution time for derivatives of isolated peak I or II (Figure 3A) was 28 min.
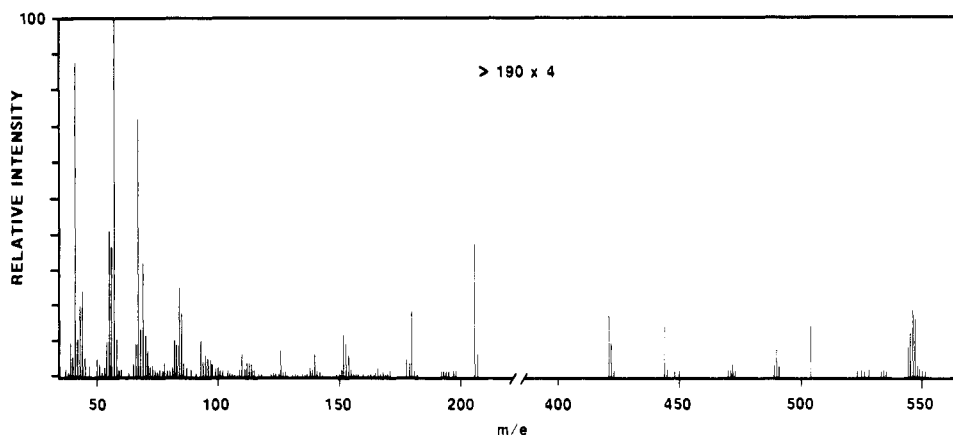

Figure 5. Mass spectrum of N-TFA-n-butyl ester derivative of isolated compound I (Figure 3A). Derivatives of isolated compound II (Figure 3A) or combined compounds I and II (Figure 4) also gave identical mass spectrum.

dehydroalanine will occur to the same extent as with dehydroalanine. Yet, the possibility should not be overlooked. Further work is needed to assess the role and behavior of substituted threonyl residues in processed high protein foods.

ACKNOWLEDGMENT

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Extraction of Important Molecular Features of Musk Compounds Using Pattern Recognition Techniques

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The relationships between molecular structure and the musk odor quality were investigated using pattern recognition techniques. A data set consisting of 60 musk odorants and 240 nonmusk compounds were coded with computer generated structural descriptors and then analyzed using a linear learning machine. After determining that the data set was linearly separable, a subset of 13 descriptors was identified and subsequently employed to predict the odor quality of nine, previously unused, musk odorants: all were correctly classified. The results of this work demonstrated the usefulness of pattern recognition techniques for studying structure–activity relationships of olfactory stimuli and elucidated some structural parameters common among musk odorants.

The perception of odors occurs in humans when airborne molecules of a volatile substance interact with some type of receptors in the olfactory region of the nose. Although the detailed mechanism of these interactions as well as the composition of the receptors remain unknown, several theories have been proposed which attempt to correlate different molecular properties with the perceived odor quality of a substance. For example, Wright (1954)

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