

Pyrolysis Gas Chromatography of Wool

Part III: Detection and Quantitation of Tyrosine

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ABSTRACT

Pyrolysis gas chromatography was used to study the content of tyrosyl residues in wool and the depletion of those residues by chemical reaction with nitrous acid. Wool samples that had undergone nitrosation of varying duration were analyzed by Curie-Point pyrolysis coupled with capillary column GC and flame ionization detection. The inertness of phenylalanyl residues to nitrosation permitted phenylalanine to be used as an internal standard. Peak areas for phenol and *para*-cresol—pyrolysis products—were compared to peak areas for phenylacetoneitrile—a pyrolysis product of phenylalanine. There was a linear relationship between the ratio of those peak areas and the molar ratio of Tyr/Phe, as determined by amino acid analysis, and another linear relationship for mixtures of known composition of polypeptides poly-tyrosine and poly-phenylalanine, but with a large difference in the slope from the line generated from the wool data. The difference was probably related to the relatively high concentration of terminal phenylalanyl groups in the polypeptide, which would result in the production of relatively larger amounts of phenylacetoneitrile in the synthetic material than in wool. The overall results demonstrated that damage to tyrosyl residues during the chemical processing of wool may be easily assessed using pyrolysis gas chromatography.

In earlier papers in this series, we demonstrated the utility of pyrolysis gas chromatography (Py-GC) for detecting and quantitating commercially applied agents on wool [4] and determining the tryptophan content of wool's protein [5]. In the latter study, we showed how Py-GC could be used to assess tryptophan depletion during chemical treatment of wool; we did this by monitoring indole, a pyrolytic product of tryptophan, and relating it to phenol and *para*-cresol, pyrolytic products of tyrosine. In that study, we assumed the lack of reactivity of tyrosine under conditions in which tryptophan might be depleted. Inasmuch as the technique might also serve as a means of monitoring the depletion of tyrosine under conditions where phenylalanine is stable, we undertook the current study to demonstrate the correlation between tyrosine : phenylalanine ratios (Tyr/Phe) determined on the one hand from Py-GC data—the ratio of the sum of phenol and *para*-cresol (both from Tyr) to phenylacetoneitrile (from Phe)—and on the other hand from conventional amino acid analysis.

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² Reference to a particular company or brand name does not constitute an endorsement by the U.S. Department of Agriculture over others not mentioned.

Experimental

MATERIAL AND METHODS

Joseph Cooper of our center provided the wool powder, which presented a pyrogram identical in pattern to that of other wool samples in the prior reports [4, 5]. Poly-L-tyrosine (mw 82,000) and poly-L-phenylalanine (mw 23,000) came from Sigma Chemical Co., St. Louis, MO.² The wool powder was depleted of its tyrosine by reaction with nitrous acid according to Cassel's method [3].

Curie Point Py-GC was accomplished using the previously reported procedure [4] but with a 30-m fused silica capillary column (0.32 mm i.d., with an 0.5 m-thick bound stationary phase of Supelcowax 10) and the following GC settings: He flow, 1.5 ml/min; He pressure, 48 kPa; He velocity, 30 cm/s. A segment of a representative chromatogram is shown in Figure 1.

Amino acids were analyzed in duplicate on a Beckman 119-CL amino acid analyzer, according to the method of Brown and Greenberg [1]. Data were recorded and processed on a Hewlett-Packard 3390 recording integrator.

DATA ANALYSIS

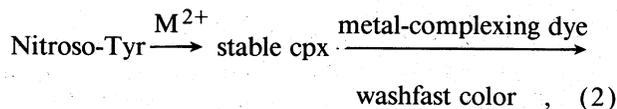
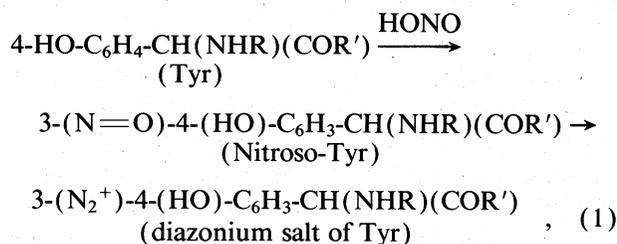
As in the previous reports [4, 5], we used an interactive reintegration method to compensate for inade-

quacies of automatic integration of GC peak data. Peaks that arise from phenol and *para*-cresol (Figure 1, 31.1 and 32.9 minutes, respectively) were used as internal standards; these compounds are pyrolytic products of tyrosyl residues. The peak area from phenylacetoneitrile (benzyl cyanide) (Figure 1, 29.6 minutes), a pyrolysis product of phenylalanyl residues, was used as an indicator of phenylalanine content.

Results and Discussion

NITROSATION

We assessed the utility of pyrolysis gas chromatography to monitor changes in wool protein's tyrosine content by analyzing wool whose tyrosine had been chemically destroyed by nitrosation. Nitrosation has been used as a means to introduce covalently bonded dyes and in the photodiazotization process for printing wool [6], as well as for mordant dyeing to produce wash-fast colors [2, 7]. Workers have used the action of nitrous acid to introduce extra crosslinking in wool to prevent supercontraction [2, 10]. The chemistry for all these processes (Equations 2-5) depends on the nitrosation and diazotization of the aromatic ring of wool's tyrosyl residues (Equation 1), though nitrous acid also reacts less specifically to deaminate amino (lysyl residues) and guanidino (arginyl residues) groups [3]:



(where M = Ni, Cu, Co)

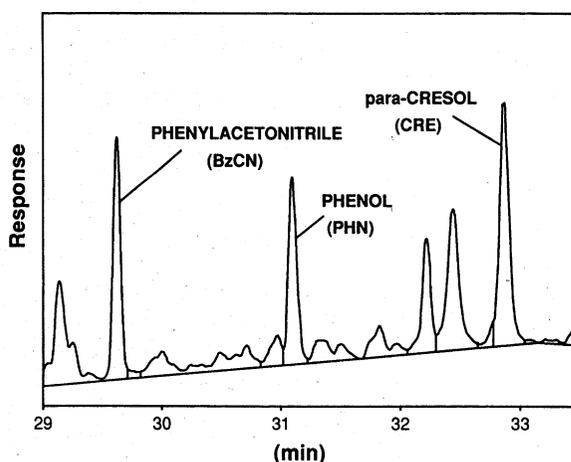
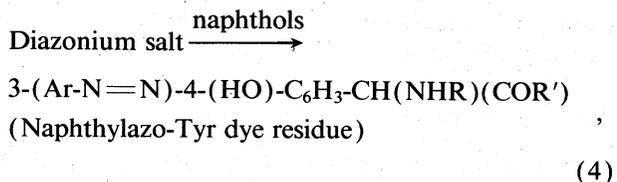
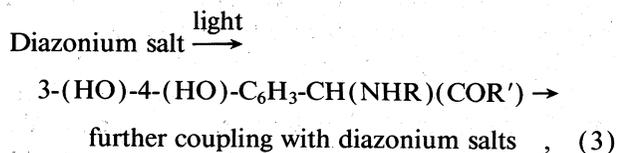
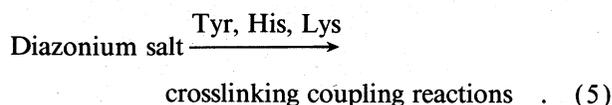


FIGURE 1. Segment of chromatogram of wool pyrolysis products. Conditions as per Experimental. Example shown is of wool after 45 hours nitrosation. Construction lines show baseline and peak separations after interactive reintegration.



Because the aromatic ring of phenylalanine is inert to the nitrosation process [3], the use of Py-GC data for determining tyrosine degradation may rely on the pyrolysis product of phenylalanyl (Phe) residues as an internal standard, relative to the pyrolytic products of tyrosyl (Tyr) residues. For this study, the phenylacetoneitrile peak served as the marker for Phe and the sum of the phenol and *p*-cresol peaks was the marker for Tyr [8, 9].

PYROLYSIS GC RESULTS

Wool that had been nitrosated over varying lengths of time was subjected to pyrolysis GC. For each duration of reaction, five samples were pyrolyzed. Peak areas for relevant peaks (means \pm standard deviations) are given in Table I. The control sample was treated in acetic acid for 1 hour in the *absence* of nitrite. Amino acid analysis of the same nitrosated wool samples was done in duplicate, and the results are given in Table II.

Pyrolysis GC results were calculated from the ratio of peak areas (phenol plus *p*-cresol) to phenylacetoneitrile. These ratios are listed in Table II and plotted in Figure 2 against the corresponding molar ratios of Tyr/Phe, as determined by amino acid analysis. Regression analysis showed a linear relationship (solid line: best fit; correlation coefficient: 0.994; slope: 10.06).

TABLE I. GC peak areas for nitrosated wool.^a

Reaction time, hours	Pyrolysis sample weight, g × 10 ⁻⁶	Peak areas × 10 ⁻³ (and retention times in minutes)		
		BzCN ^b (30)	PHN (31)	CRE (33)
0.00 ^c	203 ± 2	151.9 ± 42.8	969.4 ± 163.3	1381.4 ± 233.6
0.25	206 ± 3	128.9 ± 9.7	709.4 ± 50.9	1032.5 ± 64.5
0.50	201 ± 5	138.3 ± 21.1	745.9 ± 118.3	1044.2 ± 121.3
1.00	207 ± 3	183.4 ± 62.8	716.1 ± 139.7	1053.6 ± 199.4
4.00	206 ± 4	172.4 ± 29.8	514.0 ± 55.5	784.8 ± 82.6
45.00	206 ± 3	135.4 ± 26.0	168.9 ± 17.0	257.9 ± 17.7

^a Mean value of five determinations ± standard deviation. ^b Abbreviations: BzCN, benzyl cyanide (phenylacetoneitrile); PHN, phenol; CRE, *p*-cresol. ^c Control blank: 1 hour in acetic acid without nitrite.

TABLE II. Tyrosine depletion by nitrosation of wool.

Reaction time, hours	Molar ratio (Tyr/Phe) ^{a,b}	GC peak area ratio [(PHN + CRE)/BzCN] ^c	Percent of original tyrosine	
			From amino acid analysis	From GC data
0.00 ^d	1.49	15.80 ± 1.48	100.0%	100.0%
0.25	1.36	13.53 ± 0.66	91.3	85.4
0.50	1.27	12.99 ± 0.82	85.2	81.9
1.00	1.00	9.95 ± 1.18	67.1	62.3
4.00	0.70	7.61 ± 0.65	47.0	47.2
45.00	0.29	3.21 ± 0.41	19.5	18.8

^a By amino acid analysis (average of duplicate determinations). ^b Abbreviations: Tyr, tyrosine; Phe, phenylalanine; BzCN, PHN, CRE as per Table I. ^c Mean value of five determinations ± standard deviation. ^d Control blank: 1 hour in acetic acid without nitrite.

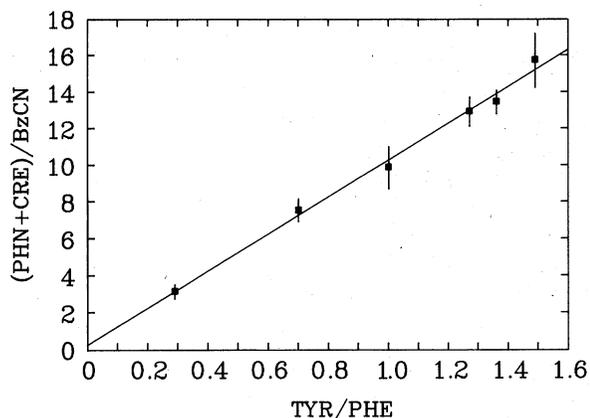


FIGURE 2. Analysis of tyrosine in nitrosated wool, showing linearity of response between pyrolysis GC and amino acid results. Ordinate: peak area ratios by pyrolysis GC; abscissa: molar ratios by amino acid analysis. Abbreviations: PHN, phenol; CRE, *p*-cresol; BzCN, benzyl cyanide (phenylacetoneitrile); Tyr, tyrosine; Phe, phenylalanine. Best fit by linear regression is indicated by solid line. Bars indicate standard deviation.

The data in Table II were used to calculate the depletion of tyrosine from each set of data (Table II, per-

cent of original tyrosine). The correlation between pyrolysis GC and amino acid analysis data is plotted in Figure 3, which shows a linear relationship (solid line: best fit; correlation coefficient: 0.996; slope: 0.977).

In addition to the nitrosation experiments on wool, we made similar trials with three mixtures of poly-ty-

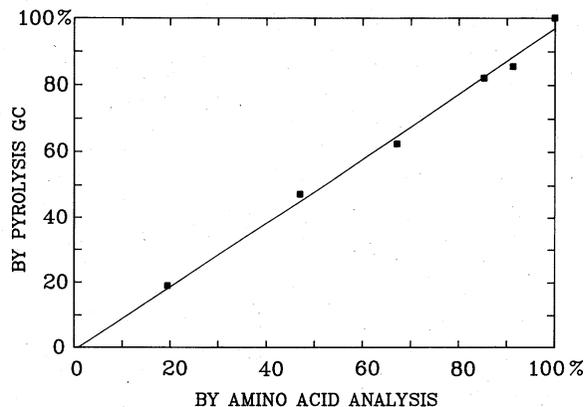


FIGURE 3. Tyrosine depletion in wool by nitrosation: correlation of results between pyrolysis GC and amino acid analysis.

rosine and poly-phenylalanine of known molar ratios (Tyr/Phe = 0.49, 0.99, 1.40). Unlike the parallel experiments we ran on poly-tryptophan versus poly-tyrosine in earlier work [5], there was poor correlation in this study between the polypeptide mixtures and the wool samples. In this work, the best fit for the polypeptide data (plotted in same way as Figure 2) showed a slope of only 5.34 versus Figure 2's slope of 10.06. This seeming anomaly is probably due to the enriched concentration of *N*-terminal phenylalanyl groups in poly-phenylalanine. Tsuge and Matsubara [9] have pointed out that nitriles such as phenylacetonitrile (BzCN) from phenylalanine arise from amines that form only from *N*-terminal residues. Wool, as a natural protein, would be expected to contain phenylalanyl residues mainly in the interior of the peptide chain. Inasmuch as the slopes in question are inversely proportional to the response of BzCN, we would expect the slope generated from the polypeptide mixture to be smaller than the slope from the wool samples.

Conclusions

Pyrolysis GC of wool gives rise to peaks because of the pyrolysis of its amino acid residues, peaks that include phenol and *para*-cresol from tyrosine as well as phenylacetonitrile from phenylalanine. The results indicate that the extent of depletion of tyrosine from wool may be determined facily from the peak areas of the pyrolytic products mentioned above. Together with earlier findings on the utility of the procedure for determining tryptophan depletion and chemical add-ons, these results support the use of Py-GC for rapid multiple analyses of wool fiber.

ACKNOWLEDGMENTS

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