The Reaction of Oxidizing Agents with Wool

4. THE REACTIVITY OF TYROSINE

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The role of tyrosine in metabolic processes and enzyme reactions has received detailed investigation, but the chemical reactivity and in particular the oxidation of this amino-acid in proteins has received little attention. The oxidation products of tyrosine itself depend on the reagent used (cf. Denis, 1912; Langheld, 1909) and indirect evidence indicates the formation of an indole derivative (Drake & Smythe, 1944; Raper, 1932). Permanganate oxidizes the tyrosine in wool to a quinone (Alexander, Carter & Hudson, 1949), and iodine forms mono- and di-iodo tyrosine in proteins (Mirsky & Anson, 1936). All the tyrosine in silk and wool was shown to be methylated by diazomethane (Rutherford, Patterson & Harris, 1940) and to be nitrated by *N*-nitric acid at 70° (Zahn & Kohler, 1950) without any oxidation of the disulphide bond occurring.

On the other hand, treatment of globular proteins with the Folin phenol reagent (Herriott & Northrup, 1935, 1936) and keten (Roverly & Desnuelle, 1948) revealed that some of the tyrosine hydroxyl groups were inaccessible to these reagents. Crammer & Neuburger (1943) showed spectroscopically that a large proportion of the tyrosine in egg albumin does not ionize at pH 12, and from the alkali-combining capacity of wool it can be concluded (Schmidt, 1944) that the tyrosine has not combined with hydroxyl ions at pH 12; at this pH the phenolic groups in tyrosine and tyrosine peptides are fully ionized. It is unlikely that the tyrosine side chains in wool are inaccessible to the small hydroxyl ion, and it appears more probable that the phenolic groups take part in hydrogen bonding which raises their *pK* so that they no longer titrate at pH 12.

In this paper it is shown that the tyrosine in wool can be divided into two fractions of widely differing reactivity with oxidizing agents, and its behaviour in this respect is very similar to that observed in the oxidation of cystine in wool (Alexander, Hudson & Fox, 1950).

EXPERIMENTAL

The preparation of the wool samples, the procedure for the oxidation by solutions of Cl_2 , KMnO_4 and peracetic acid, and also the preparation of the modified wool samples and horn were carried out exactly as described previously (Alexander, Hudson & Fox, 1950). The reactions with silk were carried

out as for wool, the samples having been previously degummed and extracted in a Soxhlet apparatus with ether, ethanol and water.

Preparation of tyrosine peptides. Three simple tyrosine peptides, glycyltyrosine, tyrosylglycine and glycyltyrosylglycine, were prepared by the method of Bailey (1949). All three of these peptides had been previously prepared and their properties reported (Fruton, 1949). The preparation of glycyltyrosine, for example, followed the following scheme. Tyrosine was first acetylated, then allowed to react at -10° with a mixture of molar quantities of triethylamine and anhydrocarboxyglycine in dioxan solution. The *N*-carboxyanhydrides used in these preparations were prepared by the Bergmann (1932) method, in which the insoluble carboxybenzoyloxy derivatives were made directly from the amino-acid and benzyl chloroformate followed by reaction with either SOCl_2 or PCl_5 . After 1 hr. the temperature was raised to 0° and then the mixture heated to $25-40^\circ$ *in vacuo* when the carbamate containing the tertiary base decomposed to leave the peptide ester.

The acetyl group was hydrolysed with 0.1 *N*-Ba(OH)₂ for 1 hr. at room temperature, followed by neutralization with H_2SO_4 , filtration and drying on a water bath. The peptides were characterized by *N* analyses and by their tyrosine content, which was determined colorimetrically with Millon's reagent (Table 1).

Table 1. *Chemical composition of tyrosine peptides*

(The calculated values are given in brackets.)

Peptide	Tyrosine (%)	Total N (%)	Amino N (%)
Tyrosylglycine	72.7 (76.04)	11.4 (11.76)	5.8 (5.88)
Glycyltyrosylglycine	50.5 (61.35)	14.3 (14.22)	4.7 (4.75)
Glycyltyrosine	73.1 (76.04)	11.9 (11.76)	5.7 (5.88)

All the amino-acids used were obtained from British Drug Houses Ltd., and polyglycine, polyalanine and polycystine were obtained by adding water to a 10% solution of the *N*-carboxyanhydride of the corresponding amino-acid in dioxan (Alexander, Bailey & Carter, 1950). The molecular weights of the polypeptides were 2000, 1200 and 1400 respectively, as estimated from amino N determinations.

Reaction of tyrosine and tyrosine peptides with chlorine solutions. An accurately known quantity of between 25 and 50 mg. of the material was dissolved in 50 ml. of a solution containing varying concentrations of Cl_2 adjusted to a pH of 2 or 10 and allowed to stand at room temperature until a sample taken from the solution no longer oxidized HI. Samples containing approximately the equivalent of 1 mg. of tyrosine were then taken and the tyrosine content deter-

mined colorimetrically. The Cl^- ion concentration in the sample was adjusted to be less than $2.5 \times 10^{-4} \text{N}$ so as not to interfere with the estimation.

To determine whether the Cl_2 had been reduced to Cl^- or reacted by substitution in the tyrosine, the total Cl^- concentration was determined after reaction by precipitation with AgNO_3 .

Rate of reaction of amino-acids and polypeptides with chlorine solutions. A solution (10 ml.) containing 0.05 g. of the amino-acid or polypeptide and 0.1 g. of Cl_2 adjusted to either pH 2 or 10 was prepared, and the rate of reaction determined by measuring the oxidizing power of samples periodically withdrawn from the solution with KI and thiosulphate. Cystine and the polypeptides were not sufficiently soluble and were added to the chlorine solution as a powder and the reaction carried out under vigorous stirring.

Oxidation of wool fractions obtained by treatment with peracetic acid. After oxidizing more than 90% of the cystine groups, wool can be fractionated into a high-molecular weight fraction soluble in NH_3 , and an insoluble fraction in the form of a membrane representing approximately 8% of the total wool substance (Alexander & Earland, 1950). These two fractions give X-ray diffraction patterns typical of α - and β -keratin respectively (Alexander, Earland & Hapley, 1950), and will be referred to as α - and β -keratose. The α -keratose, which dissolves in a buffer solution at pH 10, was made insoluble by refluxing the powder for 1 hr. with methanol containing 0.1 N-HCl (Fraenkel-Conrat & Olcott, 1945) so as to esterify the sulphonic acid groups which render the keratose soluble.

Samples of 0.5 g. of the α - or esterified α -keratose or β -keratose were added to solutions of pH 2 or 10 containing varying quantities of Cl_2 and allowed to react in a closed vessel till a sample taken from the solution no longer oxidized HI. The powder was then filtered off, washed, dried and hydrolysed and the tyrosine content determined. α -Keratose dissolved completely in solutions of pH 10 and in this case the whole of the solution was hydrolysed and the tyrosine content determined.

Combined chlorine in wool. After chlorination the wool was washed for 2 hr. with water at 80° which was shown to remove all physically held chlorine (Alexander, Gough & Hudson, 1949). The wool was then dried, weighed and heated for 2 hr. with conc. HNO_3 saturated with AgNO_3 . The wool dissolved completely and its Cl^- content was determined from the quantity of the AgCl precipitate. Experiments employing dichlorotyrosine under these conditions showed that all the chlorine was liberated as chloride.

Tyrosine analysis. The tyrosine content of virgin wool fibres has been determined by various workers, the values obtained ranging from 4.5 to 5.8%. Marston (1928) found a value of 4.8%; Block (1937), 4.5%; Block (1939), 4.8%; Rutherford *et al.* (1940), 5.8% and Middlebrook (1949), 5.43%. These workers used either the method of Thomas (1944) or some modification of the Millon reaction. On comparing the two methods with the hydrolysates of oxidized wool it was found that the Thomas method gave inconsistent and often obviously erroneous values, whereas Lugg's (1937) modification of the Millon reaction gave consistent and reproducible results. The protein samples were hydrolysed for 5 hr. with 5 N- H_2SO_4 at 120° in a sealed tube. It was necessary to employ H_2SO_4 as hydrolysing agent since it was found that more than $2.5 \times 10^{-4} \text{N-Cl}^-$ in the test solution interfered with the Lugg reaction, giving low values for tyrosine. The hydrolysate was then treated by the Lugg

procedure, the red colour being measured with a Spekker colorimeter. Untreated wool fabric samples were periodically examined and their tyrosine content found in every case to be 5.50 (± 0.15)%, a value in good agreement with those obtained in other recent work. During some of the oxidative treatments there occurred a considerable weight loss and in all cases the percentage loss of tyrosine is expressed in terms of the tyrosine content of the sample before oxidation, since no tyrosine was found in any of the reaction solutions. It was found that the three tyrosine peptides investigated gave quantitatively the same colour with Millon's reagent as tyrosine and their oxidation could be followed without hydrolysis of the samples.

According to Lugg (1937), all phenols which are not disubstituted in the *o*- or *m*-position give a positive colour with Millon's reagent. An extensive series of phenols was examined in this work and it was found that all *p*-substituted phenols and mono-*m*-substituted phenols gave a positive reaction, but that substitution in one of the *o*-positions was sufficient to prevent the formation of the colour. Thus, both mono- and di-chlorotyrosine failed to give the red colour.

RESULTS

Reaction of wool with chlorine solutions at different hydrogen-ion concentrations

Wool samples were treated with different quantities of chlorine and hypochlorite at pH 2 and 10, respectively, and the tyrosine content of the wool

Fig. 1. Disappearance of tyrosine of wool on treatment with different quantities of chlorine at pH 2 and 10. $\circ$ — $\circ$, chlorination at pH 2 at 0° ; $\times$ — $\times$, chlorination at pH 10 at 0° .

determined (Fig. 1). In solutions at pH 10 hypochlorite is expressed as chlorine. A solution of chlorine as used in these experiments consists almost entirely of Cl_2 molecules at pH 2, and almost entirely of hypochlorite ions at pH 10 (Alexander, Hudson & Fox, 1950). The rate of the reaction between chlorine solutions and wool at various pH values have been

recorded elsewhere (Alexander, Gough & Hudson, 1951). It is seen clearly that chlorine at pH 2 can, in sufficient concentration, oxidize nearly all the tyrosine of wool. Hypochlorite, however, can only oxidize about 30 % of the tyrosine and that fraction of the reagent which is in excess of the amount required for complete oxidation of this fraction, is presumably used in reactions with other groups in the wool fibres.

It was found that chlorine solutions at pH 2, 4 and 6 behaved in the same manner and the indications are that, providing sufficient oxidizing agent is employed, all the tyrosine may be oxidized (Table 2). Hypochlorite solutions at pH 7 and 8 were still capable of removing most of the tyrosine, but more chlorine was necessary for the oxidation than at lower pH values.

Table 2. *Percentage of tyrosine in wool oxidized at 0° by chlorine solutions buffered at different pH values*

Chlorine reduced (g./100 g. wool)	Tyrosine lost (% of that originally present)					
	pH 2	pH 4	pH 6	pH 7	pH 8	pH 10
2	6.61	—	—	—	—	1.3
5	12.7	—	—	7.21	8.96	9.90
10	26.9	—	—	22.4	13.5	14.9
20	41.1	—	—	—	30.6	25.9
35	—	—	—	—	—	36.5
50	76.4	87.4	72.5	59.0	—	30.0
75	90.2	—	—	—	—	—
100	—	—	—	—	68.8	—

From the change in constitution of a chlorine solution with pH (Alexander, Gough & Hudson, 1951) it appears that both molecular chlorine and hypochlorous acid can oxidize all the tyrosine of wool, whereas hypochlorite ions are able to oxidize only about 30 % of that present. At pH 7 and 8 obviously some of the oxidizing agent is used in the reaction with groups other than tyrosine and hence, greater quantities of chlorine are required than at pH 2 in order to remove the same proportion of tyrosine.

Since tyrosine containing chlorine in the *o*-position does not give a colour with Millon's reagent, it seemed possible that chlorine had reacted by substitution in the benzene ring. If all the tyrosine in wool had reacted in this way, the combined chlorine in the wool would be 1.1 or 2.2 % depending on whether the mono- or di-substituted compound had been formed. The quantities of chlorine chemically combined and not removed by washing with hot water of wool samples which have been reacted with different chlorine solutions are much too small to account for the observed loss of tyrosine by nuclear substitution (Table 3) and it must be concluded that the phenol ring is extensively oxidized by chlorine.

Table 3. *Uptake of chlorine by wool during reaction with chlorine solutions at pH 2 and 10 at 25°*

Chlorine used (g./100 g. of wool)	Chlorine remaining in the wool (g./100 g. wool)
None	0.08
35.0 at pH 2	0.14
50.0 at pH 2	0.25
35.0 at pH 10	0.18

Moreover, in every case more than 90 % of the chlorine which had reacted both at pH 2 and 10 had been reduced to chloride ions.

Oxidation with permanganate

Acid permanganate treatment is similar to alkaline chlorination in that it oxidizes only 30 % of the available tyrosine. Alkaline permanganate solutions, however, are capable of oxidizing a greater proportion of the tyrosine (see Table 4).

Table 4. *Proportion of tyrosine in wool oxidized by KMnO₄ at 0°*

KMnO ₄ reduced (g./100 g. of wool)	Tyrosine oxidized (% of that originally present)
At pH 2:	
6.25	10.5
12.50	27.9
25.00	31.7
At pH 9.2:	
6.25	15.4
12.50	26.1
25.00	43.6

Tyrosine and tyrosine peptides react rapidly and completely with both acid and alkaline permanganate (Alexander, Carter & Hudson, 1949).

Oxidation with peracetic acid

Toennies & Hommiller (1942) showed that performic acid reacts only with cystine, methionine and tryptophan, but not with other amino-acids occurring in proteins. It was found by Alexander, Hudson & Fox (1950) that, whereas aqueous solutions of performic acid were unstable, those of peracetic acid could be stored without loss of oxidizing power for many days. Preliminary experiments using paper chromatography indicated that peracetic acid exhibited the same specificity as performic acid; in the reaction with wool it was possible to oxidize exclusively the disulphide bond of cystine and the tryptophan side chains. The tyrosine contents of wool samples treated with peracetic acid solutions for periods up to 20 hr. were reduced by less than 2 % (the experimental error of the determination). The specificity of peracetic acid is thus confirmed. After oxidation with peracetic acid the

Table 5. *Oxidation by chlorine of tyrosine of wool before and after esterification*

(50 g. Chlorine/100 g. of esterified wool at pH 2 and 10 at 0°.)

Pretreatment of wool	Tyrosine content of wool			Tyrosine oxidized (% of that originally present)	
	After pretreatment	After chlorination at pH 2	After chlorination at pH 10	pH 2	pH 10
None	5.50	1.30	3.85	76.4	30.0
70% of the carboxyl groups esterified	5.14	1.39	3.20	73.0	37.7

Table 6. *Oxidation by chlorine of wool before and after acetylation*

(20 g. Chlorine/100 g. of acetylated wool at pH 2 and 10 at 0°.)

Pretreatment of wool	Tyrosine content of wool			Tyrosine oxidized (% of that originally present)	
	After pretreatment	After chlorination at pH 2.0	After chlorination at pH 10.0	pH 2	pH 10
None	5.50	3.20	4.06	41.1	25.9
80% of the amino groups acetylated	5.48	2.83	3.97	47.6	29.1

weight of the wool sample was increased by 3 % and this was corrected for when calculating the tyrosine content.

Oxidation of modified wool samples and horn

The proportion of tyrosine in wool accessible to hypochlorite ions was not significantly affected either by esterifying the carboxyl or acetylating the amino groups (Tables 5 and 6). The failure of two-thirds of the tyrosine to react with alkaline hypochlorite cannot therefore be attributed to the

proximity of charged groupings which influence the approach of ions.

In horn, also, only part of the tyrosine present was oxidized by alkaline hypochlorite, whereas nearly the whole reacted with acid chlorine solutions (Table 7). Wool and horn, though chemically alike, are very dissimilar morphologically. It is therefore very unlikely that the difference in reactivity of part of the tyrosine is due to physical inaccessibility to hypochlorite and permanganate ions.

Table 7. *Oxidation by chlorine of tyrosine in powdered horn keratin at pH 2 and 10 and 0°*

Chlorine used (g./100 g. horn)	Tyrosine content	Tyrosine oxidized (%)
None	3.97	—
At pH 10:		
10.0	3.91	1.51
35.0	3.39	14.6
50.0	2.74	31.0
At pH 2:		
50.0	1.36	66.0

The oxidation of tyrosine peptides with chlorine solutions at pH 2 and 10

The whole of the tyrosine in the three peptides examined, glycyltyrosine, tyrosylglycine and glycyltyrosylglycine was oxidized by chlorine at both pH 2 and 10. Moreover, acid and alkaline solutions were equally effective, since in both cases approximately 4–6 equivalents of chlorine were consumed per mol. of tyrosine (Table 8).

As in the case of wool, none of the chlorine had combined with the tyrosine in the peptides, but it had all been reduced to chloride ions which were precipitated quantitatively with silver nitrate.

Table 8. *Oxidation of tyrosine with chlorine at pH 2 and 10 at 25° in tyrosine, glycyltyrosine, tyrosylglycine and glycyltyrosylglycine*

Equiv. chlorine reacted/mol. of tyrosine	Tyrosine oxidized (%)							
	Tyrosylglycine		Glycyltyrosine		Glycyltyrosylglycine		Tyrosine	
	pH 2	pH 10	pH 2	pH 10	pH 2	pH 10	pH 2	pH 10
2.0	44.9	50.5	47.6	38.5	36.0	33.3	13.4*	10.6*
4.0	75.2	72.5	71.0	68.4	65.3	53.3	78.9	61.5
8.8	100	93.6	100	95.0	96.6	96.6	—	—

* In these experiments the quantity of chlorine reacted with 1.7 equiv./mol. of tyrosine.

*Reaction of silk with chlorine solutions
at pH 2 and 10*

The behaviour of the tyrosine in silk is different both from that in wool and from that in simple peptides. There was no indication of two tyrosine fractions with different reactivities, but all of it was much less readily oxidized by hypochlorite at pH 10 than by chlorine at pH 2 (Table 9). Thus, although

Table 9. *Oxidation of tyrosine in silk by chlorine at pH 2 and 10 both at 25°*

Chlorine used (g./100 g. silk)	Tyrosine oxidized (% of that originally present*)	
	pH 2	pH 10
5	22.2	6.7
10	56.7	13.3
50	95.6	52.2
75	—	67.8

* The tyrosine content of the original silk was found to be 9.00%.

all the tyrosine could be oxidized at pH 2 and 10 it required approximately five times as much chlorine to oxidize it with hypochlorite ions as with chlorine molecules. The rate of reaction of chlorine solutions with silk, when diffusion within the fibre was rate controlling, was determined in the same way as with wool (Alexander, Gough & Hudson, 1949) and was found to vary inversely with the pH of the solution over the range examined, i.e. from pH 2 to 10. For want of a better means of expressing the relative reaction rates under various conditions, the periods of half-life are used. These were found to be 3.4 min. at pH 2 and 20 min. at pH 10 using 20% of chlorine in terms of silk, with a ratio of solution to fibres of 150:1. In this respect, also, silk differs markedly from wool, the reaction rate of which is constant in the range pH 2-7 (Alexander, Gough & Hudson, 1951).

*Reaction of α - and β -keratose obtained from
peracetic acid treated wool with chlorine
solutions at pH 2 and 10*

The same proportion of tyrosine was oxidized by equal amounts of chlorine at pH 2 and 10 (Table 10) in the insoluble subcuticle of wool, β -keratose, which resembles in this respect the simple tyrosine peptides. The tyrosine in α -keratose (Alexander, Earland & Happey, 1950), the soluble high molecular weight derivative which represents between 50-60% of the wool substance, was much more readily oxidized by chlorine than by hypochlorite ions. More than twice as much chlorine was necessary at pH 10 as at pH 2 for oxidation of the same proportion of tyrosine. There was, however, no indication of the division of the tyrosine into two fractions; it is probable that all of it may eventually be oxidized by chlorine in sufficient quantity at pH 10 (Table 10) and also by potassium permanganate.

Since the α -keratose was dissolved in pH 10 buffer, it was thought that the difference in reaction might be due to the fact that at pH 10 the reaction was homogeneous, whereas at pH 2 it was heterogeneous. Accordingly, the experiments were repeated with esterified α -keratose, which was not soluble in a buffer at pH 10. However, it was still found that less chlorine was required at pH 2 than at pH 10 to oxidize the same proportion of tyrosine.

*Reaction of amino-acids and polypeptides with
chlorine solutions at pH 2 and 10*

Chromatographic analysis of wool samples in which all of the tyrosine and cystine had been oxidized by chlorine indicated that no other amino-acids had been preferentially attacked, except tryptophan which could not be detected. On the other hand, most of the free amino-acids react rapidly with chlorine solutions in acid and alkaline solution. Solutions of amino-acids at pH 2 and 10 were allowed to react with twice their weight of chlorine at 25° and the disappearance of oxidizing

Table 10. *Oxidation of tyrosine in α - and β -keratose and in esterified α -keratose by chlorine solutions at pH 2 and 10, both at 25°*

Cl ₂ used (g./100 g. of material)	Tyrosine oxidized (% of that originally present*)					
	β -Keratose		α -Keratose		Esterified α -keratose	
	pH 2	pH 10	pH 2	pH 10	pH 2	pH 10
2	—	—	25.5	18.2	28.9	16.4
5	31.6	36.8	46.8	28.3	57.0	23.4
10	53.9	40.8	54.2	36.2	77.7	52.6
20	—	—	—	41.3	—	—
35	88.0	80.5	—	—	—	62.8
50	96.1	—	89.4	60.9	95.4	76.8

* Tyrosine content of fractions before oxidation with chlorine: α -keratose, 4.70%, β -keratose, 3.80%.

power determined by periodic titration of samples; typical results are shown in Fig. 2. In cases where the reaction was rapid (e.g. alanine and tyrosine), the equivalents of chlorine consumed/mol. of amino-acid were calculated from the amount of chlorine reacted at the time when the curve flattened out; the value obtained was found to be independent of the concentrations of the reactants. The rate of reaction is expressed as the time, $t_{0.5}$, for half the chlorine reacting to be consumed.

Fig. 2. Rate of reduction of a solution of chlorine (1 g. of chlorine/100 ml. of solution at pH 2 and at 18.5°) by the amino-acids; glycine, $\otimes$ — $\otimes$; alanine, $\bigcirc$ — $\bigcirc$ and tyrosine, $\odot$ — $\odot$ (0.5 g./100 ml. of solution).

The shapes of the kinetic curves for glycine (Fig. 2), lysine and the polypeptides were such that the equivalents of chlorine reduced per mol. of amino-acid could not be calculated. In all cases, even where there was no apparent loss of oxidizing power of the solution as determined by oxidation

of iodide, chlorine disappeared rapidly from solution, probably to form a chloroamine. The concentration of free chlorine in solution was determined by extraction with carbon tetrachloride and it was found that 1 equiv. of chlorine per mol. of glycine had reacted in less than 1 min. The reaction product, a chloroamine, though insoluble in carbon tetrachloride, still oxidized iodide.

It can be seen from Table 11 that all the amino-acids, with the exception of glycine, are readily oxidized by chlorine at pH 2 and 10. Approximately the same number of equivalents of chlorine per mol. of amino-acid were required at pH 2 and 10, but the reaction was in most cases considerably slower in alkaline solution. Cystine and the polypeptides were oxidized as powders and their reaction rates are therefore not comparable with those of the other amino-acids in Table 11. Potassium permanganate is much more specific than chlorine and only tyrosine and cystine react with the former in less than 4 hr. at room temperature (Alexander, Carter & Hudson, 1949).

DISCUSSION

The experiments in the foregoing section establish clearly that the tyrosine in wool is divided into two fractions of widely differing reactivities. All the tyrosine is oxidized by chlorine and hypochlorous acid, whereas only 30% reacts with hypochlorite ions and acid solutions of permanganate. This behaviour of tyrosine is similar to that of cystine in wool of which only 25% is oxidized by hypochlorite (Alexander, Hudson & Fox, 1950). In neither case can morphological factors, such as physical inaccessibility of a portion of the fibre to certain reagents, explain the observations, since the differences persist in powdered horn which bears no histological resemblance to wool. Blocking the

Table 11. *Reaction of amino-acids and polypeptides with chlorine solutions at pH 2 and 10 at 18.5°*

Amino-acid or peptide	Equiv. chlorine reduced/mol. of amino-acid		Approx. $t_{0.5}$ of reaction (min.)	
	pH 2	pH 10	pH 2	pH 10
Glycine	*	*	>100	14
Alanine	3.4	3.1	1.8	15
Leucine	3.0	3.3	0.8	100
Serine	1.5	—	7.0	—
Methionine	3.5	—	0.3	—
Cystine	10.8	11.6	2.5	2.4
Tyrosine	8.4	7.8	1.5	7.5
Glutamic acid	3.1	4.1	0.9	40
Aspartic acid	3.0	—	1.6	32
Arginine	6.0	6.9	7.0	20
Tryptophan	—	10.2	—	1.5
Polycystine	*	*	15	60
Polyalanine	*	*	40	42
Polyglycine	*	*	>100	12

* Value could not be calculated.

charged amino or carboxyl groups fails to influence the differential behaviour of the two tyrosine and cystine fractions and the differences in reactivity can thus not be attributed to the repulsions of the oxidizing ions by charged groups in the vicinity of the cystine or tyrosine. In the case of cystine it was thought that the reactivity of the disulphide group may be dependent on induced electrical effects from neighbouring groups and that in certain combinations with amino-acids cystine may be oxidized by chlorine solutions at pH 2 and not at pH 10 (Alexander, Hudson & Fox, 1950). However, experiments, as yet unpublished, with a large number of cystine peptides failed to reveal any combination in which the disulphide bond was not oxidized equally readily by chlorine solutions at pH 2 and 10. In this paper it is shown that the tyrosine exhibits comparable reactivities in three peptides in which it was incorporated in different positions and that in every case chlorine at pH 2 and 10 is equally effective. It is therefore improbable that the differences in reactivity are produced by purely chemical factors.

There is an interesting difference in the reactivity of tyrosine and cystine in wool with alkaline permanganate. Both these amino-acids themselves or in combination in simple peptides are rapidly oxidized by this reagent; yet in wool alkaline permanganate oxidizes the tyrosine but not the disulphide bond of cystine (Alexander, Hudson & Fox, 1950). It is difficult to see why incorporation in a protein should render cystine but not tyrosine inaccessible to alkaline permanganate, especially since the two amino-acids behave alike in their reaction with solutions of acid permanganate. In general, however, the reactions of tyrosine and cystine in wool are so similar that it seems highly probable that a similar cause is responsible for their division into two fractions of different reactivities with oxidizing agents.

Besides wool there is no fibrous protein rich in cystine, the oxidation of which could therefore not be studied in other systems. A comparison is, however, possible in the case of tyrosine whose oxidation in both silk and wool was studied. The tyrosine in silk does not appear to fall into two fractions, but all of it is much less readily oxidized by hypochlorite than by chlorine (Table 10) although all of it is eventually oxidized by chlorine solutions at pH 10. The tyrosine in silk, unlike the tyrosine in peptides, exhibits a differential chemical reactivity towards chlorine and hypochlorite, although it is physically accessible to both reagents. The tyrosine in the high-molecular weight product, α -keratose, obtained from wool which had been rendered soluble by oxidizing disulphide bonds only, behaves as in silk. It cannot be divided into two fractions, but all of it reacts much less readily with hypochlorite than chlorine.

On the other hand, the tyrosine in the insoluble material, β -keratose is oxidized equally readily by chlorine solutions at pH 2 and 10.

The occurrence of different tyrosine fractions in wool can be explained in the light of the results with α -keratose and silk on the basis of the recently developed picture of molecular structure of wool. Apart from the cuticle the wool consists of spindle cells which become completely soluble in weak alkali or hydrogen bond-breaking solvents after the disulphide bonds have been split. The soluble protein can be fractionated into an electrophoretically homogeneous fraction (α -keratose) of mol. wt. 70,000 which contains relatively little sulphur and a heterogeneous fraction of low molecular weight which contains most of the sulphur in wool as cysteic acid (Alexander, Earland & Happey, 1950). There is considerable evidence from physical measurements (Alexander, 1950) that the high-molecular weight fraction forms micelles of polypeptide chains held together by hydrogen bonds and that the low-molecular weight material acts as a highly cross-linked cement between the micelles. The cortex of wool can therefore be considered as a two-phase system consisting of aggregates of long polypeptide chains mixed with relatively low-molecular weight polypeptides. Chlorine oxidizes cystine and tyrosine preferentially in both the high- and low-molecular weight fractions. The hypochlorite ion exhibits the same preferential attack in the low-molecular weight fraction and in peptides, but not in the high-molecular weight fraction or in silk where all amino-acids are slowly attacked simultaneously. In the two-phase system of wool, therefore, chlorine oxidizes all the tyrosine and cystine, whereas hypochlorite oxidizes preferentially only the tyrosine in the low-molecular weight fraction (30 % of the whole) and hypochlorite in excess of this is used up in reaction with other amino-acids in the low-molecular weight fraction in preference to a general attack on the less reactive high-molecular weight material. The 70 % of the tyrosine which is not oxidized by hypochlorite and permanganate is therefore not inaccessible, but present in a phase of the wool which is less reactive as a whole. In the 30 % fraction equal quantities of chlorine at pH 2 and 10 oxidize the same amount of tyrosine.

The reactivity of cystine can obviously not be examined in the different wool fraction since oxidation of it is necessary before a separation can be effected. It is tempting, however, to explain the division of the cystine in the same way as for tyrosine. It is difficult to understand why hypochlorite, like chlorine, attacks the tyrosine much more readily than other amino-acids in peptides and β -keratose, but that no preferential oxidation takes place in the high molecular weight fibrous proteins, silk and α -keratose.

SUMMARY

1. A study has been made of the reaction of different oxidizing agents with the tyrosine in wool, horn, silk, solubilized keratin, tyrosylglycine, glycyl-tyrosine, glycyltyrosylglycine.

2. Chlorine and hypochlorous acid oxidize all the tyrosine in wool, whereas acid permanganate and alkaline hypochlorite oxidize only 30 % of the tyrosine present. The tyrosine in wool may therefore be divided into two fractions, one of which is oxidized by the latter two reagents and the other not.

3. Peracetic acid does not oxidize the tyrosine in any of the materials studied.

4. There is no evidence for tyrosine fractions of

different reactivity in silk or the high-molecular weight keratin α -keratose rendered soluble by oxidation. More than twice as much chlorine is required at pH 10 than at pH 2 to oxidize the same amount of tyrosine in silk and α -keratose, whereas chlorine and hypochlorite are equally effective in oxidizing the tyrosine in simple peptides and low-molecular weight fractions of wool.

5. The difference in reactivity of the two tyrosine fractions in wool is explained in terms of the structure for wool proposed by Alexander (1950).

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