THE CHEMICAL COMPOSITION OF WOOL

II.* ANALYSIS OF THE MAJOR HISTOLOGICAL COMPONENTS PRODUCED BY ULTRASONIC DISINTEGRATION

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Summary

Ultrasonic disruption of powdered Merino wool in formic acid and dichloroacetic acid causes some protein to be dissolved, but the amino acid content of the residual wool is unchanged by the treatment. Cortical cells and disrupted cortical cells are found to have the same composition as the parent fibre, which is to be expected because the latter consists of about 90% cortical cells. However, the cuticle of Merino wool is different in composition from the parent fibre, being richer in cysteic acid, serine, proline, glycine, valine, and cystine, and poorer in aspartic acid, threonine, glutamic acid, methionine, isoleucine, leucine, tyrosine, phenylalanine, and arginine than the whole fibre. Thus the cuticle is considerably less polar than the fibre as a whole. With the exceptions detailed below, it is found that the first group of amino acids listed above are classified as non α-helix-forming and the second group as α-helix-forming by Blout (1962). The exceptions are isoleucine and threonine, whilst arginine and glycine are not classified. It is therefore postulated that the cuticle is amorphous because of its high content of non α-helix-forming amino acids. The cuticle of Lincoln wool shows similar differences to those already given for Merino cuticle but, in addition, contains less lysine and histidine than the whole fibre.

I. Introduction

Many chemical analyses have been made of cortical cells (Mercer, Golden, and Jeffries 1954; Ward, Binkley, and Snell 1955; Ward and Bartulovich 1956; Simmonds and Bartulovich 1958; Leveau 1957, 1958) and cuticle (Geiger 1944*a*, 1944*b*; Lustig, Kondritzer, and Moore 1945; Lindley 1947; Elliott and Roberts 1957; Elliott, Asquith, and Rawson 1959; Bradbury 1960*a*) separated from wool, but there have been large differences between the results of different workers for three reasons. Firstly, there is the possibility of inadequate identification of the histological component by microscopy; then there has been, in many cases, a lack of knowledge of the amount by which the component is chemically modified during preparation; and, finally, many of the chemical analyses have been inadequate or incomplete.

In an attempt to overcome these difficulties we have studied the ultrasonic disruption of wool (Bradbury 1960*b*; Bradbury, Rogers, and Filshie 1963), and in Part I of this series (Bradbury and Chapman 1964) have shown that disruption can be achieved and the histological components separated from one another. This paper is concerned with the amino acid analysis of the various components and the

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related important consideration of the possibility of chemical modification of the components during ultrasonic treatment in formic acid, dichloroacetic acid, and dimethyl sulphoxide.

This latter question cannot be answered by reference to the literature, because of the complete lack of information on the effect of ultrasonic irradiation in the liquids concerned. On the one hand, there is a considerable body of information concerned with the modification of amino acids and soluble proteins by ultrasonic irradiation in aqueous solution (Khenokh and Lapinskaya 1958; Nishihara and Doty 1958; Levinson and Kovrov 1959; El'piner 1960; El'piner and Zorina 1960*a*, 1960*b*; Dietrich 1962; Barany *et al.* 1963). It is generally agreed that water, on ultrasonic irradiation, is partly decomposed into free radicals (Weissler 1959) which cause modification of the soluble proteins both with regard to their molecular weight (Nishihara and Doty 1958; El'piner 1960; Barany *et al.* 1963) and biological activity (Dietrich 1962). It is also possible to protect the protein from inactivation in certain cases by the addition of radical scavengers or other protective reagents (El'piner and Zorina 1960*a*, 1960*b*; Dietrich 1962).

On the other hand, a good deal is known about the chemical reactions between proteins and formic acid. Immersion of proteins in formic acid for periods of several days at room temperature causes no appreciable amount of hydrolysis of peptide bonds (Harrap and Woods 1959, 1961; Narita 1959; Smillie and Neurath 1959), but does produce formylation of the hydroxyl groups of serine and threonine (Kienhuis, Blasse, and Matze 1959; Narita 1959). The only data that we have found with regard to stability in dichloroacetic acid is that polyglycine yields ninhydrin-positive glycine peptides in 26 hr at 37°C, whereas polyleucine is stable for 50 days (Heyns, Walter, and Grützmacher 1957). Also the viscosity of a solution of poly-γ-benzyl-L-glutamate in dichloroacetic acid decreases slowly after 3 days at 25°C (Doty, Bradbury, and Holtzer 1956). These changes are probably due to peptide bond fission. Little is known about reactions between proteins and dimethyl sulphoxide, but it seems unlikely that any reaction will occur at ordinary temperatures, owing to the inertness of the liquid (Koenig and O'Connell 1960). Because of this lack of information it has been necessary to investigate the problem of the chemical modification of the components during ultrasonic treatment.

II. Materials and Methods

(*a*) *Preparation of Wool*

The Merino 64's wool used was the same as in Part I (Bradbury and Chapman 1964). The same sample of Lincoln 36's wool processed to "top" form as that used previously by Bradbury (1960*a*) was extracted with detergent by the method of Bradbury and Chapman (1964).

(*b*) *Preparation of Histological Components*

The Merino wool was cut into short lengths and samples (1 g) were subjected to ultrasonic treatment for the appropriate time interval in 50 ml of either 98% formic acid, dichloroacetic acid, or dimethyl sulphoxide, the conditions described for

maximum breakdown being used (see Table 4 of Bradbury and Chapman 1964). The suspension was poured through a coarse sieve to remove short wool fibres; these were washed with water which was added to the suspension. The wool components were then sedimented, washed with water and ethanol, and screened by the method described by Bradbury and Chapman (1964). The histological purity of the separated components was always checked by light microscopy and that of the cuticle preparations by light and electron microscopy. Lincoln wool was subjected to ultrasonic disintegration in formic acid for 15 min and the components separated as already described.

(c) *Methods for Detection of Chemical Modification during Ultrasonic Treatment*

A sample of Merino wool was ground into a powder in a pestle and mortar containing liquid air. Samples (1 g) of the powder were ultrasonically irradiated in 50 ml of either dichloroacetic acid or formic acid, the same conditions as applied

TABLE 1

YIELD OF SEPARATED COMPONENTS OF WOOL PRODUCED BY ULTRASONIC DISINTEGRATION

Wool Component	Yield (mg) from 1 g Merino Wool Treated in:		
	Dichloroacetic Acid for 15 Min	Formic Acid for 15 Min	Dimethyl Sulphoxide for 10 Min
Cuticle	20	4·5	0·3
Cortical cells	50	8·5	Trace*
Disrupted cortical cells	22	3·5	Trace*

* This treatment produced only very slight amounts of material from the cortex. Longer treatments gave greater yields of disrupted material but the disrupted cortical cells were so small that they could not be removed effectively from the cuticle by screening methods.

to the wool samples being used. Sufficient formic acid was added to the suspension in dichloroacetic acid to reduce the density of the liquid to below that of the solid. On centrifugation the solid material sedimented and the supernatant liquid was removed and evaporated to dryness under reduced pressure in a rotary evaporator. A residue remained which consisted of material dissolved during the ultrasonic disintegration process, together with a small amount of material produced by evaporation of the pure liquid. The sedimented material, which consisted of powdered wool and wool components (cortical cells, cuticle, etc.) produced during the disintegration was washed three times with water and analysed.

(d) *Amino Acid Analysis*

The material for analysis was dried at 100°C *in vacuo* for 1 hr and about 1·5 mg weighed accurately. About 0·5 ml 6N HCl (constant boiling point acid produced by distillation of A.R. HCl) was added to the sample which was sealed in a glass tube *in vacuo* and hydrolysed for 24 hr at 110°C. Some hydrolyses were also continued for

longer periods (see below). The solution was evaporated to dryness and one-fifth of the hydrolysate loaded on an ion-exchange column (0·63 cm diameter by 125 cm length) of a Technicon amino acid analyser (Piez and Morris 1960). Known amounts of the amino acids taurine, norleucine, and α-amino-β-guanidinopropionic acid were also run routinely as internal standards to correct for small variations in behaviour of the amino acid analyser. Each hydrolysate was analysed at least twice and the results averaged.

TABLE 2

AMINO ACID ANALYSIS OF MERINO WOOL AND ITS COMPONENTS

Components (cortical cells and disrupted cortical cells) produced by ultrasonic disintegration of virgin wool for 15 min in the solvents indicated. Results expressed as μmoles amino acid per gram of dry material

Amino Acid	Virgin Wool	Cortical Cells		Disrupted Cortical Cells
		Formic Acid	Dichloroacetic Acid	Formic Acid
Cysteic acid	7	17	8	16
Aspartic acid	560	600	600	599
Threonine	572	476	513	496
Serine	902	946	883	923
Glutamic acid	1049	1038	1027	1037
Proline	522	522	531	529
Glycine	757	856	835	861
Alanine	469	499	488	497
Valine	486	520	488	510
½ Cystine	922	806	832	865
Methionine	44	42	35	40
Isoleucine	275	296	284	293
Leucine	676	689	699	676
Tyrosine	349	388	353	377
Phenylalanine	257	276	278	271
Lysine	269	251	255	252
Histidine	82	72	63	74
Arginine	600	601	610	588
Recovery of anhydroamino acids (%)	95·5	96·2	95·2	96·2

III. RESULTS

In Table 1 are shown the amounts of cuticle, cortical cells, and disrupted cortical cells produced by ultrasonic disintegration in the three liquids used. These liquids had been shown previously to give the most rapid rates of disruption of wool (Bradbury and Chapman 1964). The amino acid analyses of Merino 64's wool and the wool components produced from it are given in Tables 2 and 3. The results are expressed in micromoles of amino acid per gram of dry material and the percentage recovery of anhydroamino acid residues from the protein is also calculated. These recoveries

amount to *c.* 96% because tryptophan, which occurs to the extent of 1·9 g/100 g protein in dry wool (Simmonds 1954), has not been analysed owing to its destruction during acid hydrolysis. In addition, there is a small amount of non-protein material present in the wool fibres, probably largely in the cell membranes.

TABLE 3

AMINO ACID ANALYSIS OF CUTICLE FROM MERINO WOOL

Cuticle produced by ultrasonic disintegration of virgin wool in solvents indicated. Results expressed as μmoles amino acid per gram of dry material

Amino Acid	Formic Acid (15 min)	Dichloroacetic Acid (15 min)	Dimethyl Sulphoxide (10 min)	Mean Cuticle Analysis	Virgin Wool
Cysteic acid	65	22	25	37	7
Aspartic acid	344	404	415	388	560
Threonine	478	469	452	466	572
Serine	1289	1272	1238	1266	902
Glutamic acid	796	850	909	852	1049
Proline	886	871	742	833	522
Glycine	852	881	929	887	757
Alanine	541	554	490	528	469
Valine	629	618	560	602	486
½ Cystine	1423	1284	1184	1297	922
Methionine	31	35	42	36	44
Isoleucine	222	253	234	236	275
Leucine	541	599	569	570	676
Tyrosine	268	289	260	272	349
Phenylalanine	164	193	180	179	257
Lysine	265	290	256	270	269
Histidine	80	87	98	88	82
Arginine	422	467	461	450	600
Recovery of anhydroamino acids (%)	96·8	98·4	94·3	96·5	95·5

Powdered wool was hydrolysed for 48, 72, and 140 hr and cuticle produced by ultrasonic irradiation in dichloroacetic acid for 48 and 72 hr. The content of threonine, serine, cystine, and tyrosine decreased in both samples by the same percentage, within experimental error, owing to the prolonged hydrolysis. The corrections for decomposition during the first 24 hr of hydrolysis were obtained by linear extrapolation to zero time (Tristram and Smith 1963) and on the average they amounted to 7% for threonine, 7·5% for serine, 3% for cystine, and 5·5% for tyrosine. The results for cysteic acid were anomalous because for powdered wool the value was maximal after 72 hr, whereas with the cuticle sample the value remained approximately constant up to 72 hr. There was no evidence of incomplete liberation of amino acids after hydrolysis for 24 hr (Tristram and Smith 1963). Thus, with the exception of cysteic acid, the corrections for losses during hydrolysis are small and reproducible for the two samples of protein examined. However, one cannot be

sure that the corrections for all samples will be exactly the same, hence the tabulated results are not corrected for hydrolytic losses.

In Table 4 the results are given for the analysis of the same sample of Lincoln 36's top as was used in the earlier work of Bradbury (1960*a*). The analysis of the cuticle produced by ultrasonic irradiation is given together with the earlier values

TABLE 4

AMINO ACID ANALYSIS OF LINCOLN WOOL AND OF CUTICLE DERIVED FROM THIS WOOL

Cuticle produced by ultrasonic disintegration of virgin wool in formic acid for 15 min. Results expressed as μmoles amino acid per gram dry material

Amino Acid	Lincoln 36's Wool "Top"	Cuticle	"Cuticle-rich" Material*
Cysteic acid	12	80	—
Aspartic acid	603	318	479
Threonine	563	436	535
Serine	920	1226	1050
Glutamic acid	1130	837	844
Proline	615	1019	832
Glycine	617	732	547
Alanine	504	544	547
Valine	552	684	1582†
½ Cystine	881	1515	934
Methionine	44	24	—
Isoleucine	324	215	1582†
Leucine	708	504	1582†
Tyrosine	234	192	354
Phenylalanine	229	127	—
Lysine	252	209	296
Histidine	66	50	—
Arginine	667	440	615
Recovery of anhydroamino acids (%)	97·4	94·7	—

* Bradbury (1960*a*).

† Valine + isoleucine + leucine = 1582; corresponding value for cuticle produced by ultrasonic disintegration is 1403, and for the whole fibre is 1584.

obtained for "cuticle-rich" material, obtained by a mechanical method and analysed by the dinitrophenylation method. The results of analyses of powdered Merino wool before and after ultrasonic irradiation in formic acid and dichloroacetic acid are given in Table 5. The experimental conditions were the same as those used in the preparation of cortical cells and cuticle.

IV. DISCUSSION

Before considering the amino acid analyses of the components of wool, it is necessary to decide on the possible extent of chemical modification of those components during ultrasonic irradiation. Virtually no information is available from the literature on this question.

It is shown in Table 5 that some material is dissolved during the treatment in formic acid and dichloroacetic acid under the conditions used. Preliminary experiments have indicated that this material contains about 50% protein and that the protein constituent has an analysis similar to that of whole wool (Bradbury, unpublished data). The dissolution of a small amount of material of amino acid composition not greatly different from that of wool itself would result in a negligible change

TABLE 5

AMINO ACID ANALYSIS OF POWDERED MERINO WOOL BEFORE TREATMENT AND AFTER ULTRASONIC IRRADIATION

Powdered wool was treated for 15 min in the solvents indicated.* Results expressed as μmoles amino acid per gram dry material

Amino Acid	Amino Acid Content of Powdered Merino Wool:		
	Before Treatment	After Treatment in Formic Acid	After Treatment in Dichloroacetic Acid
Cysteic acid	8	9	11
Aspartic acid	590	570	583
Threonine	554	525	580
Serine	935	895	998
Glutamic acid	1032	1021	1064
Proline	561	560	580
Glycine	814	809	800
Alanine	499	469	504
Valine	511	492	510
½ Cystine	871	901	913
Methionine	45	44	39
Isoleucine	286	282	279
Leucine	704	699	694
Tyrosine	380	361	355
Phenylalanine	260	264	260
Lysine	273	256	263
Histidine	81	82	82
Arginine	605	615	607
Recovery of anhydroamino acid (%)	97·5	96·0	98·5

* The amount of material dissolved during the treatment amounted to 2% in formic acid and about 7% in dichloroacetic acid.

in the amino acid composition of the residual powdered wool. This is indeed the case, since the analyses of powdered wool before and after ultrasonic irradiation in formic acid and dichloroacetic acid are the same, within experimental error (see Table 5). This shows that although there is the likelihood of disruption of peptide or other chemical bonds during the treatment, any such changes do not affect the amino acid composition of the powdered wool. On this basis it is reasonable to assume that the various wool components separated after ultrasonic disintegration have the same amino acid composition as in the intact fibre. The validity of this assumption is confirmed by other results discussed below.

The amino acid analyses of cortical cells and disrupted cortical cells are compared with that of virgin wool in Table 2. It is noted that the amount of cysteic acid in the cortical cells and disrupted cortical cells produced in formic acid is greater than that of virgin wool or cortical cells produced in dichloroacetic acid. However, this result should be treated with reserve, because of the large errors involved in the determination of the area under such a small peak and also the difficulties already noted in accounting for the change of the cysteic acid content with the time of hydrolysis. The variation between duplicate analyses for histidine, methionine, threonine, and cystine is larger than that for the other amino acids. For this reason none of the differences which are observed between the results for any particular amino acid in Table 2 are considered to be significant. Therefore, the amino acid compositions of cortical cells and disrupted cortical cells are the same, within experimental error, as that of virgin wool. This result is not unexpected because of the fact that the fibre consists of approximately 90% cortical cells and 10% cuticle, and the composition of the latter is not vastly different from that of the parent fibre (see Table 3). No attempt has been made to decide whether the cortical cells are from the ortho- or paracortex or both. The general agreement between the amino acid analyses of virgin wool, disrupted cortical cells, and cortical cells produced by two different methods confirms that no changes in amino acid composition have occurred during ultrasonic irradiation.

The amino acid analysis of cuticle produced by ultrasonic irradiation of Merino wool in three different liquids is given in Table 3. The agreement between the different analyses is good for most of the amino acids, but the cysteic acid content of the cuticle produced in formic acid is much greater than that produced in the other two liquids. Also, the average deviations from the mean cuticle analysis are appreciable for the following amino acids: methionine 12%, histidine 7%, proline 7%, aspartic acid 7%, cystine 6%, and phenylalanine 6%. On closer examination of these six amino acids it is found that the cuticle produced by ultrasonic disintegration in dichloroacetic acid gives an analysis close to the average for five of the amino acids; hence in these cases the bulk of the deviations are produced by the formic acid and dimethyl sulphoxide samples. This may be due to real differences in the ratio of exocuticle to endocuticle between different preparations, since it has been shown previously (Bradbury and Chapman 1964) that the endocuticle can be eroded in some cases by the ultrasonic treatment. However, in the absence of further evidence on this question at the present time, the mean value given in Table 3 is considered to be the amino acid analysis of whole cuticle from Merino wool.

A number of considerable differences is noted between the amino acid analysis of cuticle and whole fibre both for Merino wool (Table 3) and Lincoln wool (Table 4). In the latter case the same sample of Lincoln wool has been used as in the earlier work (Bradbury 1960*a*) in which cuticle-rich material (about 50% cuticle) was produced by a laborious mechanical method and analysed by the dinitrophenylation method. Because of the inaccuracies in this method of analysis and the assumptions inherent in this earlier attempt to analyse cuticle, it is gratifying to note that the amino acids which showed significant differences in the previous work, viz. aspartic acid, glutamic acid, cystine, and proline, were present in amounts intermediate

between those of the parent fibre and the cuticle produced by the ultrasonic treatment.

The differences between the amino acid content of cuticle and the parent fibre obtained in this and previous work are summarized in Table 6. Most of the larger differences obtained in this work have been observed previously by one or more workers, and in almost all cases there has been agreement on the high cystine and low tyrosine content of cuticle as compared with the parent fibre.

Examination of the values in Table 6 shows that by far the largest percentage difference occurs with cysteic acid. This may be partly due to the higher value obtained by disintegration in formic acid as compared with dichloroacetic acid or dimethyl sulphoxide (Table 3). However, even if the formic acid result is ignored the cysteic acid content of Merino cuticle is still about 3·5 times as great as in the parent fibre. This is considered to be a real effect and is presumably due to exposure of the cuticle to the oxidative action of the weather. Similarly, it was found by Bradbury (1960*a*) that there is about four times as much cysteic acid in the tips of the fleece, which are more exposed to the weather, as in the base. It should be noted that during the hydrolysis of the protein, partial oxidation products of cystine disproportionate to cystine and cysteic acid (Leach 1960; Maclaren, Leach, and Swan 1960), hence the results given for cysteic acid represent the sum of the various oxidation products of cystine.

The amino acid composition of the cuticle protein is different from that of the high sulphur protein obtained by dissolution of wool with thioglycollate followed by coupling the thiol groups with iodoacetic acid (Gillespie 1963). Thus, although both proteins are rich in serine, proline, and cystine derivatives and poor in aspartic acid, the fraction studied by Gillespie is high in threonine and low in lysine and histidine, whereas our fraction is low in threonine and shows no change in lysine and histidine (with Merino wool) as compared with the parent fibre. In addition, our protein shows differences in eight other amino acids not mentioned above, hence it is clear that there are considerable differences between the two samples. This is not unexpected because the bulk of the high sulphur protein obtained by Gillespie (1963) must originate from the cortex, which has a different amino acid composition from that of the cuticle.

The results in Table 6 show that the cuticle of Merino wool contains considerably less aspartic acid, glutamic acid, arginine, and tyrosine than the parent fibre. Lincoln cuticle contains, in addition to the above, less lysine and histidine than the fibre. The cuticle is thus less polar than the fibre, as noted previously by Bradbury (1960*a*). This agrees with the postulate of Lindberg (1953) that the surface barrier to the diffusion of dyes and acids into wool (Millson and Turl 1950; Lindberg 1953; Medley and Andrews 1959) is electrical in nature. However, there is some evidence, reviewed by Bradbury (1960*a*), which indicates that this barrier may be caused by the thin, outermost layer of cuticle, viz. the epicuticle, which only amounts to about 5% of the total cuticle. It is, therefore, not possible to resolve this question until more information is available about the composition and properties of the separate cuticle layers and, in particular, the epicuticle.

TABLE 6

DIFFERENCES OF AMINO ACID CONTENT OF CUTICLE FROM PARENT FIBRE

Values given in the table represent percentage differences of the cuticle analysis from that of the parent fibre; the crosses represent differences observed in previous work

Amino Acids Showing		Present Work		Bradbury (1960*a*)	Elliott and Roberts (1957)	Lindley (1947)	Lustig *et al.* (1945)*	Geiger (1944*a*, 1944*b*)
Positive Differences	Negative Differences	Merino Wool	Lincoln Wool					
Cysteic acid		430	570	—	—	—	—	—
Serine		40	33	—	—	—	×	×
Proline		60	66	×	—	×	—	—
Glycine		17	19	—	—	—	—	—
Valine		33	24	—	—	—	—	—
Cystine		41	72	×	×	×	×	×
	Aspartic acid	31	47	×	—	—	—	—
	Threonine	19	23	—	—	—	×	—
	Glutamic acid	19	26	×	—	—	—	—
	Methionine	18	45	—	—	—	—	—
	Isoleucine	14	34	—	—	—	—	—
	Leucine	16	29	—	—	—	—	—
	Tyrosine	22	18	—	×	×	×	×
	Phenylalanine	30	45	—	—	—	×	—
	Lysine	—	17	—	—	—	—	—
	Histidine	—	24	—	—	—	—	—
	Arginine	25	34	—	—	×	—	×

* Hair was used in this case and the cuticle was also found to be deficient in tryptophan and histidine.

It was shown some time ago by X-ray examination that the cuticle is an amorphous protein (Woods 1938; Lustig, Kondritzer, and Moore 1945), hence the component of wool which is responsible for its X-ray diffraction pattern must be present in the cortex. This ordered component consists of α-helices which are formed together into a more complex ordered structure, about which there is considerable discussion (Fraser, Macrae, and Rogers 1962). One might therefore expect that the cuticle would be richer than the cortex (or the parent fibre) in those amino acids which have difficulty in forming an α-helix and correspondingly poorer in those amino acids which readily form an α-helix. A classification has been made of amino acids according to their ability to produce synthetic polypeptides which have an α-helical structure in the solid state and in solution (Blout *et al.* 1960; Blout 1962). When one compares this classification with Table 6, it is found that all those amino acids which are enriched in the cuticle are non α-helix-forming according to Blout (1962), with the exception of glycine which is unclassified. In addition, for Merino wool 6 out of 9 amino acids and for Lincoln wool 7 out of 11 amino acids which occur to a lesser extent in the cuticle than in the fibre are classified as α-helix-forming by Blout (1962). The others are arginine and histidine which have not been classified, isoleucine which is non α-helix-forming, and threonine which is predicted to be non α-helix-forming. Thus, out of a total of 17 amino acids in Table 6 it is seen that 12 fit the hypothesis, two (isoleucine and threonine) are in disagreement, and three others (histidine, arginine, and glycine) are unclassified.

Since the weight of evidence supports the above hypothesis, it appears that the cuticle is amorphous simply because of its amino acid composition, i.e. because it contains large amounts of non α-helix-forming amino acids. However, it should be noted that with the probable exception of proline (Kendrew 1962; Ramachandran, Ramakrishnan, and Sasisekharan 1963) and the possible exception of cystine, the so-called non α-helix-forming amino acids are able to exist in an α-helical chain which contains a variety of amino acids (Kendrew *et al.* 1961; Watson and Kendrew 1961; Kendrew 1962). Thus, in myoglobin it is found that in three different sequences of eight amino acids which occur in helical sections of the chain, there are four amino acids in each sequence which consist of valine, isoleucine, serine, or threonine. These amino acids are therefore not as important as proline and (probably) cystine in destabilizing the α-helix, but nevertheless it seems likely from the above evidence that they do play some part.

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