STUDIES ON OVALBUMIN

IV.* TRYPSIN DIGESTION AND THE CYSTINE PEPTIDES OF OVALBUMIN AND S-OVALBUMIN

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Summary

Conditions for obtaining comparable tryptic digests of denatured, alkylated ovalbumin and S-ovalbumin have been studied. The tryptic peptides soluble at pH 4·5 were fractionated by gel filtration in 1% formic acid. The cystine-peptide fraction was resolved into two cystine peptides by chromatography on DEAE-Sephadex, and by amino acid analyses these were shown to be derived from the same region in the molecule. The composition of the cystine peptides from ovalbumin and S-ovalbumin was the same. Further evidence that the disulphide cross-link is in the same position in the two proteins was obtained by comparing partial acid hydrolysates of performic-acid-oxidized cystine peptides isolated from pepsin digests.

A method for the resolution of the insoluble fraction from the tryptic digests, by polyacrylamide gel electrophoresis in 8M urea at pH 3·5, is described.

I. Introduction

It was suggested previously that ovalbumin and its stable modification, S-ovalbumin, differed in covalent structure. A shift in the position of the disulphide cross-link, resulting from an interaction with a sulphydryl group, was postulated (Smith and Back 1965). To obtain direct evidence of covalent bond alteration a comparison of the fragments produced by enzymic digestion of each protein was attempted. Because of their tendency to aggregate, a comparison of the tryptic peptides by "finger-printing" (Ingram 1958) was not feasible.

In this paper methods are described for producing comparable tryptic digests and for their preliminary separation into groups of peptides. The identification and separation of cystine peptides is described and a method is given for resolving peptides in an "insoluble" fraction. Although these methods have not revealed a difference in the two ovalbumins they are described in detail because of their value in subsequent work on the amino acid sequence of ovalbumin.

II. Materials and Methods

(*a*) *Materials*

Ovalbumin and S-ovalbumin were prepared as described previously. Trypsin (Sigma, twice crystallized, lot T61B–219) was treated with 1-tosylamide-2-phenylethylchloromethyl ketone to inactivate chymotrypsin (Kostka and Carpenter 1964). Pepsin was a 1 : 60,000 crystallized preparation from Sigma. The sulphydryl reagents 4-dimethylamino-3, 5-dinitrophenylmaleimide and 5,5′-dithiobis(2-nitrobenzoic acid) were from the Aldrich Chemical Co. Inc.

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Acrylamide, *N,N'*-methylenebisacrylamide, and *N,N,N',N'*-tetramethylethylenediamine were from Eastman Organic Chemicals.

(*b*) *Denaturation and Alkylation*

To a mixture of 1·0 g protein and 4 ml water was added 40 ml of a solution 8M in urea and 0·1N in HCl. The mixture was stirred until all the protein had dissolved, then transferred to the water-jacketed cell of the pH-stat. A solution of 0·5 g iodoacetic acid in 0·5 ml 5M NaOH and 1 ml water was added, and the pH adjusted to 8·0 with 5M NaOH. Alkylation was carried out for 15 min at 25°C, the pH being maintained at 8·0 by the automatic addition of 1M NaOH. Glacial acetic acid was then added to bring the pH to 4·8, the protein was precipitated by the addition of 60 ml of water, centrifuged, and washed three times with 60-ml lots of water.

(*c*) *Tryptic Digestion and Preparation of Soluble Fraction*

The washed precipitate was suspended in water in the pH-stat at 40°C, stirred vigorously to disperse lumps, and titrated at pH 8·15 for 15–30 min, when most of the protein had dissolved and the rate of consumption of alkali (0·5M NaOH) was very low. A stream of nitrogen was passed over the solution in the pH-stat cell to avoid absorption of atmospheric CO_2. The trypsin (2% of the weight of protein) was dissolved in a small volume of water immediately before adding. At the end of the digestion period, 25% formic acid was added to bring the pH to 4·5, and the precipitate was centrifuged at 10,000 r.p.m. for 15 min and washed twice with water. Variations in the protein concentration and digestion time will be discussed in Section III(*a*).

(*d*) *Fractionation of Soluble Tryptic Peptides*

A column 3·1 by 75 cm (bed volume 500 ml) of Sephadex G-25 was equilibrated with 1% formic acid, and 20 ml of the soluble fraction of the digest was applied and eluted with 1% formic acid, pumped through the column at the rate of 1 ml/min. The effluent was monitored continuously for its absorbance at 280 mμ by means of a Beckman model DB spectrophotometer equipped with flow cell and recorder. Fractions of 6 ml volume were collected and analysed for cystine as described below, and pooled fractions (Fig. 2) were freeze-dried.

The cystine peptides were separated on columns (100 or 180 ml bed volume) of DEAE-Sephadex type A50 equilibrated with 0·1M acetic acid–pyridine buffer of pH 5·6 (stock 1M buffer contained 60 ml of glacial acetic acid and 240 ml of pyridine in 1 litre). About 150 mg of dried peptide mixture was dispersed in 10 ml of starting buffer (it did not dissolve completely—see below) and the mixture was centrifuged and applied to the column. Elution at the rate of 0·6 ml/min was started with the 0·1M acetic acid–pyridine buffer and 6 ml fractions were collected. After 50 ml had passed through, a gradient was formed by running 0·3M acetic acid–pyridine into a mixing vessel containing 300 ml of the 0·1M buffer. Fractions were analysed for total peptide by the Lowry modification of the Folin method (Lowry *et al.* 1951) and for cystine.

(*e*) *Analysis of Fractions for Cystine Peptides*

The modified phosphotungstic acid method of Kassel and Brand (1938), as described by Spackman, Stein, and Moore (1960), was used initially to determine cystine. A more sensitive and convenient method was then developed, based on reduction of the cystine with borohydride (Brown 1960) and reaction of the thiol with 5,5'-dithiobis(2-nitrobenzoic acid), as described by Ellman (1959). The method is as follows: A 1-ml sample (containing up to 2×10^{-4}M disulphide) in 1% formic acid is neutralized with 1 ml 0·25M NaOH and 1 ml freshly prepared 2·5% sodium borohydride is added from a burette. After 5 min 2 ml acetone is added to react with excess borohydride, and is followed by 1 ml 0·5M NaH_2PO_4 and 0·1 ml 5,5'-dithiobis(2-nitrobenzoic acid) (0·4% in pH 7 phosphate buffer). The colour develops immediately and is read at 412 mμ. Cystine itself could not be used as a standard, for the reaction of cysteine with this reagent is very slow, but either oxidized or reduced glutathione was satisfactory and gave absorbance values in agreement with those of Ellman (1959). A similar method for determining disulphide in proteins has recently been described by Cavallini, Graziani, and Dupré (1966).

(*f*) *Peptic Digestion and Fractionation*

To a solution of 2 g of protein in 80 ml of 8M urea at pH 3 and 25°C, 66 mg of 4-dimethylamino-3,5-dinitrophenylmaleimide dissolved in 0·66 ml of dimethylformamide was added (allowing a 10% excess over that required to block four thiol groups/mole). The pH was adjusted to 6·0 and 1 hr later was brought to pH 2·5 with 5M HCl. The solution was dialysed overnight against two 2-litre portions of 0·01M HCl to remove most of the urea, then 10 mg pepsin was added, and the pH adjusted to 2·0. After incubating the mixture at 37°C for 8 hr the pH was brought to 3·0, a further 10 mg of pepsin added, and the digestion continued at 20°C for 22 hr. The pH was then adjusted to 4·0, insoluble peptides removed by centrifuging, and the supernatant adjusted to pH 2·5. Fractionation on Sephadex G-25 (in 1% formic acid) was as described for the tryptic digest. The peptides labelled with 4-dimethylamino-3,5-dinitrophenylmaleimide were retarded on the column and the absorbance was monitored at 440 mμ after one column volume.

The pooled cystine fractions from five Sephadex column runs were freeze-dried and oxidized with performic acid, as described by Hirs (1956). Partial acid hydrolysis of 30 mg of oxidized peptides was effected in 2 ml of 10M HCl at 37°C for 72 hr. The cysteic acid dipeptides were then separated on a column of Amberlite CG-120, type 2 (8–10% cross-linked) as described by Flavin and Anfinsen (1954).

(*g*) *Electrophoresis of Peptides*

High-voltage electrophoresis on Whatman 3 HR paper was carried out at a voltage gradient of 50 V/cm in the formic acid–acetic acid and pyridine–acetic acid buffers described by Atfield and Morris (1961). Cooling was accomplished either by pumping Varsol through a cooling coil and around the paper (suspended in a vertical tank), or by means of a water-cooled, flat-plate apparatus. Peptide spots were developed with the cadmium–ninhydrin stain of Atfield and Morris (1961).

Electrophoresis at pH 3·5 in polyacrylamide gel that was 8M in urea was found to resolve the larger and insoluble peptides which on paper either showed pronounced "tailing" or did not move at all. The apparatus was similar to that described by Raymond (1964) but was not cooled. A slab of gel 22 by 8 cm and 0·3 cm thick was cast with the apparatus horizontal. Gels contained 15% acrylamide and 0·1% methylenebisacrylamide and were prepared from the following stock solutions:

A: 75 g acrylamide, 0·5 g methylenebisacrylamide, and 120 g urea, made up to 250 ml with water, and de-ionized by stirring with 20 g MB1 resin.

B: 4 ml formic acid (99%), 20 ml 1M NaOH, 48 g urea, and 4 ml tetramethylethylenediamine, made up to 100 ml with water.

To prepare the gel, 32 ml of solution A was mixed with 8 ml of solution B, the mixture was cooled, and a freshly prepared solution of 0·08 g of ammonium persulphate, in 24 ml of de-ionized 8M urea was added. Samples were placed in slots 0·5 cm wide in the upper edge when the apparatus was set up with the long dimension vertical. The lower edge dipped into the cathode electrolyte (0·5% formic acid) and the upper edge was in contact with the anode electrolyte, 8M urea in 0·5% formic acid. The dried peptides (5 mg) were dissolved in 1 ml of the latter and mixed with enough Biogel P300 to give a thick slurry. After placing about 0·05 ml of this sample in each slot, all slots were covered with a 1-cm layer of anode electrolyte thickened with Biogel before filling the anode vessel. Runs were of 16–20 hr duration at 20°C with an applied voltage of 160 V, the current decreasing from 20 to 12 mA during the run. After removing the gel, it was stained with a 0·2% solution of amido black 10B in 7% acetic acid for 30 min and then washed with 7% acetic acid.

(*h*) *Amino Acid Analyses*

The freeze-dried peptides (5 mg) were hydrolysed in evacuated, sealed tubes with 1–4 ml of 6M HCl for 20 hr at 110°C. The acid was removed by evaporation in a continuously evacuated desiccator over KOH pellets for 4–6 hr. Aliquots of the hydrolysate were analysed in a Beckman model 120C amino acid analyser using the 4-hr accelerated system. As internal standards, DL-norleucine and L-α-amino-β-guanidopropionic acid (0·125 μmole of each) were added.

Corrections for the destruction or incomplete release of amino acids during hydrolysis were not made, since the analyses were used only for comparing related peptide fractions. Tryptophan was not determined in the fractions examined.

III. Results and Discussion

(*a*) *Denaturation, Alkylation, and Tryptic Digestion*

Trypsin does not attack native ovalbumin (Christensen 1952) so that it was necessary to use a denaturing agent before digestion. Both ovalbumin and S-ovalbumin have been shown to denature rapidly in acidic 8M urea (Smith and Back 1968), and they may then be treated with iodoacetate to alkylate the four sulphydryl groups, thus preventing subsequent oxidation or interchange reactions. The conditions were

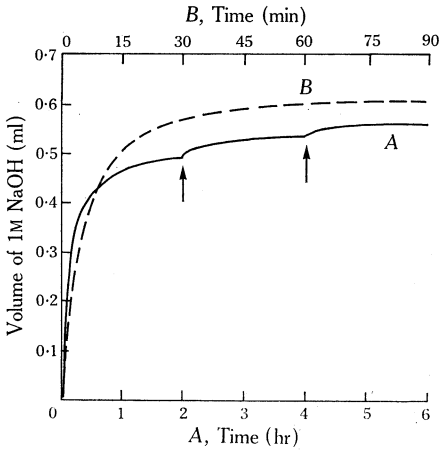

Fig. 1.—Trypsin digestion of ovalbumin and S-ovalbumin as measured in the pH-stat at 40°C, pH 8·15, with 1 g protein (denatured and alkylated), 20 mg trypsin. *A*, 10 mg trypsin added initially, 5 mg added at 2 hr and 4 hr (arrows). Protein concentration 0·5%. *B*, 20 mg trypsin added at beginning of digestion. Protein concentration 2·0%.

chosen to minimize alkylation of other side-chains, although subsequent amino acid analyses of peptide fractions containing methionine did show small amounts of *S*-carboxymethylhomocysteine, arising from the alkylation of methionine (Gundlach, Moore, and Stein 1959). The precipitation and washing of the denatured proteins, as well as removing urea and excess iodoacetate, ensured that both proteins were in a comparable state before tryptic digestion.

The rate of tryptic digestion was followed by titrating the liberated hydrogen ion in the pH-stat (Fig. 1). Assuming that 90% of the released amino groups are titrated at pH 8·15 (Richards 1955), digestion for 90 min at an initial protein concentration of 2% (Fig. 1*B*) involves breakage at an average of 32 of the total 36 lysine and arginine residues. However, the recovery of cystine from ovalbumin, based on measurements of the cystine in fractions from the Sephadex G-25 column, was only 35% under these conditions of digestion.

A number of different conditions was tried in attempts to improve the recovery. The main loss was in the residue insoluble at pH 4·5, although 70% of the material absorbing at 280 mμ was soluble. Increasing the digestion time to 180 min and adding more trypsin had little effect, but decreasing the protein concentration from

2 to 1% increased recovery to 50%. Digestion in 2·5M urea with a protein concentration of 0·5% gave 87% recovery of the cystine.

Although it was possible to increase the proportion of the cystine peptides in the soluble fractions, further difficulties were encountered in subsequent fractionation. Peaks from the Sephadex G-25 column showed increased "tailing" and lyophilized fractions became increasingly less soluble. Although protein solutions of initial concentration 2 and 1% cleared rapidly after the addition of trypsin, they became increasingly turbid after digestion for 60 min and on several occasions consumption of alkali ceased at this point. The division into "soluble" and "insoluble" fractions

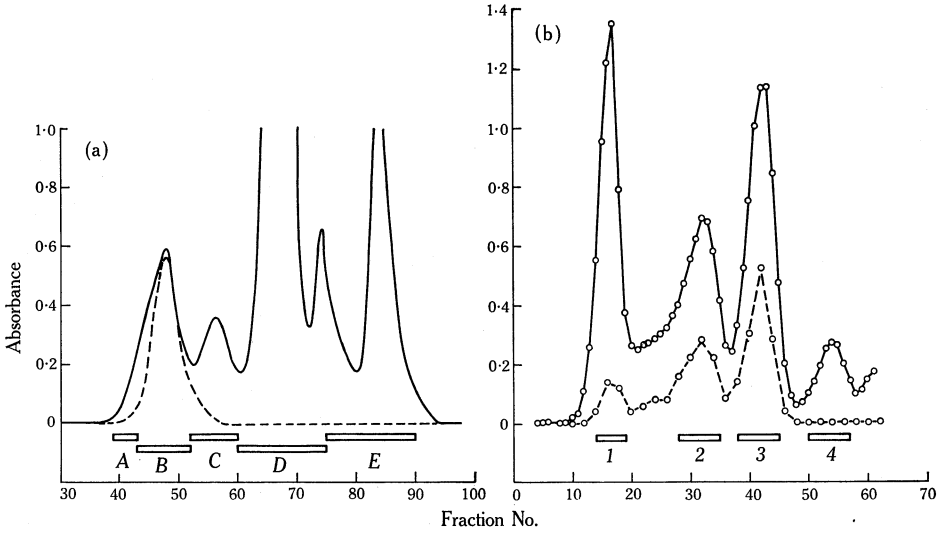

Fig. 2.—(*a*) Fractionation of soluble tryptic peptides on Sephadex G-25. ——— Absorbance at 280 mμ, monitored continuously. – – – – Disulphide estimations on fractions, absorbance at 412 mμ. (*b*) Chromatography of cystine peptides (fraction *B*) on DEAE-Sephadex. ——— Fractions analysed by Lowry–Folin method, absorbance at 720 mμ. – – – – Fractions analysed for disulphide, absorbance at 412 mμ.

was somewhat arbitrary, and the peptides apparently soluble in 1% formic acid were highly aggregated, showing a fast-moving peak in the ultracentrifuge and causing in some cases precipitation in the Sephadex column. The digestion conditions were chosen to reduce aggregation by using low protein concentrations (0·5%), and to increase the extent of attack on less susceptible bonds by a longer digestion time and by the addition of trypsin in three lots. This resulted in an increased recovery of cystine although the total digestion was apparently less (Fig. 1*A*). After the digestion, the solution was freeze-dried and the product was dissolved in 1% formic acid to give a 2% solution, from which a little insoluble material was removed by centrifuging before the solution was applied to the column.

No significant difference in rates of digestion or total alkali consumption was found between ovalbumin and S-ovalbumin, but the former generally gave a lower recovery of cystine and the peptides derived from it appeared to aggregate more readily.

(*b*) *Fractionation of Tryptic Peptides*

The soluble peptides (65–70% of the original weight of protein) yielded a highly reproducible chromatogram on gel filtration through Sephadex G-25 [Fig. 2(*a*)]. No difference between the soluble peptides from ovalbumin and S-ovalbumin was observed. The cystine peptides emerged in one peak [fraction *B*, Fig. 2(*a*)]. Fractions *A*, *B*, *C*, and *D* from each protein were compared by electrophoresis on polyacrylamide gel in 8M urea at pH 3·5, and the smaller peptides in fraction *E* by high-voltage paper electrophoresis at pH 1·9. No difference in size or position of the stained peptide spots was found. Close identity of the corresponding peptide fractions *A*, *B*, *C*, and *D* from the two proteins is also demonstrated by the amino acid analyses given in Table 1.

TABLE 1

AMINO ACID ANALYSES OF SOLUBLE TRYPTIC PEPTIDES, FRACTIONATED ON SEPHADEX G-25

Digestion time 6 hr, with a 0·5% protein solution. Fractions identified as in Figure 2(*a*). Values expressed as moles of amino acid per 100 moles total amino acids.* OA, ovalbumin; SOA, S-ovalbumin

Amino Acid	Fraction *A*		Fraction *B*		Fraction *C*		Fraction *D*	
	OA	SOA	OA	SOA	OA	SOA	OA	SOA
Lysine	4·21	4·56	3·48	3·48	3·17	3·30	8·29	8·15
Histidine	2·62	2·46	3·17	3·00	0·93	0·93	0·23	0·27
Ammonia	8·10	7·82	7·11	7·01	12·79	12·50	11·59	10·52
Arginine	2·15	1·97	3·39	3·28	4·27	4·15	6·15	5·67
SCM-cysteine†	0·88	0·84	1·03	0·91	0·31	0·25	1·38	1·23
Aspartic acid	7·36	7·47	7·42	7·42	10·36	10·34	8·99	9·22
Threonine	2·55	2·91	1·91	1·90	3·77	3·66	6·13	6·26
Serine	13·51	13·94	9·34	9·54	6·71	7·12	7·88	7·98
Glutamic acid	10·85	10·53	12·65	12·71	13·33	13·25	13·30	13·86
Proline	1·38	1·40	3·36	3·48	4·12	4·16	3·33	3·00
Glycine	5·92	5·84	6·04	6·21	5·40	5·15	2·54	2·79
Alanine	13·49	12·64	12·31	12·19	10·54	9·77	6·55	7·12
½Cystine	0	0	1·14	1·31	0	0	0	0
Valine	6·65	6·54	7·58	7·70	4·39	4·91	5·81	5·81
Methionine	2·68	2·95	1·09	1·06	1·14	1·13	3·52	3·40
Isoleucine	5·33	5·45	5·05	4·95	6·16	6·24	5·41	5·72
Leucine	6·54	6·91	6·61	6·52	5·78	5·97	4·33	4·33
Tyrosine	1·54	1·66	2·38	2·39	2·18	2·43	1·09	0·86
Phenylalanine	4·21	4·10	4·98	4·92	4·65	4·71	3·48	3·79

* Determined on aliquots equivalent to approximately 0·25 mg of peptide. Hydrolysis time 20 hr. Amounts of amino acid less than 0·1 mole % are shown as 0.

† *S*-carboxymethylcysteine.

Fractionation of the cystine peptides in fraction *B* on a 100-ml column of DEAE-Sephadex gave the chromatogram shown in Figure 2(*b*). Cystine was found in the first three peaks, but the amount present in peak *1* was low, and the electrophoresis of this fraction on polyacrylamide gel showed the presence of several peptides. Carbohydrate was also found in peak *1*. Peaks *2* and *3* gave one main band on gel electrophoresis. Fractionation on a longer column (180 ml) showed peak *1* as two partly resolved peaks but peaks *2* and *3* remained essentially the same as before.

Amino acid analyses of the four fractions, collected and pooled as shown in Figure 2(*b*), are given in Table 2. There is good agreement in the analyses for the corresponding purified fractions from ovalbumin and S-ovalbumin. The presence of two cystine peptides in the digests from each protein can also be explained by these analyses. If the histidine and threonine values for fractions *2* and *3* represent one residue of each of these amino acids, fairly close integral values for the number of residues of the other amino acids may be calculated. Peak *3* then appears as a peptide containing 49 residues, including two lysine, two arginine, and two half-cystine

TABLE 2

AMINO ACID ANALYSES OF PEPTIDE FRACTIONS FROM SEPHADEX A-50 SEPARATION OF "CYSTINE FRACTION"

Values expressed as moles of amino acid per 100 moles total amino acids*

Amino Acid	Ovalbumin Fraction 2†	Ovalbumin Fraction 3†	Ovalbumin Fraction 3‡	S-ovalbumin Fraction 3‡	Ovalbumin Fraction 4‡	S-ovalbumin Fraction 4‡
Lysine	4·52	4·02	3·99	4·06	2·33	2·43
Histidine	2·00	1·83	1·80	1·86	4·75	4·55
Ammonia	8·65	7·51	8·14	7·69	7·44	6·94
Arginine	2·41	4·18	4·54	4·35	3·70	3·75
SCM-cysteine	0	0	0	0	1·94	1·77
Aspartic acid	7·76	6·83	6·59	6·61	7·19	7·32
Threonine	2·47	2·16	2·14	2·16	0·89	0·89
Serine	9·01	7·56	7·53	7·70	8·44	8·46
Glutamic acid	10·21	13·34	13·22	13·30	13·06	13·39
Proline	7·31	6·55	6·02	5·92	2·66	2·74
Glycine	7·56	6·55	6·31	6·53	7·04	7·21
Alanine	2·81	4·37	4·33	4·42	16·74	16·17
½Cystine	4·46	3·91	3·62	3·62	0	0
Valine	7·03	6·12	6·10	6·13	8·29	8·36
Methionine	0·13	0	0	0	0	0
Isoleucine	4·74	4·21	4·18	4·25	5·29	5·37
Leucine	9·91	10·82	10·92	10·92	3·69	3·94
Tyrosine	4·18	5·73	6·34	6·23	0·57	0·70
Phenylalanine	4·86	4·30	4·25	4·26	5·97	6·01

* See first footnote to Table 1.

† 90 min digestion, 2% protein. Fractions from 100 ml Sephadex A-50 column.

‡ 6 hr digestion, 0·5% protein. Fractions from 180 ml Sephadex A-50 column.

residues. Peak *2* has 43 residues, the other six probably representing a peptide with the composition (Glu_2, Ala, Leu, Tyr, Arg) which is slowly split from the larger peptide in peak *3*. Since peak *2* has three basic residues one of these may be located next to one of the three proline residues and thus be resistant to trypsin (Hill 1965).

(*c*) *Comparison of Cysteic Acid Peptides from Peptic Digests*

Conversion of ovalbumin to S-ovalbumin during the tryptic digestion of the former at pH 8 and 40°C was thought to be unlikely when the kinetics of the reaction were considered (Smith and Back 1965). However, because of the possibility that conversion might occur faster with the denatured protein, and the further

possibility of disulphide interchange at alkaline pH (yielding three different cystine peptides), it was thought desirable also to compare peptides from a digest prepared at low pH. The results already presented and those in the following section indicate that neither eventuality need be considered, so that the examination of peptic digests is mainly confirmative.

The –SH groups were blocked with a maleimide at pH 6 and the digestion was carried out at pH 2–3. The cystine fraction was again eluted early from the Sephadex G-25 column, the peptides labelled with the coloured maleimide being retarded and completely separated from the cystine peptides. Recovery of cystine from the Sephadex column in one experiment was 64% for ovalbumin and 81% for S-ovalbumin.

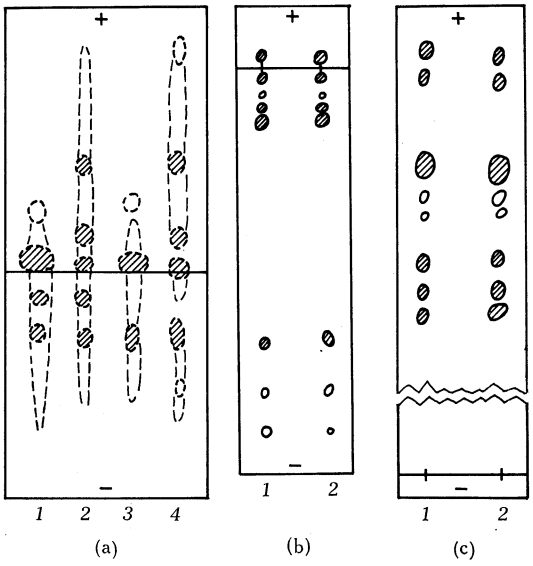

Fig. 3.—High-voltage paper electrophoresis of ovalbumin and S-ovalbumin cystine peptides from peptic digestion. (*a*) Cystine fraction from Sephadex G-25 column; electrophoresis in pyridine–acetate buffer, pH 5·2. *1*, ovalbumin; *2*, ovalbumin after oxidation with performic acid; *3*, S-ovalbumin; *4*, S-ovalbumin after oxidation with performic acid. (*b*) and (*c*) Partial acid hydrolysates of oxidized peptides after separation of cysteic acid peptides by ion-exclusion chromatography. Electrophoresis in formic acid–acetic acid buffer pH 1·9 (*b*) and in pyridine–acetate buffer pH 5·2 (*c*). *1*, ovalbumin; *2*, S-ovalbumin.

Figure 3(*a*) shows the results of high-voltage electrophoresis of the cystine fractions before and after performic acid oxidation. Two additional components moving towards the anode at pH 5·2 appear after oxidation, and presumably correspond to the two cysteic acid peptides from the two halves of a cystine peptide. Partial acid hydrolysis and separation of the cysteic acid peptides gave the mixtures shown in Figures 3(*b*) and 3(*c*). A single cystine peptide should yield four cysteic acid dipeptides after oxidation and partial hydrolysis (Flavin and Anfinsen 1954) but it is likely that some larger peptides were also present. There was no difference in the electrophoretic separation of the peptides from ovalbumin and S-ovalbumin, and this confirms the results obtained by tryptic digestion. It is concluded that there is no difference in the position of the disulphide bond in ovalbumin and S-ovalbumin.

(*d*) *Insoluble Peptide Fraction from Trypsin Digestion*

The amino acid analyses of the insoluble fraction from tryptic digests of ovalbumin and S-ovalbumin are given in Table 3. For comparison, the amino acid

analysis of whole ovalbumin (Habeeb 1961) is also shown. The main differences are the lower content of lysine and arginine in the insoluble fractions (indicating larger peptides than in the soluble fractions), their higher serine, leucine, and phenylalanine content, and their lower glutamic acid content.

Fractionation of these peptides, which contain about one-third of the total residues, was difficult because of their low solubility. A completely dispersed solution was only obtained with 8M urea at pH 3·5. Gel electrophoresis in this solvent resolved

TABLE 3

AMINO ACID ANALYSES OF INSOLUBLE FRACTIONS AFTER TRYPSIN DIGESTION, COMPARED WITH WHOLE OVALBUMIN

Values expressed as moles of amino acid per 100 moles total amino acids

Amino Acid	Insoluble Fraction from: Ovalbumin*	S-ovalbumin*	Whole Ovalbumin†
Lysine	4·26	4·27	5·54
Histidine	1·77	1·95	1·98
Arginine	2·23	2·23	4·02
SCM-cysteine	1·04	1·22	1·40‡
Aspartic acid	8·51	8·38	8·38
Threonine	3·57	3·65	3·53
Serine	10·10	10·06	7·09
Glutamic acid	12·05	11·99	13·01
Proline	3·41	3·45	3·83
Glycine	5·33	5·09	5·01
Alanine	9·15	9·25	9·69
½Cystine	0	0	0·40
Valine	8·36	8·21	8·54
Methionine	4·63	4·67	4·25
Isoleucine	6·28	6·30	6·48
Leucine	10·21	10·34	8·62
Tyrosine	2·63	2·62	2·63
Phenylalanine	6·03	6·24	5·38

* See first footnote to Table 1.

† Calculated from analysis of Habeeb (1961). Determined after heating in a sealed tube in 6M HCl for 24 hr at 105°C.

‡ As cysteine.

at least eight components that could be stained with amido black. With two digestions a component was detected in the mixture from ovalbumin which was not present in the S-ovalbumin mixture, but it is not known if this represents a difference in peptide composition.

IV. CONCLUSION

There is no difference in the peptides linked by the disulphide bond in both ovalbumin and S-ovalbumin, and the hypothesis that conversion of ovalbumin to S-ovalbumin involves a shift in the position of this bond is not tenable.

A comparison of the soluble peptides from tryptic digests did not reveal any significant differences. A difference in the aggregation properties and in the resolution

of the insoluble peptides on polyacrylamide gel was observed but no conclusions can be drawn from this observation.

V. Acknowledgments

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