STUDIES ON OVALBUMIN

V.* THE AMINO ACID COMPOSITION AND SOME PROPERTIES OF CHICKEN, DUCK, AND TURKEY OVALBUMINS

By M. B. SMITH† and JOAN F. BACK†

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Summary

The ovalbumins from chicken, duck, and turkey eggs were prepared by ammonium sulphate fractionation and purified by isoelectric focusing in a pH gradient from 3 to 6. Amino acid analyses show a closer relationship between turkey and chicken ovalbumins than between duck and chicken ovalbumins. Major differences in composition are in sulphydryl, disulphide, and methionine content (chicken 4, 1, and 15; duck 2, 1, and 23; and turkey 3, 3, and 14 groups per mole respectively). Carbohydrate is present in the three proteins in similar amounts. No *N*-terminal amino acid could be detected, but *C*-terminal proline was identified in the three proteins.

Electrophoretic properties of the purified proteins were in agreement with the results of other workers on the electrophoresis of whole egg whites, and their hydrodynamic properties indicated a close similarity in size and shape. Both duck and turkey ovalbumins were converted to more heat-stable forms when exposed to pH 10 at 40°C, conditions similar to those required to convert chicken ovalbumin to S-ovalbumin.

I. INTRODUCTION

Although comparisons have been made of the composition of egg whites and of the properties of some isolated proteins from the eggs of different avian species (e.g. Feeney *et al.* 1960), little comparative work has been done with the major protein of egg white, ovalbumin. This protein has not been shown to possess any specific biological role and is generally regarded as being present in egg white solely as a source of amino acids for the developing embryo.

In previous papers we have shown that chicken ovalbumin possesses some unusual and specific properties, viz. it may be converted to a more stable form, S-ovalbumin (Smith and Back 1965, 1968*a*), and it is specifically attacked by bacterial proteases at a point 33 residues from its *C*-terminus (Smith 1968; Sleigh *et al.* 1969). When comparing the amino acid composition of tryptic peptides containing the disulphide bond of ovalbumin and S-ovalbumin (Smith and Back 1968*b*) we became aware of the lack of published amino acid analyses of ovalbumin by modern techniques with corrections for hydrolysis losses (Neuberger and Marshall 1966). In this paper we present the results of our analyses by current techniques of ovalbumins from chicken, duck, and turkey eggs after purification by isoelectric focusing. Some observations on their electrophoretic and hydrodynamic properties and an experiment to demonstrate that duck and turkey ovalbumins undergo a change in stability in alkaline solution are also presented.

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† Division of Food Preservation, CSIRO, P.O. Box 43, Ryde, N.S.W. 2112.

A short account of this work has previously been presented (Smith and Back 1969).

II. Materials and Methods

(a) *Preparation and Purification of Ovalbumin*

Chicken, duck, and turkey eggs were obtained from local poultry farms and refrigerated within 24 hr of laying. Ovalbumin was prepared from the separated whites by fractional precipitation with ammonium sulphate (Sørensen and Høyrup 1915–17) followed by reprecipitation twice with half-saturated ammonium sulphate at pH 4·7. The chicken albumin was obtained in a crystalline form but attempts to crystallize duck and turkey albumins were unsuccessful. The proteins were dialysed against water to remove ammonium sulphate, and then freeze-dried and stored at 4°C.

The method of isoelectric focusing as described by Vesterberg and Svensson (1966) was used to purify the ovalbumins. An L.K.B. type 8102 electrofocusing column was filled with a sucrose density gradient made from 45 discrete 10-ml fractions containing 1% ampholine carrier ampholytes for a pH range of 3–6. The dry protein (50 mg) was dissolved in two 10-ml fractions near the middle of the gradient while filling the column. Water at 5°C was circulated through the jacket of the column and an increasing voltage (up to 500 V) applied over 45 hr. The column was then emptied with a peristaltic pump at a flow rate of 45 ml/hr, the solution flowing first through a flow cell in a Beckman DB spectrophotometer to monitor the absorbance at 280 nm, and then to a fraction collector (7·5-ml fractions). The pH of the fractions was measured at 20°C with a Radiometer 25SE pH-meter. Fractions were combined (as indicated in Fig. 2) and freed from sucrose and carrier ampholytes by gel filtration on Sephadex G-50 in a volatile buffer of 0·5% formic acid adjusted to pH 4·0 with ammonia. Water and buffer salts were removed by freeze-drying and the final drying was done in a vacuum desiccator over P_2O_5.

(b) *Amino Acid Analysis*

Stock solutions (1% w/v) of the purified ovalbumins in water were prepared and aliquots containing 2·5 mg of protein hydrolysed with 2 ml of 6N HCl in sealed, evacuated tubes for 20 and 70 hr at 110°C. The acid was removed in a rotary evaporator at 40°C and the residue dissolved in 10 ml of citrate buffer (pH 2·2) containing 1·25 μmoles of each of the internal standards DL-norleucine and L-α-amino-β-guanidopropionic acid. The analyses were made with a Beckman 120C analyser, using the 4 hr program.

Corrections were made for destruction of serine and threonine by extrapolation to zero hydrolysis time (Hirs, Stein, and Moore 1954). Valine and isoleucine values were taken from the 70-hr hydrolysis and tyrosine decomposition was corrected for by adding 3% of its higher value. Results for the other amino acids are the means of the values obtained at the two hydrolysis times.

To obtain the total cystine and cysteine, ovalbumin samples were first oxidized with performic acid (Hirs 1956) and analysed after a 20-hr hydrolysis. Cysteine was determined as *S*-carboxymethylcysteine in the analyser after hydrolysis of the denatured and alkylated protein, prepared as described previously (Smith and Back 1968*b*). Tyrosine and tryptophan were determined spectrophotometrically by the method of Goodwin and Morton (1946).

(c) *End Groups and Carbohydrate*

Edman degradation and hydrazinolysis of duck and turkey ovalbumins were carried out as described by Sleigh *et al.* (1969). "Dansylation" and detection of "dansyl" amino acids were carried out by the procedure of Gray (1967).

Carbohydrate was determined by the orcinol–sulphuric acid method for non-nitrogenous sugars (Francois, Marshall, and Neuberger 1962), with mannose as the standard.

(d) *Paper Electrophoresis*

The L.K.B. apparatus was used with Whatman No. 1 paper and sodium phosphate buffer of ionic strength 0·05, pH 7·5. Electrophoresis was carried out at 20°C for 17 hr at 150–170 V. The paper was stained with amido black in methanol–acetic acid–water (114 : 19 : 90 v/v) and washed with the same solvent.

(*e*) *Sedimentation Measurements*

Sedimentation coefficients were determined as described previously (Smith 1964) at 20°C with protein concentrations of 3, 5, 8, and 10 mg/ml. The solvent was 0·05 ionic strength phosphate buffer, pH 6·9, containing 0·1M NaCl.

(*f*) *Denaturation Measurements*

The relative rates of denaturation were compared by heating 0·5% solutions of each ovalbumin in 0·05 ionic strength phosphate buffer, pH 7·0, at temperatures of 68, 70, and 73·5°C. The percentage soluble protein was determined as described by Smith (1964). Conversion to a more stable form was studied at a temperature of 40°C and pH of 10, using the procedure of Smith and Back (1965).

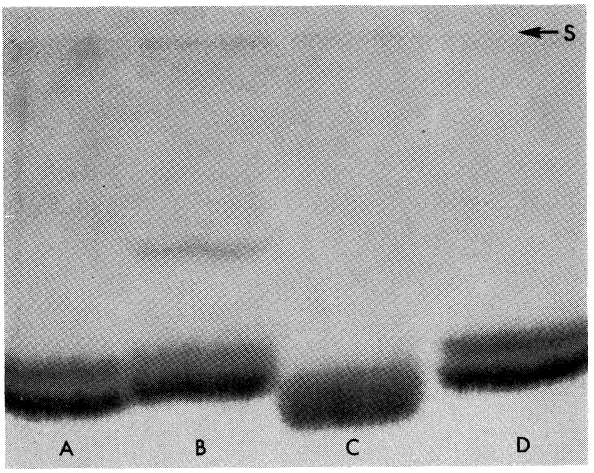

Fig. 1.—Paper electrophoresis of chicken (*A*, *D*), turkey (*B*), and duck (*C*) ovalbumins in 0·05 ionic strength phosphate buffer, pH 7·5. *S*, starting point.

III. Results and Discussion

(*a*) *Preparation and Electrophoretic Properties*

Because neither duck nor turkey ovalbumin could be induced to crystallize from ammonium sulphate solution, the homogeneity of the preparations was examined by paper electrophoresis. Figure 1 shows some contamination of one of the turkey ovalbumin preparations, probably by conalbumin. For the amino acid analyses it was desirable to use the purest preparations obtainable, and the method of isoelectric focusing was used to purify 50-mg quantities of each preparation.

Figure 2 shows the result of one run with turkey ovalbumin. The first peak is due to components absorbing at 280 nm in the ampholyte. There was no apparent resolution of the different ovalbumin components which, in the case of chicken ovalbumin, are known to be caused by differences in phosphate content (Perlmann 1952); however, adequate separation from conalbumin (pH 6·0) and ovomucoid (pH 3·9–4·3) was ensured. The mean pH values for two preparations at the centre of the eluted peak were 4·53 for chicken, 4·34 for duck, and 4·50 for turkey ovalbumin. These values are lower than the accepted isoelectric points for the major components, but show duck ovalbumin to be more acidic than turkey and chicken ovalbumins, which is also indicated by their mobility differences (Fig. 1; also Bain and Deutsch 1947).

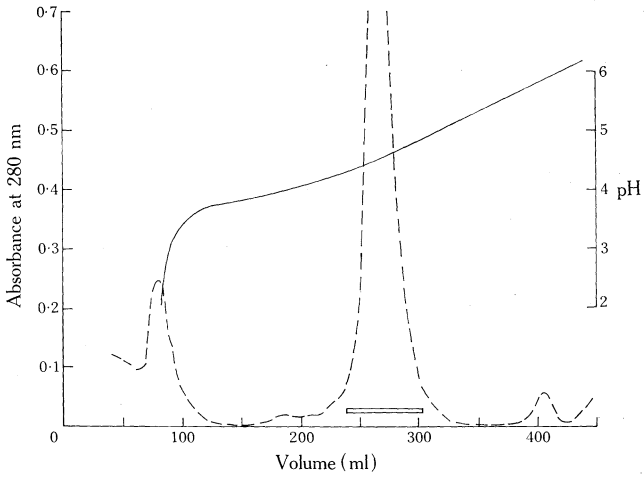

Fig. 2.—Isoelectric focusing of turkey ovalbumin:
– – – Absorbance at 280 nm, monitored continuously.
—— pH of 7·5-ml fractions.
The open rectangle indicates those fractions which were combined.

TABLE 1

AMINO ACID ANALYSES OF CHICKEN, DUCK, AND TURKEY OVALBUMINS

Values expressed as moles of amino acid per 100 moles total amino acids*, for two separate preparations

Amino Acid	Chicken		Duck		Turkey	
	1	2	1	2	1	2
Lysine	5·13	5·14	4·87	4·80	5·42	5·39
Histidine	1·74	1·74	1·55	1·45	1·42	1·56
Arginine	4·04	3·71	3·58	3·45	3·10	3·22
Aspartic acid	8·18	8·08	7·21	7·10	8·10	8·00
Threonine	3·90	3·76	5·92	5·86	4·95	5·09
Serine	9·88	9·58	11·86	11·27	9·76	10·28
Glutamic acid	13·27	13·49	13·23	13·75	12·90	12·41
Proline	3·95	3·83	3·53	3·58	3·57	3·84
Glycine	4·90	4·87	5·01	4·93	5·39	5·45
Alanine	9·16	9·03	6·90	6·74	7·58	7·74
Valine	8·16	8·31	7·21	7·34	7·42	7·21
Methionine	4·02	3·87	5·81	5·95	3·71	3·77
Isoleucine	5·96	6·35	4·64	4·96	6·63	6·45
Leucine	7·85	8·19	7·34	7·44	8·32	7·98
Tyrosine	2·58	2·62	2·61	2·56	3·30	3·31
Phenylalanine	5·01	5·08	6·55	6·63	4·98	5·01
Tryptophan†	0·80	0·90	1·09	1·11	1·22	1·09
Cysteic acid‡	1·48		1·09		2·24	
SCM-cysteine§			0·50			0·68

* Obtained from results of 20- and 70-hr hydrolyses as described in Section II.

† Determined separately by spectrophotometry.

‡ After performic acid oxidation.

§ *S*-carboxymethylcysteine, after alkylation.

(*b*) *Amino Acid Composition*

Table 1 shows the amino acid composition of separate preparations of chicken, duck, and turkey ovalbumins. Two preparations were carried out at different times with different batches of eggs, and the analyses therefore include this preparation variability. Table 2 shows the means of these analyses expressed as residues per 387 residues (corresponding to the number in chicken ovalbumin) and thus allows a comparison of the numbers of side-chain groups in the three ovalbumins. The values given by Tristram and Smith (1963) for chicken ovalbumin are also shown and are in reasonable agreement with our values.

TABLE 2

COMPOSITION OF CHICKEN, DUCK, AND TURKEY OVALBUMINS

Amino acid values expressed as residues per 387 residues (mean of two preparations). Carbohydrate expressed as a percentage of mannose by weight

Amino Acid	Chicken		Duck	Turkey
	Tristram and Smith (1963)	This Paper		
Lysine	20	20	19	21
Histidine	7	7	6	6
Arginine	15	15	14	12
Aspartic acid	32	32	28	31
Threonine	16	15	23	19
Serine	36	38	45	39
Glutamic acid	52	52	52	49
Proline	14	15	14	14
Glycine	19	19	19	21
Alanine	35	35	26	30
Valine	28	32	28	28
Methionine	16	15	23	14
Isoleucine	25	24	19	25
Leucine	32	31	29	32
Tyrosine	9	10	10	13
Phenylalanine	21	19	26	19
Tryptophan	3	3	4	4
Cystine	1	1	1	3
Cysteine	5	4	2	3
Carbohydrate (%)		2·0	2·7	3·4

The main differences in the analyses of the three ovalbumins are in the values for threonine, methionine, tyrosine, phenylalanine, cystine, and cysteine. Turkey ovalbumin appears to be more closely related in composition to chicken ovalbumin than is duck ovalbumin. This is illustrated by calculating the "difference indices" of Metzger *et al.* (1968), a method for comparing the amino acid composition of proteins. The difference index for chicken and turkey ovalbumins is 4·6, that for chicken and duck is 8·0, and that for turkey and duck is 6·8. The precision of these values

may be judged from the fact that difference indices calculated from the amino acid analyses for the different preparations of the same protein were in the range 1·3–1·5. The closer resemblance of turkey to chicken rather than to duck ovalbumin may be expected from their evolutionary relationships; this is also shown in the properties of other egg-white proteins (Miller and Feeney 1964).

(c) *Carbohydrate and End Groups*

The estimations of total hexoses with orcinol showed that both duck and turkey ovalbumins contained carbohydrate in amounts similar to that in chicken ovalbumin (Table 2). Separate hydrolyses for hexosamines were not carried out, but indication of the presence of hexosamines in both duck and turkey ovalbumins was obtained during the determinations of amino acids.

No *N*-terminal amino group could be detected either by Edman reaction or by the "dansyl" technique in duck and turkey ovalbumins, suggesting that the *N*-terminal amino group is acetylated, as it is in chicken ovalbumin. Hydrazinolysis liberated proline as *C*-terminal amino acid from both duck and turkey ovalbumins in amounts comparable to that given by chicken ovalbumin (Sleigh *et al.* 1969).

Experiments on the liberation of a *C*-terminal peptide from duck and turkey ovalbumins after limited proteolysis (M. B. Smith, J. F. Back, and R. W. Sleigh, unpublished data) suggested that duck ovalbumin has a similar *C*-terminal structure to chicken ovalbumin (Smith 1968). The duck peptide had a similar amino acid composition to the peptide from chicken ovalbumin, but lacked the two cysteine residues and contained a residue of methionine. The separation of a corresponding peptide from turkey ovalbumin was not achieved, possibly because of the presence of two extra disulphide bonds, which may have bound this segment to the rest of the molecule.

(d) *Sedimentation Coefficients*

For duck ovalbumin, the relation between sedimentation coefficient ($S_{20,w}$) and concentration (C, g/dl) was found to be:

$$S_{20,w} = 3{\cdot}29 - 0{\cdot}06C,$$

and for turkey ovalbumin:

$$S_{20,w} = 3{\cdot}48 - 0{\cdot}35C.$$

The relation for chicken ovalbumin was previously found (Smith and Back 1965) to be:

$$S_{20,w} = 3{\cdot}53 - 0{\cdot}27C.$$

(e) *Denaturation and Stability Change*

Chicken ovalbumin is 88% denatured (as measured by the loss of solubility at its isoelectric point) when heated at pH 7·0 and 73·5°C for 60 min (Smith and Back 1965). When duck and turkey ovalbumins were heated under the same conditions they were denatured 95 and 89% respectively. To better compare the changes in stability that might occur in alkaline solution, a heating temperature of 70°C was used for duck ovalbumin, giving 91% denaturation in 1 hr.

Solutions of the ovalbumins in carbonate buffer were incubated at pH 10·0 and 40°C, and samples were removed periodically, brought to pH 7·0 and heated

under the standard conditions. After 6 hr at pH 10, chicken ovalbumin had changed from 88 to 41% denaturation, duck ovalbumin from 91 to 37%, and turkey ovalbumin from 89 to 65%. Thus both duck and turkey ovalbumins appear to change in stability in alkaline solution in the same way that chicken ovalbumin is converted to the more stable derivative, S-ovalbumin (Smith and Back 1965). The kinetics of the change have not been examined in detail with duck and turkey ovalbumins, but it appears that the rate of change for duck ovalbumin is slightly faster, and for turkey ovalbumin considerably slower than that for chicken ovalbumin.

IV. Acknowledgment

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