MICROHETEROGENEITY AND PAUCIDISPERSITY OF GLYCOPROTEINS

PART II. THE CARBOHYDRATE OF OVALBUMIN FROM DIFFERENT SPECIES*†**

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ABSTRACT

L-β-Aspartamido-carbohydrate was prepared from the egg-white ovalbumins of duck, turkey, Western gull, and genetically homogeneous chickens, demonstrating the general nature of their linkage of carbohydrate to protein. In each case, the carbohydrate was paucidisperse; and the compositions of the fractions for duck and turkey were such that it is likely that more than one oligosaccharide residue per polypeptide chain is present. The results further support the hypothesis that the series of carbohydrate fractions represents the result of biosynthesis, and not that of genetic heterogeneity or subsequent degradation of one or more oligosaccharides.

INTRODUCTION

It is now well established that the single carbohydrate residue in the crystalline ovalbumin of chicken is paucidisperse[1]. It was known previously that chicken ovalbumin is microheterogeneous and that differences in the starch electrophoretic patterns are related to genetic variants[2]. To what extent the microheterogeneity of the whole protein is related to the carbohydrate paucidispersity is not yet understood. However, in any study of the structures of these carbohydrate residues, it is important to establish the origin and biological significance of their paucidispersity, which may have arisen by degradation during isolation or from the pooling of eggs from a genetically heterogeneous population. It was of interest also to analyze the analogous situation in the eggs of other birds.

This paper describes the diversity of the carbohydrate groups from six genetic variants of chicken, from other chicken ovalbumins, and from ovalbumins of duck, turkey, and Western gull.

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EXPERIMENTAL

Ovalbumins. — (*a*) Eggs of homozygous genotypes were collected daily at the Basic Research Laboratories, Hy-Line Poultry Farms, Johnson, Iowa, and refrigerated, and the ovalbumin was prepared within four days by the method described previously[3,4]. The genotypes, as described by Lush[2], are indicated in Table I, where the number of individual birds and eggs combined in the preparation are also noted. The ovalbumin of the 1b genotype was not genetically homogeneous in region II and III (ref. 2).

(*b*) Samples of chicken ovalbumin of undescribed genotype were obtained from investigators throughout the world, as acknowledged in the footnotes to Table I.

(*c*) Domestic-duck and turkey ovalbumins were kindly provided by Mr. M. B. Smith, C.S.I.R.O., Ryde, N.S.W. 2112, Australia.

(*d*) Western-gull ovalbumin was prepared from egg white kindly provided by Dr. A. Wilson, Department of Biochemistry, University of California at Berkeley, by fractionation on *O*-(carboxymethyl)cellulose[5].

Analytical methods. — Total neutral sugars, expressed as D-mannose, 2-amino-2-deoxy-D-glucose, and amino acids were determined as described previously[1].

L-β-Aspartamido-carbohydrate components of ovalbumin. — The preparative and chromatographic procedures followed those described earlier[1]. Each preparation of ovalbumin was digested four times with pronase, and the resulting glycopeptide mixture, containing up to 5 mg-equiv. of D-mannose, was fractionated on a column (0.9 × 150 cm) of AG-50 WX2 ion-exchange resin (200–400 mesh) at room temperature (20–25°). The column had been equilibrated with sodium acetate buffer, pH 2.6 and mM in Na^+, for 48 h, and the L-β-aspartamido-carbohydrate fractions* were eluted with this buffer at a flow rate of 27.5 ml/h. Glycopeptides having additional amino acid residues, principally those of L-leucine, were then eluted with 0.05M sodium acetate, pH 6.0. The eluates were continuously analyzed for total neutral sugars as described previously[6], and the percentage distribution of equivalents of D-mannose was determined from a measurement of the area under each peak. For duck and turkey, the chromatographic fractions corresponding to each peak of L-β-aspartamido-carbohydrate were combined and freeze-dried, the salt was removed by chromatography[1] on Sephadex G-25, and, without further purification, each product was analyzed for amino acid, total neutral sugar, and 2-amino-2-deoxy-D-glucose. The results are summarized in Table I.

RESULTS AND DISCUSSION

The presence of one oligosaccharide residue attached to the same unique asparaginyl residue in each polypeptide chain of chicken ovalbumin is well estab-

*The mixture of L-β-aspartamido-carbohydrate components from ovalbumins will be abbreviated AC, each of the chromatographic fractions being represented as AC-A, AC-B, etc., in their order of elution from the ion-exchange column.

TABLE I

COMPOSITION OF THE CARBOHYDRATE OF OVALBUMIN FRACTIONS RESULTING FROM PROTEOLYSIS WITH PRONASE

			Total neutral sugars (%)[a]				
Ovalbumin source	*No. of birds*	*No. of eggs*	*A*	*B*	*C*	*D*	*E*
Phenotype[b]							
IA IIA IIIA	3	8	5.9	10.5	37.9	31.1	14.6
IA IIA IIIA (H12)	1	4	7.9	13.9	40.9	23.2	14.0
IA IIA IIIB	4	11	8.2	16.5	36.8	24.5	14.0
IA IIB IIIAB	3	12	7.0	16.5	37.7	22.4	16.4
IA IIB IIIB	3	10	5.9	10.3	35.8	32.1	15.8
Ib	—	8	6.3	11.8	37.1	25.6	19.1
Pirkl[c]			6.0	11.8	37.0	30.7	14.5
Kaverzneva[d]			4.3	10.0	31.9	34.4	19.3
Marshall[e]			4.1	7.5	33.1	36.2	19.1
Yamashina[f]			4.9	9.3	42.5	28.8	15.0

[a]Normalized to total neutral sugar of AC 100%. [b]IA IIA IIIA, IA IIA IIIA (H12), ----Ib, six different genetic variants of chicken. The eggs were provided by Hy-Line Research Laboratories, and the crystalline ovalbumin was prepared by the method of Kekwick and Cannan[3]. [c]See ref. 1. [d]Provided by Dr. E. D. Kaverzneva, Department of Protein Chemistry, Academy of Sciences, Moscow, U. S. S. R. [e]Provided by Dr. R. D. Marshall, Department of Chemical Pathology, St. Mary's Hospital Medical School, London W.2., England. [f]Provided by Professor I. Yamashina, Department of Biological Chemistry, Kyoto University, Kyoto, Japan.

lished[7], as is the fact that each oligosaccharide is not the same[1,7]. Considering that most preparations of chicken ovalbumin come from pooled collections of eggs, it was however, reasonable to suppose that the variations in carbohydrate may have been due to genetic variations, which had been demonstrated by starch-gel electrophoresis[2,8].

The starch-gel electrophoretograms of chicken egg-white were analyzed by Lush[8], and showed differences in one or more regions, identified as I, II, and III. Two variants, IA and Ib, were associated with region I, which contained the ovalbumin. The other regions show variants A, B, or AB. Of these many possible combinations, those available for the isolation of ovalbumin from genetically homogeneous chickens are given in Table I. The extent of the digestion of ovalbumin by pronase to afford the L-β-aspartamido-carbohydrate is somewhat variable, resulting in somewhat differing yields of the higher glycopeptides, even from the same sample of ovalbumin. This variation is due to (*a*) the variable extents of initial denaturation under conditions that leave the ovalbumin soluble in aqueous solutions, (*b*) the random nature of the proteolytic cleavage by pronase, and (*c*) the different resistances to further hydrolysis of the glycopeptides by pronase, a property noted previously for other proteolytic enzymes[9,10]. It is, however, possible to obtain the same spectrum of L-β-aspartamido-carbohydrates from extensive digestion of the associated glycopeptides, and so the distribution of neutral sugars in AC was normalized to a total of 100%. It may be seen from Table I that the carbohydrate in all of the chicken ovalbumins is paucidisperse.

Furthermore, the distribution of carbohydrate in the AC fractions of genetically homogeneous ovalbumin and other samples of ovalbumin, which have the same average carbohydrate compositions, is not significantly different.

It has now been shown that a similar diversity is present in the carbohydrate of ovalbumin of other birds. It is known that differences exist in their immunology[11] and amino acid compositions[12,13]. The latter fact was confirmed for the present preparations of chicken, duck, and turkey ovalbumins; the results have been normalized to 32 aspartic acid residues in each case and are summarized in Table II.

TABLE II

AMINO ACID AND CARBOHYDRATE COMPOSITION OF OVALBUMINS

Component	*Number of residues*[a]		
	Chicken	*Duck*	*Turkey*
Lysine	20	22	20
Histidine	6	4	6
Arginine	15	15	13
Aspartic acid	32	32	32
Threonine	16	24	20
Serine	43	47	35
Glutamic acid	52	56	44
Proline	16	15	15
Glycine	19	22	21
Alanine	28	31	28
Half cystine	7	4	6
Valine	28	29	24
Methionine	16	11	10
Isoleucine	26	20	22
Leucine	33	34	30
Tyrosine	10	10	12
Phenylalanine	21	28	18
Total neutral sugars[b]	5	6	8
Glucosamine	3	6	6

[a]Expressed to the nearest integer for a single condition of hydrolysis (6N H^+, 24 h, 105°). Normalized to 32 residues of aspartic acid. [b]Calculated as mannose.

In general, the amino acid composition follows those reported previously[12,13,15], but more extensive studies will be required in order to establish the absolute, rather than the relative, values for the amino acid contents.

The AC of chicken, duck, turkey, and Western-gull ovalbumins was each found to be separated by ion-exchange chromatography into several fractions (see Table III). Although the chromatography was conducted under identical conditions for each AC preparation, the elution times, which reflect the molecular volumes rather than the molecular weights[1], did not correspond for each ovalbumin AC. It is impossible to interpret these chromatographic differences precisely, in view of the presence of sugars other than D-mannose and 2-acetamido-2-deoxy-D-glucose in the ovalbumins

of duck and turkey[1,3] and the dramatic effect on the elution times of substituting one sugar for another. For example, substitution of one residue of 2-acetamido-2-deoxy-D-glucose for one of D-mannose in chicken AC-C, to give AC-B[1], changes the elution time by 90 min (see Table III). Analysis of the compositions of the AC fractions of duck and turkey, without further purification, is summarized in Table IV. As L-aspartic acid was the only significant amino acid present, as in the case[1] of chicken AC, the analyses could be expressed per mole of that component. Each fraction from each ovalbumin had different ratios of neutral sugar and hexosamine, and it will be seen from Tables III and IV that the first fraction to be eluted from duck AC has an

TABLE III

FRACTIONATION OF THE AC OF OVALBUMINS

Ovalbumin source	*Total neutral sugars (%)*[a]				
	A	*B*	*C*	*D*	*E*
Chicken	6.0	11.8	37.1	25.6	19.1
	(460)[b]	(530)	(620)	(750)	(910)
Turkey	5.0[c]	13.9	36.5	15.8	28.8
	(570)	(720)	(810)	(990)	(1200)
Duck	7.6	38.1[c]	24. 6	15.1	14.6
	(900)	(1100)	(1280)	(1530)	(1830)
Western gull	8.3	19.9	37.5	25.5	8.8
	(590)	(720)	(830)	(960)	(1080)

[a]Calculated as mannose [b]The numbers in parentheses express the average elution times (in minutes) of the fractions. [c]Contains two poorly resolved peaks.

elution time lying between those of the third and fourth AC fractions of turkey, a result approximately in keeping with the carbohydrate composition. Because the molecular weights and composition of homogenous AC fractions from chicken have been established[1], and the elution times of the AC fractions of the other ovalbumins in Table III are of the same order of magnitude as those for chicken, it is presumed that all of these fractions contain one mole of L-aspartic acid (not two or more). Such being the case, the maximum number of residues of the sugars, particularly of 2-amino-2-deoxy-D-glucose (which was specifically determined), do *not* provide for the proportions found in the native-duck and turkey ovalbumins. More than half of the duck AC has ~3.5 moles of hexosamine per mole of L-aspartic acid, and, for less than one-tenth, this ratio is 4.1:1, compared to a ratio of 6:1 in the glycoprotein. About 90% of the turkey AC has less than 4 moles of hexosamine per mole of L-aspartic acid, compared to a ratio of 6:1 in the native ovalbumin. The result requires that more than one oligosaccharide residue, probably two, be present for each polypeptide chain in duck and turkey AC, which is in direct contrast to chicken AC.

A number of further questions have been resolved by the present results. The isolation, from each ovalbumin, of fractions that contain exclusively L-aspartic acid

TABLE IV

COMPOSITION OF THE L-β-ASPARTAMIDO-CARBOHYDRATES FROM DUCK AND TURKEY OVALBUMINS

Component	*AC Component*				
	A	*B*	*C*	*D*	*E*
	Duck				
Aspartic acid[a]	1.0	1.0	1.0	1.0	1.0
Threonine			T[b]		
Serine	T	T	T	T	T
Glutamic acid					T
Glycine	T	T		T	T
Glucosamine	4.1	3.7	3.4	2.4	2.8
Neutral sugars[c]	4.6	4.8	3.7	3.5	4.6
	Turkey				
Aspartic acid	1.0	1.0	1.0	1.0	1.0
Threonine	T	T	T	T	T
Serine				T	
Glutamic acid	T				
Glycine	T	T		T	T
Leucine	T				
Glucosamine	5.4	4.3	3.9	3.4	2.1
Neutral sugars[c]	6.7	5.6	5.2	4.8	4.4

[a]All analytical data were calculated with reference to one mole of aspartic acid. [b]Too little to calculate; less than 0.01 mole. [c]Calculated as mannose.

indicates the general nature of the β-L-aspartamido-carbohydrate linkage. Even where there is more than one oligosaccharide residue in each molecule, there does not appear to be a second type of linkage, as was demonstrated for A myeloma globin[16] and basement-membrane glycoprotein[17] of bovine kidney.

The AC fractions of turkey differed in their content of 2-acetamido-2-deoxy-D-glucose. As the egg white of turkey does not contain *N*-acetyl-β-D-glucosaminidase[18], it is unlikely that the diversity of carbohydrate is due to degradation in the egg white. Furthermore, in chicken ovalbumin, the relative amounts of the carbohydrate fractions are not dependent upon the age of the egg white, as judged by the analysis of the AC fractions from different eggs from the same bird.

The accumulating evidence as to the diversity of carbohydrate residues in glycoproteins strongly suggests that it is not the result of isolation artifacts[19]. It is consistent with the concept of biosynthesis in which each sugar residue is added stepwise to a growing chain[20]. Thus, the paucidispersity in the carbohydrate of ovalbumin may represent oligosaccharides in various stages of completion, or that result from transglycoslyations that do not have an absolute specificity. Furthermore, if the function of the carbohydrate in glycoproteins is related to transport of these molecules through cellular membranes[21], the process cannot be highly specific for the structure of the whole oligosaccharide unit.

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