The Subunit Structure of Prealbumin

By G. GONZALEZ* AND R. E. OFFORD

Laboratory of Molecular Biophysics, Department of Zoology, University of Oxford,
South Parks Road, Oxford OX1 3PS, U.K.

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1. Prealbumin, a thyroxine-binding protein, is known to bind also to retinol-binding protein and is suspected on X-ray-crystallographic evidence of having a quaternary structure. 2. Experiments described in this paper suggest that the molecule is a tetramer of mol.wt. about 56000, which is disaggregated with some difficulty into subunits of mol.wt. about 14000. 3. The number and staining properties of the tryptic peptides indicate that the subunits are identical or closely similar. This conclusion is reinforced by comparing the sum of the amino acid compositions of the tryptic peptides with the amino acid composition of the whole protein. All minor peptides that were isolated could be shown to be derived from major peptides. 4. No evidence has been found, either from electrophoretic experiments or from amino acid sequence determination, for any dissimilarity between the subunits.

Plasma prealbumin has been recognized as one of the three proteins responsible for the transport of L-thyroxine in the blood stream (Robbins & Rall, 1960; Ingbar, 1963; Oppenheimer, 1968). Recent reports have shown that prealbumin is also involved in the transport of vitamin A in plasma. This is done in association with a specific retinol-binding protein (Kanai, Raz & Goodman, 1968; Raz & Goodman, 1969; Raz, Shiratori & Goodman, 1970; Peterson, 1971a); the prealbumin-retinol-binding protein complex is able to combine with both thyroxine and retinol. This complex of hormone- and vitamin-binding protein is clearly of considerable interest. Prealbumin (Raz & Goodman, 1969; Oppenheimer, Surks, Smith & Squef, 1965; Purdy, Woerber, Holloway & Ingbar, 1965; Schuijze, Heimbürger & Frank, 1962) and retinol-binding protein (Raz & Goodman, 1969; Raz *et al.* 1970; Peterson & Beggard, 1971; Peterson, 1971b) have now been purified and partly characterized.

By equilibrium-dialysis studies of the thyroxine-prealbumin interaction, Raz & Goodman (1969) found a molecular weight of about 50000. These authors found the same value by using the sedimentation-equilibrium technique and obtained results indicating that prealbumin consists of a single molecular species.

Peterson (1971a), by studying the interaction between prealbumin and the retinol-binding protein, found a molecular weight of about 17000 for

prealbumin by gel filtration on a Sephadex G-200 column equilibrated with 5M-guanidine hydrochloride.

In a crystallographic study Blake *et al.* (1971) have obtained evidence for a tetrameric structure for the prealbumin molecule. Examination of the molecular symmetry made it possible to define the quaternary structure of prealbumin as two pairs of non-identical subunits or four identical subunits. (This evidence is largely independent of ours but makes some use of the chemical work described in the present paper. Similarly we have benefited by knowing of the X-ray work in advance of publication.) Thus, in addition to its interactions with other molecules, prealbumin itself forms an associated system with a defined quaternary structure.

The experiments reported here were designed to obtain information about the molecular weight of prealbumin, to confirm the possible existence of subunits in the molecule and, if subunits do exist, to determine whether or not they are identical.

MATERIALS AND METHODS

Materials. Prealbumin was a gift of Dr K. Keide of Behringwerke A.G., Marburg/Lahn, West Germany. Trypsin was obtained from Worthington Biochemicals Corp., Freehold, N.J., U.S.A. (tosyl-pb-nylalanyl chloromethyl ketone Trypsin batch OFA). Phenyl isothiocyanate and pyridine (for Edman degradations only) were redistilled from AnalaR grade materials. Iodoacetic acid was recrystallized from petroleum ether (b.p. 60–80°C). Iodo[¹⁴C]acetic acid was obtained from The Radiochemical

* On leave of absence (1968–72) from Departamento de Química, Universidad de Chile-Casilla 130-V, Valparaíso, Chile.

Centre, Amersham, Bucks., U.K. at a specific radio-activity of 20 mCi/mmol labelled on the C-2 atom. It was diluted with the non-labelled material to a specific radio-activity of 1.66 mCi/mmol and stored as a frozen solution of the sodium salt (6 mg/ml) at pH 6. Phenylthiohydantoin-glycine was obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Whatman papers were obtained from W. and R. Balston Ltd., Maidstone, Kent, U.K. Double-sided polyamide sheets were obtained from the Cheng-Chin Trading Co. Ltd., Taipei, Taiwan. All other reagents were AnalaR grade.

Gel-filtration. The protein was run in the absence of urea on a column (128 cm $\times$ 2 cm diam.) of Sephadex G-100 equilibrated with 0.05 M-tris-HCl buffer, pH 7.5, containing 0.1 M-KCl. The flow rate was 1.3 ml/min. Runs in the presence of urea were performed at 4°C on a column (34 cm $\times$ 0.75 cm) of Sephadex G-100 equilibrated with 0.1 M-tris-glycine buffer, pH 9.5, in 6 M-urea and the flow rate was 0.05 ml/min. Bovine serum albumin (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K.), ovalbumin, trypsin (Worthington) and ribonuclease (Sigma) were used as marker proteins in separate runs to estimate the approximate molecular weight (Andrews, 1964).

Polyacrylamide-gel electrophoresis. (a) In 6 M-urea. Polyacrylamide-gel electrophoresis in 0.1 M-tris-glycine buffer, pH 9.5, in 6 M-urea (final concentration) was performed as described by Davis (1964). The gel concentration used was 7.5% in acrylamide. Electrophoresis was performed at 30.7 V/cm for 30 min. The gels were stained after electrophoresis as described by Weber & Osborn (1969).

(b) Hybridization. Hybridization experiments were performed by a method similar to that described by Markert (1963). Prealbumin samples dissolved in NaCl solutions were frozen at -70°C for 30 min and then incubated for 30 min at 37°C. This procedure was repeated three times. Different concentrations of protein (0.1, 1.0 and 10.0 mg/ml) and NaCl (0, 1, 2 and 4 M) were employed. After this treatment the samples were submitted to polyacrylamide-gel electrophoresis at pH 9.5 as described above, but without urea.

(c) In sodium dodecyl sulphate. Polyacrylamide-gel electrophoresis in sodium dodecyl sulphate was performed as described by Weber & Osborn (1969). Trypsin (Worthington), myoglobin, α -lactalbumin (a gift of Dr R. Aschaffenburg) and pig insulin were used as marker proteins.

Reduction and carboxymethylation. Urea was added to protein (5 mg/ml) in 0.2 M-tris-EDTA buffer, pH 8.6, until the solution was saturated. Dithiothreitol (1 mg/50 mg of protein) was added and N₂ was bubbled into the solution to remove dissolved O₂. The solution was allowed to stand for 1 h at room temperature under a N₂ atmosphere to achieve full reduction. Solid iodoacetic acid was added as alkylating agent (1 mg/5 mg of protein) and allowed to react for 20 min at room temperature. Excess of iodoacetic acid was eliminated by the addition of mercaptoethanol (1 mg/mg of iodoacetic acid). Extensive dialysis was performed before freeze-drying. The ¹⁴C-labelled iodoacetate solution was used in a similar manner.

Amino acid analyses. Amino acid analyses were performed on samples hydrolysed in 6 M-HCl under N₂-flushed vacuum at 108°C. The method used was that of Benson &

Patterson (1965) by using a 120C Beckman Spinco automatic amino acid analyser.

N-Terminal amino acid analysis of the protein. Qualitative N-terminal amino acid analyses were performed by the dansyl technique described by Gray & Hartley (1963a,b). The reaction was performed in a urea-saturated solution; urea was largely removed by dialysis before acid hydrolysis of the sample. Identification was by two-dimensional chromatography on polyamide sheets (Woods & Wang, 1969).

Quantitative N-terminal amino acid analyses were performed by using the Edman reaction modified as described by Konisberg (1967). Aq. 75% (v/v) pyridine was used as the solvent for the reaction with phenyl isothiocyanate. The cyclization reaction was achieved by using anhydrous trifluoroacetic acid at room temperature for 80 min. The cyclized product was dried, then dissolved in water. The extraction of the N-terminal amino acid derivative was performed three times with equal volumes of ethyl acetate. A measured sample of the cyclized material was taken for hydrolysis and amino acid analysis to determine the weight of protein present. The amount of N-terminal amino acid derivative was determined by recovery of the free amino acid after acid hydrolysis in 6 M-HCl for 24 h at 108°C.

Tryptic digestion. It was found necessary to denature the carboxymethylated protein by boiling a solution (3 mg/ml in water) for 3 min, before cleavage by trypsin. After cooling the boiled suspension, 0.5 g of solid NH₄HCO₃ was added/100 ml. Tosyl-phenylalanyl chloromethyl ketone-treated trypsin was added in the proportion 1:100 (w/w) to the protein. The mixture was incubated at 37°C for 4 h and then freeze-dried. If denaturation was not performed, very little tryptic digestion occurred. This indicates the lack of any lasting effect of the denaturing conditions used in the alkylation.

Purification of peptides. (a) Gel filtration. The first step in the purification of the tryptic peptides was gel filtration on Sephadex G-50 in 0.5% NH₄HCO₃. Up to 300 mg of the peptide mixture, dissolved in 10 ml of 0.5% NH₄HCO₃, was put on the column (190 cm $\times$ 4.2 cm), which was eluted with the same buffer at a flow rate of about 3 ml/min. The eluate was collected in 7 ml fractions and peptides were located by the measurement of *E*₂₈₀ and by electrophoretic separation on paper at pH 6.5 of portions of every third fraction (see Fig. 3). The effluent was pooled into eight fractions, each of which was freeze-dried and submitted first to high-voltage paper electrophoresis at pH 6.5.

(b) High-voltage paper electrophoresis. Peptide mixtures were separated by paper electrophoresis at 60–100 V/cm in an apparatus similar to that described by Michl (1951), on Whatman 3MM and no. 1 papers at pH 1.9, 3.5, 6.5 or 8.9. Buffers and equipment were as described by, e.g., Ambler (1963). Cooling of the papers was by white spirit 100 (Esso Ltd.) with, in the case of the pH 6.5 system, an admixture of pyridine 8% (v/v).

(c) Paper chromatography. Peptides were separated by descending chromatography with the solvent mixture butan-1-ol-acetic acid-water-pyridine (15:3:12:10, by vol.; Waley & Watson, 1953) overnight at room temperature.

Paper chromatography was never used as the last step because of reports that peptides so prepared suffered

destruction of amino acids on hydrolysis (Ambler, 1963). In this laboratory, similar problems have been experienced with some peptides in which electrophoresis at pH 6.5 is used as the final purification step.

Peptide maps. About 0.1 μ mol of the tryptic digest was separated by electrophoresis as a 1 cm band on Whatman 3MM paper at pH 6.5. The strip of paper containing the separated peptides was then sewn for separation in a second dimension by descending chromatography as described above. A number of duplicate maps were prepared for use with the different staining reactions.

Location of peptides on paper. Peptides were located on paper as described by Offord (1969). Ilford Industrial 'G' X-ray film was used for radioautography.

Sequential Edman degradation of peptides. This technique was used with dansylation as described by Gray (1967).

RESULTS AND DISCUSSION

Gel filtration. The elution position of a protein on a Sephadex column may give an approximate measure of the molecular weight (Andrews, 1964). In the absence of urea, prealbumin was eluted at a position corresponding to a molecular weight of 57 000. In the presence of urea, prealbumin and carboxymethyl-prealbumin eluted at a position corresponding to mol.wt. either 30 000 or 57 000 depending on the mildness of the previous treatment of the protein. Remarkably, the lower-molecular-weight peak predominated in those samples that had been mildly treated whereas the higher-molecular-weight peak predominated after the more severe treatment (such as brief boiling or boiling in saturated urea).

Polyacrylamide-gel electrophoresis. Electrophoresis of prealbumin in 6M-urea showed no duplication of bands that might be ascribed to dissimilarity of charge between subunits. As with the gel filtration, the more severely the samples were treated before electrophoresis, the larger the apparent molecular weight. The single band (mobility 3.6 cm/h toward the anode at 30.7 V/cm) shown by samples without pretreatment was replaced by a band of least mobility 10.8 cm/h at 30.7 V/cm if the samples were boiled before electrophoresis.

Samples of eluate from the protein-peak tubes of the gel-filtration experiments were run direct on polyacrylamide gels (the buffers were the same). Material from the 30 000 mol.wt. position gave only the fast band whereas material from the 57 000 mol.wt. position gave only the slow band.

We are unable to explain the apparent increase of molecular weight after treatment that should have decreased the molecular weight. However, the purpose of the experiments was to look for evidence of charge differences between subunits and there was certainly none.

Hybridization. As the previous experiments

showed no charge differences between subunits, it was not surprising that there was no evidence for formation of hybrid molecules under any of the conditions tried. In each case freezing and thawing did not alter the electrophoretic behaviour of the protein.

Polyacrylamide-gel electrophoresis in sodium dodecyl sulphate. Fig. 1 shows the results of a typical run. Under the disaggregating conditions of the experiment the molecular weight of prealbumin was decreased to about 14 000. This is about one-quarter of the value determined by gel filtration and by X-ray crystallography (Blake *et al.* 1971) and is consistent with the existence of four subunits for the prealbumin molecule. The peak (mol.wt. 30 000) seen in 6M-urea could thus be a dimer.

Amino acid composition. The results of the amino acid analysis of prealbumin are given in Table 1. Molar ratios are given on the basis of 16 histidine residues, a value which brings the total molecular weight (56 000) nearest to that beginning to become apparent for the whole molecule. Values are also given for a total chain length of one-quarter of this value, which is suggested for the subunit size by the gel-electrophoresis experiments.

It appears, therefore, that urea is inadequate for the complete disaggregation of the molecule. It has already been mentioned that urea treatment does not cause any lasting disruption of the tertiary structure.

N-Terminal amino acid determination. The N-terminal amino acid of prealbumin was identified

Fig. 1. Determination of the molecular weight of the subunits of prealbumin by sodium dodecyl sulphate electrophoresis. The arrow indicates the mobility of prealbumin. The interpolated value for the molecular weight of the prealbumin subunits is 14 000. (1) Trypsin; (2) myoglobin; (3) α -lactalbumin; (4) insulin. Experimental details are given in the text.

Table 1. *Amino acid composition of prealbumin*

For experimental details see the text. The corrected distribution is the average value for all the amino acids except for threonine and serine (for which the observed values were extrapolated back to zero time), and for valine and isoleucine (for which the observed values were plotted against $1/t$ and extrapolated to $1/t = 0$).

Amino acid Hydrolysis time ...	Content (nmol)			Corrected distribution (nmol)	Number of residues relative to histidine	
	24 h	48 h	96 h		His = 16	His = 4
Lys	204	194	201	200	34.8	8.7
His	94	90	94	92	16.0	4.0
Arg	94	90	90	91	15.8	3.9
Asp	178	194	192	188	32.7	8.2
Thr	258	252	249	260	45.2	11.3
Ser	220	222	192	225	39.1	9.8
Glu	310	309	313	309	53.8	13.4
Pro	190	189	203	193	33.6	8.4
Gly	232	230	236	233	40.5	10.1
Ala	280	289	279	282	49.1	12.3
Cys					4.0*	1.0*
Val	241	266	284	291	50.6	12.6
Met					4.0*	1.0*
Ile	105	109	113	115	20.0	5.0
Leu	168	168	147	161	28.0	7.0
Tyr	107	108	106	107	18.6	4.6
Phe	111	113	108	117	19.3	4.8
Trp					16.6†	4.1†

* Values obtained by analysis of three more samples (24h hydrolysed), after performate oxidation at 0°C (Hirs, 1956).

† Trp/Tyr ratio = 1:1.1. Method of Goodwin & Morton (1946).

Fig. 2. Peptide 'map' after tryptic digestion of prealbumin. Broken lines indicate minor peptides. The symbols representing the different amino acids stained for, and the symbol showing the radioactive peptide, are indicated on the figure. Experimental details are given in the text.

Table 2. *Peptide counting*

For experimental details see the text.

	Predicted value		Observed value
	No subunits (or $\alpha\beta\gamma\delta$)	Four identical subunits	
Total no. of tryptic peptides	52*	14*	14 major 7 minor†
Histidine staining	16	4	3
Arginine staining	16	4	3
Tyrosine staining	18	5	3
Tryptophan staining	16	4	4
Radioactive†	4	1	1 major 1 minor‡

* Number of unique lysine residues + number of unique arginine residues + 1.

† With iodo[^{14}C]acetate.

‡ See Table 5.

Fig. 3. Gel filtration of a tryptic digest of carboxymethylated prealbumin. Samples of effluent fractions were submitted to electrophoresis at pH 6.5 and the pattern of the peptides is shown below the effluent curve. The eluates were combined to give eight separate fractions as indicated. Fraction F0 corresponds to undigested prealbumin, some of which always remained even after the denaturation process described in the Materials and Methods section. For experimental details see the text.

by using the dansyl method; the reaction was performed in the presence of urea in an attempt to maximize the access of the reagent to the terminal part of the polypeptide chain. The result showed glycine to be the sole amino acid in prealbumin with a free α -amino group.

The Edman reaction was used for the quantitative determination of glycine at the *N*-terminus of the protein. The method chosen was the release of glycine as its phenylthiohydantoin derivative, from which the free amino acid was regenerated by acid hydrolysis. The recovery of glycine was estimated by submitting a spectrophotometrically determined sample of phenylthiohydantoin-glycine (previously shown to be uncontaminated by the free amino acid) to the same conditions of hydrolysis as the experimental sample. The yield obtained from this sample was 19%. The uncorrected recovery of glycine from the experimental sample was 1 mol/76900 g of protein; that is, after correction, one residue of *N*-terminal glycine/15380 g of protein. This implied value for the subunit size corresponds approximately to one-quarter of the molecular weight of the undissociated protein.

Peptide 'maps'. The pitfalls of using peptide 'maps' to determine molecular weights are well known (Harris & Hindley, 1965; Fraenkel-Conrat, 1965). However, with suitable safeguards, peptide 'maps' (Fig. 2) could be used in the present case to give at least a preliminary estimate of the size and number of the subunits.

Table 2 shows the number of peptides to be expected (a) if the total of 56000 is made up of a single chain, or four completely dissimilar subunits ($\alpha\beta\gamma\delta$); (b) if there were four identical subunits (α_4). Other cases, such two pairs of identical sub-

units ($\alpha_2\beta_2$) will give values intermediate between these extremes depending on the extent to which sequences are duplicated between subunits.

There is very little insoluble material in the digest and thus a common source of error in counting the peptides (the accumulation of a number of different peptides in a single spot at the origin) may be assumed to be absent. Therefore the count is certainly sufficient to eliminate the possibility that prealbumin is a single chain of mol.wt. 56000, or four dissimilar subunits, or even the total dissimilarity of α and β in the event of the structure being $\alpha_1\beta_2$.

It is not possible to conclude more than that the prealbumin structure approximates to α_4 or a close variant (a result also suggested by the sodium dodecyl sulphate electrophoresis and by the *N*-terminal work) without isolating the individual peptides and determining whether or not they are all unique sequences. This is a necessary preliminary to the determination of the complete amino acid sequence of the protein, to which we are committed as a necessary adjunct to the interpretation of high-resolution X-ray results being obtained in this laboratory.

Purification of the tryptic fragments. The methods used for purification are described in the Materials and Methods section. Fig. 3 shows the elution pattern of the initial gel filtration. Table 3 indicates the sequence of steps used to purify the major peptides.

Nature of the tryptic fragments. Table 4 shows the amino acid composition of the major unique fragments. A number of other fragments isolated had compositions related to those of the major fragments (Table 5).

Table 3. *Purification scheme for the tryptic peptides*

For experimental details see the text. Solvent BAWP is butanol-acetic acid-water-pyridine (15:3:12:10, by vol.).

Purification steps (numbers indicate the order followed)

Peptide	Sephadex G-50	Electrophoresis at pH 6.5	Paper chromatography in solvent BAWP	Electrophoresis at pH 3.5	Electrophoresis at pH 8.9
F4N3	1	2	3		4
F4N4	1	2	3	4	
F5N1	1	2	3	4	
F5N5-3	1	2	3	4	5
F6N5	1	2	3	4	
F5B5	1	2	3	4	
F5B7-2	1	2	3		4
F8B2	1	2	3	4	
F2A1	1	2			3
F2A2	1	2	3	4	
F5A5-2	1	2	3	4	
F5A1	1	2			
F6B1	1	2			

Table 4. *Amino acid composition of the tryptic peptides*

Hydrolyses were performed in 6M-HCl at 108°C for 24 h. The values given are the numbers of residues (those below 0.3 have been omitted). No corrections for destruction, incomplete hydrolysis or impurities have been made. The tryptophan analysis is omitted. Cysteine is in the carboxymethyl form (CMCys).

Amino acid	F6B1	F4N4	F4N3	F5N1	F5N5-3	F6N5	F5B5	F5B7-2	F8B2	F2A1	F2A2	F5A5-2	F5A1	Peptide sum	Prealbumin (4)
Lys		1.0		1.0	1.0		1.0	1.0	1.0	1.0	1.0	1.0		9.0	8.7
His			2.0					1.0		0.9				4.0	4.0
Arg	1.0*		1.0			1.0								2.0	3.9
Asp		1.2	2.2			1.0		2.0			1.0	2.2		9.0	8.2
Thr		3.1	1.0	2.0				0.9		2.5	1.0	1.0		11-12	11.3
Ser		2.7	2.2	1.0			1.0	2.1	1.0	1.7		1.0		13.0	9.8
Glu		1.1	2.4	1.0						5.9	1.0	1.2	1.0*	12-13	13.4
Pro		2.2	2.0	1.0	1.0			1.2				0.9		8.0	8.4
Gly		0.6	2.4	3.0			1.0	2.2		2.7		1.2		13.0	10.1
Ala		2.0	3.6			1.0	1.0	2.3				3.0		13.0	12.3
CMCys					1.0									1.0	1.0
Val		1.1	1.7		1.0	2.0		0.6		1.1	1.0			9.0	12.6
Met					1.0									1.0	1.0
Ile		0.3	1.0					1.0		0.9	1.0			4-5	5.0
Leu		2.0	1.0		1.0	1.0				1.7				7.0	7.0
Tyr		1.8							1.0	0.9				4.0	4.6
Phe			2.0							0.9		0.9		4.0	4.8

* Not a di- or higher peptide by electrophoretic and chromatographic mobility in all systems and amino acid analysis before hydrolysis.

Table 5. *Minor peptides obtained from the tryptic digest*

Peptide F5N4 was radioactive before hydrolysis. Experimental details are given in the text. CMCys, *S*-carboxymethylcysteine.

Minor peptide	Composition	Related major peptide	Reason for occurrence
F5A63	Lys _{2.0} Asp _{2.4} Thr _{1.0} Ser _{1.0} Glu _{1.2} Pro _{1.7} Gly _{1.3} Ala _{3.7} Phe _{1.1}	F5A52	Shown to have extra Lys at <i>N</i> -terminus.
F5B4	Lys _{1.0} Val _{1.0}	F5N53	Chymotryptic split after Met.
F5A43	CMCys _{1.0} Pro _{1.0} Leu _{1.0} Met _{1.0}	F5N53	Chymotryptic split after Met.
F5A32	Asp _{1.1} Glu _{0.9} Ala _{2.0} Phe _{1.7}	F4N3	Chymotryptic split?
F5N4	Lys _{1.0} Pro _{1.0} Val _{1.0} Met _{1.0} Leu _{1.0}	F5N5-3	Atmospheric oxidation of CMCys residue (e.g. Harris, 1967) causing change of mobility.

Table 6. *Preliminary sequence data on the tryptic peptides*

For experimental details see the text.

Peptide	Sequence (complete or partial) determined by dansyl-Edman degradation
F4N3	Ala-Leu-Gly-Ile-Ser
F4N4	Ala-Leu-Leu
F5N1	Gly-Pro-Thr-Gly-Thr-Gly-Glu-Ser-Lys
F5N5-3	CMCys-Pro-Leu-Met-Val-Lys
F6N5	Val-Leu-Asp-Ala-Val-Arg
F5B5	Ala-Ser-Gly-Lys
F5B7-2	Gly-Ser
F8B2	Ser-Tyr-Trp-Lys
F2A1	Thr-Ser-Glx-Ser-Gly-Glx-Leu-Glx-Gly
F2A2	Val-Glu-Ile-Asp-Thr-Lys
F5A5-2	Ala-Ala-Asx-Asx-Thr

Of the fragments present in sufficient quantity to be isolated, none had amino acid compositions irreconcilable with those of the major fragments. Before a complete overlapping sequence is obtained it is not possible to be sure that every part of the molecule had been recovered in the form of a tryptic peptide. However, the summation in Table 4 makes it seem unlikely that any great portion can be missing. Taken with all the other evidence, the summation indicates that the molecule must be made up of four subunits and that, if the subunits differ in sequence, they can do so only slightly. This conclusion is strengthened by the sequence information so far obtained (Table 6). If there were slight differences between subunits one might expect to find peptides with almost identical sequences, or alternatively a difference could occur that might not be sufficient to permit separation of two forms of a peptide. In the latter case there should be ambiguities in the sequences at the position of the difference. The results in Table 6 do not indicate that such ambiguities occur although they cannot be absolutely ruled out until the sequence work is

complete. [A parallel study of the sequence of prealbumin is being performed by using an automatic Edman degradation machine (R. E. Canfield & F. J. Morgan, personal communication). We have seen the preliminary results of the above work and, for regions where both groups have sequence results, find no great discrepancy between the results obtained by the two approaches.]

Conclusion. The results presented above indicate that prealbumin is composed of four polypeptide chains, each having a molecular weight of about 14000. There is no evidence inconsistent with the view that the four chains have an identical amino acid sequence but the possibility that there are small dissimilarities cannot be eliminated until the complete sequence is determined.

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