also the Carmarthenshire County Council for a Scholarship to R. J. H. W. and the Science Research Council for substantial research grants.

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Biochem. J. (1965) **97**, 32c

The C-Terminal Peptide of the Heavy Chain of the Rabbit Immunoglobulin IgG

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(Received 20 September 1965)

Much progress has been reported recently on the determination of the amino acid sequence of the Bence-Jones proteins (Hilschmann & Craig, 1965; Milstein, 1965; Titani, Whitley, Avogardo & Putnam, 1965), which are equivalent to the light chains of human myeloma globulins (Edelman & Gally, 1962). There has also been a report on the separation of the tryptic peptides of rabbit IgG* heavy chains (Nelson, Noelken, Buckley, Tanford & Hill, 1965). We now report the cleavage of these heavy chains with CNBr (Gross & Witkop, 1962) and the isolation and sequence of the C-terminal peptide that was obtained.

After incubation of the heavy chain (500 mg.) with CNBr (1 g.) in 25 ml. of 70% formic acid (Steers, Craven, Anfinsen & Bethune, 1965) for 24 hr. at 25° and 4° the conversion of methionine into homoserine was 95 and 92% respectively. Electrophoresis of the product on starch gel (urea-formate) revealed six bands in both preparations, whereas the control (heavy chain incubated with 70% formic acid under the same conditions) did not show any significant cleavage. The components that appear on the starch gel could be partially separated by gel filtration on a Sephadex G-100 column (200 cm. × 3 cm.) in N-acetic acid. The first peak (C-1), which comprises about 50% of the material, corresponds in its mobility on starch gel to fragment Fd obtained from papain piece I (Fleischman, Porter & Press, 1963), and it is also very similar to it in its amino

acid composition and in the absence of a free N-terminal amino acid. The second fraction (C-2) was obtained in a very low yield and has not been characterized. Fraction C-3 gave three fast-moving bands on the starch gel, and fraction C-4 gave two bands with a mobility between those of fractions C-3 and C-1. All the bands on the starch gel that were obtained from fractions C-3 and C-4 could be similarly observed in a CNBr digest of papain piece Fc and none of them was observed in the CNBr digest of fragment Fd. The last fraction (C-5) did not stain after the electrophoresis in the starch gel. However, ninhydrin staining, after high-voltage electrophoresis on paper at pH 3.5, showed a single component moving quickly towards the cathode. This peptide could be detected on paper from the CNBr digest of the whole heavy chain or of fragment Fc, but not from that of fragment Fd.

Composition and sequence of the peptide C-5. The peptide C-5, as obtained from the Sephadex G-100 column, appeared to be slightly impure, as judged by analysis, and could be further purified on a Sephadex G-50 column. It gave no colour with Ehrlich's reagent and therefore does not contain tryptophan. Its amino acid composition (Table 1) shows that it contains 18 amino acid residues, including 3 of the 5 histidine residues that are present in fragment Fc. The molecular weight of peptide C-5 from its amino composition is 2062 and it was obtained in 80% yield from the CNBr digest of the heavy chain (16 mg. out of 480 mg.). This peptide does not contain homoserine. Its C-terminal amino acid was found by hydrazinolysis to be glycine, the same as was found for the whole heavy chain. We

* Abbreviations: IgG, immunoglobulin; the nomenclature is that recommended by the World Health Organisation (1964); PTH, phenylthiohydantoin.

Table 1. *Amino acid composition of the peptide C-5 and the peptides obtained from it*

	Amino acid composition (residues/mol.)						
	Peptide C-5	Peptides obtained by tryptic hydrolysis of peptide C-5			Peptides obtained by chymotryptic hydrolysis of peptide T ₁		
		Peptide T ₁	Peptide T ₂	Peptide T ₃	Peptide T ₁ C ₁	Peptide T ₁ C ₂	Peptide T ₁ C ₃
Lys	1.00	1.0	—	—	—	0.95	—
His	2.74	2.8	—	—	1.94	—	1.0
Arg	0.94	—	1.0	—	—	—	—
Asp	1.06	1.0	—	—	1.0	—	—
Thr	0.95	0.94	—	—	—	0.91	—
Ser	2.79	—	1.9	1.0	—	—	—
Glu	1.92	1.80	—	—	—	1.0	1.08
Pro	1.09	—	—	0.86	—	—	—
Gly	1.03	—	—	1.0	—	—	—
Ala	1.05	1.08	—	—	—	—	1.0
Ile	0.91	—	1.0	—	—	—	—
Leu	1.06	0.92	—	—	—	—	1.0
Tyr	0.89	0.8	—	—	0.5	—	—
Remarks							
Colour by ninhydrin-collidine	Violet	Violet	Grey-yellow	Yellow	Grey	Violet	Grey
Sakaguchi	+	—	+	—	—	—	—
Pauli	+	+	—	—	+	—	+
Nitroso-naphthol	+	+	—	—	+	—	—

therefore conclude that C-5 is the *C*-terminal peptide of the heavy chain. The *N*-terminal amino acid of peptide C-5 was found to be histidine by the dinitrophenylation technique and by the Edman degradation method (Margoliash, 1962). After the incubation of peptide C-5 with trypsin for 3 hr. at 37° only three peptides were separated by high-voltage electrophoresis on paper (Table 1). Peptide T₁ (11 amino acid residues) could be separated from peptides T₂ and T₃ (4 and 3 amino acid residues respectively) on a Sephadex G-25 (fine) column in 1% formic acid. Peptides T₂ and T₃ were separated by paper electrophoresis at pH 3.5 (4kv, 40min.) and were eluted with the pH 3.5 pyridine-acetate buffer in a 45% (T₂) and a 75% (T₃) yield. From the *C*- and *N*-terminal groups of peptide C-5, the amino acid composition of the three peptides and the known specificity of trypsin, it was possible to assign the order of these peptides as follows:

Peptide T₃. By the Edman degradation the *N*-terminal group is serine (42% yield on the basis of the 270mμ absorption of the PTH-serine). The second step showed PTH-Pro (74%), and therefore the sequence of peptide T₃ is:

Ser-Pro-Gly

Peptide T₂. By the Edman degradation the first step showed PTH-Ser (65% yield), the second step showed PTH-Ile (70%) and the third step showed PTH-Ser. The residual material, when analysed on the amino acid analyser without hydrolysis, revealed only arginine. Therefore the sequence of peptide T₂ is:

Ser-Ile-Ser-Arg

Peptide T₁. Three successive Edman steps on the whole C-5 peptide showed an *N*-terminal sequence His-Glu-Ala. Two Edman steps on peptide T₁ showed an *N*-terminal sequence His-Glu. After digestion of peptide T₁ at 37° for 3 hr. by chymotrypsin, only three peptides could be separated by paper electrophoresis (Table 1). The peptides were eluted from the paper by water and, by knowing the *N*-terminal sequence of the whole peptide, it is possible to arrange them in the following order:

Peptide T_1C_3 . The first step of the Edman degradation showed PTH-His (45%) and the second step showed PTH-Glu (40%). *C*-Terminal leucine was found by hydrazinolysis and its sequence is:

in agreement with the *N*-terminal sequence of peptide C-5.

Peptide T_1C_1 . The first step of the Edman degradation showed PTH-His (72%) and the second step showed PTH-Asp (36%). *C*-Terminal tyrosine was determined by digestion with carboxypeptidase A and hydrazinolysis. Therefore its sequence is:

Peptide T_1C_2 . Its *N*-terminal residue was found by Edman degradation to be threonine (90%) and its sequence is therefore:

Another Edman step was not attempted since it was found to contain glutamine, which might cyclize to the pyrrolidone.

Since each one of the above three peptides contains only 1 glutamic acid or aspartic acid residue and they do not contain serine, it was possible to determine if the glutamic acid or aspartic acid is amidated by analysing the amino acids released from the isolated peptides after digestion with leucine aminopeptidase. Peptide T_1C_2 has glutamine, peptide T_1C_1 has asparagine and peptide T_1C_3 has glutamic acid.

The summary of these results gives the following

sequence for the C-5 peptide, which is probably bound to the rest of the heavy chain through methionine:

Considering the high recovery of this peptide from the heavy chain it can be concluded that in rabbit γ -globulin, which is prepared by a chromatography on DEAE-Sephadex, both heavy chains have predominantly the same unique sequence at their *C*-terminal end. A parallel work on human myeloma heavy chain has shown that it contains a very similar *C*-terminal octadecapeptide with only two replacements (P. J. Piggot, personal communication). Amino acid sequence studies of tryptic peptides by R. L. Hill and collaborators (unpublished work) are in agreement with the sequence reported here.

D. G. thanks the Wellcome Trust for the award of a Wellcome Research Fellowship.

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Biochem. J. (1965) **97**, 34c

Subcellular Distribution of some Folic Acid-Linked Enzymes in Rat Liver

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(Received 20 September 1965)

Although much is known about the folic acid-linked enzymes of liver (Friedkin, 1963), work on their intracellular location has not been reported. The distribution of three such enzymes, tetrahydrofolate dehydrogenase (EC 1.5.1.3), methyltrans-

ferase and thymidylate synthetase, is examined here. Tetrahydrofolate-dehydrogenase activity results in the production of reduced forms of folic acid; methyltransferase and thymidylate synthetase are concerned with the utilization of reduced