An Addition at the C-Terminus of Water-Buffalo Immunoglobulin Lambda Chains

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The amino acid sequence of the C-terminal tryptic peptide of pooled water-buffalo immunoglobulin lambda chains was determined as Thr-Val-Lys-Pro-Ser-Glu-Cys-Pro-Ser. This sequence is closely homologous to equivalent sequences from other species, but shows an additional amino acid on the C-terminal side of the interchain half-cystine residue.

Bovine serum immunoglobulins have been shown to consist mainly of two IgG $\dagger$ subclasses, IgG1 and IgG2 (Pierce & Feinstein, 1965; Milstein & Feinstein, 1968) and to have light chains almost entirely of the λ -type (Hood *et al.*, 1967). The serum immunoglobulins of the water-buffalo (*Bubalus bubalus*) may be similarly fractionated into two major components by DEAE-cellulose column chromatography. Further analysis of these components (Svasti *et al.*, 1976) by physicochemical methods, including the chemical typing procedure (Frangione *et al.*, 1969), analytical ultracentrifugation, isoelectric focusing and hexose determinations, suggests that water-buffalo immunoglobulins also consist of two IgG subclasses, named IgG1 and IgG2 in an analogous manner to the bovine IgG subclasses.

Further, chemical typing of water-buffalo light chains has revealed the presence of two radioactive peptides of similar electrophoretic mobility to those of bovine light chains, suggesting that they are also of the λ -type. The present investigation describes the isolation and amino acid sequence of the C-terminal tryptic peptide of water-buffalo light chains.

Materials and Methods

Water-buffalo serum was prepared from blood obtained from the local slaughter-house. Immunoglobulins were prepared from 100 ml of pooled serum by precipitation with $(\text{NH}_4)_2\text{SO}_4$ to 43% saturation, followed by chromatography of the resuspended precipitate (after dialysis against 0.01 M- Na_2HPO_4) on a column (4 cm $\times$ 40 cm) of DEAE-cellulose (Bio-Rad Laboratories, Richmond, CA, U.S.A.) equilibrated with 0.01 M- Na_2HPO_4 (Svasti

et al., 1976). After washing with 600 ml of the same buffer, adsorbed protein was eluted out with a gradient formed from 600 ml of 0.01 M- Na_2HPO_4 and 600 ml of 0.3 M- NaH_2PO_4 . Selected tubes were analysed by electrophoresis in 7.5% polyacrylamide gels in 0.38 M-Tris/glycine buffer, pH 9.5 (Hjerten *et al.*, 1965), and pools were made accordingly. The IgG2 pool was obtained from the breakthrough peak and the IgG1 pool from the first part of the gradient.

Selective reduction of interchain disulphide bonds and alkylation with iodo[1- ^{14}C]acetic acid (New England Nuclear Corp., Boston, MA, U.S.A.) was carried out by the procedure of Frangione *et al.* (1969). Light chains were separately prepared from selectively reduced and [^{14}C]carboxymethylated IgG1 (150 mg) and IgG2 (75 mg) by chromatography on a column (4 cm $\times$ 90 cm) of Sephadex G-100, equilibrated with 5% (v/v) formic acid. The purity of these preparations was checked by electrophoresis in 7.5% polyacrylamide gels in 0.1% sodium dodecyl sulphate/0.1 M-sodium phosphate buffer, pH 7.2 (Weber *et al.*, 1972). Isolated light chains were then totally reduced (Pink *et al.*, 1970) and alkylated with non-radioactive iodoacetic acid. After exhaustive dialysis against 0.5% NH_4HCO_3 , the samples were digested with trypsin (Sigma Chemical Co., St. Louis, MO, U.S.A.) at an enzyme/substrate ratio of 1:50 (w/w) for 4 h at 37°C.

High-voltage paper electrophoresis was performed as described by Milstein (1966): electrophoretic mobilities at pH 6.5 and at pH 3.5 were measured relative to aspartic acid. Radioactive peptides were detected by radioautography by using Kodak NS-2T No-Screen X-ray film. Performic acid oxidation was carried out as described by Brown & Hartley (1966). For the determination of amino acid compositions, peptides were hydrolysed with 6 M-HCl (Pro Analysis grade; E. Merck, Darmstadt, Germany) under vacuum for 20 h at 105°C. Amino acid analyses were kindly performed by Mr. K. Edwards and Dr. C.

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$\dagger$ Abbreviation: IgG, immunoglobulin G.

Milstein of the MRC Laboratory of Molecular Biology, Cambridge, U.K., using a Durrum amino acid analyser. Amino acid sequences were performed by the rapid dansyl-Edman procedure of Gray (1972), by using sequencer-grade phenyl isothiocyanate (Eastman Organic Chemicals, Rochester, NY, U.S.A.).

Results and Discussion

When the tryptic digests of light chains derived from water-buffalo IgG1 and IgG2 were fractionated by electrophoresis at pH6.5, a single radioactive peptide (termed 1LR and 2LR respectively) was obtained in each case, with an electrophoretic mobility of +0.23. Both peptides were further purified by electrophoresis of pH3.5, followed by performic acid oxidation and a further electrophoresis at pH3.5. Light chains from both sources gave single radioactive spots at each step of identical electrophoretic mobilities at pH3.5 of -0.78 and of -0.40 before and after performic acid oxidation respectively.

The amino acid composition of the radioactive peptide isolated from the light chains of water-buffalo IgG1 is shown in Table 1; the equivalent peptide from IgG2 had a very similar composition. Since the peptide has a net negative charge at pH6.5, the Glx residue must be glutamic acid.

The amino acid sequence of peptide 1LR was determined and shows obvious similarities to the C-terminal peptides of λ light chains from other species (Fig. 1). Since no other radioactive peptides were detectable on electrophoresis at pH6.5, this indicates that, like those of other artiodactyls (Hood *et al.*, 1967), water-buffalo immunoglobulins have light chains almost entirely of the λ type. The sequence of the water-buffalo peptide is most similar to that

Table 1. *Amino acid composition of peptide 1LR*
For details see the text. Abbreviation: CmCys(O₂), carboxymethylcysteine sulphone.

	Content (residues/molecule)	
	Analysis	Integer
CmCys(O ₂)	1.03*	1
Thr	0.90	1
Ser	1.98	2
Glu	1.18	1
Pro	1.65	2
Val	1.04	1
Lys	1.00	1
Others	≤0.15	—

* From radioactivity.

Fig. 1. *C-Terminal tryptic peptide of lambda chains*

(a) Determination of the amino acid sequence of the water-buffalo λ -chain C-terminal tryptic peptide, which was obtained as the S-[¹⁴C]carboxymethyl sulphone derivative (peptide 1LR, Table 1). Full arrows (→) show dansyl-Edman results; broken arrow (→) indicates that the identification of the dansyl derivative was rather dubious; double arrow (⇒) indicates that the dansyl-amino acid was obtained without hydrolysis, showing the release of a free C-terminal residue. (b) Comparison of the water-buffalo sequence with equivalent sequences from other species: bovine (Beale & Squires, 1970), goat (Strausbauch *et al.*, 1971), human (Milstein, 1965) and chicken (Grant *et al.*, 1971). Amino acids identical with those of water-buffalo λ -chains are shown by a solid line; a gap in sequence is indicated by ▨.

of other artiodactyls, particularly the goat and bovine species, but shows an additional amino acid on the C-terminal side of the interchain half-cystine residue. Although a deletion occurs at the C-terminus of chicken λ chains (Grant *et al.*, 1971) and an addition occurs at the C-terminus of pig κ chains (Novotny & Franek, 1968), the water-buffalo is the first species to show an addition at the C-terminus of the λ chain.

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