position 190 is indicated by the fact that it is present only when the arginine-containing peptide T3 is absent.

One of the proteins (Mz) contained two variants, one in peptide T8 (valine instead of alanine) and the other in peptide T4/T5 (see Fig. 1). The variant in peptide T8 was located at residue 143. This was shown by isolating a subtilisin peptide derived from peptide T8 with the sequence Val-Val-Thr. This was confirmed by determining the *N*-terminus of peptide T8 (alanine) and isolating a subtilisin peptide Val-Ala, which accounts for the two alanine residues of peptide T8 of protein Mz. The variant peptide of T4/T5 appears to involve a substitution of lysine for asparagine at position 171, giving only one peptide instead of the two observed in the other proteins studied. Asparagine rather than aspartic acid is indicated by the fact that the peptide was neutral at pH 6.5. Apart from these two variants the composition of all the tryptic peptides of this protein (Mz) corresponding to the *C*-terminal half was in full agreement with the sequence depicted in Table 1.

The results suggest that position 190 of human chains may be present in the population in two forms, one containing lysine and the other arginine. It is noteworthy that the Inv character of κ -chains is correlated with a leucine/valine substitution at position 191.

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The Amino Acid Sequence of the *N*-Terminal 84 Residues of a Human Heavy Chain of Immunoglobulin G (Daw)

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The chemical structure of the heavy chain of a human pathological immunoglobulin G has been studied by splitting with cyanogen bromide at the four methionine residues and separation of the resulting five fragments. The method of isolation, the composition and the order of the five fragments have been reported, together with the amino acid sequence of the *N*-terminal fragment (2b) consisting of 34 residues (Piggot & Press, 1967).

The next fragment from the *N*-terminal end is fragment 4, having 50 residues, and the amino acid sequence of this fragment has now been determined by using the methods described by Piggot & Press (1967). The α -amino group of this fragment is blocked.

Tryptic peptides. Fragment 4 was digested with trypsin (200 μ g./ μ mole) for 5 hr. at 37° at pH 8.3, and the digest fractionated on a column of Sephadex G-25 in aq. 20mN-NH₃. Five fractions were

obtained and from these five peptides were isolated; one fraction was pure (peptide 4T1) and the remaining fractions were purified by paper electrophoresis at pH 3.5. The compositions and mobilities of the peptides are given in Table 1.

Peptide 4T2 α contained homoserine and is therefore the *C*-terminal peptide. The partial sequence was established by the 'dansyl'-Edman technique to be: Asx*-Glx-Val-(Val,Leu,Ser)-homoserine.

Peptide 4T2 β was neutral at pH 6.5 and the sequence was established by the 'dansyl'-Edman technique to be: Asp-Thr-Ser-Lys.

Peptide 4T3 was shown by the 'dansyl'-Edman technique to have the sequence: Leu-Ala-Val-Ser-Lys.

Peptide 4T5 contained tryptophan and was

*Abbreviations: Asx, aspartic acid or asparagine; Glx, glutamic acid or glutamine; CM-Cys, *S*-carboxymethylcysteine; PCA, pyrrolid-2-one-5-carboxylic acid.

Table 1. *Amino acid composition of significant peptides from fragment 4 of Daw heavy chain*

The analysis of fragment 4 was based on 1 homoserine residue/mol. The analyses of the peptides were calculated on the assumption that the average of the amounts of each amino acid was integral.

	Fragment 4	Tryptic peptides						Chymotryptic peptides of peptide 4T1		
		4T2 α	4T2 β	4T3	4T5	4T1	Sum	4T1C2 γ	4T1C1 γ	4T1C3 γ
Lys	2.9	—	0.9	1.0	—	1.0	3	—	1.1	—
Arg	1.7	—	—	—	1.0	0.9	2	—	—	1.0
Asp	6.7	1.1	1.0	—	—	3.9	6	—	4.3	—
Thr	3.0	—	0.9	—	—	1.3	2	—	—	1.0
Ser	4.8	1.2	1.2	1.0	—	1.3	4	—	—	1.1
Glu	5.6	1.0	—	—	—	3.6	5	2.8	—	1.0
Pro	3.1	—	—	—	—	2.0	2	2.2	—	—
Gly	2.7	—	—	—	—	2.1	2	1.0	—	1.0
Ala	5.5	—	—	1.0	1.1	3.0	5	1.0	1.1	1.0
Val	4.0	1.7	—	1.0	1.1	0.3	4	—	—	—
Ile	2.2	—	—	—	1.0	1.1	2	—	1.0	—
Leu	7.4	1.0	—	1.0	—	4.0	6	1.0	1.8	0.9
Tyr	2.4	—	—	—	—	1.8	2	—	1.7	—
CM-Cys	0.9	—	—	—	0.8	—	1	—	—	—
Trp†	3.0	—	—	—	1.0	2.0	3	1.0	1.0	—
Homoserine	1.0	1.0	—	—	—	—	1	—	—	—
Total residues	56.9	7	4	5	6	28	50	9	12	7
Mobility at pH 3.5*		Neutral (0)	Basic (0.4)	Basic (0.6)	Neutral (0)			Neutral and acidic (0) (0.3)		Basic (0.5)

* Acidic peptides, mobility relative to aspartic acid; basic peptides, mobility relative to lysine.

† Tryptophan, estimated from u.v. absorption at 280 m μ .

ninhydrin-negative, and the *N*-terminal residue could not be determined by the 'dansyl' method. Two peptides were isolated from a chymotryptic digest: peptide 4T5C β , whose sequence was Ile-Arg, and peptide 4T5C α , which was shown by carboxypeptidase A digestion and hydrazinolysis to be CM-Cys-Val-Ala-Trp. The latter peptide was acidic, although the cysteine residue had been alkylated with iodoacetamide; presumably the α -amino group was blocked, perhaps by a ring-formation with the side chain. Since this is a tryptic digest, arginine must be *C*-terminal and the sequence is: CM-Cys-Val-Ala-Trp-Ile-Arg.

Peptide 4T1 gave a low yield of glutamic acid as the *N*-terminal residue. It was digested with chymotrypsin (400 μ g./ μ mole) at 37° at pH 8 for 2 hr. and the digest fractionated on a column of Sephadex G-25 in aq. 20mN-NH₃. Three pure peptides were isolated by paper electrophoresis at pH 3.5 (peptides 4T1C2 γ and 4T1C3 γ) or by chromatography (peptide 4T1C1 γ) in pyridine-3-methylbutan-1-ol-water (7:7:6, by vol.). These peptides accounted for the whole of peptide 4T1 (see Table 1).

Peptide 4T1C2 γ . Two peptides were obtained having the same composition, but one was nin-

hydrin-negative and acidic, whereas the other was weakly acidic at pH 3.5 and with an *N*-terminal glutamic residue. It was apparent that the α -amino group was blocked by formation of the pyrrolidone ring in the former peptide and that this is probably the *N*-terminal peptide of peptide 4T1. The 'dansyl'-Edman technique on the latter peptide established a partial sequence. Papain digestion at pH 4.3 cleaved both peptides into three constituent peptides: a neutral peptide (Gln,Pro₂,Gly) or an acidic peptide (PCA,Pro₂,Gly) and two acidic peptides (Glu,Ala) and (Leu,Glu,Trp), and from these the sequence was established as: Gln-Pro-Pro-Gly-Glu-Ala-Leu-Glu-Trp.

Peptide 4T1C1 had been resolved into three peptides by paper chromatography in pyridine-3-methylbutan-1-ol-water (7:7:6, by vol.). They differed from each other only in tyrosine content. Peptide 4T1C1 γ had two tyrosine residues (Table 1), peptide 4T1C1 β had one tyrosine residue and peptide 4T1C1 α had no tyrosine. Leucine was *N*-terminal in all three and tyrosine *C*-terminal in peptides 4T1C1 β and 4T1C1 γ . Redigestion of peptide 4T1C1 β for 18 hr. with chymotrypsin resulted in cleavage to give three acidic peptides: (a) Leu-(Ala,Trp,Asp,Ile,Leu), (b) Asn-Asp-Asp-Lys-

Tyr and (c) Asp-Asp-Lys-Tyr; the sequences of the last two were readily determined by the 'dansyl'-Edman method. Peptide (a) was subjected to sequence analysis by digestion for various times with leucine aminopeptidase and carboxypeptidase A. The complete sequence of peptide 4T1C1 γ is: Leu-Ala-Trp-Asp-Ile-Leu-Asn-Asp-Asp-Lys-Tyr-Tyr.

Peptide 4T1C3 γ was shown by the 'dansyl'-Edman method to have the sequence: Gly-Ala-Ser-Leu-Glu-Thr-Arg. It was a neutral peptide at pH 6.5. This peptide was presumed to be the C-terminal peptide of peptide 4T1 because of C-terminal arginine, and the order of the three peptides was confirmed by isolation of other peptides in low yield that overlapped the three main chymotryptic peptides. The order is therefore: 4T1C2 γ -4T1C1 γ -4T1C3 γ .

Chymotryptic peptides. The order of the five tryptic peptides was determined by isolation and partial sequence analysis of three chymotryptic peptides. Fragment 4 was digested with chymotrypsin (500 μ g./ μ mole) for 2½ hr. at 37° at pH 8.0, and the digest fractionated on a column of Sephadex G-25 in aq. 20mM-NH₃.

Peptide 4C2 δ was isolated by paper electrophoresis at pH 6.5. From its composition it was deduced to contain peptide 4T1C2 γ and the C-terminal two residues of peptide 4T5. The partial sequence confirmed this: Ile-Arg-Glx-(Pro,Pro,Gly, Glu,Ala,Leu,Glu,Trp).

Peptide 4C3 δ was purified by paper electrophoresis at pH 3.5 and its sequence, Glu-Thr-Arg-Leu, proved that peptide 4T1 was followed by peptide 4T3.

Peptide 4C2 α was separated by paper electrophoresis at pH 6.5. It had alanine as the N-terminal residue and from its composition contained peptide 4T2 β and part of peptide 4T3 and the N-terminal asparagine residue of peptide 4T2 α . This was confirmed by tryptic digestion and isolation of peptide 4T2 β , asparagine (N-terminal residue of peptide 4T2 α) and a peptide Ala-(Val,Ser,Lys) that is part of peptide 4T3.

Two further chymotryptic peptides, 4C3 γ ,

Gln-Val-Val-Leu, which was neutral, and 4C3 β , Ser-homoserine, were separated by electrophoresis at pH 3.5 and these must be derived from peptide 4T2 α and thus complete the sequence of the latter. An acidic ninhydrin-negative peptide, 4C6 α , was isolated by electrophoresis at pH 6.5, having the sequence CM-Cys-Val-Ala-Trp, which completed the alignment of all the tryptic peptides as shown in Fig. 1.

The isolation of peptides having the N-terminal residue *S*-carboxyamidomethylcysteine from both tryptic and chymotryptic digests of fragment 4 suggested that this was indeed the N-terminal residue of fragment 4. However, the sum of the residues of the five tryptic peptides is several residues less than that of fragment 4 (Table 1). Also, it had not been possible to establish that *S*-carboxyamethylcysteine was N-terminal in fragment 4, even when this cysteine residue was alkylated with iodoacetic acid. It was therefore necessary to establish that this cysteine residue did in fact follow the methionine residue at position 34 in the whole heavy chain by isolating a peptide overlapping the cyanogen bromide fragments 2b and 4. A fragment, 4a, had been obtained in low yield (8% of theoretical) from the cyanogen bromide cleavage of the heavy chain, which was a partial cleavage product consisting of fragments 2b and 4. A peptide was isolated from the chymotryptic digest of fragment 4a, having the partial sequence: Ser-(Leu,Ser,Gly,Gln,Thr,'Met')-CM-Cys-Val-Ala-Trp. The first seven residues are the same as the C-terminal chymotryptic peptide of fragment 2b (Piggot & Press, 1967) and this confirms that the sequence of 50 residues shown below is the complete sequence of fragment 4. Methionine was not detected in fragment 4a nor in the peptide isolated from the chymotryptic digest of fragment 4a, but, as 0.2mole of homoserine was found, cyanogen bromide had presumably reacted with a methionine residue, but cleavage had not occurred.

The sequence of the first 84 residues of Daw heavy chain is therefore: PCA-Val-Thr-Leu-Arg-Glu-Ser-Gly-Pro-Ala-Leu-Val-Arg-Pro-Thr-Gln-Thr-Leu-

Fig. 1. Amino acid sequence of fragment of 4 Daw heavy chain.

Thr-Leu-Thr-Cys-Thr-Phe-Ser-Gly-Phe-Ser-Leu-Ser-Gly-Glu-Thr-Met-Cys-Val-Ala-Trp-Ile-Arg-Gln-Pro-Pro-Gly-Glu-Ala-Leu-Glu-Trp-Leu-Ala-Trp-Asp-Ile-Leu-Asn-Asp-Asp-Lys-Tyr-Tyr-Gly-Ala-Ser-Leu-Glu-Thr-Arg-Leu-Ala-Val-Ser-Lys-Asp-Thr-Ser-Lys-Asn-Gln-Val-Val-Leu-Ser-Met.

Chemical studies of Bence-Jones proteins have shown that the sequence of the C-terminal half of the chains is common to all proteins within a type, but the N-terminal half of the chains shows many variations (see Cohen & Milstein, 1967). Similarly in the heavy chains the occurrence of regions of common and variable sequence have been deduced from comparative peptide 'maps' (Frangione, Prelli & Franklin, 1967). Comparison of the

N-terminal sequence reported here with that of other heavy chains of the same Gm group will enable the extent of variability to be defined.

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The Thiol Groups and Sub-unit Structure of Creatine Kinase

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Creatine kinase (EC 2.7.3.2) from rabbit skeletal muscle has a molecular weight of 81000–82000, and its amino acid composition is known. Alkylation with iodoacetate in the presence of sodium dodecyl sulphate yielded 6.9 moles of S-carboxymethylcysteine/mole, whereas 7.9 moles of cysteic acid are found/mole of performic acid-oxidized protein, and there are 8.3 g.atoms of non-methionine sulphur/mole. Amperometric titration with Ag⁺ in the presence of 8M-urea gave 6.3 cysteine residues/mol. in agreement with the value of 6.5 thiol groups/mol. reported by Benesch, Lardy & Benesch (1955). From these analytical values it was concluded that the enzyme contained 6 cysteine residues (2 reactive and 4 unreactive) and a single disulphide bridge (for review see Kuby & Noltman, 1962).

More recently, values of 6–7 cysteine residues/mol. have been reported (see review by Kuby & Noltman, 1962; Thomson, Eveleigh & Miles, 1964). Further, it was shown by Thomson *et al.* (1964) and by Mahowald (1965) that the enzyme contains two active sites/mol. with two identical sequences, Thr-Cys-Pro-Ser-Asn, about the reactive cysteine

residues. Since tryptic digests of the denatured enzyme yield only half the expected number of peptides (Dance & Watts, 1962; Thomson *et al.* 1964), and since the reduced and alkylated enzyme has a molecular weight of about 40000 (Eveleigh & Thomson, 1965), it seems likely that the native enzyme consists of two sub-units of similar composition.

The present work reports further studies on the thiol groups of creatine kinase aimed at deciding whether the two sub-units are linked by a disulphide bridge. The analytical methods used enabled thiol and disulphide groups to be clearly distinguished.

Methods. The creatine kinase used was purified to the precrystalline stage from rabbit skeletal muscle by method B (Kuby & Noltman, 1962). It had a specific activity of 60–70 units/mg. by the labile-phosphate assay.

Thiol groups were determined by (i) reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) by the method of Ellman (1959), and (ii) amperometric titration at the dropping-mercury electrode with phenylmercuric hydroxide (Allison & Cecil, 1958) by using the apparatus of Cecil & Snow (1962). The latter method also enabled disulphide groups to be determined after reaction with sulphite (Cecil &

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