Biological Chemistry Hoppe-Seyler

Volume 366 – First half year

THE OFFICIAL ORGAN OF THE GESELLSCHAFT FÜR BIOLOGISCHE CHEMIE

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1985

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Biological Chemistry Hoppe-Seyler

This Journal was founded in 1877 as *Zeitschrift für Physiologische Chemie* by F. Hoppe-Seyler and was continued after his death under the editorship of A. Kossel, F. Knoop, K. Thomas, F. Lynen, A. Butenandt and G. Weitzel as *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* [Volume 21 (1895)–Volume 365 (1984)].

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Vol. 366, pp. 395–402, April 1985

Primary Structure of the Hemoglobins from the Greater Kudu Antelope *(Tragelaphus strepsiceros)**

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(Received 17 January 1985)

Summary: The adult greater Kudu antelope has two hemoglobin components, Hb A and Hb B, with one α and two β chains. The complete amino-acid sequences of these three chains are presented. The two β chains differ only in one residue at position 16 (Gly→Ser) and may be the product of two allelic genes. The primary structure of the chains was determined by sequencing the tryptic peptides after their isolation from the tryptic digest of the chains by high performance liquid chromatography. The alignment of these peptides was deduced from homology with the chains of bovine hemoglobin. Between the Kudu hemoglobins and those of cattle a high degree of homology was found.

Primärstruktur der Hämoglobine des großen Kudu (Tragelaphus strepsiceros)

Zusammenfassung: Die Erythrozyten des großen Kudu enthalten zwei Hämoglobin-Komponenten, A und B, mit einer α- und zwei β-Ketten. Die vollständige Aminosäuresequenz der drei Ketten wird angegeben. Die zwei β-Ketten unterscheiden sich nur in einer Aminosäure in der Position 16 (Gly→Ser) und könnten das Produkt zweier Allelgene sein. Die Primärstruktur der Ketten wurde durch Sequenzierung der tryptischen Peptide bestimmt. Diese konnten mit Hilfe der Hochdruckflüssigkeitschromatographie aus den tryptischen Hydrolysaten der Ketten gewonnen werden. Die Zuordnung dieser Peptide erfolgte aufgrund der Homologie zu den Ketten vom Rind. Die Kudu-Hämoglobine und die der Rinder erwiesen sich als sehr homolog.

Key words: Tetrameric hemoglobin, greater Kudu antelope, primary structure, evolution.

The systematic investigation of hemoglobins has provided important progress in solving questions such as the control of gene expression, structure-function relationships of proteins, and the pathophysiology of abnormal hemoglobins. Moreover, the physiological and structural differences can be related to the evolutionary development; such information was used to construct evolutionary trees which affirm or contradict the classical taxonomy.
The greater Kudu antelope *(Tragelaphus strepsiceros)* was classified, based on its morphology and behavioural pattern, as a member of the order of Artiodactyla, suborder Ruminantia, family Bovidae, genus Tragelaphini; i.e., the Kudu antelope appears to be a close relative of

Abbreviations:
Hb = Hemoglobin; $TosPheCh_2Cl$ = (*N*-tosyl-L-phenylalanyl)chloromethane; Tp = tryptic peptide; Quadrol = *N,N,N',N'*-tetrakis-(2-hydroxypropyl)ethylenediamine, Reagent I = 4-(isothiocyanato)benzenesulphonate, sodium salt; Reagent IV = 7-(isothiocyanato)naphthalene-1,3,5-trisulfonate, trisodium salt; 2,3-Glyc-P_2 = D-glycerate-2,3-bis-(phosphate).

* 78th Communication on hemoglobins; for 77th communication see ref.[1].

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the cow and goat. Expressed in terms of molecular evolution, the hemoglobins of all these animals should have a great degree of homology and similar physiologic properties. The primary structures of the chains from bovine hemoglobins with variants and from goat have already been reported[2–7]. The present investigation was designed to determine the relationship between Kudu hemoglobins and other ruminant hemoglobins.

Material and Methods

Isolation of hemoglobin and chain separation

Blood of an adult Kudu antelope was obtained from the Hellabrunn Zoo in Munich. After separation of the erythrocytes from the plasma by centrifugation and three washings with physiological saline, hemolysis was performed by lysing the cell with distilled water containing 5% CCl_4. After centrifugation of the cell debris, the hemoglobin solution was analysed by disc electrophoresis with Triton X-100 and urea[8] for the number of chains (Fig. 1). The polypeptides were isolated directly from hemoglobin by reversed-phase high performance liquid chromatography on a RP2 Lichrosorb (Merck) column (4.6 x 250 mm), equilibrated with 30% acetonitrile (Baker) in 50mM ammonium acetate with 5% formic acid. The concentration of acetonitrile was increased linearly from 30 to 60% for 60 min (0.5%/min) at a flow rate of 0.5 ml/min (Fig. 2). After the evaporation of acetonitrile, the protein peaks were first lyophilized and then desalted on Sephadex G-25 medium in 1M acetic acid and again freeze-dried.

Enzymatic cleavage and separation of peptides

The chains (1 μmol) were digested at pH 9 with 2% (w/w) trypsin (Worthington, TosPheCH_2Cl-treated) at room temperature for 4 h. The reaction was stopped with 0.5M HCl. At pH 6.6 for the α and 6.5 for the β chains the "cores" were precipitated, removed by centrifugation and lyophilized after washing with water. The tryptic digests from both chains were titrated to pH 3 with 0.5M HCl and then prefractionated on a Sephadex G-25 fine column (1.6 x 180 cm) equilibrated and eluted with 0.1M acetic acid. The peaks containing several peptides were rechromatographed on a RP2 Lichrosorb column (4.6 x 250 mm), equilibrated with 50mM ammonium acetate pH 6 and eluted with a gradient of acetonitrile from 0 to 60% in 60 min at a flow rate of 1 ml/min. The "core" peptides were also isolated by reversed-phase high performance liquid chromatography on a RP18 Vydac column (4.6 x 250 mm) as follows:

1) The α-peptides in 50mM ammonium acetate, adjusted with ammonia to pH 7, and a gradient of 0 to 60% acetonitrile with a change in the concentration rate of 1.2%/(min x ml) (Fig. 3);
2) The β-peptides in 0.1% trifluoroacetic acid and a gradient of 0 to 60% acetonitrile in 30 min at a flow rate of 1 ml/min.

In addition preparative thin-layer electrophoresis on plates at pH 5[9] was the subsequent purification step of peptides. In Tables 1 and 2 the amino-acid analyses of the tryptic peptides of both chains are shown.

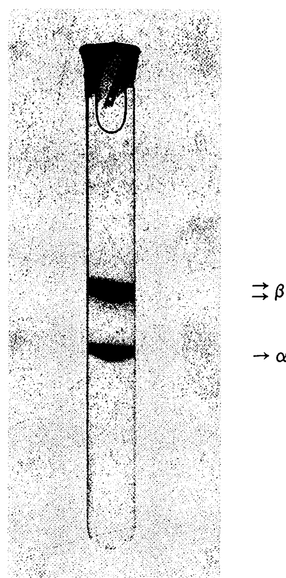

Fig. 1. Acidic disc electrophoresis with Triton and urea[8] of the Kudu hemoglobins.

Like Schroeder et al.[3] we also observed the unanticipated tryptic cleavage of the Asn-Ala bond at position 139–140 of the β chains, but already after 4 h of enzymatic digestion.

Chemical cleavage and isolation of the peptides

Asp-Pro cleavage of the chains

In order to obtain overlapping peptides in the C-terminal region of the chains, the polypeptides were also cleaved with 70% formic acid in the presence of 6M guanidine/HCl[10]. The separation of the peptides was achieved by gel filtration on a Sephadex G-50 fine column (2.5 x 180 cm) with 8M urea in 10% formic acid. The isolation of a pure β Pro-peptide was possible only by chromatography of the acidic digest (0.7 μmol) on a CM-Sephacel column (0.6 x 20 cm), equilibrated with 50mM sodium acetate/HCl buffer in 8M urea pH 5, containing 100 mg dithiothreitol/l, and eluted with a linear gradient of 100 ml equilibrating buffer and 100 ml of equilibrating buffer plus 0.1M NaCl. The flow rate was 7.5 ml/h. This method permitted the separation of the Pro-peptide (pos 100–146) from fragment 58–98, produced by the unexpected cleavage of the Asn-Pro bond, position 57–58; this cleavage has been observed previously (T. Kleinschmidt, personal communication). The two Pro-peptides were partially sequenced using Method B (see below).

Analytical techniques

Amino-acid analysis

1–5 nmol peptides were hydrolysed at 110 °C with 6M HCl for 20 h. The acidic hydrolysates were dried in a speed vac concentrator connected to a refrigerated condensation trap (both from Bachofer) and a high vacuum pump, and after solubilisation in 0.1N citrate buffer pH 2.2, analysed in an amino-acid analyser LC 5000 from Biotronik.

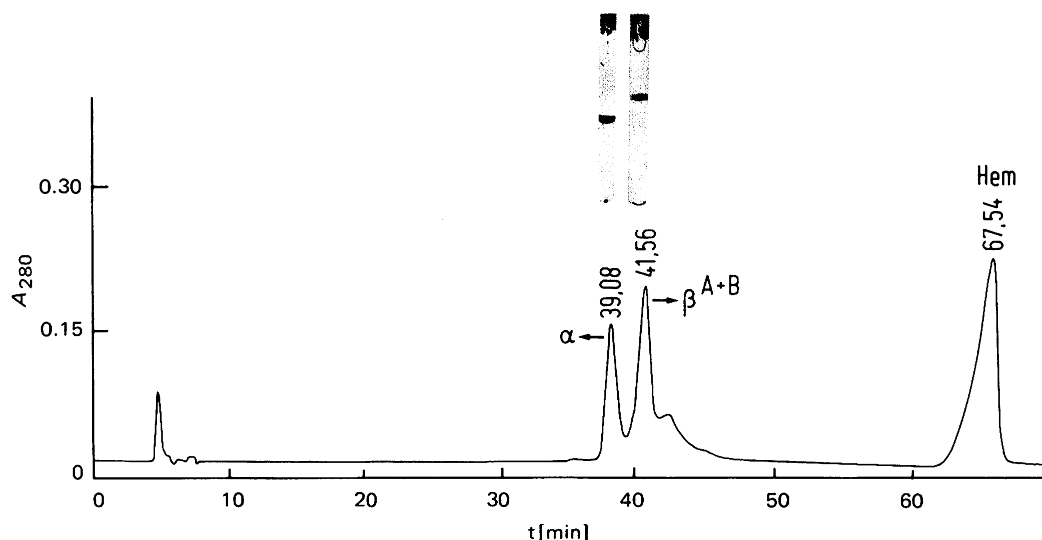

Fig. 2. Separation of the chains of Kudu hemoglobins by reversed-phase high performance liquid chromatography. Column (4.6 x 250 mm); Lichrosorb RP2; 50mM ammonium acetate with 5% formic acid plus 30% acetonitrile as the start buffer; acetonitrile gradient from 30% to 60% in 60 min. Flow rate 0.5 ml/min. Sample: 1.2 mg hemoglobin/ 20 μl protein solution diluted to 100 μl with start buffer.

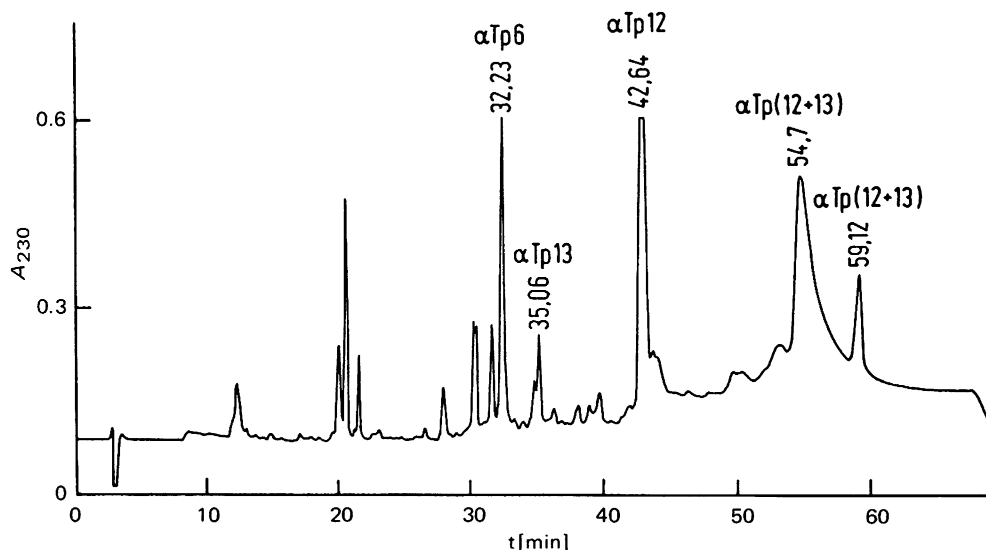

Fig. 3. Separation of the "core" peptides of the α chains by reversed-phase high performance liquid chromatography. Column (4.6 x 250 mm); Vydac RP18; 50mM ammonium acetate adjusted with NH_3 to pH 7; acetonitrile gradient from 0 to 60% with an increase in concentration of 1.2%/(min x ml). Sample: 20nmol "core" peptides/20 μl 50mM ammonium acetate buffer pH 7.5 (with NH_3).

Thin-layer electrophoresis

This technique was used to check the purity of peptides and in some cases to purify them preparative as previously described[9].

Sequence analysis

The partial sequences of the intact chains and the entire sequences of the peptide were determined by automatic Edman degradation[11] in a Beckman sequencer (Palo Alto, Calif.) using two programs:

a) Method A, sequencer 890 C and quadrol concentration of 0.25M for intact chains, large peptides and lysine peptides which have been reacted with reagent IV[12].

b) Method B, sequencer 890 B and 3-(diethylamino)-propyne[13] for arginine peptides and lysine peptides which have been reacted with reagent I[14].

The identification of the phenylthiohydantoin derivatives of amino acids was achieved by thin-layer[15,16] and reversed-phase high performance liquid chromatography[17] using a Hewlett Packard 1084 B Liquid Chromatograph.

Results and Discussion

Triton urea gel electrophoresis (Fig. 1) revealed three bands corresponding to one α and two β chains. The β chain bands migrate close to each

Table 1. Amino-acid composition of tryptic peptides of α chains determined after 20 h hydrolysis.

Pos.	Tp1 1-7	Tp2 8-11	Tp3 12-16	Tp4 17-31	Tp5 32-40	Tp6 41-56	Tp7+8 57-61
Asp	1.07	-	-	-	-	0.96	-
Thr	-	-	-	-	1.87	1.03	-
Ser	1.07	-	-	1.00	1.05	1.97	-
Glu	-	-	-	2.94	-	1.00	-
Pro	-	-	-	-	1.13	1.12	-
Gly	-	1.06	1.23(1)	1.94	-	0.99	1.92
Ala	1.96	-	1.81	3.84	-	1.01	1.06
Cys	-	-	-	-	-	-	-
Val	0.75(1)	0.90	-	1.08	-	1.01	-
Met	-	-	-	-	0.70(1)	-	-
Ile	-	-	-	-	-	-	-
Leu	0.91	-	-	1.07	1.07	1.07	-
Tyr	-	-	-	1.02	-	0.90	-
Phe	-	-	-	-	1.83	1.96	-
Trp	-	-	(1)	-	-	-	-
His	-	0.98	-	1.06	-	1.88	0.96
Lys	1.05	1.02	0.94	-	1.00	0.99	1.06
Arg	-	-	-	1.02	-	-	-
Sum	7	4	5	15	9	16	5

Pos.	Tp9a 62-68	Tp9b 69-90	Tp10 91-92	Tp11 93-99	Tp12 100-127	Tp13 128-139	Tp14 140-141
Asp	-	4.51(5)	-	1.87	1.93	1.19	-
Thr	1.01	-	-	-	2.04	1.70	-
Ser	-	2.01	-	-	4.17	2.06	-
Glu	-	-	-	-	-	-	-
Pro	-	1.21	-	1.06	2.20	-	-
Gly	-	1.21	-	-	1.45(1)	-	-
Ala	2.88	2.94	-	-	2.74	1.23(1)	-
Cys	-	-	-	-	-	-	-
Val	0.97	1.01	-	2.02	1.74	1.50(2)	-
Met	-	-	-	-	-	-	-
Ile	-	-	-	-	-	-	-
Leu	1.04	4.84	1.10	-	7.38(7)	1.77	-
Tyr	-	-	-	-	-	-	1.07
Phe	-	-	-	0.98	1.21	1.43(1)	-
Trp	-	-	-	-	-	-	-
His	-	2.77	-	-	2.74	-	-
Lys	1.10	1.06	-	1.04	1.27(1)	1.16	-
Arg	-	-	0.99	-	-	-	0.93
Sum	7	22	2	7	28	12	2

Table 2. Amino-acid composition of the tryptic peptides of β chains determined after 20 h hydrolysis.

The Tp1 peptide is a heptapeptide with one deletion in position two. The Tp2* corresponds to the second β chain (β^B) with Ser in position 16 instead of Gly.

Pos.	Tp1 1-8	Tp2 9-17	Tp2* 9-17	Tp3a 18-19	Tp3b 20-30	Tp4 31-40	Tp5 41-59	Tp6 60-61	Tp7 62-65	Tp8 66
Asp	-	-	-	-	1.04	-	3.64(4)	-	-	-
Thr	1.03	0.99	1.00	-	-	1.09	0.96	-	-	-
Ser	-	-	1.05	-	-	-	1.67(2)	-	-	-
Glu	1.73	-	-	-	1.84	1.16	1.05	-	-	-
Pro	-	-	-	-	-	1.05	1.11	-	-	-
Gly	-	1.17	-	-	2.82	-	1.25(1)	-	0.96	-
Ala	1.12	2.90	2.86	-	1.19	-	2.02	-	0.70	-
Cys	-	-	-	-	-	-	-	-	-	-
Val	-	0.98	1.01	1.01	1.99	1.74	0.99	1.01	-	-
Met	0.49(1)	-	-	-	-	-	0.70	-	-	-
Ile	-	-	-	-	-	-	-	-	-	-
Leu	1.08	-	-	-	1.22	2.13	1.09	-	-	-
Tyr	-	-	-	-	-	0.71	-	-	-	-
Phe	-	0.97	1.02	-	-	-	3.25	-	-	-
Trp	-	(1)	(1)	-	-	(1)	-	-	-	
His	-	-	-	-	-	-	-	-	1.17	-
Lys	1.05	1.00	1.04	0.99	-	-	1.17	0.99	1.16	(1)
Arg	-	-	-	-	0.89	1.09	-	-	-	-
Sum	7	9	9	2	11	10	19	2	4	1

Pos.	Tp9a 67-76	Tp9b 77-82	Tp10+11 83-104	Tp12a 105-116	Tp12b 117-120	Tp13 121-132	Tp14 133-144	Tp15 145-146
Asp	1.94	2.00	2.55(3)	0.91	-	0.99	1.02	-
Thr	-	-	1.29(1)	-	-	0.97	0.98	-
Ser	1.90	-	1.24(1)	-	-	-	-	-
Glu	-	-	2.04	-	-	3.75	-	-
Pro	-	-	1.05	-	-	0.99	-	-
Gly	1.06	-	1.43(1)	1.09	1.05	-	1.02	-
Ala	-	-	2.04	1.15	-	1.08	3.22	-
Cys	-	-	(1)	-	-	-	-	-
Val	0.98	-	0.92	3.83	-	-	2.69	-
Met	0.81	-	-	-	-	-	-	-
Ile	-	-	-	-	-	-	-	-
Leu	1.23	2.00	2.96	3.99	-	0.98	1.02	-
Tyr	-	-	-	-	-	0.96	-	1.00
Phe	1.06	-	1.94	-	1.03	1.07	-	-
Trp	-	-	-	-	-	-	-	-
His	-	0.96	1.99	-	0.85	-	1.02	1.00
Lys	1.03	1.00	1.66(2)	-	1.07	1.10	-	-
Arg	-	-	-	0.99	-	-	1.02	-
Sum	10	6	22	12	4	12	12	2

other, suggesting only very few neutral exchanges in their primary structures. Using the RP2 Lichrosorb HPLC-support for the chain separation, we could resolve only the α from the β polypeptides, but not the two β chains. Therefore the β polypeptides were sequenced together.

The determination of the chain sequences was carried out according to the usual scheme: sequencing of the N-terminal sections of the chains (42 steps) and of the Pro-peptides (35 steps for the α and 23 steps for the β chains) and sequencing of the tryptic peptides from the middle and the C-terminal end of the chains. The isolation of the β tryptic peptides permitted the identification of the single difference between these two chains, β^A and β^B. This is located at position 16 in the chain. The two tryptic peptides could be separated without difficulty by reversed-phase high performance liquid chromatography (Fig. 4) and were sequenced by Method A. At position 16 (8 in the Tp2 peptides) Gly and Ser were determined. The two peptides were isolated in roughly equal quantities, indicating that the two β chains are also produced in roughly equal amounts. Thus the Kudu erythrocytes contain hemoglobins with one α and two β chains.

Owing to the fact that hybrid hemoglobins of type $\alpha_2\beta^A\beta^B$ are not stable in mammals[18], we can assume that the Kudu antelope contains only two hemoglobin components, Hb A and Hb B.

The same ambiguity: Gly/Ser is found in the bovine β chains and constitutes one of the three differences between the two polypeptides of cow hemoglobins. The Kudu chains have the same lengths as the bovine chains, 141 residues for the α and 145 for the β chains (Fig. 5). A comparison of the sequences of Kudu and bovine chains (Tab. 3 and 4) reveals only few differences. The α chain from Kudu differs from that of the cow only in five positions: 9 His→Asn. 19 Ser→Gly, 71 Asp→Glu, 82 Asp→Glu, and 115 Gly→Ser. The Kudu α chain contains no cysteine. The cysteine at position 104 of the

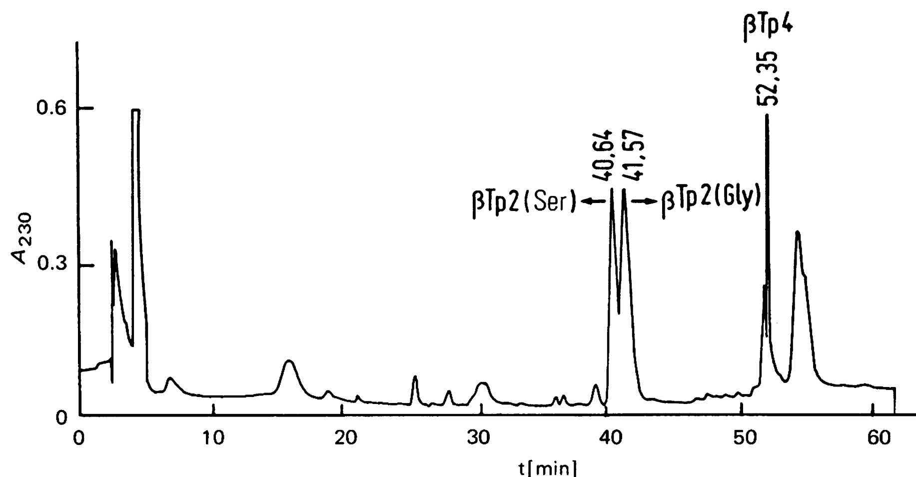

Fig. 4. Separation of the two Tp2 peptides of the β chains by reversed-phase high performance liquid chromatography. Column (4.6 x 250 mm); Lichrosorb RP2; 50mM ammonium acetate pH 6; acetonitrile gradient from 0 to 60% in 60 min with a hold of 15 min at the 23rd min. Flow rate 1 ml/min. Sample: the Sephadex fraction SIV (see Materials and Methods) 15 nmol/100 μl.

human α chain is substituted for a serine in the Kudu and bovine chains.

The β^A chains from Kudu show five differences as compared to bovine β^A chains (Tab. 4) at positions 73 Asn→Asp, 117 His→Asn, 125 Glu→ Val, 130 Tyr→Phe, and 135 Thr→Ala. The same chains differ from the bovine β^B chain by three additional exchanges at positions 16 Gly→Ser, 19 Lys→His, and 120 Lys→Asn. The β^B chains of Kudu contain six and seven different residues, as compared with the bovine β^A and β^B chains, respectively. (According to Schimenti and Duncan[31], the code triplett in the DNA sequence for the amino-acid residue in position 73 of the bovine β chains is one coding for an asparagine. Consequently the number of exchanged residues can be reduced by one in all the cases).

None of these residues is involved in the heme or $\alpha 1\beta 2$ contacts and all substitutions, except the change at position β 19, could have taken place by single base substitutions in the triplets coding for these amino acids. Assuming that nucleotide substitutions in the globin genes occur randomly, a preponderance of neutral and conservative substitutions would be expected. In fact the exchanged residues in the Kudu hemoglobin do not seem to influence the functioning of the molecule, as compared with the other hemoglobins from Bovidae. It is interesting to note that more differences were found between the two β chains of hemoglobins of the domesticated bovine than between the two β chains of Kudu, a wild animal. Another fact to be pointed out is the presence of threonine in position β 135 of Kudu hemoglobin, as in all γ chains of fetal hemoglobins of bovine[32], goat[33], sheep[30,33] and human[34], in the β^B chain of the adult aoudad[35] and in the β^{II}, β^{IV} and β^{V} chains of the adult white-tailed deer[22].

In the Kudu β chains $\beta 2$ His is also deleted, as in the bovine chains. It is known that among the Artiodactylis species the Bovidae and Cervidae have low levels of red cell 2,3-Glyc-P_2 as well as hemoglobins with a low affinity for this compound and oxygen. The lack of response to 2,3-Glyc-P_2 can be explained by two facts:

a) the deletion of one of the binding sites for organic phosphates[36], $\beta 2$ His, and

b) the N-termini of the β polypeptides of the deoxyhemoglobin, as a result of this chain shortening, are too far apart to bind 2,3-Glyc-P_2[37].

The oxygen affinity of the Kudu hemoglobins has not yet been determined, but we suppose that their respiratory characteristics could not be very different from those of other ruminants. Recently the investigations of Vyazova et al.[38] and those of Fronticelli et al.[39,40] showed that the allosteric effectors of the bovine hemoglobins in vivo are most probably the $Cl^{\ominus}$ ions. The lower oxygen affinity of these hemoglobins under normal physiological conditions is due to an enhanced sensitivity of this system to concentrations of $Cl^{\ominus}$ ions below 0.07M. It seems that in the bovine hemoglobin tetramer some additional salt bridges, formed via $Cl^{\ominus}$ ions in the deoxy form, especially stabilise this structure. Thus, these anions together with the steric effect of the N-terminal methionine of the β chains[37] may substitute for the organic phos-

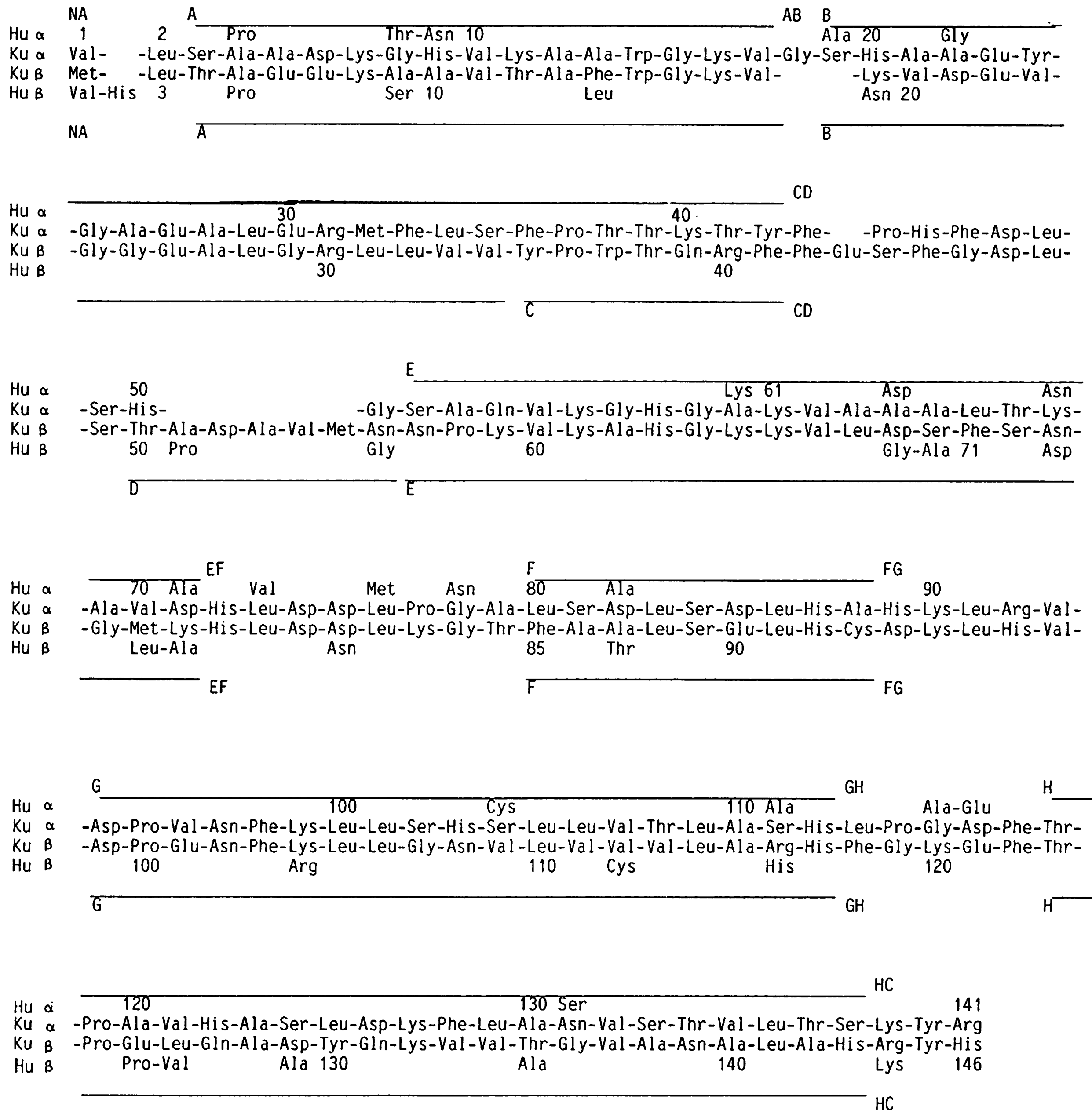

Fig. 5. The amino-acid sequences of α and β chains of Kudu hemoglobins *(Tragelaphus strepsiceros)*.

The sequences are aligned in homology to those of human hemoglobin[19]: the absence of helix D in the α chains is marked by a gap of 5 amino acids in the CD region; the β chains of Kudu are shorter by one residue than the human β chains. This is marked by a gap of one amino acid at the N-terminus in the NA region; another gap of one residue in α NA2 and a gap of two amino acids at the end of the β A helix were also necessary to align the α and β chains.

phates in their role as intracellular allosteric effectors. Due to the very similar primary structures of the bovine and Kudu hemoglobins (Tab. 3 and 4), this may be a common property of all these proteins.

The heterogeneity among the hemoglobins of Bovidae has been investigated thoroughly during the past 35 years. The screening studies on hemoglobin in several populations, as well as the comparative analyses of the primary structures of the globin chains led to the assumption that all these hemoglobin variations are the products of different genetic mechanisms, including allelism, nonallelism and activation of a silent nonallelic gene. It is well known today that the bovine hemoglobins are the products of allelic genes, but in the case of the Kudu, the lack of a screening investigation on more animals does not permit such a conclusion. We can only suppose, based on the striking homology of their hemoglobins and the similarity of their morphology with that of Bovidae, that the two Kudu hemoglobins could also have been pro-

Table 3. Exchanges in the α chains of hemoglobins of the Ruminantia species as compared with the Kudu hemoglobins.
The amino-acids in parentheses indicate that as no complete sequences were available, the residues obtained from the amino-acid analysis were taken for these positions according to the homology with the other chains. The chains of the animals below the dotted line have more substitutions than the other polypeptides, but these additional replacements were not taken into consideration because of their random distribution and small number. Abbreviations: Ku = Kudu α or β chains; Bo = Bovine chains[2–5]; Yak = Yak chains[1]; Gay = Gayal chains[20]; Bis = European Bison chains (manuscript in preparation, G. Mazur & G. Braunitzer); Elk = Elkdeer chains[21]; WTD = White-tailed Deer chains[22,23]; GO = Goat chains[6,7,24,25]; She = Sheep chains[26–30].

Pos. in the chain	8	9	15	19	20	23	50	60	71	75	79	82	104	111	115
Ku	Gly	His	Gly	Ser	His	Glu	His	Ala	Asp	Asp	Ala	Asp	Ser	Ser	Gly
Bo		Asn		Gly					Glu			Glu			Ser
Yak I		Asn		Gly			Gln		Glu			Glu			Ser
Yak II		Asn		Gly					Gly			Glu			Ser
Gay		Asn		Asp					Gly			Glu			Asn
Bis I		Asn		Asp					Gly			Glu			Asn

Elk	Ser	Asn		Gly	Asn	Ala		Glu	Gly		Thr		Thr	Ala	Ser
WTD I	( )	( )	( )	(Gly)	(Asn)	(Pro)	( )	(Glx)	(Gly)	( )	(Thr)	(Asx)	(Thr)	( )	(Asx)
WTD II	(Ser)	(Asx)	( )	(Gly)	(Lys)	(Pro)	( )	( )	(Gly)	( )	(Thr)	(Asx)	(Thr)	( )	(Asx)
Go I	(Ser)	Asx		Gly	Asx	(Gly)	( )	Glx	(Gly)	(Asx)	(Thr)	(Asx)		Cys	(Asx)
Go IB	Ser	Asx		Gly	Asx	Gly		Glx	Gly	Tyr	Thr	Asx		Cys	Asx
Go II	Ser	Asx		Gly	Asx	Gly		Glx	Gly		Thr	Asx		Cys	Ser
She I	Ser	Asx		Gly	Asx	Gly		Gln	Gly		Thr	Asx	Thr	Cys	Asx
She ID	Ser	Asx	Asp	Gly	Asx	Gly		Gln	Gly		Thr	Asx	Thr	Cys	Asx

Table 4. Exchanges in the β-chains of hemoglobin of Ruminantia species as compared with the Kudu hemoglobin.

Pos.* in the chain	16	19	21	44	50	73	117	120	125	130	132	135
Ku A	Gly	Lys	Asp	Ser	Thr	Asn	His	Lys	Glu	Tyr	Lys	Thr
Ku B	Ser											
Bo A						Asp	Asn		Val	Phe		Ala
Bo B	Ser	His				Asp	Asn	Asn	Val	Phe		Ala
Bo C						Asp	Asn		Val	Phe	Gln	Ala
Bo D			Gly	Thr		Asp	Asn		Val	Phe		Ala
Bo X		His				Asp	Asn		Val	Phe		Ala
Yak I							Asn		Val	Phe		Ala
Yak II					Ser				Val	Phe		Val
Gay		His							Val	Phe		Ala
Bis		His			Ser				Val	Phe		

Elk				His		Glu						
WTD II		Asn		(His)	(Ser)	Glu	(Asx)	(Gly)	(Leu)	(Phe)	( )	
WTD III		Asn		His	Ser	Glu	Asn	Gly	Leu	Phe		Ala
WTD IV		Asn		(His)	(Ser)	(Glx)	(Asx)	(Gly)	(Leu)	(Phe)	( )	( )
WTD V	Ser			His	( )	(Asx)	?		(Leu)	(Phe)	( )	( )
WTD VII	Ser			His	( )	(Asx)	(Asx)	(Asx)	(Leu)	(Ala)	( )	(Asx)
Go A				His	Ser			Ser	Leu	Phe		Ala
Go D			His	His	Ser			Ser	Leu	Phe		Ala
Go E				His	Ser			Ser	Val	Phe		Ala
She A				His	Ser	Asx		Ser	Val	Phe		Ala
She B				His	Asx	Asx		Asn	Val	Phe		Ala

* Numbering as for human β chains. Abbreviations and meaning of the dotted line as in Table 3.

duced by allelic genes. If this is true, the animal investigated in this study was a heterozygote.

Another question to be clarified is the evolutionary path of the Bovidae hemoglobins. The eldest known bovide is the Eotragus from the Early-Miocene (Burdigal) of Europe. From this ancestor several families and genera developed of which the Tragelaphini spread mainly in Asia and Africa. Today these animals live only in Africa. From the biological point of view the Tragelaphini are very close relatives of the cow. The very low number of mutations in the sequences of the globin chains of several Bovidae, such as

cow[2–5], yak[1,41,42], gayal[20], European bison (G. Mazur, personal communication) and greater Kudu (Tab. 3 and 4) is one of the evidences for this. Furthermore, the majority of the differences in Tables 3 and 4 are located at the same positions in the chains of Bovidae and in those of Cervidae. Regarding the replacements at 16, 19, 21, 44, 120 and 132, which constitute the differences between the bovine β chains, Table 4 shows that with one exception of position 132, exchanges can be found in all positions mentioned above of the other hemoglobins. This suggests that the β chain variants could have existed in wild species before the domestication of these animals was completed[5]. For this reason we regard the primary structure of Kudu hemoglobins as an additional evidence of the common origin of these animals.

We thank Ms *B. Schrank*, Mr *C. Krombach*, Mr *A. Stangl* and Ms *R. Gautsch* for their excellent technical work.

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