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The purification and properties of the second component of guinea-pig complement

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A method has been developed for the purification to homogeneity of guinea-pig complement component C2. Contrary to previous reports, guinea-pig C2 is a single polypeptide chain with apparent mol.wt. of 102000, the same as human C2. It is cleaved by $C\bar{1}s$ to yield fragments C2a (apparent mol.wt. 74000) and C2b (apparent mol.wt. 34000). The amino acid composition and *N*-terminal sequences of these fragments are similar to those of human C2a and C2b. Human and guinea-pig C2 show more extensive sequence homology to Factor $\bar{B}$ than previously identified. The known homology around the sites of cleavage by $C\bar{1}s$ and Factor D has now been extended by a stretch of ten identical or conservatively substituted residues. Sequence homology has now been identified at the *N*-terminal of C2b and Factor Ba. The properties of the classical-pathway C3 convertases assembled from human C4b, $C\bar{1}s$ and human or guinea-pig C2 have been compared. The rates of cleavage of human and guinea-pig C2 by $C\bar{1}s$ (and therefore the rates of assembly of the C3 convertases) are similar. The rate of decay of the activity of the C3 convertase formed from guinea-pig C2 is 10-fold lower than for human C2. This greater stability reflects a higher affinity of guinea-pig C2a for human C4b. The presence of C2b is not necessary for C3 convertase activity.

The classical pathway of complement incorporates an enzyme cascade that is activated principally by the aggregation of certain classes of immunoglobulin on binding antigen. The binding of subcomponent Clq to the antibody–antigen complex then results in the sequential activation of two serine proteinases, C1r and $C\bar{1}s$. The activated $C\bar{1}s$ cleaves C4 and C2 forming a proteolytic enzyme termed C3 convertase, which is able to cleave C3, the central protein of the complement system. In the presence of the product of this cleavage, C3b, the same enzyme is then able to cleave C5, leading to the assembly of a macromolecular complex of the late components of complement (C5–C9) and causing the lysis of certain target cells. The coating of target cells with fragments of C3 stimulates their ingestion by phagocytic leucocytes, and the generation of the small peptides C3a and C5a, which possess vasoactive and chemotactic properties, are other important functions of the complement system. [For reviews, see Porter & Reid (1979) and Muller-Eberhard (1978).]

The identification of the individual components of the complement system and elucidation of the order of their interaction came mainly from the study of proteins isolated from guinea-pig serum (see Mayer, 1977). However, with the identification of the same proteins in human serum and their subsequent purification, it has been the human proteins that have been used for most of the structural studies and for investigation of their interaction at the molecular level. Nevertheless, guinea-pig complement is used routinely in cytotoxicity assays, and functionally pure guinea-pig components (particularly C2) are still used in haemolytic assays for other complement components.

The reason for the use of guinea-pig components in these assays is the high haemolytic titre of guinea-pig serum and in particular the high titre of guinea-pig C2 (Ngan *et al.*, 1977; Opferkuch *et al.*, 1971). It is believed that this higher haemolytic titre is due mainly to the relative stability of the C3

Abbreviations used: the nomenclature of complement components is that recommended by the World Health Organisation (1968); iPr_2P-F, di-isopropyl phosphorofluoridate; SDS, sodium dodecyl sulphate.

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convertase enzyme which is formed on cleavage of human or guinea-pig C4 and guinea-pig C2 by C1s. The enzyme formed from human C4 and human C2 is highly labile, the activity decaying with a half-life of less than 1min (Kerr, 1980). The same enzyme formed from human C4 and guinea-pig C2 loses activity at one-tenth of this rate. In an haemolytic assay this would allow more C3 activation and therefore result in a far higher haemolytic titre.

We have developed a technique for the purification of milligram amounts of human C2 (Kerr & Porter, 1978) which has allowed preliminary characterization of the structure of the molecule (Kerr, 1979) and has allowed a study of the interaction of the C2 and C4 in the C3 convertase (Kerr, 1980). The greater stability of the same enzyme assembled from guinea-pig C2 will facilitate further investigation of the structure and function of this complex enzyme. Identification of the differences between guinea-pig and human C2, which lead to these differences in stability, would be expected to shed some light on the mechanism of interaction between C4 and C2. Interestingly, guinea-pig C4 and human C2 upon activation by C1s do not form any enzyme able to cleave C3 (Nelson, 1965; Austen & Russell, 1966).

In view of the fact that previous reports (Stroud *et al.*, 1966; Borsos & Rapp, 1962; Wagner & Rollinghoff, 1970) had suggested considerable differences between the physicochemical properties of guinea-pig and human C2, we have developed a method for the purification of milligram amounts of guinea-pig C2 which have allowed a more extensive comparison of the physicochemical and biological properties of the human and guinea-pig proteins.

Materials and methods

Materials

The sources of materials used in protein purification and characterization have been described previously (Kerr & Porter, 1978; Kerr, 1979).

Guinea-pig serum collected over a period of 3 years was stored at −70°C until used. Guinea-pig blood had been obtained by cardiac puncture, the blood allowed to clot at room temperature and the serum decanted after retraction of the clot at 4°C overnight.

Methods

Guinea-pig C2. This was purified by a method developed from that used for the purification of human C2 (Kerr & Porter, 1978; Kerr, 1979).

Euglobulin precipitation. Frozen serum (1 litre) was thawed and, after addition of 2ml of 2.5M-iPr_2P-F in propan-2-ol, was centrifuged at 23000*g* for 30min at 4°C. The supernatant was adjusted to pH 7.4 and then dialysed overnight at 4°C against 8 litres of 5mM-$CaCl_2$/0.5% benzamidine hydrochloride. The suspension was then centrifuged at 23000*g* for 30min and the supernatant (pseudoglobulin fraction) decanted. The euglobulin precipitate, which was used as the source of functionally pure C1, was resuspended in 5mM-$CaCl_2$, centrifuged at 23000*g* for 30min at 4°C and the pellet redissolved in 5mM-veronal buffer (pH 7.5)/150mM-NaCl/0.5mM-$CaCl_2$/2.0mM-$MgCl_2$. After incubation at 37°C for 30min to ensure activation of the C1, the solution was frozen in 1ml portions at −70°C. The pseudoglobulin fraction was on some occasions frozen at −70°C before fractionation for C2.

Chromatography of pseudoglobulin fraction on CM-Sephadex C-50. A 10ml portion of 0.2M-EDTA, pH 6.0, and 50ml of 0.4M-sodium phosphate were added to the pseudoglobulin fraction and the pH adjusted to 6.0 with 1M-NaOH. The mixture was loaded on to a column (10cm × 8cm) of CM-Sephadex C-50 equilibrated in 0.1M-sodium phosphate (pH 6.0)/0.5% benzamidine hydrochloride. The column was then washed with 1 litre of 0.1M-sodium phosphate (pH 6.0)/0.5% benzamidine hydrochloride and then developed with a linear gradient formed from 2 litres of 0.1M-sodium phosphate, pH 6.0, and 2 litres of 0.25M-sodium phosphate, pH 6.0, both containing 0.5% benzamidine hydrochloride; all solutions were pumped through the column at a rate of about 1.5–2 litres/h. C2 was eluted in the middle of the gradient.

Precipitation with $(NH_4)_2SO_4$. Fractions from the CM-Sephadex column showing C2 haemolytic activity were pooled (2 litres) and $(NH_4)_2SO_4$ added to 50% saturation (291g/litre). After being stirred for 1h at 4°C the solution was centrifuged at 23000*g* for 30min. The supernatant was decanted, filtered through Whatman no. 1 filter paper and $(NH_4)_2SO_4$ added (159g/litre) to give 75% saturation. The suspension was then stirred and left to warm to room temperature (15–20°C) before centrifugation at 23000*g* for 90min at 20°C. The pellet was redissolved in about 50ml of 0.4M-sodium phosphate, pH 6.0, and stored overnight after the addition of 0.1ml of 2.5M-iPr_2P-F.

Chromatography on 'aged' CNBr-activated Sepharose 4B. The cloudy suspension was centrifuged at 26000*g* for 30min and the pellet discarded. The supernatant was passed through a column (30cm × 4cm) of Sephadex G-25 equilibrated in 5mM-veronal buffer (pH 8.5)/0.5mM-$CaCl_2$/2.0mM-$MgCl_2$/40mM-NaCl and then applied to a column (15cm × 3cm) of 'aged' CNBr-activated Sepharose 4B (Kerr & Porter, 1978) equilibrated in the same buffer. The column was washed with the same buffer until the A_{280} of the eluate was less than 0.05 and then the C2 was eluted with 250ml of 0.4M-sodium phosphate, pH 6.0 (Fig. 1*a*).

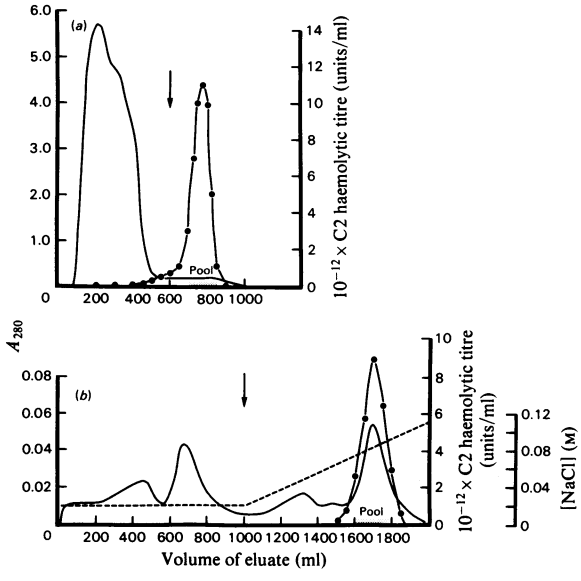

Fig. 1. *Chromatography of guinea-pig C2*

(*a*) The partially purified C2 precipitated at 75%-satd. $(NH_4)_2SO_4$ was equilibrated with 5mM-veronal buffer (pH 8.5)/0.04M-NaCl/2mM-$MgCl_2$/0.5mM-$CaCl_2$ and loaded on to a column (15cm × 3cm) containing 'aged' CNBr-activated Sepharose 4B. The column was washed with the same buffer and the C2 eluted with 250ml of 0.4M-sodium phosphate, pH 6.0 (the arrow indicates start of elution). (*b*) The protein eluted from the 'aged' CNBr-activated Sepharose 4B column was equilibrated with 5mM-veronal buffer (pH 8.5)/0.04M-NaCl/2mM-$MgCl_2$/0.5mM-$CaCl_2$ and loaded on to a column (50cm × 5cm) of DEAE-Sepharose equilibrated in the same buffer. After washing, the column was developed with a linear salt gradient from 40mM- to 120mM-NaCl in this buffer, as described in the Materials and methods section. ——, A_{280}; ●, guinea-pig C2 haemolytic activity; ----, [NaCl].

Chromatography on DEAE-Sepharose. The active fractions from the 'aged' CNBr-activated Sepharose column were pooled and equilibrated with 5mM-veronal buffer (pH 8.5)/0.5mM-$CaCl_2$/2.0mM-$MgCl_2$/40mM-NaCl by passage through a column (50cm × 5cm) of Sephadex G-25 in that buffer. The protein was then applied to a column (30cm × 2.5cm) of DEAE-Sepharose equilibrated in the same buffer; the column was washed with 100ml of the veronal buffer and developed with a linear gradient made from 500ml of 5mM-veronal buffer (pH 8.5)/0.5mM-$CaCl_2$/2.0mM-$MgCl_2$/40mM-NaCl and 500ml of 5mM-veronal buffer (pH 8.5)/0.5mM-$CaCl_2$/2.0mM-$MgCl_2$/120mM-NaCl (Fig. 1*b*).

Active fractions that were homogeneous by the criterion of SDS/polyacrylamide-gel electrophoresis were pooled and concentrated by ultrafiltration before storage at −70°C.

Other methods. Human C4 and C3 were purified as described in Kerr (1980), and human C1s by the method of Gigli *et al.* (1976). Haemolytic assays for C4, C2 and C3 were carried out as described by Kerr (1980). Amino acid analyses with a Durrum D500 analyser were carried out on samples hydrolysed as described by Kerr (1979). Methods of automatic sequence analysis were those detailed by Johnson *et al.* (1980).

Results

Purification of C2

The purification scheme has been developed with only minor modifications from that used routinely in this laboratory for the preparation of milligram amounts of human C2 and Factor B for amino-acid-sequence studies. Euglobulin precipitation is used to remove C1 from the serum, which is then used as a functionally pure reagent for the preparation of the haemolytic intermediates for the assay of other complement components. Pseudoglobulin from such precipitations can be stored frozen for several months before fractionation to yield C2. Then 99% of the pseudoglobulin proteins are removed by chromatography on CM-Sephadex C-50 and precipitation with $(NH_4)_2SO_4$. The material at this stage, after dialysis or gel filtration into veronal buffer, can be used as functionally pure C2 in haemolytic assays of C1, C4 and C3. The functionally pure C2 has no detectable C4 activity and only low levels of C1, which, because of the high yield of haemolytic titre of C2, can be diluted out. Although guinea-pig C2 has proven in practice to be much more stable than human C2, benzamidine was added to all buffers during these early stages of purification to minimize losses of C2 by cleavage by C1s.

During attempts to purify human C2 by affinity chromatography it was observed that C2 was preferentially adsorbed to a column of Sepharose 4B that had been activated with CNBr and the active groups then allowed to hydrolyse under mildly alkaline conditions. Although the basis of this affinity of C2 for the resin was not understood, this property was a major reason for the ability to purify C2 in high yield. The same affinity for 'aged' CNBr-activated Sepharose was observed with guinea-pig C2, again allowing over 30-fold purification in a single step.

Chromatography of the proteins eluted from the 'aged' Sepharose column on a column of DEAE-Sepharose allows purification of C2 to homogeneity. Guinea-pig C2 requires higher salt concentration for elution from this column than does human C2 and is free of Factor B. The purification

Table 1. *Purification of C2 from guinea-pig serum*
For details of purification, see the text. ND, not determined.

Fraction	Volume (ml)	10^{-14} × Total activity (units)	Total protein (mg)	10^{-11} × Specific activity units/mg	Activity yield (%)
Serum	1500	35	86400	0.41	100
Pseudoglobulin	1580	29.7	ND	—	85
CM-Sephadex pool	2230	28.8	ND	—	82
75%-satd.-$(NH_4)_2SO_4$ pellet	58	23	1219	18.9	66
'Aged' CNBr-activated Sepharose 4B pool	150	18.7	33.5	558.2	53
DEAE-Sepharose pool	240	9.9	7.5	1320.0	28

scheme, which is summarized in Table 1, allows a purification of greater than 3000-fold from serum. The product is homogeneous by the criteria of SDS/polyacrylamide-gel electrophoresis and by *N*-terminal analysis. It is stable on storage at 4°C at neutral pH without the addition of proteolytic-enzyme inhibitors and can be concentrated by ultrafiltration and stored frozen. Slight activation of C2 is observed on incubation at 37°C for 30 min (Fig. 2). The 28% yield (5 mg/litre) is slightly higher than that for human C2 purified by the similar technique. It suggests a serum concentration for guinea-pig C2 of 20 mg/litre, which is similar to that of human C2, despite the much higher C2 haemolytic titre in guinea-pig serum.

Characterization of guinea-pig C2

Guinea-pig C2 is a single polypeptide chain with an apparent molecular weight by SDS/polyacrylamide-gel electrophoresis of 102000 and showing the same mobility as human C2 under reducing conditions but slightly greater mobility under non-reducing conditions (Fig. 2). Alanine was identified as the *N*-terminal by the dansyl (5-dimethylamino-naphthalene-1-sulphonyl) method. The amino acid composition is given in Table 2.

Guinea-pig C2 is cleaved rapidly by human or guinea-pig $C\bar{1}s$ to yield fragments C2a (apparent mol.wt. 74000) and C2b (apparent mol.wt. 34000). Guinea-pig C2a has identical mobility on SDS/polyacrylamide gels to human C2a under reducing conditions, though slightly lower under non-reducing conditions. Guinea-pig C2b has slightly lower mobility than human C2b under reducing conditions, but identical under non-reducing conditions. These slight differences in mobility may reflect slight differences in glycosylation or in the disulphide bonding of the proteins. On higher loading of the gels, C2b was seen characteristically as a doublet, the second minor band having a lower mobility. Upon extended incubation of C2 with $C\bar{1}s$, a smaller C2b fragment was observed (apparent mol.wt. 32000); C2a was apparently unchanged. This pattern was identical with that obtained on digestion of human C2 with $C\bar{1}s$ (Kerr, 1979).

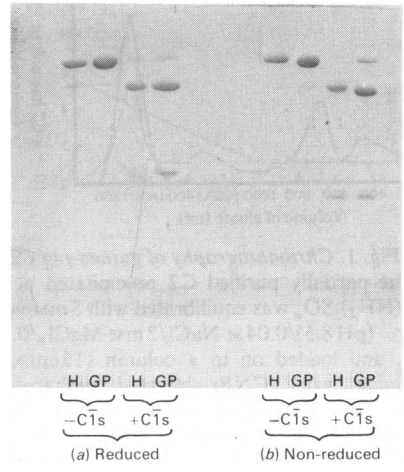

Fig. 2. *SDS/polyacrylamide gels (run as described by Laemmli, 1970) of purified human (H) and guinea-pig (GP) C2 before and after cleavage with human $C\bar{1}s$*
Human (10 μg) or guinea-pig C2 (15 μg) in 100 μl of 5 mM-veronal buffer (pH 8.5)/0.04 M-NaCl/2 mM-$MgCl_2$/0.5 mM-$CaCl_2$ was incubated at 37°C for 30 min in the presence (+) or absence (−) of purified human $C\bar{1}s$ (200 ng). The reaction was stopped by the addition of 100 μl of 40 mM-dithiothreitol/8 M-urea/2% SDS in 0.2 M-Tris/HCl, pH 8.0 (*a*, reducing conditions) or 40 mM-iodoacetamide/8 M-urea/2% SDS in 0.2 M-Tris/HCl, pH 8.0 (*b*, non-reducing conditions) before loading on the gel.

The fragments C2a and C2b are not covalently linked and can be separated completely without the need for denaturation by gel filtration on Sephadex G-100 in 0.1 M-NH_4HCO_3 buffer. The amino acid analyses of guinea-pig C2a and C2b purified in this manner are shown in Table 2. The analyses are very similar to those of human C2a and C2b, the C2a being rich in aspartic and glutamic acid residues and in the hydrophobic amino acids and C2b being very rich in the small uncharged amino acids, glycine (12.9%), serine (12.5%), proline (8.1%) and cysteine (5.3%).

Table 2. *Amino acid composition of human and guinea-pig C2, C2a and C2b*

Amino acid compositions were calculated from duplicate 24, 48 and 72h HCl hydrolysates. Corrections were made for destruction of serine and threonine by extrapolation to zero hydrolysis time. Cysteine was estimated from performic acid-oxidized samples (Hirs, 1956). Tryptophan was not determined.

		Amino acid composition (residues/100 residues)					
	Species ...	Guinea pig			Human		
Amino acid	Component ...	C2	C2a	C2b	C2	C2a	C2b
Cys		2.6	1.8	5.3	3.2	2.7	5.3
Asx		10.2	11.0	8.6	11.2	12.7	8.4
Thr		5.6	4.3	8.7	5.0	4.3	4.9
Ser		8.1	5.7	12.5	7.9	7.3	8.7
Glx		10.4	11.5	6.6	10.4	10.5	8.7
Pro		5.1	4.4	8.1	5.7	4.6	9.8
Gly		9.6	8.2	12.9	9.2	6.6	13.4
Ala		8.3	8.9	7.8	6.3	6.2	6.5
Val		5.5	6.1	6.2	6.8	6.7	7.0
Met		1.5	1.7	0.9	1.9	2.7	0.8
Ile		4.6	6.3	1.2	4.2	4.9	2.9
Leu		8.9	9.9	6.8	9.4	10.3	6.3
Tyr		2.4	2.4	3.0	2.7	1.6	3.4
Phe		3.2	3.7	2.8	4.4	4.4	4.0
His		2.6	3.4	1.6	2.7	2.8	2.1
Lys		4.8	5.8	2.6	5.1	5.9	2.5
Arg		5.4	5.8	6.1	5.3	5.5	5.3

The *N*-terminal sequences of guinea-pig C2a and C2b are shown in Table 3. C2a ran as a single sequence, allowing unequivocal identification of residues 1–27. C2b ran as a single sequence which developed considerable overlap after the proline residue at position 5. Nevertheless, identification of residues 1–26 was possible with three exceptions. *N*-Terminal sequences of human C2a and C2b are also shown in Table 3. The sequence of human C2a extends that previously published (Kerr, 1979) by two residues and includes a correction with residue 8 being identified as glycine. The sequence of C2b is extended from 10 to 26 residues. Residue 6 is corrected to glutamine from glutamic acid.

Assembly of classical-pathway C3 convertase using guinea-pig and human C2

Guinea-pig C2, human C4b and $C\bar{1}s$ (molar proportions 50:50:1) were incubated for various times at 37°C in 5mM-veronal buffer (pH 7.5)/2mM-$MgCl_2$/0.5mM-$CaCl_2$/40mM-NaCl. Samples were removed and added to C3 [150μg/ml in 5mM-veronal buffer (pH 7.5)/40mM-EDTA] and after incubation at 37°C for a further 15min the C3 destruction was determined by the loss of haemolytic activity. The results are shown in Fig. 3. C3 convertase activity was generated rapidly, reaching a maximum at around 4min. The activity then decayed with a half-life of about 4min until at 30min no activity was detectable. When the same experiment was repeated with human C2, less C3 convertase activity was generated. Maximum activity was observed at an earlier time and the observed decay rate was higher. The rates of cleavage of guinea-pig and human C2 by the $C\bar{1}s$ during these experiments were, however, similar. The C2 cleavage followed first-order kinetics, allowing determination of the rate constants $k_{+1} = 0.30\,min^{-1}$ for the cleavage of guinea-pig C2 and $k_{+1} = 0.23\,min^{-1}$ for human C2. The results for human C2 are in agreement with our previous studies.

When guinea-pig C2, human C4b and $C\bar{1}s$ in larger amounts were incubated at 37°C for 5min, then after cooling to 4°C subjected to gel filtration on Sephadex G-200 in 5mM-veronal buffer (pH 7.5)/0.5mM-$CaCl_2$/2mM-$MgCl_2$/0.15M-NaCl, C3 convertase activity could be detected as a single symmetrical peak slightly included in the column ($V_e/V_0 = 1.18$). Analysis of the fractions of this peak by SDS/polyacrylamide-gel electrophoresis showed the presence of C4b and C2a. Most of the C4b and C2a was, however, eluted later, as would be expected from their molecular weights (C4b: $V_e/V_0 = 1.27$; C2a: $V_e/V_0 = 1.64$). No C2b was detected in the C3 convertase peak; all the C2b eluted as a single symmetrical peak ($V_e/V_0 = 1.86$). In agreement with our previous studies, when human C2,

Table 3. *Amino-acid-sequence analysis of the N-terminal sequences of human and guinea-pig C2a and C2b*

Residue no.	Species ...	Amino acid identified: Guinea pig		Human	
	Component ...	C2a	C2b	C2a	C2b
1		Lys	Ala	Lys	Ala
2		Ile	Ala	Ile	Pro
3		Gln	Thr	Gln	Ser
4		Ile	Ser	Ile	Xaa*
5		Gln	Pro	Gln	Pro
6		Arg	Arg	Ser	Gln
7		Ser	Asn	Xaa	Asn
8		Gly	Val	Gly	Val
9		His	Xaa	Xaa	Xaa
10		Leu	Ile	Lys	Leu
11		Asn	Thr	Asn	Xaa
12		Leu	Gly	Leu	Gly
13		Tyr	Gly	Tyr	Gly
14		Leu	Ser		Ser
15		Leu	Phe		Phe
16		Leu	Thr		Thr
17		Asp	Leu		Leu
18		Ala	Xaa		Ser
19		Ser	Gln		Xaa
20		Lys	Gly		Gly
21		Ser	Ala		Xaa
22		Val/Met	Ala		Ala
23		Ser	Pro		Pro
24		Glu	Gly		Gly
25		Glu	Xaa		Xaa
26		Asp	Val		Leu
27		Ile			
Amount used in sequencer (nmol)	...	35	32	60	30
Recovery on 1st step (nmol)	...	11.2	11.5	14.0	6.0

* Xaa represents an unidentified residue.

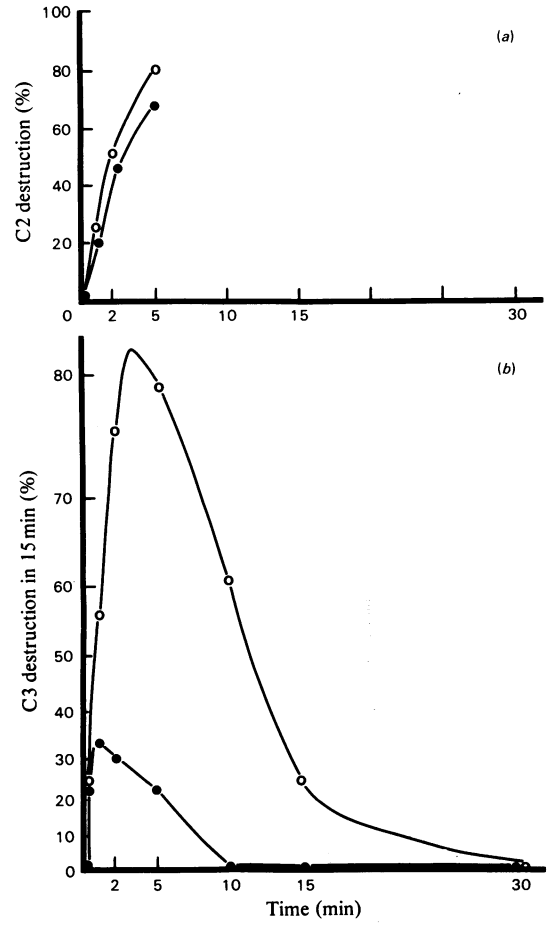

Fig. 3. *Formation and decay of C3 convertase activity using human or guinea-pig C2*

Human C4b (20μg), human C2 or guinea-pig C2 (10μg) and human $C\bar{1}s$ (200ng) in 250μl of 5mM-veronal buffer (pH 8.5)/0.5mM-$CaCl_2$/2.0mM-$MgCl_2$/40mM-NaCl were incubated at 37°C. (*a*) At different times, samples (1μl) were removed and assayed haemolytically for C2 (○, guinea-pig C2; ●, human C2). (*b*) Samples (1μl) were simultaneously assayed for C3 convertase activity by addition to 50μl of C3 (200μg/ml) in 5mM-veronal buffer/40mM-EDTA, pH 7.5. After incubation at 37°C for 15min the degree of C3 destruction was determined by haemolytic assay.

C4b and $C\bar{1}s$ were treated in the same way, no C3 convertase could be detected from the column. C4b, C2a and C2b were eluted separately in their predicted positions. The C3 convertase activity formed on incubation of human C4b, $C\bar{1}s$ and human C2 that had been treated with 10μM-iodine was eluted from the column in the same position as guinea-pig C3 convertase and contained the same proteins, C4b and C2a.

Discussion

The purification scheme described above allows the preparation of milligram quantities of stable guinea-pig C2 with yield and purity much improved over those reported for previous methods (Mayer *et al.*, 1970; Wagner & Rollinghoff, 1970). Partial completion of the purification scheme allows the preparation of functionally pure C1 and C2, which are essential requirements in the haemolytic assays of most complement components. Preliminary studies also suggest that this purification scheme, like the one for human C2 from which it was developed, can be used as the starting point for the purification of C3, C4 and C5 and Factor B and Factor D.

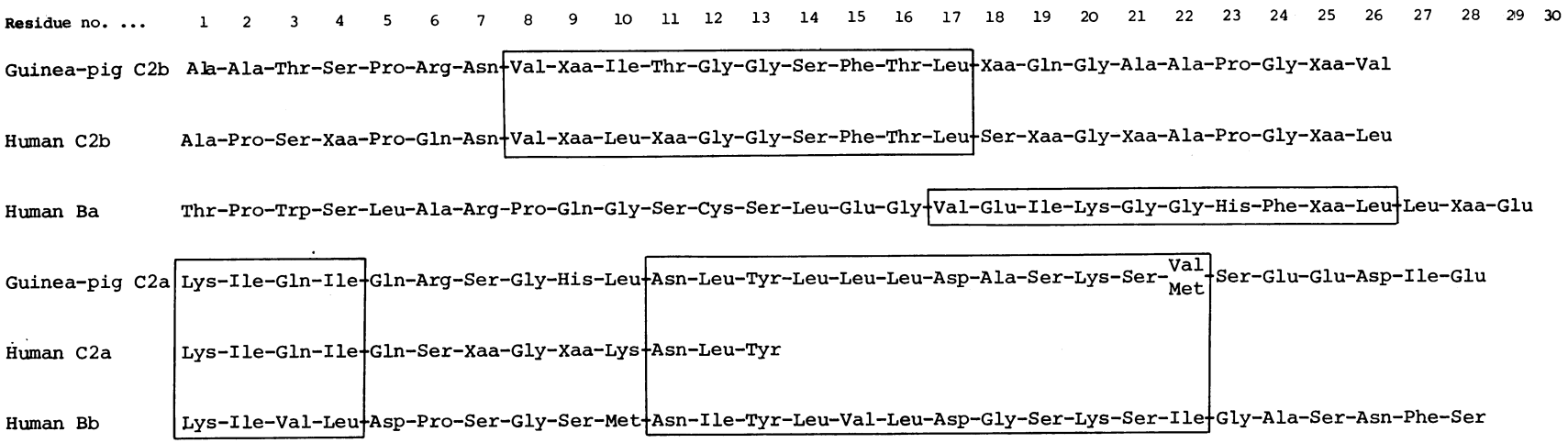

Fig. 4. *Sequence homology between human and guinea-pig C2 and human Factor B*

Comparison of the *N*-terminal amino acid sequences of human and guinea-pig C2a and C2b with those of human Factor Ba and Bb. Sequence data for Factor Ba are from Christie & Gagnon (1982). The sequence of Bb is from Christie *et al.* (1980). Regions of homology are shown in boxes. Xaa, 'unknown' amino acid.

The availability of these quantities of guinea-pig C2 has allowed a detailed comparison of the properties of the protein with those of human C2. Previous results had suggested a molecular weight for guinea-pig C2 of 130000–150000 using gel-filtration or sedimentation in sucrose density gradients (Stroud *et al.*, 1966; Borsos & Rapp, 1962; Wagner & Rollinghoff, 1970). A contemporary estimate of the molecular weight of human C2 obtained by using similar techniques (Muller-Eberhard *et al.*, 1967) was 117000. Since then, Nagasawa & Stroud (1977) reported a mol.wt. of 102000 for human C2 using the more reliable techniques of sedimentation equilibrium and SDS/polyacrylamide-gel electrophoresis. Similar results were obtained by Kerr & Porter (1978). Our present data show both human and guinea-pig C2 to have the same mol.wt. (102000), and both are cleaved by C1s to fragments of the same size C2a (apparent mol.wt. 74000) and C2b (apparent mol.wt. 34000). Although we have not attempted any estimation of their molecular size by gel filtration, human and guinea-pig C2 run identically under the same conditions on Sephadex G-200; it is therefore likely that previous differences reflected differences in column buffer or contamination with C4b, which markedly alters the gel-filtration and sedimentation properties of C2 (Kerr, 1980; Muller-Eberhard *et al.*, 1967).

Our characterization of the proteins suggests no major structural differences between guinea-pig C2 and human C2 which might explain their functional differences. The amino acid compositions of the two proteins and the fragments obtained on cleavage with C1s are similar. The amino acid sequences of guinea-pig C2a and human C2a show 10 identities in the first 13 residues, and for C2b, 17 in the first 26 residues.

One of the more obvious differences in amino acid composition between the two proteins is the difference in cysteine content (determined as cysteic acid after performic acid oxidation of the proteins). It has been shown that the haemolytic activity of human, but not guinea-pig, C2 is markedly enhanced by treatment with low concentrations of iodine (Polley & Muller-Eberhard, 1967, 1968). It has been reported that this enhancement of activity, which reflects increased stability of the C3 convertase, is due to the oxidation of two free thiol groups in human C2. Since increased stability of the enzyme must be due to modification of the C2a fragment (Muller-Eberhard *et al.*, 1968; Kerr, 1980), it is noteworthy that the differences in cysteine content between human and guinea-pig C2 are due to differences in the C2a part of the molecules. This could reflect a difference in free thiol content between human and guinea-pig C2a.

The specific haemolytic titre of guinea pig C2 is 10 times that of human C2 when assayed using sheep erythrocytes sensitized with rabbit antibody bearing guinea-pig component C1 and human C4 and using EDTA-treated guinea-pig serum as a source of components C3–C9. This is the same ratio as that seen in whole sera and is in agreement with previous studies (Opferkuch *et al.*, 1971). The higher haemolytic activity is reflected in the higher levels of fluid-phase C3 convertase that can be formed with guinea-pig C2.

The amount of C3 convertase at any time can be considered to be the result of first-order processes of assembly and decay which can be summarized as:

$$\mathrm{C4b + C2 = C4b \cdot C2\ complex} \xrightarrow[\text{Assembly}]{k_{+1}} \mathrm{C4b(C2b)C2a}$$

$$\mathrm{C4b(C2b)C2a} \xrightarrow[\text{Decay}]{k_{+2}} \mathrm{C4b(C2b) + C2a}$$

In such a model the concentration of convertase (Y) varies as:

$$Y = \frac{[\mathrm{C2}]k_{+1}}{k_{+2} - k_{+1}} (e^{-k_{+1}t} - e^{-k_{+2}t})$$

and the time at which maximal C3 convertase activity is formed ($t_{\mathrm{max.}}$) is given by:

$$t_{\mathrm{max.}} = \frac{1}{k_{+1} - k_{+2}} \ln \frac{k_{+1}}{k_{+2}}$$

By using k_{+1} as the value for the apparent first-order rate of cleavage of C2 by C1s, it is possible from the determined $t_{\mathrm{max.}}$ values in Fig. 3 to estimate decay-rate constants for the C3 convertases formed from guinea-pig and human C2. These are 0.2 and 2.0 $\mathrm{min^{-1}}$ respectively. After incubation for 5 min, the time used routinely in haemolytic assays, the amount of C3 convertase formed from guinea-pig C2, human C4b and C1s would be theoretically ten times greater than that formed from human C2.

C2 and Factor B are apparently members of a novel group of serine proteinases whose mechanism of action appears to be the same as that of other serine proteinases but whose activation is different. The *C*-terminal portion of Factor B shows extensive sequence homology with typical serine proteinases, but the amino acid sequence of the *N*-terminal of Factor Bb derived from activation of Factor B shows no homology with the highly conserved *N*-terminal sequences of serine proteinases (Christie *et al.*, 1980).

A previous study showed a limited amount of sequence homology between C2 and Factor B around the site of cleavage during activation (the *N*-termini of C2a and Bb), but no sequence homology at the *N*-terminal of the whole molecules. From the extended sequences of guinea-pig and

human C2a and C2b and the extended sequences of human Factor Ba and Bb shown in Fig. 4, it is now clear that C2 and Factor B show more homology than previously suggested. The *N*-terminal of C2a and Bb contains a stretch of ten identical or conservatively substituted amino acids. C2b and Factor Ba and hence the intact molecules C2 and Factor B show clear homology with a similar stretch of sequence identity. Interestingly, Factor B, the slightly smaller molecule, shows an *N*-terminal extension of nine amino acids relative to C2. The homology throughout the molecules is confirmation that the proteins arose through gene duplication and is in accord with their similarity of function and their close location in the major histocompatibility complex on chromosome 6 in man.

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