Immunology 1985 **56** 561

Cleavage of the second component of complement by plasma proteases: implications in hereditary C1-inhibitor deficiency

MAJORIE A. SMITH & M. A. KERR *Department of Pathology, University of Dundee, Ninewells Hospital and Medical School, Dundee*

Accepted for publication 18 July 1985

Summary. EDTA plasma from patients with hereditary angioedema (HAE), the genetic deficiency of C1-inhibitor, when incubated at 37° produces a kinin-like activity which can induce contraction of oestrus rat uterus. The second component of complement (C2) has previously been suggested to be the source of this kinin-like activity, with the implication that C2-kinin is a normal product of complement activation. Our results show that purified human C2 is cleaved rapidly to C2a and C2b when added to HAE plasma, but not normal plasma or plasma from a danazol-treated HAE patient. However, the addition to HAE plasma of C2 at 20× normal plasma concentration had no effect on the kinin activity generated on incubation at 37°. In the presence of soya bean trypsin inhibitor, the rate of C2 cleavage and products were unaltered but no kinin activity was generated.

C2 was cleaved by purified $C\bar{1}s$ to C2a and C2b. Incubation of C2 with trypsin resulted in cleavage to C2a and C2b followed by more extensive cleavage of both C2a and C2b. Kallikrein cleaved C2 to C2a and C2b but plasmin had no effect on C2. In no case was kinin activity generated. When C2 was cleaved by $C\bar{1}s$ to C2a and C2b then incubated with trypsin, kallikrein, or plasmin, no kinin activity was generated: only trypsin cleaved the C2 fragments further. The results suggest that C2 is not the source of the kinin-like activity generated in hereditary angioedema plasma.

Correspondence: Dr M. A. Kerr, Dept. Pathology, Ninewells Hospital and Medical School, Dundee, Tayside DD2 1UB, U.K.

INTRODUCTION

Hereditary angioedema (HAE), the hereditary deficiency of the serum control protein C1-esterase inhibitor (C1INH), is inherited as an autosomal dominant trait recognized biochemically by persistently low levels of C1INH, C4 and C2 but normal levels of C3 (Donaldson & Evans, 1968; Donaldson & Rosen, 1964; Austen & Sheffer, 1965). Clinically, the disease is manifested by infrequent attacks of localized, non-inflammatory oedema (reviewed by Frank, Gelfand & Atkinson, 1976; Ballogh & Whaley, 1980). C1INH is a plasma protein first recognized as an inhibitor of C1 esterase by Ratnoff & Lepow (1957). C1INH was subsequently shown to be the same protein as the inhibitor of the permeability factor, kallikrein, which had been shown by Landerman *et al.* (1962) to be deficient in HAE plasma. C1INH has now been shown to inhibit several other plasma proteases, including plasmin, factor XIIa, factor XIa, $C\bar{1}r$ and $C\bar{1}s$ (Gigli *et al.*, 1976; Ratnoff *et al.*, 1969; Forbes, Pensky and Ratnoff 1970; Schreiber, Kaplan & Austen, 1973). Most of these enzymes are also inhibited by other plasma protease inhibitors, such as α_2-macroglobulin, α_1-proteinase inhibitor or α_2-antiplasmin (Travis & Salvesen, 1983). C1INH is, however, the only inhibitor of $C\bar{1}r$ and $C\bar{1}s$ (Arlaud *et al.*, 1979; Sim *et al.* 1979), and therefore a deficiency of C1INH is most clearly observed by the lack of control of the complement system. In the complement system, C1INH controls activation of the classical pathway by complexing with

$C\bar{1}r$ and $C\bar{1}s$. In the absence of C1INH, C4 and C2 are continuously activated by $C\bar{1}s$ but, since C3 convertase assembly in the fluid phase is highly inefficient, C3 levels remain unchanged.

Early studies by Landerman *et al.* (1962) identified in the plasma of patients with active hereditary angioedema a permeability increasing factor. This factor could also be generated from plasma taken from patients in remission when the plasma was incubated at 37°. This activity was attributed to a peptide with kinin activity since HAE plasma, on incubation at 37°, induces contraction of the isolated oestrus rat uterus, a smooth muscle preparation especially sensitive to kinins. The markedly low levels of C2 and C4 in HAE plasmas led to investigations on the possible role of these proteins in kinin generation. Complement proteins were first implicated as the source of kinin by Donaldson, Rosen & Bing (1977) who found that mixtures of $C\bar{1}s$, C4, C2 and plasmin caused contraction of the isolated rat uterus. Although attempts, by a number of other groups, to repeat these experiments have been unsuccessful (Curd *et al.*, 1982; Fields, Ghebrehiwet & Kaplan, 1983; Smith & Kerr, 1983), other experiments still implicate complement proteins, especially C2, in kinin formation. For example, $C\bar{1}s$ injected into normal and HAE volunteers was found to cause oedematous wheals, but in individuals having a deficiency of C2 (Klemperer, Donaldson & Rosen, 1967) and in C4-deficient guinea-pigs, this response was markedly decreased (Donaldson *et al.*, 1969). Similarly, HAE-plasma, drawn into antiserum to C2 or C4 but not to C3, failed to produce kinin on incubation at 37° (Donaldson *et al.*, 1977).

A crude preparation of kinin was isolated by Donaldson *et al.* (1969) but it has not been characterized extensively. Although the activity had some of the properties of bradykinin, for example heat stability, sensitivity to carboxypeptidase B and inability to induce tachyphylaxis, the activity differed from bradykinin in its sensitivity to trypsin and lack of a pain reaction when injected into volunteers. More recently, Bourgarit *et al.* (1983) have reported the isolation of a vasoactive peptide from the urine of HAE patients which was not bradykinin. The same group has also reported that, under specific conditions, they can generate a similar kinin from C2 (Lopez-Trascasa *et al.*, 1982). It is frequently implied that C2-kinin is a normal product of complement activation, although this has never been shown.

The role of C1INH in inhibiting not only the classical complement pathway but also enzymes of the plasminolytic and kinin pathways, has led others to postulate that the kinin produced in HAE is in fact bradykinin, generated from high molecular weight kininogen on activation by kallikrein. Increased kallikrein levels have been found in blister fluids from HAE patients (Curd, Prograis & Cochrane (1980), and prekallikrein activation and high molecular weight kininogen consumption have been found in HAE attack plasma (Schapira *et al.*, 1983). Curd *et al.* (1982) have also reported that all the kinin in HAE plasma coelutes with bradykinin on high pressure liquid chromatography.

Studies on the possible role of C2 in the generation of kinin activity have, in the past, been hampered by the inavailability of sufficient quantities of highly purified C2. We have in recent years developed methods for the purification of milligramme quantities of C2 which have led to an understanding of the structure and function of the molecule (Kerr & Porter, 1978; Kerr, 1980; Kerr & Gagnon, 1982; Parkes, Gagnon & Kerr, 1983; Kerr & Parkes, 1984). In this publication, we have studied the cleavage of such quantities of C2 by HAE plasma, plasma proteases inhibitable by C1INH and by trypsin in an attempt to define the role, if any, of C2 in kinin formation.

MATERIALS AND METHODS

Proteins

Complement proteins C2 (Kerr, 1981), $C\bar{1}s$ and $C\bar{1}r$ (Sim, 1980) were isolated as described previously. Kallikrein and plasminogen were isolated by affinity chromatography according to published methods (Nagasawa & Barrett, 1981; Castellino & Powell, 1980). The specific activities of the kallikrein and the plasmin measured using the synthetic substrates S2302 and S2251 (Kabi Diagnostics, Stockholm, Sweden) were similar to those reported by the manufacturers. Plasminogen was converted to plasmin using streptokinase (Jackson, Easmon & Tang, 1980). C2 was converted to C2a and C2b using purified $C\bar{1}s$ and the fragments separated where necessary by gel filtration on Sephadex G100 (Kerr, 1979). TPCK (L-(tosylamido 2-phenyl) ethyl chloro-methyl ketone) treated trypsin and soya bean trypsin inhibitor were purchased from Worthington (Lorne Lab Ltd, Twyford, Berks). Soya bean trypsin inhibitor (10 mg/ml in 0·15 M NaCl) was diluted to 1 mg/ml in incubation mixtures.

Table 1. Plasma samples used in this study

Plasma	Diagnosis	C1INH	C3 mg/dl	C4 mg/dl	C2 (% normal)
Normal		28	100	19	100
DC	HAE-danazol therapy	21	126	14	88
DH	HAE-asymptomatic	7	91	6	54
CB	HAE/attack phase	7	94	7	60
Normal range		15–35	90–150	20–40	

C1INH, C3 and C4 were measured by radial immunodiffusion, C2 by haemolytic activity.

Radiolabelling of C2 and analysis of C2 digests
C2 was radiolabelled with Na ^{125}I (Amersham International, Amersham, Bucks) using chloramine T. C2 cleavage in serum was followed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (Laemmli, 1970, Dodds *et al.*, 1978). Autoradiography of dried, Coomassie blue R250 stained gels was carried out using Kodak direct exposure film (Kodak Eastman, Hemel Hempstead, Herts) as described by the manufacturers.

Haemolytic assays
Cellular intermediates and assays were prepared and carried out as described previously (Kerr, 1980).

EDTA-plasma
Blood was drawn with minimum trauma and transferred immediately into 50-ml polypropylene tubes (Alpha Laboratories, Eastleigh, Hants) previously siliconized using dichloro-dimethy-silane as described by the manufacturers and containing one-ninth blood volume 0·13 M-trisodium citrate pH 5·3/0·006 M Na_2H_2 EDTA. The blood and anticoagulant were mixed and then centrifuged at 1000 ***g*** for 10 min. Plasma was removed using a siliconized pipette and 'snap' frozen and stored at −70° in 50-μl aliquots in siliconized 1-ml polypropylene conical minifuge tubes (Alpha Laboratories). Details of plasma samples used in this study are given in Table 1.

Kinin assay
Oestrus was induced in a female Wistar rat (120–160 g) by intraperitoneal injection of diethylstiboestrol dissolved to 20 mg/ml in 20% (v/v) ethanol in saline. Ten mg stilboestrol were administered per 100 g body weight and, 20–24 hr later, the condition of the uterus was determined following microscopic examination of a trypan-blue stained vaginal smear. Oestrus rats were killed and the 3-cm sections of uterine horn suspended in 10 ml organ baths containing de Jalon's buffer (90 g–NaCl, 4·2 g–KCl, 5 g–glucose, 5g–$NaHCO_3$ and 0·3 g–$CaCl_2$ in 10 litres of distilled water) at 29–32° and aereated with 5% CO_2 in O_2 (Garcia de Jalon, Bayo Bayo & Garcia de Jalon, 1945). Contractions indicating the presence of kinins were measured using a kymograph. A dose-response curve for bradykinin (Sigma Chemical Company, St Louis, MO) was obtained in the range 10^{-11}–10^{-6} M for each experiment.

RESULTS

Cleavage of C2 by HAE plasma

When HAE plasma, diluted with an equal volume of Veronal buffer, was incubated in siliconized tubes at 37°, the remaining C2 haemolytic activity was destroyed. Normal plasmas or plasma from an HAE patient receiving danazol therapy showed no loss of C2 activity under the same conditions. When purified C2 was added to each plasma to a final concentration of 400 μg/ml (20 × normal plasma concentration) and the samples incubated, C2 haemolytic activity was again destroyed in the untreated HAE plasmas but not in normal plasma or in the plasma from the danazol-treated patient. The rate of C2 cleavage was similar in the plasma from the asymptomatic patient and the attack phase plasma (Fig. 1).

When the same samples were assayed for kinin activity by the induction of contraction of oestrus rat uterus, kinin activity was detected in the three HAE samples after incubation for 30 min and was maximal after 1 hr. The amount of activity was the same after 3 or 6 hr incubation (Fig. 2).

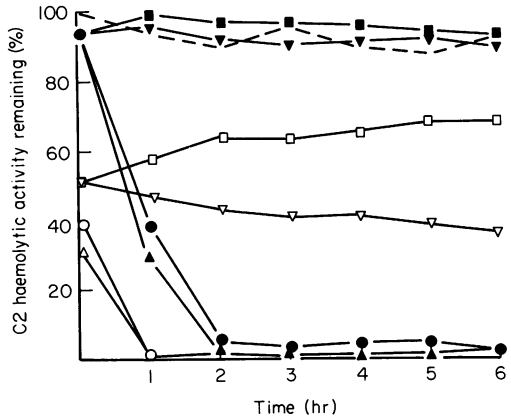

Figure 1. The effect of incubation of purified C2 at 37° with normal or HAE EDTA-plasmas: 50 μl EDTA plasmas from HAE patients or normal plasma were incubated at 37° with 50 μl 5 mM Veronal buffer, 150 mM NaCl pH 7·4 (open symbols) or 50 μl purified C2, 1 mg/ml in the same buffer (closed symbols) and the C2 remaining assayed by haemolytic activity. The plasmas were: (□) normal; (○) attack phase HAE; (△) asymptomatic HAE or (▽) danazol-treated HAE. C2 incubated alone lost no haemolytic activity (– – –).

Normal plasma showed no increase in kinin activity on incubation for 3 hr, although, on prolonged incubation, activity was generated in this sample. The addition of C2 to the samples prior to incubation had no effect on the generation of kinin activity. The amount of kinin generated in the HAE plasmas was the same as that generated on incubation of HAE or normal plasmas in non-siliconized tubes. The kinin activity was well within the range of sensitivity of the rat uterus used in this assay (Fig. 2 insert) and is similar to the level of activity determined in HAE plasmas by other workers using the same assay.

^{125}I-labelled C2 incubated for up to 6 hr with HAE plasma was cleaved to C2a and C2b, but no other breakdown products were detected on autoradiography. [^{125}I]C2 was not cleaved in normal plasma and a mixture of [^{125}I]C2a and C2b was not cleaved further by any of the plasma tested.

The effect of soya bean trypsin inhibitor on C2 cleavage and kinin generation

Soya bean trypsin inhibitor had no effect on the rate of cleavage of endogenous or added C2 in HAE-plasmas (Fig. 3). However, in agreement with all earlier studies, soya bean trypsin inhibitor was observed to abolish all kinin-generating activity in the HAE plasmas. The

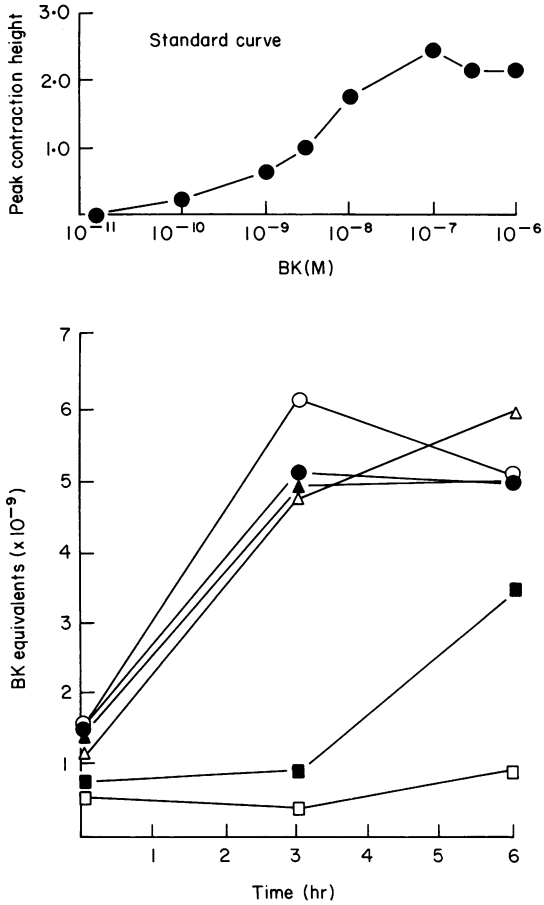

Figure. 2. The effect of purified C2 on the kinin activity generated on incubation of HAE or normal plasma: 50 μl EDTA plasmas from HAE patients or normal plasma were incubated at 37° with 50 μl 5 mM Veronal buffer, 150 mM NaCl pH 7·4 (open symbols) or 50 μl C2, 1 mg/ml in the same buffer (closed symbols) and the kinin activity generated measured by the oestrus rat uterus assay. The samples which were the same as those assayed for C2 haemolytic activity in Fig. 1 were (□) normal plasma; (○) attack phase HAE or (△) asymptomatic HAE. None of the plasmas incubated in the presence of soyabean trypsin inhibitor generated any detectable kinin activity. The bradykinin (BK) dose-response curve for the oestrus uterus used in this assay is shown above.

soya bean trypsin inhibitor also inhibited the low level of kinin activity detectable on incubation of normal plasma at 37° for 3 hr and the low level detectable in HAE plasma prior to incubation. The addition of soya bean trypsin inhibitor had no effect on the kinin activity when added after incubation of the HAE plasma nor on the dose-response curve of purified bradykinin.

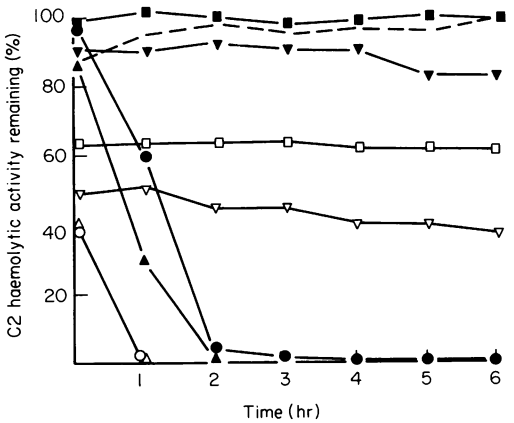

Figure 3. The effect of incubation of purified C2 at 37° with normal or HAE EDTA-plasmas in the presence of soya bean trypsin inhibitor. Soyabean trypsin inhibitor (200 μg in 20 μl saline) was added to 50 μl EDTA from HAE patients or normal plasma. The plasmas were then incubated at 37° with 50 μl 5 mM Veronal buffer, 150 mM NaCl pH 7·4 (open symbols) or 50 μl purified C2, 1 mg/ml in the same buffer (closed symbols) and the C2 remaining assayed by haemolytic activity. The plasmas were (□) normal; (○) attack phase HAE; (△) asymptomatic HAE or (▽) danazol-treated HAE.

Digestion of C2 with proteases

Digestion of C2 (1 mg/ml) by C1̄s at an enzyme to substrate ratio of 1:100 for up to 60 min gave no products identifiable by SDS polyacrylamide gel electrophoresis other than C2a and C2b (Fig. 4a). Digestion of C2 with trypsin (Fig. 4b) at an enzyme to substrate ratio of 1:100 resulted in the initial appearance of C2a and C2b. This C2a had identical mobility on the gels to the trace of C2a which contaminated the C2 preparation used in this study. Extended digestion led to the further cleavage of C2a to produce a slightly smaller fragment followed by cleavage of the remainder of the C2a to fragments of apparent molecular weight 61,000 and 15,000. When C2 was incubated with kallikrein it was cleaved to C2a and C2b only. The rate of cleavage was much slower than that obtained with C1̄s or trypsin under identical conditions. The C2a and C2b produced had identical electrophoretic mobility to that obtained on cleavage of C2 by C1̄s. Plasmin had no effect on C2 as could be seen from both SDS-polyacrylamide gel analysis and from haemolytic activity measurements (Fig. 5). Samples (200 μg) of C2 were also removed per time-point from each digest and assayed on the oestrus rat uterus. No kinin activity could be detected from any of these samples.

Digestion of C2a/C2b and purified C2b with proteases

Milligram quantities of C2 were digested with C1̄s at an enzyme to substrate ratio of 1:100 and the resultant C2a and C2b fragments incubated further with C1̄s, plasmin, trypsin or kallikrein. Samples were removed at specific time-points for SDS-polyacrylamide gel analysis shown in Fig. 6. Samples (200 μg) were also

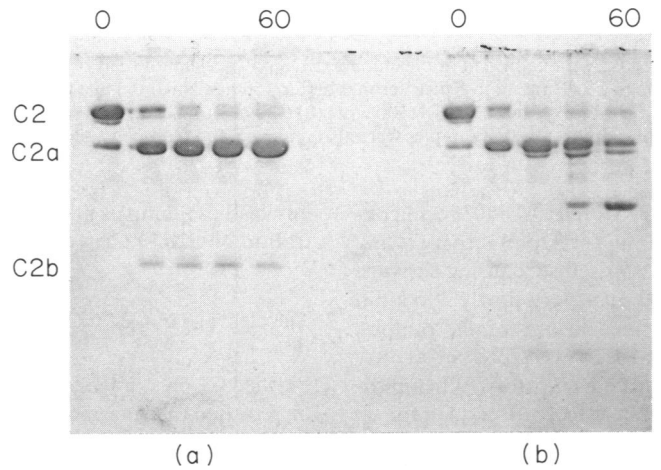

Figure 4. Digestion of purified C2 with C1̄s or trypsin. SDS polyacrylamide gels of 25-μl samples taken at 0, 1, 10, 30 and 60 min from incubations of C2 (1 mg/ml in 5 mM Veronal buffer/150 mM NaCl/0·5 mM $CaCl_2$/2·0 mM $MgCl_2$ pH 7·4) with (a) C1̄s or (b) trypsin at an enzyme to substrate ratio of 1:100 w/w. 100-μl samples were also removed and, after the addition of excess soyabean trypsin inhibitor, frozen at −70° prior to assay for kinin acitivity.

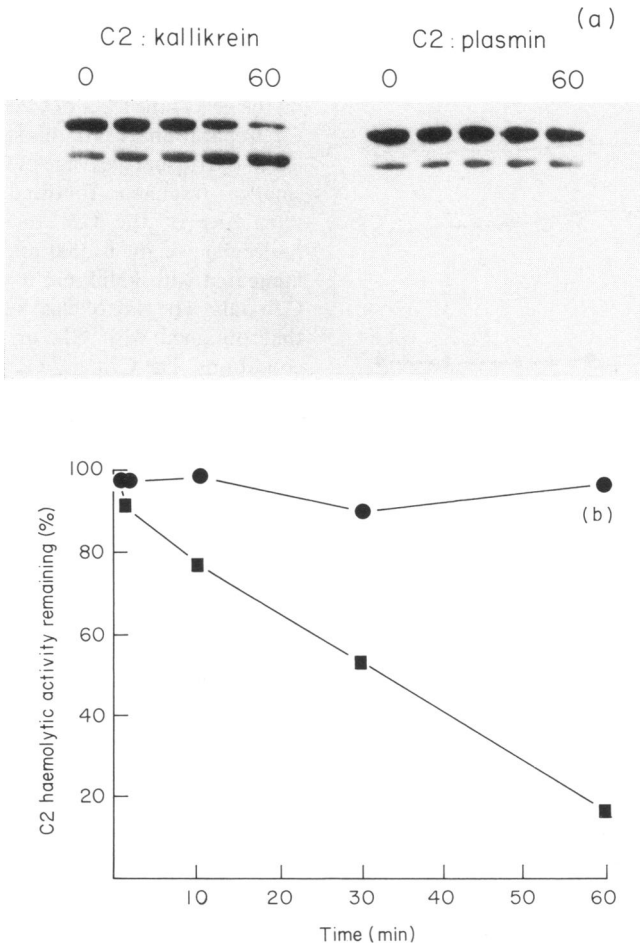

Figure 5. Digestion of purified C2 with kallikrein or plasmin: (a) SDS polyacrylamide gels of 25-μl samples taken at 0, 1, 10, 30 and 60 min from incubations of C2 (1 mg/ml in 5 mM Veronal buffer/150 mM NaCl/0·5 mM $CaCl_2$/2·0 mM $MgCl_2$ pH 7·4) with kallikrein or plasmin at enzyme to substrate ratio of 1:100 w/w; (b) C2 haemolytic assay of samples (1-μl) taken from the same incubation. Kallikrein (■), plasmin (●). 100 μl samples were also removed and frozen at $-70°$ prior to assay for kinin activity.

removed, snap frozen and stored at $-70°$ in siliconized tubes prior to analysis for kinin activity. Only trypsin was found to have any effect, causing cleavage of both C2a and C2b, although very slowly. No kinin activity could be determined in any of the samples tested, although C2a and C2b were present at fifty times their normal plasma concentration. When purified C2b (1 mg/ml) was incubated with trypsin for up to 64 min (Fig. 7) at an enzyme to substrate ratio of 1:100, C2b (34,000 molecular weight) was cleaved to a slightly smaller (32,000) fragment similar to that produced on extended incubation of C2b and $C\bar{1}s$. Nevertheless, no kinin activity could be detected in any of these samples at 100 times the plasma concentration of C2b in C2.

The cleavage of C2 by $C\bar{1}s$ requires a native conformation

Purified C2 on storage for prolonged times at $-70°$ or on repeated freezing and thawing has been observed to lose haemoytic activity. This loss of activity is due to the formation of a species of C2 which cannot be cleaved to $C\bar{1}s$ and is similar to that observed on treatment of C2 with excess iodine or iodine and various nucleophiles (Kerr & Parkes, 1984). The

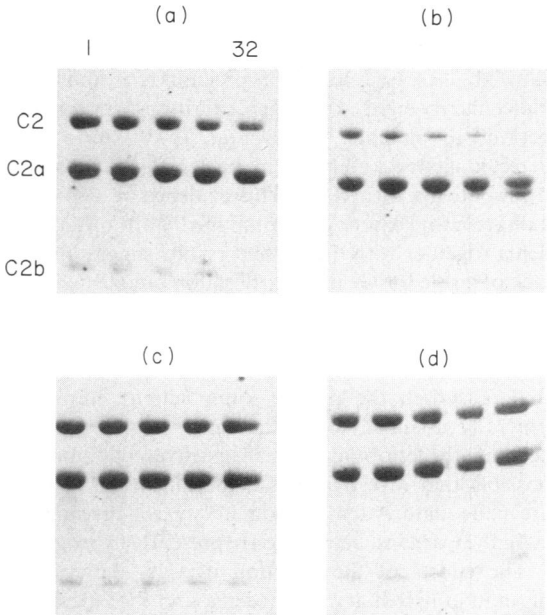

Figure 6. Digestion of a mixture of C2a, C2b and haemolytically inactive C2 with $C\bar{1}s$, trypsin, kallikrein or plasmin. SDS polyacrylamide gels of samples (25-μl) taken at different times (1, 2, 4, 16 and 32 min) from incubations of a mixture of C2a, C2b and haemolytically inactive C2 (total 1 mg/ml in 5 mM Veronal buffer 150 mM NaCl/0·5 mM $CaCl_2$/2·0 mM $MgCl_2$ pH 7·4) with (a) $C\bar{1}s$, (b) trypsin, (c) kallikrein or (d) plasmin. 100-μl samples were also removed and frozen at $-70°$ prior to assay for kinin activity. Excess soyabean trypsin inhibitor was added to the trypsin-treated samples before freezing.

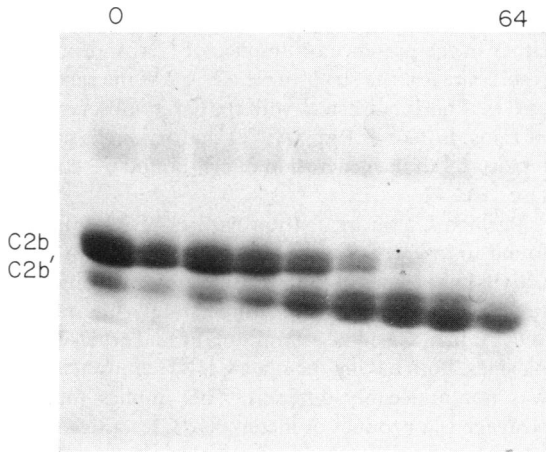

Figure 7. Digestion of C2b with trypsin. SDS-polyacrylamide gels of unreduced samples (100-μg) taken at different times (0, 1, 2, 4, 8, 16, 32 and 64 min) from incubations of C2b (1·0 mg/ml in 5 mM Veronal buffer/150 mM NaCl/0·5 mM $Ca–Cl_2$/2·0 mM $MgCl_2$ pH 7·4) with trypsin at an enzyme to substrate ratio of 1:100. Samples (200-μg) were also removed and after the addition of excess soyabean trypsin inhibitor assayed for kinin activity.

species is therefore analogous to the haemolytically inactive forms of C3 and C4 which are formed on discharge of the thiol ester group. Although C2 does not contain this group, it is interesting that here also a cysteine residue appears to be involved. The preparation of C2 used for the generation of C2a and C2b in the experiment shown in Fig. 6 contains some 40% of this inactive C2. It can be seen that this species is also uncleaved by kallikrein but is cleaved by trypsin at a similar rate and to give similar fragments to that of native C2. These results can be compared with those in Figs 4 and 5 where the C2 preparation contained very little of the inactive species.

DISCUSSION

Although previous studies have implicated C2 as the source of kinin in HAE-plasma, our studies using highly purified C2 at concentrations many times the normal plasma concentration have failed to support this. C2 incubated with plasma deficient in C1INH or with kallikrein, plasmin or $C\bar{1}s$, three plasma proteases inhibited by C1INH, failed to produce kinin activity. Similarly, C2 incubated first with $C\bar{1}s$ to produce C2 and C2b and then with plasmin or kallikrein failed to produce kinin activity. On no occasion during this extended study was any kinin detected in any incubation containing C2. In all cases, the smooth muscle preparation detected kinin activity in HAE plasma and was sensitive to bradykinin at levels comparable to those in other published reports (Donaldson *et al.*, 1977; Fields *et al.*, 1983).

The first direct evidence of a role for C2 in kinin generation was reported by Donaldson *et al.* (1977), who observed that incubation of preparations of C2, $C\bar{1}s$ and plasmin, but not any two of these proteins, produced kinin activity. The present results have shown plasmin to have no effect on C2 or on C2a or C2b produced by $C\bar{1}s$ cleavage of C2. No kinin activity was generated in these mixtures. More recently, Sobel and colleagues (Lopez-Trascasa *et al.*, 1982; Bourgarit *et al.*, 1983) have reported the generation of kinin activity from C2 incubated with $C\bar{1}s$ and then briefly with trypsin. Again, all three proteins were needed. Our results have shown that, although C2 is cleaved

extensively by trypsin, no kinin activity was generated either in the presence or absence of $C\bar{1}s$. Indeed, our results suggest that trypsin cleaves C2 at the same site as $C\bar{1}s$. This is consistent with the earlier observations of Loos, Borsos & Rapp (1972) that trypsin is able to replace $C\bar{1}$ in the generation of cell-bound C3 convertase, SAC42.

Purified C2 on incubation with HAE plasma was found to lose activity after 2 hr. No increase in kinin activity was found in these plasmas following incubation for up to 6 hr at 37°. Interestingly, the rate of activation of C2 in the asymptomatic and attack phase plasmas, both having the same C1INH concentration, was not noticeably different. This implies that C2 cleavage is a product of incomplete $C\bar{1}$ control but is not related to the phase of the disease or to kinin production.

Soya bean trypsin inhibitor, which has been shown in this and all previous studies to abolish kinin generation in HAE plasmas, does not affect the rate of cleavage of C2 or the products formed. Taken together, these results suggest that it is unlikely that C2 is the source of kinin activity that can be generated in HAE plasma. This is consistent with the presence of low C4 and C2 levels in HAE plasma, regardless of the disease phase, but the highly episodic and localized nature of the attacks of oedema.

The observations that mild trauma is often the cause of onset of an attack had led to suggestions that oedema is the result of activation of the contact processes. Our own results (Smith & Kerr, 1983) have shown that no kinin activity could be generated from kallikrein or kininogen deficient plasmas depleted of C1INH by immunoabsorption on anti-C1INH IgG-Sepharose, whereas considerable activity was generated from both normal and C2-deficient plasmas treated in the same way. The experiments were not conclusive, since the CNBr activation of the Sepharose produced groups on the resin which were highly efficient at activating the contact process and therefore generating bradykinin from normal plasma. However, the results do suggest that *all* the kinin activity generated in C1INH-depleted plasma is dependent on the kallikrein/kininogen system. This is further supported by the effect of soya bean trypsin inhibitor in abolishing the generation of kinin activity in C1INH-depleted or deficient (HAE) plasmas. Soya bean trypsin inhibitor is a potent inhibitor of kallikrein (Negasawa & Barrett, 1981) and can be used as it was in this study for the selective affinity purification of kallikrein from activated plasma.

These observations are consistent with the results of Schapira *et al.* (1983) and Witzke *et al.* (1983) who showed decreased levels of prekallikrein and high molecular weight kininogen during symptomatic periods in patients. Curd *et al.* (1980) have also detected active kallikrein in blister fluids obtained from patients with HAE. This evidence of a role for kallikrein and kininogen in the mediation of angioedema together with the present results suggesting the lack of a role for C2 in the generation of plasma kinin activity, implies the need for a reassessment of previous data implicating the complement system in the pathogenesis of HAE. It should, however, be noted that, although the plasma kinin activity has been generally assumed to be the same as that causing oedema, the lesions in HAE are extravascular and it is possible that investigations of plasma kinin activity are inadequate. Attempts to identify vasoactive material in the extravascular space are presently in progress.

The effects of the injection of $C\bar{1}s$, the role of plasmin in hereditary angioedema and the efficacy of inhibition of plasminogen activation such as ε-aminocaproic acid or tranexamic acid in the treatment of the disease remain unclear. However, it is possible that, since plasmin is present in the highest concentration in plasma of all the C1INH inhibitable enzymes, it will be the activation of plasmin which will deplete C1INH most readily. If, as has been suggested for the complement system (Ziccardi, 1983), control of the kinin system also requires a critical concentration of C1INH, then depletion of C1INH by plasmin or $C\bar{1}s$, indirectly and therefore sporadically and in a localized manner, will allow the activation of kinin-producing enzymes.

ACKNOWLEDGMENTS

We are most grateful to Drs Wilkinson, Campbell and Whaley for samples of HAE plasma. We thank Mrs R. Mitchell, Mr R. Fawkes and Mr A. Grant for assistance in the preparation of the manuscript. This work was supported by a grant from the Scottish Home and Health Department.

REFERENCES

Arlaud G.J., Reboul A., Sim R.B. & Colomb M.G. (1979) The interaction of C1INH with $C\bar{1}r$ and $C\bar{1}s$ subcomponents in human C1. *Biochem. biophysica Acta*, **576**, 151.

AUSTEN K.F. & SHEFFER A.L. (1965) Detection of hereditary angioneurotic edema by demonstration of a reduction of the second component of human complement. *N. Engl. J. Med.* **272,** 649.

BALLOGH A. & WHALEY K. (1980) Hereditary angio-oedema. Its pathogenesis and management. *Scott. med. J.* **25,** 187.

BOURGARIT J.J., HESLAN J.M., BRANELLES A., MOISY M., SOBEL A.T. & LAGRUE G. (1983) Characterization of a vasoactive peptide in the urine of HAE patients. *Immunobiology*, **164,** 215.

BOURGARIT J.J., LOPEZ-TRASCASA M., MOISY M. & SOBEL A.T. (1983) Identification of C2k, a C2-derived vasoactive peptide. *Fed. Proc.* **42,** 1234.

CASTELLINO F.J. & POWELL J.R. (1980) Human plasminogen. *Meth. Enzymol.* **80,** 365.

CURD J.G., PROGRAIS L.J. & COCHRANE C.G. (1980) Detection of active kallikrein in induced blister fluids of hereditary angioedema patients. *J. exp. Med.* **152,** 742.

CURD J.G., YELVINGTON M., BURRIDGE N., STIMLER N.P., GERARD C., PROGRAIS L.J., COCHRANE C.G. & MÜLLER-EBERHARD H.J. (1982) Generation of bradykinin during incubation of hereditary angioedema plasma. *Molec. Immunol.* **19,** 1365.

DODDS A.W., SIM R.B., PORTER R.R. & KERR M.A. (1978) Activation of the first component of human complement (C1) by antibody antigen aggregates. *Biochem. J.* **175,** 383.

DONALDSON V.H. & EVANS R.R. (1968) A biochemical abnormality in hereditary angio-neurotic edema. *Amer. J. Med.* **35,** 37.

DONALDSON V.H., RATNOFF O.D., DIAS DA SILVA W. & ROSEN F.S. (1969) Permeability-increasing activity in hereditary angioneurotic oedema plasma. *J. clin. Invest.* **48,** 642.

DONALDSON V.H. & ROSEN F.S. (1964) Action of complement in hereditary angioneurotic edema. The role of C1 esterase. *J. clin. Invest.* **43,** 2204.

DONALDSON V.H., ROSEN F.S. & BING D.H. (1977) Role of the second component of complement (C2) and plasmin in kinin release in hereditary angioneurotic edema (HANE) plasma. *Trans. Assoc. Amer. Phys.* **60,** 174.

FIELDS T., GHEBREHIWET B. & KAPLAN A.P. (1983) Kinin formation in hereditary angiodema plasma: evidence against kinin derivation from C2 and in support of 'spontaneous' formation of bradykinin. *Allergy Clin. Immunol.* **72,** 54.

FORBES C.D., PENSKY J. & RATNOFF O.D. (1970) Inhibition of activated Hageman factor and activated plasma thromboplastin antecedent by purified C1-inactivator. *J. Lab. clin. Med.* **76,** 809.

FRANK M.M., GELFAND J.A. & ATKINSON J.P. (1976) Hereditary angioedema: the clinical syndrome and its management. *Annals. Int. Med.* **84,** 580.

GARCIA DE JALON P., BAYO BAYO J. & GARCIA DE JALON M. (1945) Sensible y neuvo metodo de valoracion de adrenalina en utero aislado de mata. *Farmacoterapia Actual.* **2,** 313.

GIGLI I., MASON J.W., COLMAN R.W. & AUSTEN K.R. (1976) Interaction of plasma kallikrein with C1-inhibitor. *J. Immunol.* **104,** 574.

JACKSON K.W., EASMON N. & TANG J. (1980) Streptokinase and staphylokinase. *Meth. Enzymol.* **80,** 387.

KERR M.A. (1979) Limited proteolysis of complement components C2 and Factor B. *Biochem. J.* **183,** 615.

KERR M.A. (1980) The human complement system: assembly of the classical pathway C3 convertase. *Biochem. J.* **189,** 173.

KERR M.A. (1981) The second component of human complement. *Meth. Enzymol.* **80,** 56.

KERR M.A. & GAGNON J. (1982) The purification and properties of the second component of guinea pig complement. *Biochem. J.* **205,** 59.

KERR M.A. & PARKES C. (1984) The effects of iodine and thiol blocking agents on complement component C2 and on the assembly of the classical pathway C3 convertase. *Biochem. J.* **219,** 391.

KERR M.A. & PORTER R.R. (1978) The purification and properties of the second component of human complement. *Biochem. J.* **171,** 99.

KLEMPERER M.R., DONALDSON V.H. & ROSEN F.A. (1967) The vasopermeability response in man to purified C1-esterase. *J. clin. Invest.* **46,** 1079.

LAEMMLI U.K. (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.)*, **227,** 680.

LANDERMAN N.S., WEBSTER M.E., BECKER E.D. & RATCLIFFE H.E. (1962) Hereditary angioneurotic oedema. II. Deficiency of an inhibitor for serum globulin permeability factor and/or plasma kallikrein. *J. Allergy*, **33,** 330.

LOOS M., BORSOS T. & RAPP H.J. (1972) Immune hemolysis and the functional properties of the second (C2) and fourth (C4) components of complement. IV. Formation of EAC 42 by treatment of C2 with trypsin in the presence of EAC 4. *J. Immunol.* **109,** 434.

LOPEZ-TRASCASA M., MOISY M., PIROTZKY E., BLOUGUIT Y., BLANC C. & SOBEL A.T. (1982) Isolation and characterization of a biologically active C2-derived oligopeptide. *Molec. Immunol.* **19,** 1403.

NAGASAWA H. & BARRETT A.J. (1981) Human plasma kallikrein: a rapid purification method with high yield. *Biochem. J.* **193,** 187.

PARKES C., GAGNON J. & KERR M.A. (1983) The reaction of iodine and thiol blocking reagents with complement components C2 and Factor B. *Biochem. J.* **213,** 201.

RATNOFF O.D. & LEPOW I.H. (1957) Some properties of an esterase derived from preparations of the first component of complement. *J. exp. Med.* **106,** 327.

RATNOFF O.D., PENSKY J., OGSTON D. & NAFF G.B. (1969) The inhibition of plasmin, plasma kallikrein, plasma permeability factor and the C1r subcomponent of the first component of complement by serum C1 esterase inhibitor. *J. exp. Med.* **129,** 315.

SCHAPIRA M., SILVER L.D., SCOTT C.F., SCHMAIER A.H., PROGRAIS L.J., CURD J.G. & COLMAN R.W. (1983) Prekallikrein activation and high-molecular-weight kininogen consumption in hereditary angioedema. *N. Engl. J. Med.* **308,** 1050.

SCHREIBER O.D., KAPLAN A.P. & AUSTEN K.F. (1973) Inhibition by C1 INH of Hageman factor fragment activation of coagulation, fibrinolysis, and kinin generation *J. clin. Invest.* **52,** 1402.

SIM R.B. (1980) The human complement system serine proteases C1r and C1s and their proenzymes. *Meth. Enzymol.* **80,** 26.

SIM R.B., REBOUL A., ARLAUO G.J., VILLIERS C.L. & COLOMB M.G. (1979) Interaction of ^{125}I-labelled complement components $C\bar{1}r$ and $C\bar{1}s$ with protease inhibitors in plasma. *FEBS Lett.* **97,** 111.

SMITH M.A. & KERR M.A. (1983) The kinin activity generated in C1INH-deficient plasma is not derived from C2 and originates from the kallikrein-kinogen pathway. *Immunobiology*, **164,** 299.

TRAVIS J. & SALVESEN G.S. (1983) Human plasma proteinase inhibitors. *Ann. Rev. Biochem.* **52,** 655.

WITZE G., BORK K., BENES P. & BÖCKERS M. (1983) Hereditary angioneurotic oedema and blood-coagulation: interaction between C1-esterase inhibitor and the activation factors of the proteolytic enzyme system. *Klin. Wochenschr.* **61,** 1131.

ZICCARDI R.J. (1983) The first component of complement (C1). Activation and control. *Springer Seminars in Immunopathology*, **6,** 195.