Clin Exp Immunol 1996; **106**:416–422

The behaviour of human vitronectin *in vivo*: effects of complement activation, conformation and phosphorylation

P. W. PEAKE, J. D. GREENSTEIN, B. A. PUSSELL & J. A. CHARLESWORTH *Renal Laboratory, The Prince Henry Hospital, Sydney, Australia*

(Accepted for publication 28 June 1996)

SUMMARY

We examined the behaviour *in vivo* of native, specifically phosphorylated, and multimeric vitronectin to determine the effects of these modifications on its turnover, distribution and molecular behaviour. In normal rabbits, the plasma half-life ($T_{1/2}$) of antigenically detected vitronectin was $8{\cdot}00 \pm 1{\cdot}26$ h (mean ± s.d.), with a fractional catabolic rate (FCR) of $18{\cdot}77 \pm 1{\cdot}57$%/h and extravascular/intravascular ratio (EV/IV) of 1·00 (0·48–1·60, median and range). For vitronectin selectively phosphorylated by protein kinase A, $T_{1/2}$ was $8{\cdot}87 \pm 0{\cdot}48$ h, with a significantly smaller FCR of $10{\cdot}85 \pm 0{\cdot}71$%/h ($P<0{\cdot}005$) and an EV/IV of 0·28 (0·15–0·36) ($P<0{\cdot}05$ compared with antigenically detected vitronectin). *In vitro*, phosphorylation had no effect on the affinity of vitronectin for heparin–Sepharose, while complement activation with cobra venom factor (CVF) led to a two-fold enrichment of ^{32}P-vitronectin within the SC5b-9 complex. *In vivo* CVF caused a rapid decrease in the circulating levels of ^{32}P-vitronectin and was accompanied by the prompt appearance of a high mol. wt species consistent with SC5b-9. Despite specific incorporation of ^{32}P-vitronectin into SC5b-9, both forms of the molecule had similar inhibitory effects on C9-mediated haemolysis of EAC1-7 cells. Urea-activated vitronectin was rapidly cleared from circulation with less than 15% remaining after 1 h while protein-bound label accumulated in the spleen, lung and liver. These results demonstrate that vitronectin is a rapidly metabolized protein whose *in vivo* behaviour is markedly altered when phosphorylated or activated to form multimers and SC5b-9.

Keywords vitronectin metabolism phosphorylation human

INTRODUCTION

Vitronectin, also known as S-protein [1], is a multi-functional glycoprotein of 75 kD with well-defined roles in cell adhesion, inhibition of the membrane-attack complex (MAC) of complement and coagulation. Specific domains of the molecule have affinities for β-endorphin, plasminogen activator inhibitor 1 (PAI-1), heparin and integrins [2,3]. A high degree of structural flexibility mediates many of vitronectin's biological functions: a number of agents including foreign surfaces, chaotropes and macromolecules such as C5b-9 and thrombin–antithrombin III are able to 'open' its structure and expose functionally important epitopes such as the cryptic heparin-binding domain, while spontaneous multimerization enhances binding to PAI-1, cell sufaces and extracellular matrices [4–11]. Within the MAC, vitronectin occupies the metastable membrane-binding site of the C5b-7 assembly, thus preventing its insertion into membranes and completion of the lytic C5b-9 pore [12]. The resultant soluble complex binds C8 and C9 to form SC5b-9 which is normally rapidly cleared from the circulation [13] but may also deposit on tissue surfaces [14]. Polymerization of C9 is thought to be inhibited by the heparin-binding domain of vitronectin [15].

A cyclic AMP-dependent kinase released from activated platelets selectively phosphorylates vitronectin on Ser 378 which is within the heparin-binding domain [16]. Insertion of a phosphate into this highly basic region of the molecule could influence its conformation, self-association and interaction with other molecules; it has been shown, for example, to decrease the affinity of vitronectin for PAI-1 [17].

The majority of observations on the biochemical behaviour of vitronectin have been made *in vitro*. Little is known of its kinetics *in vivo*. However, changes in metabolic behaviour and regional distribution in response to complement activation or biochemical modification could influence the regulatory properties of the molecule under both physiological and pathological conditions. Hence, we have investigated the metabolic behaviour in normal rabbits of native, phosphorylated and multimeric forms of vitronectin, and examined the response *in vivo* of vitronectin to complement activation.

Correspondence: J. A. Charlesworth, Renal Laboratory, Clinical Sciences Building, The Prince Henry Hospital, Anzac Parade, Little Bay, Sydney, NSW, Australia 2036.

MATERIALS AND METHODS

Purification of vitronectin

Human vitronectin was purified by heparin affinity chromatography following treatment by urea [2], with the exception that no reducing agent was included. For some experiments the method of Hayashi and Mosher [18] was used to purify the molecule in native form.

Radiolabelling of vitronectin

Phosphorylation. Vitronectin in fresh human plasma was selectively phosphorylated by a mixture of γ-^{32}P-ATP (Amersham, Sydney, Australia) (approximately 10^{-8} M) and a 3,5-cAMP-dependent kinase from rabbit muscle (Sigma) as described [19]. Purified vitronectin was similarly labelled after transfer to HEPES buffer via a G25 column. For quantitative labelling, 20 mM ATP was added to the mixture, and the quantity of kinase increased [19].

Iodination. Purified native vitronectin was labelled with ^{125}I using lactoperoxidase [20]. Vitronectin purified by urea treatment and elution from heparin–Sepharose was labelled by the iodogen procedure [21]. Subsequently it was repurified by elution from heparin–Sepharose using a 0·15–1·0 M gradient of NaCl. Rabbit serum albumin (RSA) was labelled with ^{131}I by the iodogen procedure.

Quantification of vitronectin

Phosphorylated vitronectin was quantified by measuring Cerenkov radiation by a liquid scintillation counter (Packard, Sydney, Australia). Vitronectin labelled with both ^{125}I and ^{32}P was quantified by liquid scintillation counting in an ACS II scintillant (Amersham). There was 2% crossover of ^{32}P radiation into the ^{125}I channel. ^{125}I-vitronectin and ^{131}I-RSA were measured by a Packard Minaxi gamma counter and allowance made for 16·8% crossover of ^{131}I into the ^{125}I channel.

During purification of vitronectin from plasma, the elution pattern of the molecule was determined by radial immunodiffusion in agarose gels containing polyclonal sheep anti-human vitronectin (The Binding Site, Birmingham, UK).

The behaviour of vitronectin on various column matriceswas determined by a monoclonal capture ELISA on SDS-denatured preparations [22]. Samples (50 μl) were added to wells coated with 2 μg/ml MoAb 24.4 [23] in PBS and vitronectin detected by adding suitably diluted sheep anti-human vitronectin followed by donkey anti-sheep IgG conjugated with alkaline phosphatase (Sigma). The assay was calibrated against serial dilutions of human plasma in BSA.

Native human vitronectin in rabbit plasma was assayed using -MoAb 24.4 [23] capture and polyclonal sheep anti-vitronectin detection. The assay was similar to that described above except that there was no denaturation procedure, and all blocking and dilution buffers contained 1% milk powder (Carnation, Sydney, Australia) to block non-specific binding. Denaturation of human plasma diluted in rabbit plasma as described in the previous assay led to a loss of specific signal. The results were calibrated against serial dilutions of human plasma in rabbit plasma which were similarly treated. ELISAs were done at least twice with duplicate points.

Characterization of phosphorylated vitronectin

Affinity for heparin–Sepharose. The behaviour of phosphorylated vitronectin in plasma was compared with that of all antigenically detectable vitronectin on a heparin–Sepharose column using a gradient of 20–500 mM NaCl in Tris buffer. The results were monitored by Cerenkov counting and ELISA using denatured preparations as described above.

Effect on complement. Serum or heparin-purified vitronectin in HEPES/Mg^{2+} buffer was quantitatively phosphorylated with 20 mM ATP as before, while control incubations were done in the absence of protein kinase A. The ability of vitronectin to prevent the lysis of antibody-sensitized erythrocytes by complement components in the sera, and that of phosphorylated purified vitronectin to inhibit the C9-mediated lysis of EAC1-7 cells was also determined as described [24]. This assay quantified the lysis of EAC1-7 cells (generated by incubation with C8 deficient sera (Sigma)) by pure C8 and limiting C9 (Sigma) in the presence of varying amounts of vitronectin.

Effect of cobra venom factor. Phosphorylated plasma vitronectin was equilibrated with complement fixation diluent (CFD, Oxoid) by passage down a G25 Sephadex column. Subsequently 0·5 ml aliquots were incubated for 2 h at 37°C with 40 μl of CFD or 1 mg/ml cobra venom factor (CVF, Sigma), and the results analysed on an FPLC Superose 12 column. In other experiments, a native vitronectin preparation labelled with ^{125}I and ^{32}P was added to normal human serum, and incubated in the presence and absence of CVF. Specifically, dual-labelled vitronectin was applied to an FPLC Superose 12 column, and 200 μl of the monomeric form of the molecule mixed with 50 μl serum, 100 μl CFD and 8 μl of 1 mg/ml CVF (or PBS) and incubated for 3 h at 37°C before reanalysis on the Superose column.

The proportion of radiolabelled vitronectin of high mol. wt (approx. 1000 kD) which was present as CVF-generated SC5b-9 rather than as aggregates, for example, was determined by immunoprecipitation with a MoAb specific for a neo-epitope on polymerized C9 (aE11, Dako, Carpinteria, CA) bound to Gamma-Bind Sepharose beads (Amrad Pharmacia, Sydney, Australia).

Studies of vitronectin in vivo

The behaviour of human plasma vitronectin and its phosphorylated form was studied *in vivo* using 2·5–3·5 kg adult male New Zealand rabbits. Approximately 3 ml of diluted plasma containing vitronectin trace-labelled with γ-^{32}P-ATP was injected via a marginal ear vein. This material was approximately 1/10 of the original plasma protein concentration and had an activity of 10^6 ct/min. Blood samples in ethylenediaminetetra-acetic acid (EDTA) were drawn from the contralateral ear at 10 min and subsequently 3–4 times daily until levels of protein-bound radioactivity were less than 8% of the initial dose. Protein-bound radioactivity was determined by precipitation with 12% trichloroacetic acid (TCA). The labelled preparations of vitronectin used in these experiments were less than 3 days old and thawed once only. The experimental protocols are outlined below:

(1) Four rabbits received 3 ml human plasma containing trace-phosphorylated vitronectin.

(2) Four rabbits received 3 ml human plasma containing trace-phosphorylated vitronectin followed by 0·25 mg of purified CVF at 10 min. Control animals received an equal volume of PBS (approx. 1·0 ml) instead of CVF.

(3) Two rabbits received 3 ml human plasma containing trace-phosphorylated vitronectin which had been activated by incubation with 8 M urea for 2 h at 37°C followed by dialysis against PBS. Concurrently, 2×10^6 ct/min ^{131}I-RSA was also injected.

(4) Two rabbits received 3×10^6 ct/min of ^{125}I-vitronectin

which had been treated with urea and 2×10^6 ct/min ^{131}I-RSA as before. Animals were killed at 5 h and samples of their plasma, liver, spleen, kidneys, lungs, heart and skeletal muscle obtained for counting radioactivity.

(5) Two rabbits received 3×10^6 ct/min of ^{125}I-vitronectin which had been purified under native conditions but which had partially multimerized on storage for 3 weeks at 4°C as shown by FPLC gel filtration (Superose 12, Amrad Pharmacia).

The amount of plasma protein-bound radioactivity was plotted against time after injection, with the assumption that radioactivity at 10 min represented 97% of the injected dose [13]. Circulating human vitronectin was determined antigenically by a monoclonal–polyclonal capture ELISA for the native form and a monoclonal–monoclonal capture ELISA for SDS-denatured samples. The analysis of data by the Mathews method was performed as previously described [13]. Plasma volumes were calculated by isotope dilution. Ethics Committee approval was obtained for these experiments.

Tissues. Immediately following a lethal dose of pentobarbitone, liver, spleen, kidneys, lungs, heart and samples of skeletal muscle were removed, washed in saline, cleared of surrounding fat, and a portion of each tissue weighed and placed into polystyrene tubes to count total radioactivity. A further sample of tissue was homogenized in PBS containing 25 mM benzamidine, 0·5 mM phenylmethylsulphonylfluoride, and 4.4 mM Na_2EDTA. A 0·5 ml aliquot of organ homogenate was added to 1·5 ml of 24% TCA, centrifuged at 2500 ***g*** for 20 min then filtered through packed nylon wool and the supernatant (containing free iodide) counted.

Statistical analysis

For normally distributed data the results were expressed as mean ± s.d. and a non-paired Student's *t*-test was used to assess the difference between groups. Otherwise, the median, range and the Mann–Whitney *U*-test were used to compare data.

RESULTS

Purified vitronectin

Vitronectin purified either by column chromatography or heparin affinity techniques migrated as a doublet on reducing SDS gels with the molecular weight of visible bands being 75 and 65 kD. Coomassie staining indicated that the preparations were more than 95% pure. On analysis by autoradiography of reducing SDS gels, plasma incubated with cAMP dependent kinase and γ-^{32}P-ATP showed a single labelled band of 75 kD (Fig. 1a) as did the purified molecule when similarly treated (not shown). Typically this procedure resulted in the labelling of 0·5% of vitronectin molecules. Quantitative labelling of vitronectin typically led to the incorporation of a molar ratio of phosphate : vitronectin of 0·25 : 1 [19]. In each such experiment phosphorylation was confirmed by the incorporation of ^{32}P into the preparation. Vitronectin purified by urea treatment and heparin–Sepharose chromatography and subsequently iodinated contained only two significant labelled species (of 75 and 65 kD) on autoradiography of reducing SDS gels (Fig. 1b).

Chromatographic analysis of modified vitronectin

The effect of phosphorylation on the chromatographic behaviour of vitronectin was examined for both the native and urea-treated forms. On elution from heparin–Sepharose under native conditions using a salt gradient, phosphorylated vitronectin was not significantly separated from the antigenic peak. Other experiments revealed no significant differences between the elution profiles of the two forms of the native molecule on analysis by hydrophobic interaction and hydroxylapatite chromatography. Both iodinated and phosphorylated urea-treated vitronectin bound heparin–Sepharose tightly. Urea treated vitronectin consisted of a number of species displaying higher than normal molecular weights of up to 10^6 kD as previously reported [25] (not shown).

Incorporation into SC5b-9

In CVF-activated serum, ^{32}P-vitronectin was partially incorporated into a high mol. wt complex not seen in the control experiment, where serum was incubated with PBS alone (Fig. 2a). A MoAb specific for an epitope on neo-C9 (aE11) immunoprecipitated 84% of CVF-generated ^{32}P-vitronectin species of high mol. wt, confirming that this material was SC5b-9. When the peak fractions of the high mol. wt (1000 kD) and monomeric species were separately pooled and quantified by ELISA on SDS denatured preparations, there was a 2·2-fold specific increase in the proportion of ^{32}P-vitronectin to antigenic vitronectin in the high mol. wt region of the column profile. Purified ^{125}I and ^{32}P-labelled vitronectin were used to confirm this observation. The monomeric fractions of such preparations were added to serum, and incubated with and without added CVF. While some high mol. wt complexes formed in the control incubation (Fig. 2b), under the influence of CVF activation there was a significant (2·9-fold) increase in the ^{32}P : ^{125}I ratio in this region, when compared with that in the monomer (Fig. 2c).

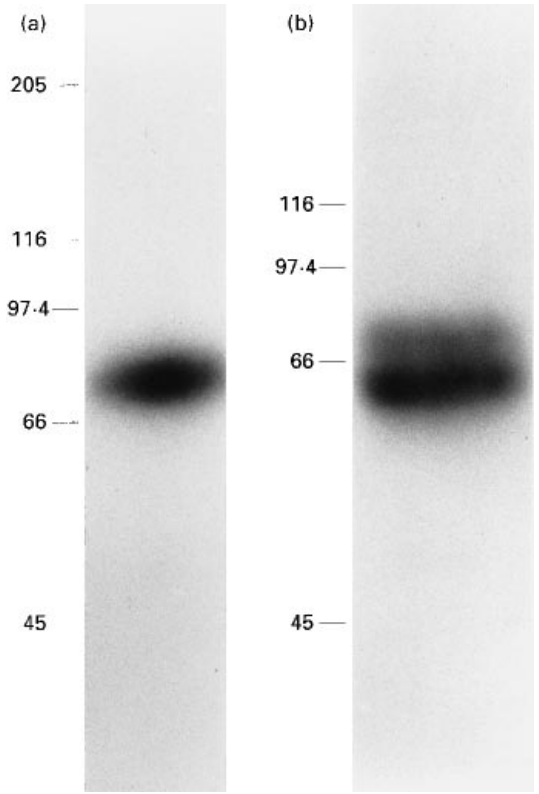

Fig. 1. Autoradiographs of reducing SDS gel analyses of (a) vitronectin selectively labelled in plasma by protein kinase A and γ-^{32}P-ATP, and of (b) vitronectin purified by heparin–Sepharose chromatography and labelled with ^{125}I.

Behaviour in vivo

Figure 3 shows the rate of disappearance of antigenically detectable human vitronectin and plasma protein-bound ^{32}P radioactivity in rabbits, one group of which received 0·25 mg of CVF immediately after the first blood sampling. In each case, there was an initial fall in concentration of the circulating protein, followed by a phase during which there was a slower, linear decline. The plasma $T_{1/2}$ of ^{32}P-vitronectin in control animals was $8{\cdot}87 \pm 0{\cdot}49$ h and did not differ significantly from the $T_{1/2}$ of all antigenically detectable molecules, i.e. $8{\cdot}00 \pm 1{\cdot}26$ h (Fig. 3a). However, the FCR of ^{32}P-vitronectin was significantly lower than that for vitronectin detectable by ELISA ($10{\cdot}85 \pm 0{\cdot}71$ %/h *versus* $18{\cdot}77 \pm 1{\cdot}57$ %/h, $P < 0{\cdot}005$). Similarly, ^{32}P-vitronectin had a significantly smaller extravascular distribution compared with the antigenically detectable species (i.e. EV/IV, median (range): 0·28 (0·15–0·36) *versus* 1·00 (0·48–1·6), $P < 0{\cdot}05$). Free ^{32}P represented approximately 10% of total plasma radioactivity in all animals and was released at a steady rate throughout the study period. Plasma volume was $37{\cdot}5 \pm 1{\cdot}8$ ml/kg ($n = 6$).

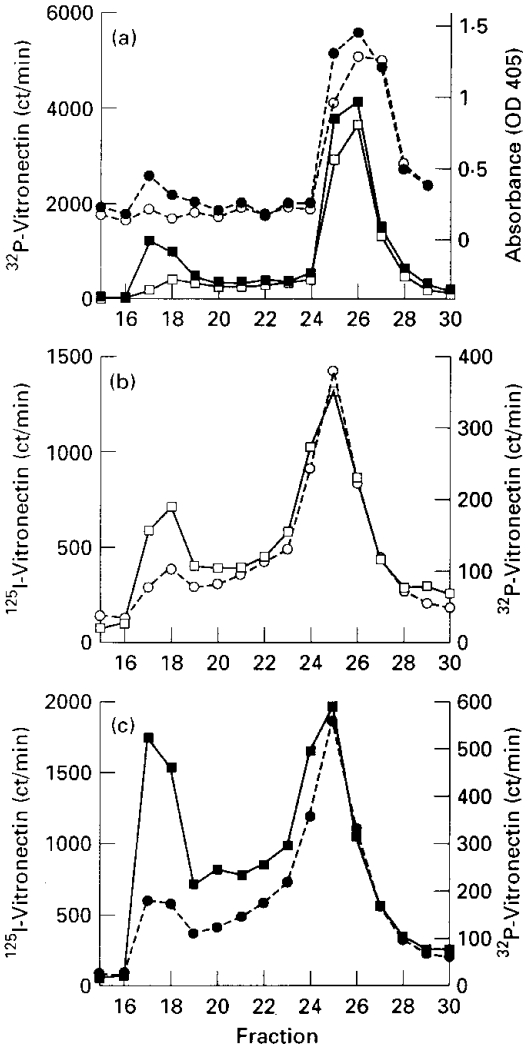

Fig. 2. The mol. wt distribution of phosphorylated vitronectin in serum before and after incubation with cobra venom factor (CVF) as analysed by elution with phosphate buffer from a Superose 12 size-sorting column. V_o is at fractions 16–17. (a) The distribution of ^{32}P-vitronectin (□) and antigenic vitronectin (○) in phosphorylated serum after incubation with PBS and for ^{32}P-vitronectin (■) and antigenic vitronectin (●) in phosphorylated serum after incubation with CVF. (b) The distribution of ^{32}P-vitronectin (□) and ^{125}I-vitronectin (○) in serum spiked with dual-labelled vitronectin after incubation with PBS. (c) The distribution of ^{32}P-vitronectin (■) and ^{125}I-vitronectin (●) in serum spiked with dual-labelled vitronectin after incubation with CVF.

After the injection of CVF, plasma disappearance of ^{32}P-vitronectin markedly increased, although the slopes of the final exponentials were similar to those for antigenically detected vitronectin. The behaviour of the latter in these animals was more variable and showed considerable overlap with normal animals (Fig. 3b,c). Analysis of plasma from control rabbits showed that the circulating ^{32}P-vitronectin remained predominantly monomeric for at least 7 h following injection, with no evidence of breakdown products. However, within 2 h of injection of CVF, a fraction of the ^{32}P-vitronectin was also present in a high mol. wt form (approximately 1000 kD; not shown).

Vitronectin which had been urea-activated was rapidly cleared from circulation, unlike native ^{131}I-RSA which followed a much slower time course in the same animals. This rapid clearance was observed for ^{125}I-vitronectin (Fig. 4), coinciding with the release of free ^{125}I, as well as for ^{32}P and antigenically detected species of urea-treated molecules (not shown). When a ^{125}I-labelled but partially multimeric preparation of native vitronectin was injected into two rabbits, there was also a large initial fall (70% of injected dose in 4 h) in circulating levels of vitronectin, with a concomitant increase in the release of free ^{125}I (up to 40%).

Tissue distribution

In two normal rabbits given urea-treated ^{125}I-vitronectin and native ^{131}I-RSA (as a marker of the plasma space), the concentration (ct/min) of protein-bound ^{125}I in the organs tested (i.e. liver, spleen, kidneys, lungs, heart, and skeletal muscle) varied between 2 and 29 times the plasma concentration (ct/min) at 6 h following injection (Fig. 5). ^{131}I-RSA concentration within organs was many-fold lower. The percentage of free ^{125}I in plasma rose to over 90% while free ^{131}I remained low (<10%). There was marked accumulation of protein-bound ^{125}I in the spleen (up to 29 × plasma concentration), the lung and the liver.

Effect on complement.

Complement-mediated lysis of erythrocytes was measured in sera where vitronectin had been quantitatively phosphorylated. No significant difference in CH50 was observed between sera where the vitronectin had been phosphorylated or incubated in the absence of kinase as a control. Similarly, purified urea-treated vitronectin retained unaltered its ability to inhibit C9-mediated lysis of EAC1-7 cells irrespective of prior quantitative phosphorylation (not shown).

DISCUSSION

These data show that vitronectin is a rapidly metabolized protein whose *in vivo* behaviour is influenced by complement activation, phosphorylation or prior activation *in vitro*. This rapid rate of disappearance was unlikely to be explained by denaturation as there was minimal *in vitro* manipulation of the preparation and no disproportionate release of ^{32}P immediately following injection. Both plasma vitronectin and the subset of phosphorylated vitronectin molecules were shown to be monomeric and to bind

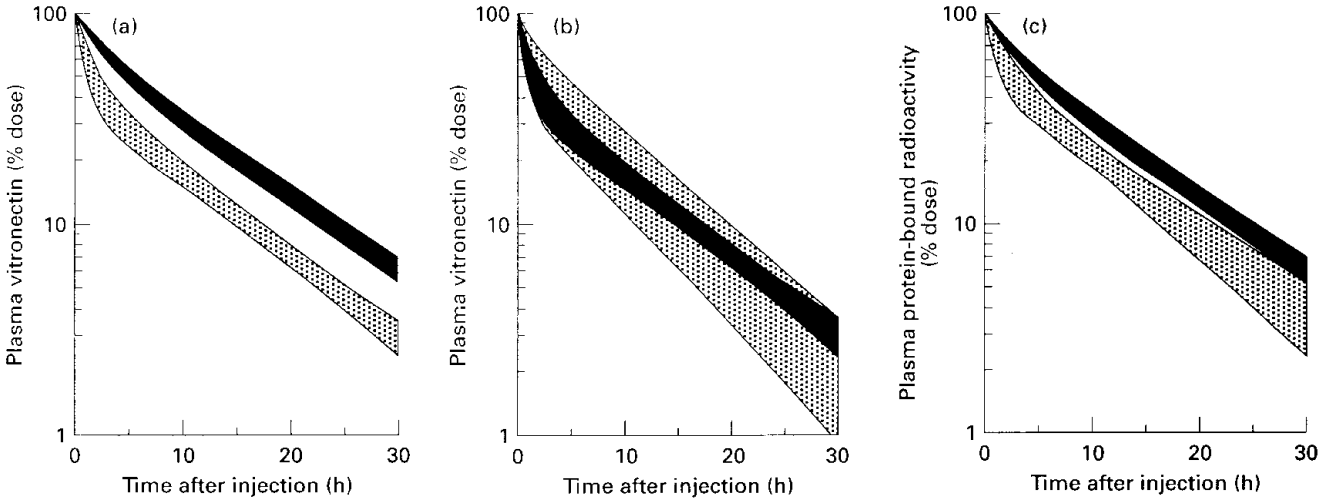

Fig. 3. (a) Plasma disappearance curves for vitronectin from phosphorylated plasma injected into rabbits at $t = 0$. Data shown are the ranges (mean ± s.d.) for protein-bound ^{32}P in four control rabbits (■), and for antigenically detected vitronectin in the same rabbits (▨). (b) Plasma disappearance curves for vitronectin from human plasma injected into rabbits. Data shown are the ranges (mean ± s.d.) for antigenically detected vitronectin in four rabbits injected at 10 min with PBS (■), or with CVF (▨). (c) Plasma disappearance curves for vitronectin from phosphorylated plasma injected into rabbits. Data shown are the ranges (mean ± s.d.) for protein-bound ^{32}P in four rabbits injected at 10 min with PBS (■), or with CVF (▨).

heparin–Sepharose poorly, so that the cryptic heparin-binding domain was not exposed as is the case for 'activated' forms of the molecule. Further, the low EV/IV ratio suggested that true catabolism rather than compartmental redistribution led to this result. FPLC gel filtration of plasma showed the phosphorylated vitronectin monomer to be the only major radioprotein present throughout the turnover period. Presumably this rapid plasma disappearance reflects the multifunctional nature of vitronectin with its capacity to be activated by a number of agents to form multimolecular complexes. In comparison with results obtained previously for other human complement proteins in rabbits such as C3 ($T_{1/2}$, 35–40 h [26]), and C9 ($T_{1/2}$, 20·6–29·5 h [13]), the plasma disappearance of vitronectin was more rapid. However, human fibronectin is another adhesive multifunctional protein which also has a short plasma $T_{1/2}$ (approx. 16 h) in rabbits [27].

The final exponentials for the decay of phosphorylated and antigenically-detectable plasma vitronectin were similar, but antigenically detectable vitronectin was more rapidly cleared from the intravascular space than the phosphorylated species (or was modified to a conformation not detectable by this ELISA). It is known that the phosphorylation of vitronectin is sensitive to its tertiary structure [28], and evidence shown in Fig. 1 demonstrated, for example, that the nicked 65 + 10 kD form of the molecule was present but not labelled with ^{32}P. Thus phosphorylation identifies a subset of all vitronectin species present, as may occur *in vivo* [11]. Nevertheless, it remains possible that phosphorylation altered the native molecule's functional characteristics, although not its

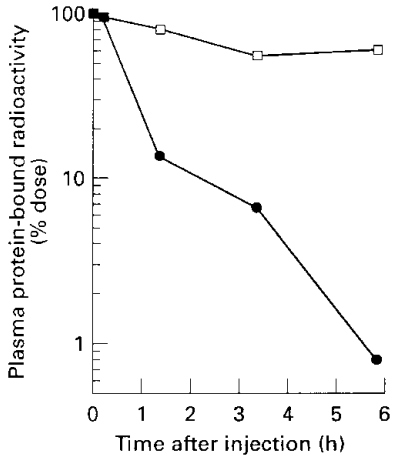

Fig. 4. Plasma disappearance curves for urea-treated ^{125}I-vitronectin (●) and ^{131}I-RSA (□) in a rabbit.

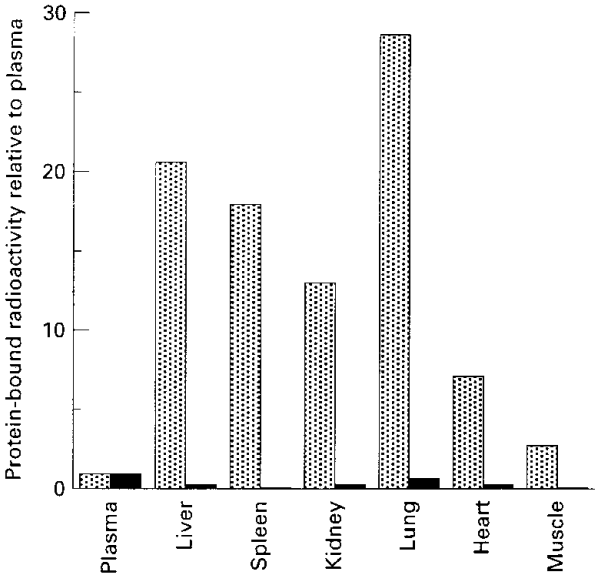

Fig. 5. Tissue protein-bound ^{125}I (▨) and ^{131}I (■) in a rabbit given urea-treated ^{125}I-vitronectin and ^{131}I-RSA and killed at 5 h. The results are expressed as (ct/min per g fresh tissue)/(ct/min per ml plasma).

affinity for heparin, so influencing its distribution and fractional catabolism. *In vitro*, phosphorylated vitronectin shows reduced affinity for PAI-1 [17]. The heparin-binding domain where phosphorylation of vitronectin by protein kinase A occurs is necessary for multimer formation and might stabilize the tertiary structure of the native form [25].

When the complement system was activated *in vivo* by CVF, there was a rapid fall in plasma levels of ^{32}P-labelled vitronectin. A high mol. wt species (approx. 1000 kD) consistent with SC5b-9 [13] and containing ^{32}P was also generated. These data are consistent with the observation of enhanced participation by phosphorylated vitronectin in the formation of SC5b-9 *in vitro*. Previous studies have demonstrated a similarity between the subunit composition of rabbit and human SC5b-9 [29]. Phosphorylated, iodinated and unlabelled native vitronectin were all capable of forming SC5b-9 *in vitro*. However, the degree of complement-mediated cell lysis by sera in which vitronectin had been quantitatively phosphorylated was essentially the same as that in unaltered sera, while phosphorylated purified vitronectin inhibited C9-mediated lysis in a similar fashion to the unmodified form. While the heparin-binding domain of vitronectin is involved in the inhibition of complement-mediated lysis [12], we have identified a major complement inhibitory region outside the heparin-binding domain in a 43 kD CNBr fragment (30). This polypeptide strongly inhibited the binding of C5b-7 to vitronectin as well as C5b-8-induced polymerization of C9. The inability of phosphorylation of a serine residue outside this fragment to strongly affect the interaction of vitronectin with complement is consistent with this finding.

Urea-treated vitronectin has been used in many studies [5,8,25] to mimic the activated form of the molecule and was shown in this and other studies to inhibit complement activity and to bind strongly to heparin–Sepharose. *In vivo*, the spleen, lung and liver were major sites of sequestration of urea-activated ^{125}I-vitronectin when compared with the distribution of ^{131}I-RSA. Antigenically detected and ^{32}P-labelled urea-activated preparations behaved similarly. We were unable, however, to calculate the true plasma $T_{1/2}$, FCR and EV/IV distribution for urea-activated vitronectin due to its rapid clearance and lack of equilibration across body compartments. A native but spontaneously multimerized ^{125}I-labelled preparation was also rapidly cleared from the circulation, with a concomitant increase in the release of free ^{125}I, so that the rapid clearance of urea activated vitronectin was not simply a function of its treatment. These and other data [13,26] demonstrate the sensitivity of *in vivo* metabolic parameters of proteins to their structure. We did not specifically investigate the fate of vitronectin sequestered by organs, but it remains likely that the spleen, lung and liver, or the reticuloendothelial elements therein, are sites of its metabolism.

Previous studies have shown SC5b-9 to be rapidly catabolized and taken up into the reticuloendothelial system [13]. Thus its behaviour is similar to that shown here for multimeric vitronectin, the form of the molecule active in binding PAI-1, cells, and matrices, and providing it with important roles in haemostasis, cell adhesion and migration. This rapid clearance and catabolism of vitronectin complexes is consistent with vitronectin's putative role as a scavenging molecule for activated ligands [3].

ACKNOWLEDGMENTS

We wish to acknowledge the National Health and Medical Research Council of Australia and the Australian Kidney Foundation for their financial support of this work.

REFERENCES

1 Preissner KT, Heimburger EA, Anders E, Muller-Berghaus G Physicochemical, immunochemical and functional comparison of human S-protein and vitronectin. Evidence for the identity of both plasma proteins. Biochem Biophys Res Commun 1986; **134**:951–6.

2 Hayashi M. Vitronectins: from vertebrates to invertebrates. In: Preissner KT, Rosenblatt S, Kost C, Wegerhoff, Mosher D, eds. Biology of vitronectins and their receptors. Amsterdam: Elsevier, 1993; 3–11.

3 Preissner KT, Jenne D. Structure of vitronectin and its biological role in haemostasis. Thromb Haemostasis 1991; **66**:123–32.

4 Preissner KT, Muller-Berghaus G. Neutralization and binding of heparin by S protein/vitronectin in the inhibition of Factor Xa by antithrombin III. Involvement of an inducible heparin binding domain of S protein/vitronectin. J Biol Chem 1987; **262**:12247–53.

5 Hess S, Stockmann A, Volker W, Preissner KT. Multimeric vitronectin: structure and function. In: Preissner KT, Rosenblatt S, Kost C, Wegerhoff J, Mosher D, eds. Biology of vitronectins and their receptors. Amsterdam: Elsevier. 1993; 21–9.

6 Bale MD, Wohlfahrt LA, Mosher D, Tomasini B, Sutton RC. Identification of vitronectin as a major plasma protein absorbed on polymer surfaces of different copolymer composition. Blood 1989; **74**:2698–706.

7 Yatohgo T, Izumi M, Kashiwagi H, Hayashi M. Novel purification of vitronectin from human plasma by heparin affinity chromatography. Cell Struct Funct 1988; **13**:281–92.

8 Volker W, Hess S, Vischer P, Preissner KT. Binding and processing of multimeric vitronectin by vascular endothelial cells. J Histochem. Cytochem. 1993; **41**:1823–32.

9 Declerck PJ, De Mol M, Alessi MC, Baudner S, Paques EP, Preissner KT, Muller-Berghaus G, Collen D. Purification and characterisation of a plasminogen activator inhibitor 1 binding protein from human plasma. Identification as a multimeric form of S protein (vitronectin). J Biol Chem 1988; **263**:25454–61.

10 Lawrence DA, Naski MC, Berkenpas MB, Palaniappan S, Podor TJ, Mosher DF, Ginsburg D. The interaction of plasminogen activator inhibitor-1 with vitronectin. In: Preissner KT, Rosenblatt S, Kost C, Wegerhoff J, Mosher D, eds. Biology of vitronectins and their receptors. Amsterdam: Elsevier, 1993; 303–10.

11 Shaltiel S, Schvartz I, Gechtman Z, Kreizman T. Covalent modulation of vitronectin structure of the control of plasminogen activation by PAI-1. In: Preissner KT, Rosenblatt S, Kost C, Wegerhoff J, Mosher D, eds. Biology of vitronectins and their receptors. Amsterdam: Elsevier. 1993; 311–24.

12 Podack ER, Muller-Eberhard HJ. Isolation of human S-protein, an inhibitor of the membrane attack complex of complement. J Biol Chem 1979; **254**:9908–14.

13 Greenstein JD, Peake PW, Charlesworth JA. The kinetics and distribution of C9 and SC5b-9 *in vivo*: effects of complement activation. Clin Exp Immunol 1995; **100**:40–6.

14 Bariety J, Hinglais N, Bhakdi S., Mandet C, Rouchon M, Kazatchkine M. Immunohistochemical study of complement S protein (vitronectin) in normal and diseased human kidneys: relationship to neoantigens of the C5b-9 complex. Clin Exp. Immunol. 1989; **75**:76–81.

15 Tschopp J, Masson D, Schafer S, Peitsch M, Preissner KT. The heparin binding domain of S-protein/vitronectin binds to complement components C7, C8, and C9 and perforin from cytolytic T-cells and inhibits their lytic activities. Biochemistry 1988; **27**:4103–9

16 McGuire EA, Peacock ME, Inhorn RC, Siegel NR, Tollefsen DM. Phosphorylation of vitronectin by a protein kinase in human plasma. Identification of a unique phosphorylation site in the heparin binding domain. J Biol Chem 1988; **263**:1942–5.

17 Shaltiel, S., Schvartz I., Korc-Grodzicki B., and Kreizman T. Evidence

for an extra-cellular function for protein kinase A. Mol Cell Biochem 1993; **128**:283–91.

18 Hayashi I, Mosher DF. Purification of stabilised native vitronectin. In: Preissner KT, Rosenblatt S, Kost C, Wegerhoff J, Mosher D. eds. Biology of vitronectins and their receptors. Amsterdam: Elsevier. 1993; 13–20.

19 Korc-Grodzicki B, Chain D, Kreizman T, Shaltiel S. An enzymatic assay for vitronectin based on its selective phosphorylation by protein kinase A. Anal Biochem 1990; **188**:288–94.

20 Marchalonis JJ. An enzymic method for the trace iodination of immunoglobulins and other proteins. Biochem J 1969; **113**:299–305.

21 McConahey PJ, Dixon FJ. A method of trace iodination of proteins for immunologic studies. Int Arch Allergy 1966; **29**:185–9.

22 Akama T, Yamada KM, Sero N, Matsumoto I, Koni I, Kashiwagi H, Fumaki T, Hayashi M. Immunological characterisation of human vitronectin and its binding to glycosaminoglycans. J Biochem 1986; **100**:1343–51.

23 Morris CA, Underwood PA, Bean PA, Sheehan M, Charlesworth JA. Relative topography of biologically active domains of human vitronectin. Evidence from monoclonal antibody epitope and denaturation studies. J Biol Chem. 1994; **269:**23845–52.

24 Milis L, Morris CA, Sheehan MC, Charlesworth JA, Pussell BA. Vitronectin-mediated inhibition of complement: evidence for different binding sites for C5b-7 and C9. Clin. Exp. Immunol. 1993; **92**:114–9.

25 Stockmann A, Hess S, Declerck R, Timpl R, Preissner KT. Multimeric vitronectin. Identification and characterisation of conformation-dependent self-association of the adhesive protein. J Biol Chem. 1993; **268**:22874–82.

26 Charlesworth JA, Timmermans V, Golding J, Campbell LV, Peake P, Pussell BA, Wakefield D, Howard N. The complement system in type 1 (insulin-dependent) diabetes. Diabetologia 1987; **30**:372–9.

27 Pussell BA, Peake PW, Brown MA, Charlesworth JA. Human fibronectin metabolism. J Clin Invest 1985; **76**:143–8,

28 Chain D, Korc-Grodzicki B, Kreizman T, Shaltiel S. The phosphorylation of the two-chain form of vitronectin by protein kinase A is heparin dependent. FEBS Lett. 1990; **269**:221–5.

29 Bhakdi S-., Hugo F, Tranum-Jensen J. Functions and relevance of the terminal complement sequence. Blut 1990; **60**:309-18.

30 Sheehan M, Morris, CA, Pussell, BA, Charlesworth JA. Complement inhibition by human vitronectin involves non-heparin binding domains. Clin Exp Immunol 1995; **101**:136–41.