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ARTICLE *in* BIOCHIMICA ET BIOPHYSICA ACTA · AUGUST 2001

Impact Factor: 4.66 · DOI: 10.1016/S0167-4838(01)00215-1 · Source: PubMed

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Kringles of the plasminogen–prothrombin gene family share conformational epitopes with recombinant apolipoprotein (a): specificity of the fibrin-binding site

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Received 15 February 2001; received in revised form 27 April 2001; accepted 2 May 2001

Abstract

Monoclonal antibodies directed against recombinant apolipoprotein (a) (r-apo(a)) lacking plasminogen-like KIV-2 repeats were used to identify structurally related conformational epitopes in various members of the plasminogen–prothrombin gene family. A number of procedures including a fibrin-binding inhibition immunoassay and surface plasmon resonance studies were used. Two antibodies (A10.1 and A10.4) recognised common conformational structures in r-apo(a), prothrombin, factor XII, plasminogen and its tissue-type and urokinase-type activators. In contrast, two other antibodies recognised specifically an epitope comprising residues of the lysine-binding site (A10.2) or close to it (A10.5) and inhibited the fibrin-binding function of r-apo(a) ($IC_{50} = 36$ pmol/l and 9.76 nmol/l, respectively). Interestingly, these antibodies distinctly recognised the elastase-derived fragments of plasminogen K4 (A10.2) and K1+2+3 (A10.5) without affecting plasminogen binding to fibrin. These results suggest that highly conserved conformational regions are common to various proteins of the plasminogen–prothrombin gene family and are in agreement with the concept that these proteins constitute a monophyletic group derived from an ancestral gene.

Keywords: Plasminogen; Apolipoprotein(a); Monoclonal antibodies; Lysine-binding site; Apo(a) inhibition; Kringle domain

1. Introduction

The apolipoprotein apo(a), plasminogen and other members of the plasminogen–prothrombin family involved in blood coagulation and fibrinolysis (prothrombin, factor XII, tissue- and urokinase-type plasminogen activators (t-PA and u-PA)) contain a structure termed 'kringle' (K) [1,2]. Kringles are modules with a common evolutionary origin, consisting of approx. 80 amino acid residues arranged in a triple-loop tertiary structure stabilised by three disulphide bridges. Their number and sequence identity shows variation among the different kringle-containing proteins (Table 1). Plasminogen, for instance, contains five different kringles among which K1 and K4 ensure its binding to fibrin and cell mem-

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0167-4838/01/$ – see front matter
PII: S0167-4838(01)00215-1

Table 1
Kringle-containing proteins of clotting and fibrinolysis systems

Protein	Number of kringles	LBS function
Plasminogen	5	K1, K4
Apo(a)	≈10 to ≈40	KIV-10/KIV-5 to KIV-8
Prothrombin	2	–
t-PA	2	K2
Pro-urokinase	1	–
Factor XII	1	–

Plasminogen-like apo(a) kringle IV (KIV) repeats are classified in 10 different types: KIV-1 to KIV-10.

brane proteins through lysine-binding sites (LBS) [3], thus allowing the formation of plasmin and thereby fibrinolysis and pericellular proteolysis [4]. Apo(a) contains multiple copies of plasminogen-like K4 followed by a single copy of K5 and a serine-protease domain (approx. 94% sequence identity) [5,6]. However, in contrast to plasminogen apo(a) cannot be transformed into an active proteolytic enzyme and is therefore not considered a zymogen [7]. Plasminogen-like kringle 4 copies in apo(a) are not identical; 10 different types, hereafter referred to as KIV-1 to KIV-10, have been recognised [8] and at least one of them, KIV-10, contains an LBS similar to that of plasminogen K4. The different KIV types are present in single copies in apo(a) except KIV-2, which varies in number giving rise to a series of isoforms of variable molecular weight [9]. Because of these structural homologies and enzymatic differences, apo(a) isoforms may be of pathophysiological relevance as they can bind to fibrin and cell surfaces and inhibit thereby plasmin formation [10,11]. Reagents that may specifically interfere with this inhibitory activity, i.e. monoclonal antibodies (mAbs), may be useful to preserve normal fibrinolysis. However, the large heterogeneity of apo(a) and its remarkable structural analogy with plasminogen may constitute an impairment to the development of specific mAbs. The structural particularities of apo(a) must therefore be considered in the choice of the antigen and in the development of specific and selective tests for hybridoma screening. Using a recombinant apo(a) lacking the KIV-2 repetitive sequence for the immunisation of mice, an ELISA test and a well characterised solid-fibrin immunoassay [12] for the selection of hybridomas, we have obtained a panel of five monoclonal antibodies to kringle modules of recombinant apo(a). We report here their reactivity with recombinant (r)-apo(a), plasminogen and its elastase-derived fragments and with other kringle-containing proteins, as well as its effects on the binding of r-apo(a) and plasminogen to fibrin.

2. Material and methods

2.1. Production and purification of recombinant apo(a)

The recombinant apo(a) A10 used for immunisation and screening of hybridomas was produced as described [13,14]. Briefly, a stable transfection of the cDNA expression plasmid pCMV-A10 encoding for the r-apo(a) A10 was prepared in the human embryonic kidney cell line 293 and cultured in a hollow fibre bioreactor. The expressed r-apo(a) A10 consists of a plasminogen-like serine-protease domain, kringle 5, and nine KIV repeats representing each of the KIV types (type 1 and types 3–10) except KIV-2 (Fig. 1). The culture medium was supplemented

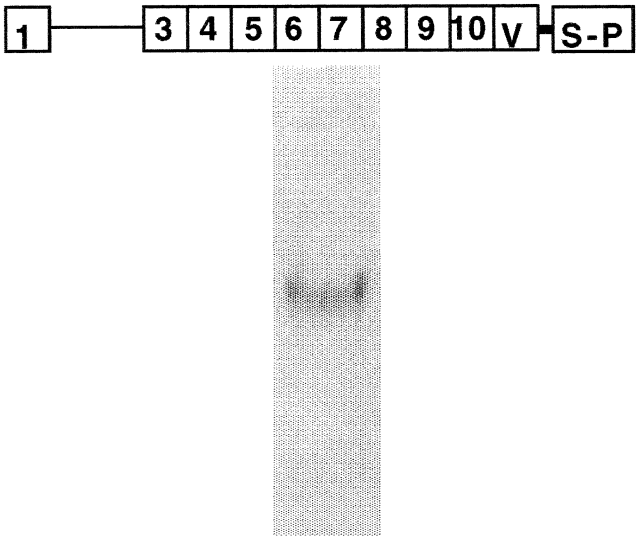

Fig. 1. Schematic representation and SDS–PAGE of apolipoprotein(a). The protein band represents purified r-apo(a) A10 stained with Coomassie Blue. Boxes and numbers represent the different types of plasminogen-like kringle 4 (KIV), kringle 5 (V) and the serine-proteinase (S-P) domain. The r-apo(a) A10 consists of the serine-protease domain, one copy of KV and nine KIV repeats including all KIV types but KIV-2.

with serine-proteinase inhibitors (20 kIU/ml aprotinin, 1 mmol/l aminoethylbenzenesulphonyl fluoride, 2 mmol/l EDTA and 0.01% (w/v) NaN_3, final concentrations) and used for the isolation of r-apo(a) by affinity chromatography on a Sepharose 4B-immobilised mAb (mAb a3) directed against human native apo(a) [15]. After sample application and washing, bound r-apo(a) was eluted with 20 mmol/l glycine–HCl, pH 2, containing 100 mmol/l NaCl, and fractions were collected in tubes containing a volume of 10 mmol/l Tris, pH 10, enough to neutralise the pH. Fractions containing the r-apo(a) were pooled, concentrated on dried polyethylene glycol 20,000 (Serva, France), dialysed against buffer A containing 2 mmol/l EDTA, and the concentration determined by the method of Lowry et al. [16] using purified human plasminogen as standard. The r-apo(a) preparation was more than 99% pure as assessed by SDS–PAGE followed by Coomassie blue staining, and by amino-terminal sequencing analysis using a microsequanator Applied Biosystem model equipped with a model 610A data analysis system.

2.2. Development of monoclonal antibodies

Balb/C mice were immunised with three subcutaneous injections of 30 μg r-apo(a) A10 at 2 week intervals. Serum titres were evaluated in a direct binding ELISA. Three days before fusion, the mouse with the highest antibody titre received a final intraperitoneal booster of 30 μg of r-apo(a) A10 in PBS. Spleen cells from this mouse were fused with the non-secreting mouse myeloma cell line P3/X63-Ag8.653 and hybridoma lines were grown and selected according to standard procedures [17]. Antibody-producing hybridomas were identified with a direct binding ELISA and positive clones were further analysed in an r-apo(a) fibrin-binding inhibition assay (see below). The cell lines of interest were cloned repeatedly by limiting dilution and expanded in large scale cultures using a bioreactor. Monoclonal antibodies were purified from the bioreactor extracapillary culture medium on immobilised protein A-Sepharose as described [18] with minor modifications. The isotypes of mAbs were determined by immunodiffusion using antisera directed against specific mouse immunoglobulins (IgG) (IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3) (Sigma) (Table 2).

Table 2
Isotype and affinity of monoclonal antibodies for r-apo(a) and plasminogen

mAb	Isotype	Dissociation constant (K_d, nmol/l)	
		r-apo(a)	Plasminogen
A10.1	IgG_{2b}	0.30 ± 0.05	2.21 ± 0.2
A10.2	IgG_1	0.19 ± 0.04	0.25 ± 0.03
A10.3	IgG_{2b}	8.86 ± 0.06	9.40 ± 0.1
A10.4	IgG_1	0.23 ± 0.03	15.78 ± 0.2
A10.5	IgG_1	0.17 ± 0.02	0.63 ± 0.08

2.3. Direct binding and fibrin-binding inhibition assays

The direct binding assay was performed using r-apo(a) immobilised on microtitre plates as follows. The r-apo(a) A10 was adjusted to a concentration of 4 μg/ml in 100 mmol/l bicarbonate buffer pH 8.5, and 100 μl of the solution was incubated overnight at 4°C on the wells of flexible microtitre polyvinyl chloride plates. Unbound protein was discarded and 150 μl of 1 mg/ml BSA in buffer A was added per well to saturate the plastic surface. A volume of 50 μl per well of culture supernatants was then incubated with the immobilised r-apo(a) A10. After 1 h at 37°C unbound antibody was removed and the surface was probed with an appropriate dilution (1/10 000) of a peroxidase-conjugated goat anti-mouse IgG (GamIg-PO). After 1 h incubation at 37°C, unbound conjugate was removed, a solution of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS, 1 mg/ml) was used as a substrate for colour development and the absorbance at A_{405nm} measured in a microplate counter. To explore if monoclonal antibodies may block the lysine-binding site of r-apo(a), positive clones were further analysed in a fibrin-binding inhibition immunoassay. For this purpose, intact fibrin and plasmin-degraded surfaces were prepared and characterised as previously described [19]. Briefly, purified fibrinogen [20,21] was covalently bound to immobilised polyglutaraldehyde derivatives, transformed to a fibrin surface by treatment with thrombin, and partially degraded with plasmin to expose carboxy-terminal lysines residues. Plasminogen and the r-apo(a) bind to these residues through LBS present in K4 and KIV-10, respectively. In a typical experiment recombinant apo(a) A10 (50 nmol/l) in

buffer A containing 40 g/l BSA was incubated for 30 min at room temperature with the hybridoma culture supernatants or with 200 mmol/l of 6-aminohexanoic acid (6-Ahx), a lysine analogue. The culture supernatant of the myeloma cell line P3/X63 was used as control. A volume of 50 μl/well of the different samples was then incubated for 1 h at 37°C with solid-phase fibrin to allow binding of residual r-apo(a). The plates were then washed and the amount of r-apo(a) bound detected with a sheep antibody directed against r-apo(a) A10 prepared at the Institut National de la Recherche Agronomique (Centre de Clermont-Ferrand-Theix, France) by immunising the animal with three subcutaneous injections of purified r-apo(a) A10. The secondary antibody was a peroxidase-labelled rabbit anti-sheep IgG and colour development was measured as indicated above. For each sample, specific inhibition of the binding of r-apo(a) to fibrin was determined by reference to the amount bound in the presence of P3/X63 culture medium. Complete inhibition of the binding was estimated by reference to the absorbance measured in the presence of 6-Ahx.

2.4. Analysis of the binding of mAbs to r-apo(a) and plasminogen

The assay was performed using r-apo(a) A10 and plasminogen immobilised on microtitre plates at 40 nmol/l as indicated above. A volume of 50 μl per well of purified monoclonal antibodies at varying concentrations in buffer A containing 40 mg/ml BSA was then incubated with the immobilised proteins. After 1 h at 37°C unbound antibody was removed by washing, the surface was probed with a GamIg-PO and colour development was measured as indicated above. Original raw data (A_{405nm}/min vs. mAb concentrations) were fitted to the Langmuir adsorption equation

$$(S{\cdot}X) = S_0\frac{K[X]}{1+K[X]} = \frac{S_0[X]}{[X]+K^{-1}} \quad (1)$$

for bimolecular interactions at heterogeneous interfaces as described, where $S{\cdot}X$ represents the equilibrium fraction of mAb bound to the immobilised antigens, S_0 the total amount of antigen binding sites, K the association constant of the mAb/antigen interaction and X the total input of mAb.

2.5. Reactivity of mAbs with kringle-containing proteins

All proteins were of human origin. Plasminogen, purified from fresh-frozen human plasma as previously described [22] with modifications [19], was lipoprotein (a) (Lp(a))-free and more than 99% pure as assessed by SDS–PAGE. Elastase-derived plasminogen fragments (K1+2+3, K4 and K5-serine-proteinase (K5-SP)) were prepared according to Sottrup-Jensen et al. [23] as previously described [24]. Prothrombin and its isolated kringle 2 fragment purified as described [25,26] were kindly provided by M.C. Guillin. Single-chain urokinase was a kind gift from H.R. Lijnen. Single-chain t-PA and factor XII were obtained from Biopool (Sweden) and Enzyme Research Laboratories (UK), respectively.

The proteins of interest, r-apo(a) A10, plasminogen, plasminogen fragments K1+2+3, K4 and K5-SP, prothrombin, factor XII, urokinase and t-PA were adjusted to 4 μg/ml in 100 mmol/l bicarbonate buffer, pH 8.5, and immobilised on flexible polyvinyl chloride microtitre plates as indicated above. A volume of 50 μl/well of the different mAbs at 10 μg/ml in buffer A containing 40 mg/ml BSA was incubated with the immobilised proteins for 1 h at 37°C. After washing the plates with assay buffer, bound mAbs were detected by adding 50 μl/well of the GamIg-PO solution as described above.

2.6. Biosensor studies

To further investigate the specificity of mAbs A10.2 and A10.5 on the inhibition of r-apo(a) binding to fibrin, we analysed the effect of 6-Ahx on the recognition of r-apo(a) by these mAbs using a BIAcore 2000 system. Sensor chips CM5, amine coupling reagents and an immunosorbent purified rabbit IgG directed against mouse Fc (RamFc), all from Biacore (Uppsala, Sweden) were used. RamFc was coupled to the sensor chip as suggested by the manufacturer. An analytical immunocapture cycle consisted of injection of mAbs (1 μg/ml) followed by a 10 μl injection over the immobilised mAb of r-apo(a) at 50 nmol/l previously preincubated (30 min at 22°C) with 0–50 mmol/l of 6-Ahx. After each cycle the sensor chip was regenerated by injection of 10 μl of 40 mmol/l HCl. This treatment did not denature the

RamFc immobilised onto the sensor surface as shown by equivalent signals on repetitive mAb injections. Experiments were performed at 25°C and a flow rate of 10 μl/min using 10 mmol/l HEPES, pH 7.4, containing 150 mmol/l NaCl, 3.4 mmol/l EDTA and 0.005% (v/v) Tween 20. Controls were performed in parallel using an unrelated mAb (7D4, directed against PAI-1) [27]. In all experiments, the number of RU (amount bound) at equilibrium and at 30 s after the end of the injection was recorded and the RU counts of the control subtracted.

3. Results

3.1. Characteristics of the binding of mAbs to r-apo(a) A10 and plasminogen

Five mAbs were selected, purified and characterised (Table 2). Binding parameters of these mAbs to r-apo(a) and plasminogen were determined using trace amounts of immobilised antigen relative to the varying concentrations of mAbs in solution. Apparent dissociation constants (K_d) were calculated by fitting raw data to the simple Langmuir equation for interaction at interfaces as described by Fleury and Anglés-Cano [19]. The calculated dissociation constants are depicted in Table 2. The affinity of most mAbs for r-apo(a) was in the same order of magnitude (10^{-10} M) except for mAb A10.3 which was 30–50-fold lower. All mAbs recognised plasminogen immobilised on microtitre plates and showed a large variation in affinity that was ranked as follows: A10.2 > A10.5 > A10.1 > A10.3 > A10.4. However, mAbs A10.2 and A10.3 displayed differences in affinity for plasminogen that were similar to those found for r-apo(a). The affinity of mAb A10.5 for plasminogen was close to the affinity of mAb A10.2 whereas the affinities of mAb A10.1 and A10.4 were 10–60-fold lower. Reduction of r-apo(a) resulted in complete loss of reactivity with the mAbs as assessed by immunoblotting (data not shown), thus suggesting that all target epitopes are conformational and that linear sequences are not identified with these mAbs.

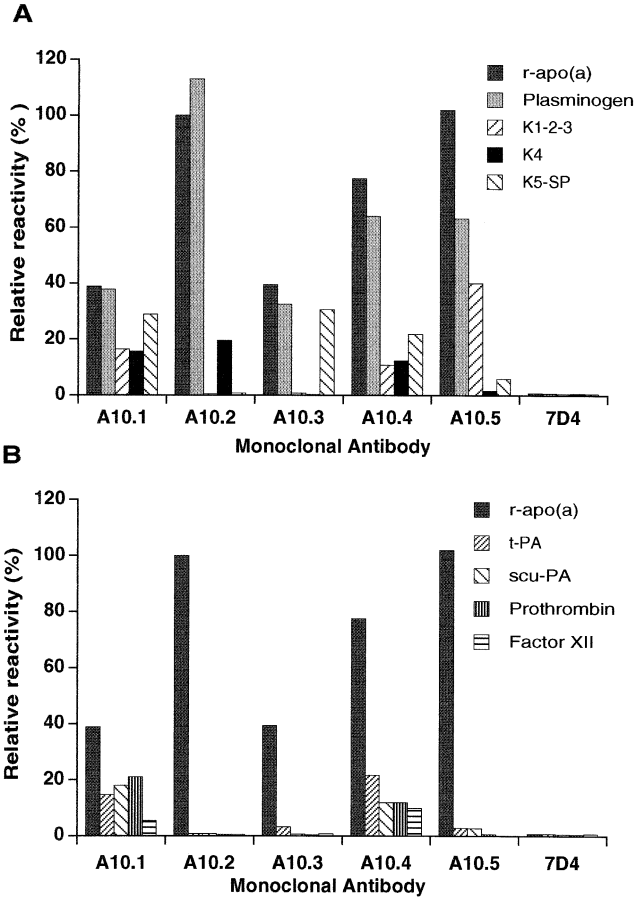

Fig. 2. Binding of mAbs raised against r-apo(a) A10 to kringle-containing proteins. Antigens passively adsorbed to plastic (2.5 μg/ml) were r-apo(a), plasminogen and its fragments (K1+2+3, K4, K5-SP) (A), single-chain urokinase- and tissue-type plasminogen activators (scu-PA, t-PA), prothrombin and factor XII (B). The immobilised proteins were incubated with mAbs at 10 μg/ml. An unrelated mAb (7D4) directed against PAI-1 was used as control [27]. Bound antibodies were detected with a peroxidase-conjugated goat anti-mouse antibody. Results are expressed by the relative reactivity (%) of mAbs compared to the reactivity of mAb A10.2.

Table 3
Inhibition of r-apo(a) and plasminogen binding to fibrin (% inhibition)

mAbs	A10.1	A10.2	A10.3	A10.4	A10.5	6-Ahx
r-apo(a) A10	5.8	98	4.5	4.6	27	100
Plasminogen	5.5	0	2.3	3.9	0	100

Digits represent the relative inhibition (%) of r-apo(a) and plasminogen binding to fibrin by reference to total (100%) inhibition of binding in the presence of 6-Ahx, a lysine analogue. Plasminogen and r-apo(a) at 66 nmol/l were incubated with the mAbs at 130 nmol/l or 6-Ahx at 0.2 mol/l, and then with fibrin. Bound proteins were detected as indicated in Section 2. Coefficient of variation of the assay was approx. 8%.

3.2. Cross-reactivity of mAbs with kringle-containing proteins

Data on the reactivity of purified mAbs towards various kringle-containing proteins immobilised on microtitre plates are represented in Fig. 2. Immobilisation of antigens to the plates was verified using polyclonal antibodies directed against the proteins of interest. The assay was then performed using mAbs at a concentration (10 μg/ml) at least 250-fold greater than their corresponding K_d for r-apo(a) A10 (Table 1). This concentration ensures detection of low affinity interactions with the kringle-containing proteins. Under these conditions it could be established, as shown in Fig. 2 and as expected from the data in Table 1, that all anti-r-apo(a) mAbs also recognised plasminogen. It can be observed (Fig. 2A) that using elastase-derived plasminogen fragments, specific epitopes were localised on isolated K4 for mAb A10.2, on fragment K1+2+3 for mAb A10.5 and on fragment K5-SP for mAb A10.3. The lower reactivity of A10.2 observed for isolated K4 as compared to plasminogen and r-apo(a) is likely due to its weaker binding to the plate. The specific reactivity of mAb A10.2 with isolated K4 was verified in surface plasmon resonance studies using A10.2 immobilised onto a RamFc sensor chip. In contrast to the restricted specificity of mAb A10.2, mAbs A10.1 and A10.4 recognised with a variable avidity an antigenic determinant present in all tested kringle-containing proteins (urokinase, t-PA, prothrombin, factor XII) except prothrombin fragment 2 (data not shown) (Fig. 2B).

3.3. Effect of mAbs on the binding of r-apo(a) to fibrin

To further explore if the mAbs reacting with r-apo(a) may block the lysine-binding site of this molecule, fibrin-binding experiments were performed as indicated in Section 2. The r-apo(a) and plasminogen were incubated with varying concentrations of the different mAbs. The unrelated mAb 7D4 directed against anti-PAI-1 was used as control. These solutions were then incubated with a fibrin surface and the amount of r-apo(a) and plasminogen bound detected with specific polyclonal antibodies. Results for r-apo(a) and plasminogen are shown in Table 3. The amount of these proteins bound to fibrin in the presence of mAb 7D4 was considered 100% binding and used as reference to calculate the percent inhibition of the binding by the mAbs. The binding of plasminogen was not affected. In contrast, mAb A10.5 and mAb A10.2 produced, respectively, partial and com-

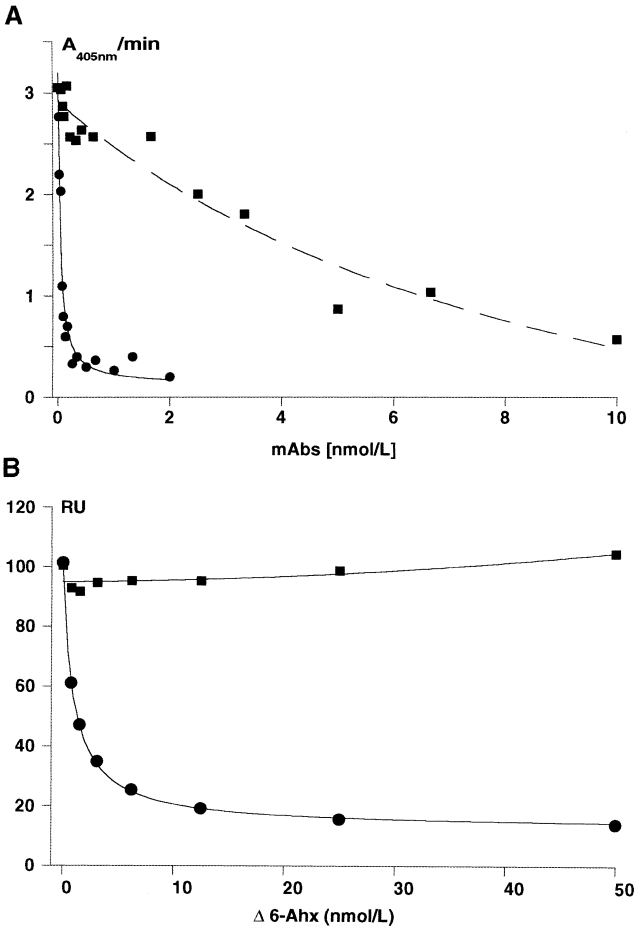

Fig. 3. Fibrin-binding inhibition immunoassay and surface plasmon resonance analysis. (A) Inhibition of the binding of r-apo(a) A10 to fibrin by mAbs A10.2 (●) and A10.5 (■). Serial dilution of each mAb and a fixed concentration of r-apo(a) A10 (1 nmol/l) were incubated with fibrin and the amount of r-apo(a) bound detected. Data were fitted to a hyperbolic decay equation which allows calculation of the concentration of mAb that inhibits 50% of the binding: $IC_{50} = 36$ pmol/l (A10.2) and 9.76 nmol/l (A10.5). (B) Effect of 6-Ahx on the interaction of r-apo(a) with mAbs A10.2 (●) and A10.5 (■) analysed by surface plasmon resonance. Increased concentrations of 6-Ahx were incubated with r-apo(a) A10 (50 nmol/l) for 30 min at 25°C and then injected on a RamFc sensor chip containing the immobilised mAbs. The graph shows a complete inhibition of the interaction of r-apo(a) with immobilised A10.2 by 6-Ahx ($IC_{50} = 0.98$ mmol/l).

plete inhibition of the binding of r-apo(a) to fibrin, thus suggesting differences in their inhibitory mechanism. Fig. 3A shows that inhibition of the binding of r-apo(a) to fibrin was dose-dependent with differences in the inhibitory strength (IC_{50} = 36 pmol/l for mAb A10.2 and 9.76 nmol/l for mAb A10.5). Only mAb A10.2 was able to produce an inhibition similar to that produced by 6-Ahx, thus suggesting a similar interaction with r-apo(a). To further investigate this hypothesis, competitive experiments with the lysine analogue were performed using biosensor analysis. Fig. 3B shows that 6-Ahx was able to produce a complete inhibition of the interaction of mAb A10.2 with r-apo(a) (IC_{50} = 0.98 mmol/l) whereas no effect on the interaction with mAb A10.5 could be detected (Fig. 3B). These data indicate that mAb A10.2 interacted directly with the LBS in r-apo(a) whereas mAb A10.5 recognised an epitope outside of the LBS but close enough to induce a weak effect on the binding of r-apo(a) to fibrin.

4. Discussion

Monoclonal antibodies directed against apo(a) have been produced by several groups and used in either assay methods for measurement of Lp(a) [28–30] or as analytical tools [31–33]. In the present study our aim was to develop a strategy that may allow production of Lp(a)-specific inhibitory mAbs. Because in the Lp(a) particle the variable number of KIV-2 repeats may modulate the lysine binding function of apo(a) [34–36], the type and number of kringles was considered critical in the choice of a suitable apo(a) preparation for immunisation. Since separation of apo(a) from apo B100 with reducing agents may modify antigenic determinants of interest, a r-apo(a) which lacks the repetitive sequence of plasminogen-like KIV-2 was purposively selected as antigen. The production of mAbs directed against KIV types present as single copies in the sequence of this molecule (Fig. 1) may thus be favoured. Another key aspect in this work was the combination of a classical ELISA with a fibrin-binding inhibition assay for the screening of hybridomas. This system has already been successfully used for (1) the screening of mAbs directed against proteins interacting with fibrin [12] and (2) the detection of autoantibodies directed against receptor-induced neoepitopes in t-PA and plasminogen [37]. Five different mAbs (A10.1–A10.5) which recognise r-apo(a) and plasminogen were thus produced and selected. The affinity of these mAbs but one (A10.3) for r-apo(a) was similar. A moderate range variation in affinity was, however, observed for plasminogen (Table 2) which was targeted at specific kringle domains by three mAbs (mAb/plasminogen fragment: A10.2/K4, A10.3/K5-SP, A10.5/K1+2+3). Of note is that even if plasminogen kringles K1–K3 are not represented in apo(a), mAb A10.5 was able to recognise an epitope present in plasminogen fragment K1+2+3. Two other mAbs (A10.1, A10.4) cross-reacted with epitopes common to different kringle-containing proteins including t-PA, u-PA, prothrombin and factor XII, thus indicating that epitopes present in r-apo(a) kringles are not only close to those of plasminogen but are also present in other kringle-containing proteins. Furthermore, the absence of reactivity with prothrombin fragment 2 (data not shown) suggests that these mAbs recognise an antigenic determinant present in the single kringle of urokinase and factor XII, and in prothrombin kringle 1. All epitopes targeted by these mAbs were conformational as suggested by the absence of reactivity with reduced proteins. These data suggest that a high degree of conservation of some residues in these kringles resulted in conformational epitopes similar to those of plasminogen-like kringles in apo(a) which are recognised by mAbs A10.1 and A10.4. The present study is the first report of shared conformational epitopes among kringles of the plasminogen–prothrombin gene family. These data provide biological support to the statistically low sequence homology concept that kringles present in different clotting and fibrinolytic proteins of the plasminogen–prothrombin family constitute a monophyletic group derived from an ancestral gene containing a single-kringle and serine-protease regions [2,38]. In contrast, the development of a specialised binding function is a distinctive characteristic of some kringles as indicated by the ability of mAb A10.2 to inhibit r-apo(a) binding to fibrin with high affinity (IC_{50} = 36 pmol/l) (Fig. 3A). Of note, plasminogen binding to fibrin was not affected by this mAb (Table 3), most probably as a result of the LBS activity of kringle 1 which is not a target for mAb A10.2. Occupancy of the LBS of r-apo(a) KIV-10 with 6-Ahx

(IC_{50} = 0.98 mmol/l) completely blocked the interaction of r-apo(a) with mAb A10.2 immobilised on a RamFc sensor chip, thus indicating that the epitope is localised within the LBS. The fibrin-binding function of r-apo(a) was weakly inhibited by mAb A10.5 (IC_{50} = 9.76 nmol/l). However, 6-Ahx did not affect the interaction with r-apo(a), thus suggesting that inhibition of fibrin binding was due to conformational changes affecting the LBS function and not to a direct interaction with the LBS. In conclusion, our data indicate that these proteins share highly conserved conformational regions, and emphasise the structural specificity of functional sites in some kringles.

Acknowledgements

This research was supported by project 4N001B (Réseau Nord-Sud) from the Institut National de la Santé et de la Recherche Médicale and by grant ACCSV No. 9 from the Ministère de la Recherche (France). M. Dominguez is a recipient of a research scholarship from CONACyT (Mexico). We are grateful to D. Durand (Institut National de Recherche Agronomique, Centre de Clermont-Ferrant-Theix, France) for the preparation of the polyclonal antibody against r-apo(a). We gratefully acknowledge J. Lechevalier for his editorial assistance.

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