PROTEINS: Structure, Function, and Genetics 12:188–199 (1992)

A Structural Assessment of the Apo[a] Protein of Human Lipoprotein[a]

Juan Guevara, Jr.,[1] Roger D. Knapp,[1] Sandra Honda,[2] S. Robert Northup,[1] and Joel D. Morrisett[3]
Departments of [1]Medicine, [2]Cell Biology, and [3]Biochemistry, Baylor College of Medicine, Houston, Texas 77030

ABSTRACT **Apolipoprotein[a], the highly glycosylated, hydrophilic apoprotein of lipoprotein[a] (Lp[a]), is generally considered to be a multimeric homologue of plasminogen, and to exhibit atherogenic/thrombogenic properties. The cDNA-inferred amino acid sequence of apo[a] indicates that apo[a], like plasminogen and some zymogens, is composed of a kringle domain and a serine protease domain. To gain insight into possible positive functions of Lp[a], we have examined the apo[a] primary structure by comparing its sequence with those of other proteins involved in coagulation and fibrinolysis, and its secondary structure by using a combination of structure prediction algorithms. The kringle domain encompasses 11 distinct types of repeating units, 9 of which contain 114 residues. These units, called kringles, are similar but not identical to each other or to PGK4. Each apo[a] kringle type was compared with kringles which have been shown to bind lysine and fibrin, and with bovine prothrombin kringle 1. Apo[a] kringles are linked by serine/threonine- and proline-rich stretches similar to regions in immunoglobulins, adhesion molecules, glycoprotein Ib-α subunit, and kininogen. In comparing the protease domains of apo[a] and plasmin, apo[a] contains a region between positions 4470 and 4492 where 8 substitutions, 9 deletions, and 1 insertion are apparent. Our analysis suggests that apo[a] kringle-type 10 has a high probability of binding to lysine in the same way as PGK4. In the only human apo[a] polymorph sequenced to date, position 4308 is occupied by serine, whereas the homologous position in plasmin is occupied by arginine and is an important site for proteolytic cleavage and activation. An alternative site for the proteolytic activation of human apo[a] is proposed.**

Key words: apolipoprotein[a], lipoprotein[a], plasminogen, kringle, prothrombin, lysine-binding site

INTRODUCTION

In man, elevated serum levels of lipoprotein[a] (Lp[a]) have been correlated with the incidence of coronary and cerebral artery disease,[1–4] however, the physiologic role(s) of Lp[a] remains unknown, although several positive functions for it have been suggested.[5] Lp[a] contains two distinctly different proteins. Apo[a][6–11] is a very large, highly glycosylated protein which appears to have little, if any, affinity for lipid; it is disulfide-linked to one molecule of apo B-100, possibly at CYS_{3734}.[12] Apo B-100[13–17] is highly hydrophobic and is partly imbedded in a lipid-rich, pseudo-micellar particle containing mostly phospholipid and cholesteryl ester. Apo[a] occurs in at least 11 different polymorphic forms, ranging in molecular weight from about 419,000 to 838,000.[11] The mass polymorphism of apo[a] may reflect varying degrees of glycosylation (up to 35%),[13,18] and/or differing lengths of the polypeptide.[19,20] The amino acid sequence of a polymorph of about 530 kDa has been inferred from the nucleotide sequence of the cDNA.[21]

The primary structure of apo[a] consists of two distinct polypeptide domains: a kringle-containing domain, and a protease domain. The interkringle segments of apo[a] have some similarity to threonine- and proline-rich regions contained in adhesion glycoproteins and immunoglobulin proteins. Further, the smaller arginine–glycine–aspartate ("RGD") adhesion motif present in many of the macromolecules involved in thrombosis[22–24] is also present in one of the kringles of apo[a]. The presence of these sequence motifs and structural features in apo[a] suggests a role for Lp[a] in thrombosis and/or fibrinolysis. Several reports have described results indicating that Lp[a] reduces binding of plasminogen to its receptor, to fibrin, and to enzymes which cleave plasminogen to form active plasmin, thereby

Received January 29, 1991; revision accepted May 31, 1991.
Address reprint requests to Dr. Joel D. Morrisett, The Methodist Hospital, MS A601, 6565 Fannin Street, Houston, TX 77030.

Abbreviations: Apo[a], apoprotein[a]; BPTK1, bovine prothrombin kringle 1; CF1, Chou–Fasman algorithm; F12K1, Factor XII kringle 1; GAR, Garnier; TAY, Taylor; G/R, Garnier/Robson; GPIb-α, glycoprotein Ib-α chain; HGF, human hepatocyte growth; HGFK1, human hepatocyte growth factor kringle 1; HPTK1, human prothrombin kringle 1; HU, human; ICAM, intercellular adhesion molecule; IgA, immunoglobulin A; IgG(CL), immunoglobulin G light chain; KNGH, kininogen high-molecular mass; LPaK1, lipoprotein[a] kringle 1; PGK1, plasminogen kringle 1; PLM, plasmin; tPA, tissue plasminogen activator; tPAK1, tissue plasminogen activator kringle 1; UK, urokinase; and UKK1, urokinase kringle 1.

interfering with the normal process of fibrinolysis.[25–30] Based on these results one might conclude that Lp[a] is strictly an atherogenic, perhaps suicidal, protein.

In the kringle domain of the 530-kDa apo[a] polymorph, there are 11 different kringle types. The second kringle type to occur (Lpak2) is repeated 28-fold. A kringle is a noncatalytic, recognition and binding polypeptide which forms a highly conserved, tri-loop structure stabilized by three disulfide bridges. Kringles of proteins involved in thrombosis and fibrinolysis contain 78–82 residues.[31–34] Apo[a] kringle types 1 through 10 (Lpak1-Lpak10) are highly homologous but not identical to plasminogen kringle 4 (PGK4). The kringles are separated by a Ser/Thr- and Pro-rich region.

In an effort to elucidate a pathophysiologic role for Lp[a], our laboratory has demonstrated recently that plasma Lp[a] levels are positively correlated with documented coronary artery disease,[3] and are markedly reduced in aggressively treated type II diabetics,[35] and in patients who have undergone cardiac transplantation.[36] Apo[a] has been found to colocalize with apoB in aortocoronary bypass vein grafts[37] and the aorta,[38] but the identification of a positive role for Lp[a] has been elusive. To gain insight into possible positive functions of the apo[a] protein of Lp[a], we have systematically examined its primary structure by sequence comparison and its secondary structure by using a combination of predictive algorithms. We have compared each apo[a] kringle with kringles which have been shown to bind lysine (Lys), ϵ-aminocaproic acid (EACA), and fibrin,[39–45] and with prothrombin kringle 1.[46,47]

SYSTEMS AND METHODS

The cDNA inferred amino acid sequence of one polymorph of apo[a][21] was examined by sequence alignment and compared with that of human PGK1, PGK4, PGK5, tissue plasminogen activator kringle 2 (tPAK2), and bovine prothrombin kringle 1 (BPTK1). In addition, several structural prediction algorithms[48–50] were used to determine the probability for secondary structure within the apo[a] kringles. First, the method was applied to BPTK1 in order to compare predicted secondary structures with known structures determined previously by X-ray crystallography.[46,47] In our analysis, secondary structure probability was determined on the basis of average values for 15 amino acids comprising a moving window, i.e., seven residues preceding and seven succeeding a selected amino acid. The reliability value for the predicted β-turn or β-sheet was determined by multiplying the number of residues in the segment by 4, the number of different algorithms used, then dividing the number of accurate predictions by that number. For example in BPTK1, β-turn 4 composed of YPHK generates 16 values, however, only the modified version of the Chou–Fasman algorithm, CF1, predicts the involvement of YP in a turn. Therefore, 2/16 yields a reliability factor of 12.5% for the occurrence of a turn in this segment of BPTK1. A zero probability for β-sheet is predicted for this segment.

RESULTS AND DISCUSSION

The sequence of the apo[a] that has been cloned is believed to be composed of two distinct domains: a kringle domain and a protease domain. The kringle domain contains 11 distinct types of repeating units called kringles, which are similar but not identical to each other or to PGK4 (Fig. 1). Each kringle is flanked by a leading and a trailing peptide which all together span 114 residues; we have designated this structure as an extended kringle and have divided it into seven segments. Segment 1 is the leading 11-residue polypeptide which is followed by a 78- or 80-residue kringle that contains segments 2 through 6. Segment 7 is the 25-residue, postkringle polypeptide. The Ser/Thr- and Pro-rich interkringle regions are composed of segments 7 and 1.

Kringles

A systematic comparison based on sequence alignment of apo[a] kringles with other kringles in proteins involved in hemostasis and fibrinolysis was made at two levels. The first comparison, shown in Table I, is based on similarity to PGK1 and other kringles known to have binding affinity for lysine and/or fibrin.[51–53] In Table I, positions are numbered to account for insertions and best residue alignment to allow comparison of apo[a] kringles with other known human kringles and BPTK1. Apo[a] kringle types 1 through 10 (numbered in order of their occurrence in the sequence) contain 78 amino acids between the first and sixth Cys, while kringle type 11 contains 80 amino acids between these two sites and is 94% similar to plasminogen kringle 5. Apo[a] kringles are listed in order of decreasing homology with other kringles such as PGK1, PGK4, tPAK2, and PGK5, which are known to bind lysine and/or fibrin.

Our comparative analysis was used to identify nonconservative alterations such as deletions, insertions, and/or substitutions in the sequence which might influence intrakringle interactions and ligand binding. We have found at least 32 residues in these kringles that are conserved and may be essential for the intrakringle interactions involved in binding lysine, EACA, and/or fibrin. PGK1 and PGK4, kringles which bind lysine and/or fibrin with relatively high affinity, share 35 residues. Furthermore in PGK1, Arg-32, Arg-34, Asp-61, and Arg-76 are known to be essential for high affinity-binding of lysine and/or fibrin.[44,45,54] Conservation of these critical residues was used as a criterion for assessing the probability that a kringle binds lysine and/or

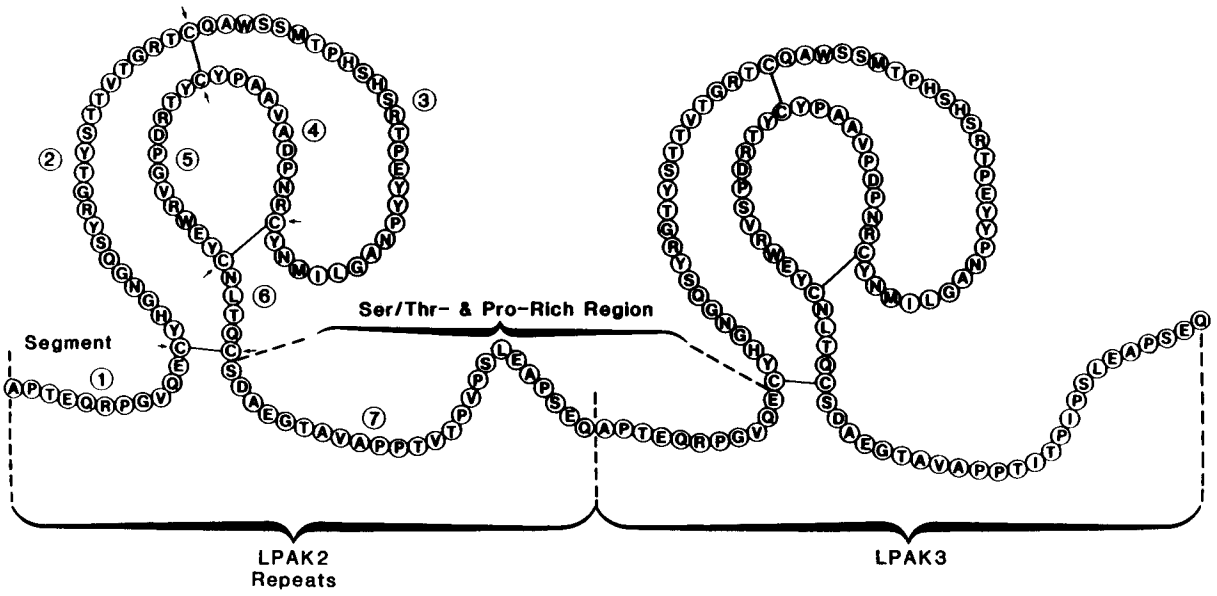

Fig. 1. Proposed Lp[a] kringle units. The typical Lp[a] kringle unit can be divided into seven segments: The prekringle segment 1 consists of 11 amino acids; segments 2 through 6 form the 78-residue kringle with the proposed three-disulfide bonds characteristically observed in other kringles; and the postkringle segment 7 consists of 25 amino acids. Segments 7 and 1, the interkringle polypeptide sequences, are rich in Ser, Thr, and Pro residues. Illustrated in this figure are Lp[a] kringle types 2 and 3, LPAK2 and LPAK3, respectively. LPAK2 is a highly repeated unit within the apo[a] protein of Lp[a].

TABLE I. Comparison of Lysine-Binding Kringles to Other Kringles in Human Proteins and Bovine Prothrombin Kringle 1*

```
          1        10        20        30        40        50        60        70        80
PGK1      CKTGDGKNYRGTMSKTKNGITCQKWSSTSPHRPR-FS---PATHPSEGLE-ENYCRNPDNDPQGPWCYITDPEK-R-YDYCDILEC
PGK4      CYHGDGQSYRGTSSTTTTGKKCQSWSSMTPHRHQK-T---PENYPNAGLT-MNYCRNPDADK-GPWCFTTDPS-VR-WEYCNLKKC
PGK5      CMFGNGKGYRGKRATTVTGTPCQDWAAQEPHRHSIFT---PETNPRAGLE-KNYCRNPDGDVGGPWCYTTNPRKL--YDYCDVPQC
tPAK2     CYFGNGSAYRGTHSLTESGASCLPWNSMILIGKV-YTAQNPSAQA-LGLGKHNYCRNPDGDAK-PWCHVLKNRRL-TWEYCDVPSC
LPaK10    CYHGNGQSYRGTFSTTVTGRTCQSWSSMTPHRHQR-T---PENYPNDGLT-MNYCRNPDAD-TGPWCFTMDPS-IR-WEYCNLTRC
LPaK11    CMFGNGKGYRGKKATTVTGTPCQEWAAQEPHRHSTFI---PGTNKWAGLE-KNYCRNPDGDINGPWCYTMNPRKL--FDYCDIPLC
F12K1     CYDGRGLSYRGLARTTLSGAPCQPWASEATYRNVTA----EQARNW-GLGGHAFCRNPDNDIR-PWCFVLNRDRL-SWEYCDLAQC
LPaK8     CYRGDGQSYRGTLSTTITGRTCQSWSSMTPHWHRR-I---PLYYPNAGLT-RNYCRNPDAEIR-PWCYTMDPS-VR-WEYCNLTRC
LPaK6     CYHGDGQSYRGSFSTTVTGRTCQSWSSMTPHWHQR-T---TEYYPNGGLT-RNYCRNPDAEIS-PWCYTMDPN-VR-WEYCNLTQC
LPaK7     CYHGDGQSYRGSFSTTVTGRTCQSWSSMTPHWHQR-T---TEYYPNGGLT-RNYCRNPDAEIR-PWCYTMDPS-VR-WEYCNLTQC
LPaK5     CYYHYGQSYRGTYSTTVTGRTCQAWSSMTPHQHSR-T---PENYPNAGLT-RNYCRNPDAEIR-PWCYTMDPS-VR-WEYCNLTQC
LPaK2     CYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSR-T---PEYYPNAGLI-MNYCRNPDA-VAAPYCYTRDP-GVR-WEYCNLTQC
LPaK3     CYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSR-T---PEYYPNAGLI-MNYCRNPDP-VAAPYCYTRDPS-VR-WEYCNLTQC
LPaK9     CYHGDGRSYRGISSTTVTGRTCQSWSSMIPHWHQR-T---PENYPNAGLT-ENYCRNPDSGKQ-PWCYTTDPC-VR-WEYCNLTQC
LPaK1     CYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHQHNR-T---TENYPNAGLI-MNYCRNPDA-VAAPYCYTRDP-GVR-WEYCNLTQC
LPaK4     CYHGNGQSYQGTYFITVTGRTCQAWSSMTPHSHSR-T---PAYYPNAGLI-KNYCRNPDP-VAAPWCYTTDPS-VR-WEYCNLTRC
tPAK1     CYEDQGISYRGTWSTAESGAECTNWNSSALAQKP-YSGRRPDAI-RLGLGNHNYCRNPDRDSK-PWCYVAGKPG--DFGYCSTPAC
PGK3      CKLGTGENYRGNVAVTVSGHTCQHWSAQTPHTHNR-T---PENFPCKNLD-ENYCRNPDGK-RAPWCHTTNNSQVR-WEYCKIPSC
BPTK1     CAEGVGMNYRGNVSVTRSGIECQLWRSRYPHKPEINS----TTHPGADLR-ENFCRNPDGSITGPWCYTTSPT-LRR-EECSVPVC
HPTK1     CAEGLGTNYRGHVNITRSGIECQLWRSRYPHKPEINS----TTHPGADLQ-ENFCRNPDSSITGPWCYTTDPT-VRR-QECSIPVC
PGK2      CMHCSGENYDGKISKTMSGLECQAWDSQSPHAHG-YI---PSKFPNKNLK-KNYCRNPDRELR-PWCFTTDPNK-R-WELCDIPRC
HGFK4     CYRGNGKNYMGNLSQTRSGLTCSMWDKNMEDLHRHIF-WEPDAS--K-LN-ENYCRNPDDDAHGPWCYTGNPL-IP-WDYCPISRC
UKK1      CYEGNGHFYRGKASTDTMGRPCLPWNSATVLQQT-YHAHRSDAL-QLGLGKNNYCRNPDNRRR-PWCYVQVGLK-PLVQECMVHDC
HGFK3     CIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDM-T---P-ENFKCKDLRENYCRNPDGSES-PWCFTTDPN-IRVGYCSQIPNC
HGFK1     CIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHS-FL---PSSYRGKDLQ-ENYCRNPRGEEGGPWCFTSNPE-VR-YEVCDIPQC
HGFK2     CMTCNGESYRGLMDHTESGKICQRWDHQTPHRHK-FL---PERYPDKGFD-DNYCRNPDGQPR-PWCYTLDPHT-R-WEYCAIKTC
HPTK2     CVPDRGQQYQGRLAVTTHGLPCLAWASAQAKALSK-----HQDFNSAVQLVENFCRNPDGDEEGVWCYVAGKPG--DPGYCDLNYC
```

*See abbreviations footnote.

fibrin (Table II). For example, in PGK4, Arg-34 is missing but Arg-32, Asp-61, Arg-76, and 35 of PGK1's essential residues are conserved; the retention of these amino acids may be the basis for PGK4's capacity to bind free lysine and EACA.[45] In LPaK10, Arg-32, Asp-61, Arg-76, and 35 of PGK1's

TABLE II. Most Conserved Residues in Human Kringles and Bovine Prothombin Kringle 1*

	1	10	20	30	40	50	60	70	80
PGK1	C G G	YRG	T G C	W S	R R S	P	GL	NYCRNPD D	PWCY	R-YDYC I C
PGK4	C G G	YRG	T G C	W S	R K T	P	GL	NYCRNPD D	PWCF	R-WEYC L C
PGK5	C G G	YRG	T G C	W	R T	P	GL	NYCRNPD D	PWCY	YDYC V C
TPAK2	C G G	YRG	T G C	W S	K T	P	GL	NYCRNPD D	PWC	WEYC V C
LPaK10	C G G	YRG	T G C	W S	R R T	P	GL	NYCRNPD D	PWCF	R-WEYC L C
LPaK11	C G G	YRG	T G C	W	R	P	GL	NYCRNPD D	PWCY	DYC I C
F12K1	C G G	YRG	T G C	W S	R		GL	FCRNPD D	PWCF	WEYC L C
LPaK8	C G G	YRG	T G C	W S	RR	P	GL	NYCRNPD E	PWCY	R-WEYC L C
LPaK6	C G G	YRG	T G C	W S	R T		GL	NYCRNPD E	PWCY	R-WEYC L C
LPaK7	C G G	YRG	T G C	W S	R T		GL	NYCRNPD E	PWCY	R-WEYC L C
LPaK5	C G	YRG	T G C	W S	R T	P	GL	NYCRNPD E	PWCY	R-WEYC L C
LPaK2	C G G	YRG	T G C	W S	R T	P	GL	NYCRNPD	PYCY	R-WEYC L C
LPaK3	C G G	YRG	T G C	W S	R T	P	GL	NYCRNPD	PYCY	R-WEYC L C
LPaK9	C G G	YRG	T G C	W S	R T	P	GL	NYCRNPD	PYCY	R-WEYC L C
LPaK1	C G G	YRG	T G C	W S	R T		GL	NYCRNPD	PYCY	R-WEYC L C
LPaK4	C G G	Y G	T G C	W S	R T	P	GL	NYCRNPD	PWCY	R-WEYC L C
tPAK1	C G	YRG	G C	W S	K S	P	GL	NYCRNPD D	PWCY	YC C
PGK3	C G G	YRG	T G C	W	R T	P	L	NYCRNPD	PWC	R-WEYC I C
BPTK1	C G G	YRG	T G C	W S	K S		L	NFCRNPD	PWCY	RR E C V C
HPTK1	C G G	YRG	T G C	W S	K S		L	NFCRNPD	PWCY	RR C I C
PGK2	C G	Y G	T G C	W S		P	L	NYCRNPD E	PWCF	R-WE C I C
HGFK2	C G G	Y G	T G C	W	R	P	L	WYCRNPD D	PWCY	WDCY I C
UKK1	C G G	YRG	G C	W S			GL	NYCRNPD	PWCY	C V C
HGFK3	C G G	YRG	G C	W S	T	P		NYCRNPD	PWCF	R YC I C
HGFK1	C G G	Y G	T G C	W S		P	L	NYCRNP E	PWCF	R YEVC I C
HGFK2	C G	YRG	T G C	W	R K	P	G	NYCRNPD Q	PWCY	R WEYC I C
HPTK2	C G	Y G	T G C	W S	K K			NFCRNPD D	WCY	YC L C

*See abbreviations footnote.

36 essential residues are conserved. Although Arg-34 is not conserved, LPaK10 does have an Arg at position 35 which may give this kringle the capacity to bind fibrin. An Asp-61→Glu-61 replacement is seen in LPaK5, LPaK6, and LPaK8. Arg-76 is conserved in LPaK1 through LPaK10, but not in LPaK11. LPaK8 contains Arg-34 but not Arg-32, and retains 34 of the PGK1 consensus residues. Nonconservative substitutions for Asp-61 occur in LPaK1, LPaK2, LPaK3, LPaK4, and LPaK9. Like PGK5 which binds lysine weakly, LPaK11 has Arg-32 and Asp-61, but is also missing Arg-34 and Arg-76; LPaK11 retains 31 of the 36 consensus residues in PGK1. This type of sequence analysis suggests that apo[a] kringles can be categorized in different ways: (1) those with 78 residues and those with 80 residues, (2) those which have relatively high homology with PGK4, and (3) those that lack certain amino acids which impart lysine binding capacity to other kringles.

A more stringent comparison was based on conservation of nineteen residues which were identified previously as essential for lysine binding in PGK4.[44] We compared these residues with those in apo[a] kringles and PGK1 (Table III). There are three differences between PGK1 and PGK4, which suggest that there can be wide flexibility in positions 33, 60, and 62 while the other 16 amino acids must be conserved for lysine binding. Therefore, ranking of apo[a] kringles according to their probability for binding lysine changes slightly to the order shown in Table III.

A second comparison of kringles is based on conservation of residues, which impart secondary structure comparable to that of bovine prothrombin kringle 1 (BPTK1). Tulinsky and co-workers[46,47] have studied this hemostatic protein extensively, and have elucidated its tertiary structure. BPTK1, however, does not bind Lys/fibrin and is used here for comparing its *known* secondary structure with that *predicted* for apo[a] kringles. Their studies indicate that the main chain tertiary structure of PGK4 is very similar to that of BPTK1.[44] Differences between these two kringles are attributed to side group substitutions and amino acid insertions which result in the formation of different hydrogen bonding interactions.[44] Although apo[a] kringles are sequentially similar to PGK4, their tertiary structures are probably almost identical. Hence, sequence differences may be expressed as differences in secondary structure and/or intrakringle microdomains.

BPTK1 is comprised of 79 amino acids with N-glycosylation sites at positions 12 and 36. The sequence homology between BPTK1 and apo[a] kringles is about 52.3±1%. Apo[a] kringles and PGK4 are about 45% homologous in sequence to BPTK1 between their first and third half-cystines. PGK1 and PGK4 contain 11 and 10 residues, respectively, between the third and fourth half-cystines, with Asp-59 and Asp-61 forming the anionic base of the ligand-binding pocket. BPTK1 contains 11 residues in this segment including the two aspartate residues which are apparently involved in an intrakringle

TABLE III. Comparison of PGK4 Lys-Binding Residues With Apo[a] Kringles and PGK1

	PGK4	LPAK10	LPAK11	LPAK5	LPAK6	LPAK7	LPAK8	LPAK9	LPAK4	LPAK2	LPAK3	LPAK1	PGK1
31	H	H	H	H	H	H	H	H	H	H	H	H	H
32	R	R	R	Q	W	W	W	W	S	S	S	Q	R
33	H	H	H	H	H	H	H	H	H	H	H	H	P
34	Q	Q	S	S	Q	Q	R	Q	S	S	S	N	R
35	K	R	T	R	R	R	R	R	R	R	R	R	F
58	P	P	P	P	P	P	P	P	P	P	P	P	P
59	D	D	D	D	D	D	D	D	D	D	D	D	D
60	A	A	G	A	A	A	A	S	P	A	P	A	N
61	D	D	D	E	E	E	E	G	V	V	V	V	D
62	K	T (63)	I	I	I	I	I	K	A	A	A	A	P
65	P	P	P	P	P	P	P	P	P	P	P	P	P
66	W	W	W	W	W	W	W	W	W	Y	Y	Y	W
67	C	C	C	C	C	C	C	C	C	C	C	C	C
68	F	F	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
76	R	R	L	R	R	R	R	R	R	R	R	R	R
77	—	—	—	—	—	—	—	—	—	—	—	—	—
78	W	W	F	W	W	W	W	W	W	W	W	W	Y
79	E	E	D	E	E	E	E	E	E	E	E	E	D
80	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
81	C	C	C	C	C	C	C	C	C	C	C	C	C

association and are not available for binding to the ϵ-amino group of lysine.[44] Apo[a] kringle type 1 through 10, like plasminogen kringles 2, 3, and 4, have 10 residues between the third and fourth half-cystines, while apo[a] kringle 11 has 11 residues in this segment and an additional amino acid inserted into segment 3. The average sequence homology within the ligand-binding region of apo[a] kringles 1 through 10 and BPTK1 is 65%. In this region the sequence homology between apo[a] kringle type 11 and BPTK1 is 82%. For segment 4, the ligand-binding site, the average sequence homology between PGK4 and apo[a] kringle types 1 and 2 is 64%; between PGK4 and types 3, 4, and 9 it is 55%; between PGK4 and types 5 through 8 it is 73%. Segment 4 in apo[a] kringle type 10 is 82% sequentially homologous to segment 4 of PGK4. The average homology between this segment of PGK4 and that of all other known kringles is about 73%.

The greatest difference (75%) between BPTK1, PGK4, and apo[a] kringles occurs between the fifth and sixth half-cystines, i.e., segment 6. In this segment only LPaK11 is as high as 75% homologous to BPTK1. This difference may be attributed to the possible location of asparagine-glycosylation sites in LPaK1 through LPaK10 but is not conserved in this segment of LPaK11. Asn-77 and Asn-101, designated here as residues 12 and 36, are the carbohydrate-bearing residues in BPTK1.[45] Although it is well known that apo[a] is highly glycosylated,[9,13,18] little is known about the function or actual location of the carbohydrate moieties amongst the kringles of apo[a]. LPaK1-LPaK10 each have one N-glycosylation site (position 76) which would appear to be distant from the glycosylation site in BPTK1. However, if the half-cystines in apo[a] kringles are cross-linked in the same way as in BPTK1, and the kringle polypeptide structure is likewise similar, then the N-linked carbohydrate moiety in apo[a] kringles may project out from the same region of the kringle on its surface and perhaps in the same direction.[45] Apo[a] kringle type 1 has a second possible N-glycosylation site at position 34, which is sequentially similar to Asn36 in BPTK1. A major difference between apo[a] kringles and PGK4 is that the latter does not contain a potential N-glycosylation residue. In this respect, apo[a] kringles are more like BPTK1 than PGK4.

Most of the conserved residues identified in kringles (Table II) are involved in β-turns or β-sheet

TABLE IV. Comparison of Predicted and Known Secondary Structures in Bovine Prothrombin Kringle 1

Position #	10	20	30	40	50	60	70	80
BPTK1	CAEGVGMNYRGNVSVTRSGIECQLWRSRYPHKPEINS----TTHPGADLR-ENFCRNPDGSITGPWCYTTSPT-LRR-EECSVPVC							
X-ray	bbb bbbbbb				b bb bb	bbbbb	b bb bb	
X-ray	tttt tttt tttt tttt t				tttttt t ttt	tttttttt	ttt t	
CF1	bb bbbbbb bbb				bb	bbbb bbbbbb		bbbbb
CF1	tttt tttttt tt				ttttt	ttttt		
GAR	bbbbbbbbb bbbbbb bbbbb bbb				b bb bb	bbb bb b	bb	bbbb
GAR	tt tt tttt tt			t	ttt tttt	t tt	ttt	
TAY	bbbb bbbbb bbbb							bbbb
TAY	t ttt ttt ttttt	ttttt		t	ttttttttttt	ttttt	tt tttt	
G/R	bbb bbb bbb				b b	b		bbbb
G/R	tt tttt tttt ttttt	ttttt		t t	tttt ttttt	tt tt	tt tttt	

(t)	1	2	3	4	5	6	7	8	9	10
	EGVG	TRSGI	LWRS	YPHK	STTH	PGA	ENFC	NPDG	SITG	SPTL
p Value	18.75%	65%	87.5%	12.5%	50%	41.7%	31.5%	89.3%	31.3%	0%

(b)	1		2		3		4		5	
	SVT	IEC	QLW	REN	RN	PW	CYT	REE	VP	VT
p Value	90%	83%	>90%	67%	0%	25%	42%	<10%	100%	100%

structures in BPTK1.[46] We have used a combination of algorithms to assign probabilities to amino acid residues for their occurrence in β-sheets and β-turns of BPTK1. These predicted structures were then compared with known structures determined from X-ray crystallographic studies for BPTK1; the comparisons are shown in Table IV. Structures reported by Tulinsky and co-workers[44,46] demonstrate clearly that BPTK1 and PGK4 are devoid of α-helicity. This characteristic is predicted by our analysis as well as that of others.[55,56] In addition, BPTK1 contains 10 β-turns and 5 short β-sheet regions (less than four residue per side). In our analysis, turns 8, 3, and 2 were predicted with high accuracy. Turn 10 is not predicted by any of our programs. The most accurate predictions were obtained using the Chou–Fasman-based[48] and Garnier/Robeson[49] programs, which were about 65% accurate for BPTK1. Hence, we used these programs to compare the known structural features of BPTK1 with those predicted for apo[a] kringles and PGK4 (Table V). Stretches of α-helicity were not predicted for apo[a] kringles or PGK4. BPTK1 turn 1 is predicted to occur in the analogous region of all apo[a] kringles and of PGK4. Hence, the only adhesion motif in LPaK8, YRGDG, is predicted to occur in a β-turn. The second β-turn shown to occur in BPTK1 is also predicted for an analogous region of PGK4; but in all apo[a] kringles, this region is predicted as β-sheet. β-Turn 3 in BPTK1 is not predicted to occur in LPaK1 through LPaK5 or in LPaK11, but is predicted for LPaK6 through LPaK10. All apo[a] kringles are predicted to have β-turns corresponding to turns 4, 5, 6, 8, and 10 known to be present in BPTK1. The β-turn 6 region of BPTK1 is predicted to occur as β-sheet in LPaK1, 2, 3, 4, and 10, as a turn in LPaK5 through LPaK9, and as random structure in LPaK11. β-Turn 9 of BPTK1 is predicted to occur as a turn in LPaK9, LPaK10 and LPaK11. Random structure is predicted for this region in kringles LPaK1 through LPaK8. Although predictive algorithms for secondary structure have inherent weaknesses, our observations suggest that subtle alterations in the primary structure of kringles may influence how the intrakringle domains differ among the kringles.

The Chou–Fasman-based algorithms, as well as more recently developed algorithms, do not predict turn 1 in BPTK1 which contains the sequence EGVG. These programs are designed to predict low β-turn probability for a polypeptide sequence containing valine, isoleucine, or leucine. A β-turn is predicted for corresponding sequences in apo[a] kringles and PGK4 which do not contain either Val, Leu, or Ile. In a region of apo[a] kringles coincident to turn 2 in BPTK1, β-sheet is predicted instead of β-turn because a valine residue occurs in the polypeptide stretch. The corresponding position in PGK4 contains threonine, which favors the predicted β-turn.

X-Ray crystallographic data show that residues between the third and fourth half-cystines in BPTK1 and in PGK4 which are in β-turns 8 and 9,[44,46] and are analogous to residues in LPaK9, LPaK10, and LPaK11, have a high probability of occurring in a β-turn also. BPTK1 turn 8 is predicted with moderate probability to occur also in LPaK1 through LPaK8, but BPTK1 turn 9 is not predicted in these kringles. Asp-61, an amino acid essential for the kringle to bind the ε-amino moiety of lysine, is replaced by valine or glutamate in apo[a] kringles types 1–8. This difference may preclude binding to lysine and/or fibrin by these kringles.

TABLE V. Comparison of Known β-Sheet and β-Turn Residues in Bovine Prothrombin Kringle 1 and Predicted β-Sheet and β-Turn Residues in Apo[a] Kringles and Plasminogen Kringle 4

Position #	10	20	30	40	50	60	70	80
BPTK1	CAEGVGMNYRGNVSVTRSGIECQLWRSRYPHKPEINS----TTHPGADLR-ENFCRNPDGSITGPWCYTTSPT-LRR-EECSVPVC							
X-ray	bbb bbbbbb				b bb bb	bbbbb	b bb bb	
X-ray	tttt ttttt tttt tttt t			tttttt t ttt	tttttttt	ttt t		
LPaK1	CYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHQHNR-T---TENYPNAGLI-MNYCRNPDA-VAAPYCYTRDP-GVR-WEYCNLTQC							
	ttttttttt bbbbbbb bbb	ttttt		ttttttbbbb b	tttt	bbbtttt	bbbbbbttt	
LPaK2	CYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSR-T---PEYYPNAGLI-MNYCRNPDA-VAAPYCYTRDP-GVR-WEYCNLTQC							
	ttttttttt bbbbbbb bbb	ttttttt t		tt tttbbbb b	tttt	bbbtttt	bbbbbbttt	
LPaK3	CYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSR-T---PEYYPNAGLI-MNYCRNPDP-VAAPYCYTRDPS-VR-WEYCNLTQC							
	ttttttttt bbbbbbb bbb	ttttttt t		tt tttbbbb b	tttt	bbbtttt	bbbbbbttt	
LPaK4	CYHGNGQSYQGTYFITVTGRTCQAWSSMTPHSHSR-T---PAYYPNAGLI-KNYCRNPDP-VAAPWCYTTDPS-VR-WEYCNLTRC							
	ttttttttbbbbbbbbb bbb	ttttttt t		tbbtttbbbb b	tttt	bbbbtttt	bbbbbbttt	
LPaK5	CYYHYGQSYRGTYSTTVTGRTCQAWSSMTPHQHSR-T---PENYPNAGLT-RNYCRNPDAEIR-PWCYTMDPS-VR-WEYCNLTQC							
	ttttttt bbbbbbb bbb	ttttttt t		tttttttt t tt ttt		bbbbtttt	bbbbbbb	
LPaK6	CYHGDGQSYRGSFSTTVTGRTCQSWSSMTPHWHQR-T---TEYYPNGGLT-RNYCRNPDAEIS-PWCYTMDPN-VR-WEYCNLTQC							
	ttttttttttttbbbbbb bbttttttttbbbb b			bbbttttt t tt ttt		bbbbtttt	bbbbbbbbb	
LPaK7	CYHGDGQSYRGSFSTTVTGRTCQSWSSMTPHWHQR-T---TEYYPNGGLT-RNYCRNPDAEIR-PWCYTMDPS-VR-WEYCNLTQC							
	ttttttttttttbbbbbb bbttttttttbbbb b			bbbttttt t tt ttt		bbbbtttt	bbbbbbbbb	
LPaK8	CYRGDGQSYRGTLSTTITGRTCQSWSSMTPHWHRR-I---PLYYPNAGLT-RNYCRNPDAEIR-PWCYTMDPS-VR-WEYCNLTRC							
	ttttttttttbbbbbbb bbttttttttttbbb b			bbbttttt t tt ttt		bbbbtttt	bbbbbbbbb	
LPaK9	CYHGDGRSYRGISSTTVTGRTCQSWSSMIPHWHQR-T---PENYPNAGLT-ENYCRNPDSGKQ-PWCYTTDPC-VR-WEYCNLTQC							
	ttttttttbbbb bbbbb bbttttttbbbbbbb t			ttttttt t tt ttttttttt	bbbbtttbb		bbbbbbttt	
LPaK10	CYHGNGQSYRGTFSTTVTGRTCQSWSSMTPHRHQR-T---PENYPNDGLT-MNYCRNPDAD-TGPWCFTMDPS-IR-WEYCNLTRC							
	ttttttttbbbbbbbbb bbttttttttttttt t			ttttttbbb b	tttttt ttbbb	bb	bbbbbbttt	
LPaK11	CMFGNGKGYRGKKATTVTGTPCQEWAAQEPHRHSTFI---PGTNKWAGLE-KNYCRNPDGDINGPWCYTMNPRKL--FDYCDIPLC							
	bbttttttt bbbbbb	ttttbbbt		ttt	ttttttttttbbbbtttt	b	btbbbbbbt	
PGK4	CYHGDGQSYRGTSSTTTTGKKCQSWSSMTPHRHQK-T---PENYPNAGLT-MNYCRNPDADK-GPWCFTTDPS-VR-WEYCNLKKC							
	ttttttttttttbbttttt ttttttttt	t		ttttttbbbb b	tttt tt	tbbbbtttt	bbbb	

Among the 11 kringle types present in the cloned polymorph of apo[a], LPaK10 has the highest probability for binding lysine and/or fibrin; its probability equals that of PGK1. This would suggest that Lp[a] binds to fibrin(ogen) perhaps in the same manner as plasminogen except that, like PGK4, LPaK10 is located near the carboxy-terminal region and may not be protected like PGK4.[41] The large conformational change induced in plasminogen by the presence of either 6-aminohexanoic acid or lysine[57–60] has not been documented for LP[a] or apo[a]. LPaK11 has a lower probability for binding lysine, approaching that of PGK5. LPaK8, LPaK6, LPaK7, and LPaK5, in this order, have low and decreasing probabilities of binding lysine, and are comparable to that of PGK2 and PGK3. LPaK1, LPaK2, LPaK3, LPaK4, and LPaK9 are not predicted to bind lysine. We have observed and others have reported[61,62] that Lp[a] binds to lysine-Sepharose and can be dissociated with high ionic strength buffers. Binding of highly sialylated Lp[a] may be a result of strong ionic interactions as well as weak specific interactions.

Interkringle Regions

Since the amino acid sequence of one apo[a] polymorph was first inferred by McLean et al.,[21] great significance has been attributed to the kringles, but the Ser/Thr- and Pro-rich interkringle regions of apo[a] have received minimal attention. These regions contain the postkringle segment 7 and the prekringle segment 1. Except for the regions between LPaK6 and LPaK7, and between LPaK10 and LPaK11, which are composed of 28 and 26 residues, respectively, regions between the other apo[a] kringles span 36 amino acid residues. These regions are rich in serine, threonine, and proline and appear to be similar to Thr/Pro domains identified in other proteins. For example, there is some sequence homology with the Thr- and Pro-rich repeat region in the α-chain of the platelet membrane glycoprotein Ib.[63] There also appears to be some sequence homology with the "hinge" region of myeloma immunoglobulin A.[64] However, the corresponding regions of apo[a] (Table VI) have greater homology with a segment of an IgG light chain (CL)[65] as well as with the T-cell receptor C domain.[66] Furthermore, several shorter sequences (Table VII) are similar to sequences present in intercellular-adhesion molecules, ICAM's 1, and 2,[67,68] neural cell adhesion molecule, NCAM,[69] and high-molecular-weight kininogen.[70] As stated above, a single copy of the adhesion motif, RGD, is contained in segment 2 of LPaK8 immediately after the first half-cystine. This is an interesting comparison which may be only coincidental, but it does suggest the possibility that the Ser/Thr- and Pro-rich interkringle regions and segments 2 of the apo[a] kringle domain where other analogues of RGD occur, may be involved in forming a receptor recognition and binding site. The secondary structure of the RGD sequence as a recognition site in proteins involved in adhesion is predicted to

TABLE VI. Comparison of the Ser/Thr- and Pro-Rich Interkringle Regions of Apo[a] With the Amino-Terminal Region of an IgG Light Chain*

```
IgG(CL)                          VLG--QPK--ANPTVTLFP-P----SSEELQA---NKATLV--C
1-11                                   AAP---------------E-Q--S--H--VVQDC
LPaK1-LPaK2                SDAE---GTA---V-APPTVT--PVPSLEAPSE--QAPTEQRPG-VQEC
LPaK2-LPaK3                SDAE---GTA---V-APPTVT--PVPSLEAPSE--QAPTEQRPG-VQEC
LPaK3-LPaK4                SDAE---GTA---V-APPTIT--PIPSLEAPSE--QAPTEQRPG-VQEC
LPaK4-LPaK5                SDAEW---TAF--V--PPNVILA--PSLEAFFE--QALTEETPG-VQDC
LPaK5-LPaK6          LVTESS----VL---------A--TLTVVPDPSTEASSEE--APTEQSPG-VQDC
LPaK6-LPaK7          PVTESS----VL---------A--TSTAV--------SE--QAPTEQSPT-VQDC
LPaK7-LPaK8          PVMEST----LL-T---------TPTVVPVPSTELPSEE--APTENSTG-VQDC
LPaK8-LPaK9          PVTESS----VT-T---------TPTVAPVPSTEAPSE--QAPPEKSP-VVQDC
LPaK9-LPaK10               S--E----TESG-VLE--TPTVVPVPSMEAHSE-A-APTEQTP-VVRQC
LPaK10-LPaK11              SD------TE-GTVVAPPT--VIQVPSLGPPSE--Q----------DC
```

*Residues are spaces to achieve best alignment.

TABLE VII. Comparison of Apo[a] Ser/Thr- and Pro-Rich Region Fragments With Common Sequence Fragments in Platelet Glycoprotein Ib-α-Subunit (GPIb), Intercellular Adhesion Molecules 1 and 2 (ICAMs), Neural Cell Adhesion Molecule (NCAM), and High-Molecular-Weight Kininogen (KNGH)*

```
GPIb-α      MESITFSKTPKSTTEPT PSPTT SEPVPEPAPNMTTLEPTPSPTTPEPSTEPAPSPTTPEPTPIPTIA TSPTI
Apo[a]      mes               pDpst   pvp                 pdpste          tpipsl  Qsptv
GPIb-α      LVSATSLITP
Apo[a]      lvtEssvl
ICAM-1      LAPLPS   DTQGTVV    TVT  F-PAPNVILTKPEV
Apo[a]      vapvps   dtegtvv    tvt  fVp-pnvilA-pSl
ICAM-2      PPRQVIL--TLQ
Apo[a]      pp-nvilapsle
NCAM        PIPSI    ISSEE  LPPTII
Apo[a]      pipsl    assee  apptvi
IgA         PVPST  PPT  PSPSTPPTP
Apo[1]      pvpst  ppt  pDpst
KNGH        ASSSEDSTTPSAQ TQEKTEG PTPIPSLA KPGV TVT FSDFQDSDLIATMMPPISPAPIQSD
Apo[1]             ttptv  teqr    Itpipsl  rpgv tvt
```

*Apo[a] sequences (shown in lower case) are not in order of occurrence in the Ser/Thr and Pro-rich region of the apo[a] molecule. Nonconservative substitutions are in upper case for apo[a].

occur in either β-bends or β-turns.[71] In LPaK8, the β-turn positions of RGDG sequence would occur as Arg_1-Gly_2-Asp_3-Gly_4 if the predicted secondary structure (Table V) is accurate and similar to that of BPTK1.

The Protease Domain

An interesting feature of the serine protease domain in apo[a] is the high degree of sequence homology between residues bounding its catalytic triad (His_{4371}, Asp_{4414}, Ser_{4500}) and the corresponding residues in plasmin (His_{602}, Asp_{645}, Ser_{740}) (Fig. 2) and other serine proteases. Two groups have reported that isolated apo[a] possesses amidolytic activity.[72,73] Nevertheless, zymogen activation by another serine protease of either the blood coagulation cascade or of the fibrinolytic process, (e.g., plasminogen-activators such as tPA, urokinase, or streptokinase), has not been reported. tPA, urokinase, and streptokinase were used in efforts to activate purified apo[a] or LP[a] but these attempt have been unsuccessful.[21,61] Because the protease domains of plasmin and apo[a] are very similar in amino acid sequence, and since the half-cystine residues of plasmin are absolutely conserved in the apo[a] protease domain, this suggests that the two proteins might also share common or at least related functions. The structural similarities of the two proteins together with apo[a]'s nonactivated protease and multikringle domain suggest only an atherogenic role for Lp[a]. Binding studies reported by Edelberg et al.[29] have demonstrated an interaction between streptokinase and Lp[a] which causes inhibition of plasminogen activation. This evidence suggests that the sequences and tertiary structures around the activation site of plasminogen and the apo[a] protease are similar. Similar sequence and structural features may also be common to the unrelated lactate-dehydrogenase subunit-M (LD-M), which also binds to streptokinase in perhaps the same way.[74] It is possible that the interactions between streptokinase and apo[a] are due to the common epitope also present in plasmin and LD-M, and may be solely an in vitro phenomenon. This possibility is supported by a study in which high plasma levels of LP[a] were not correlated with low level fibrinolysis in patients

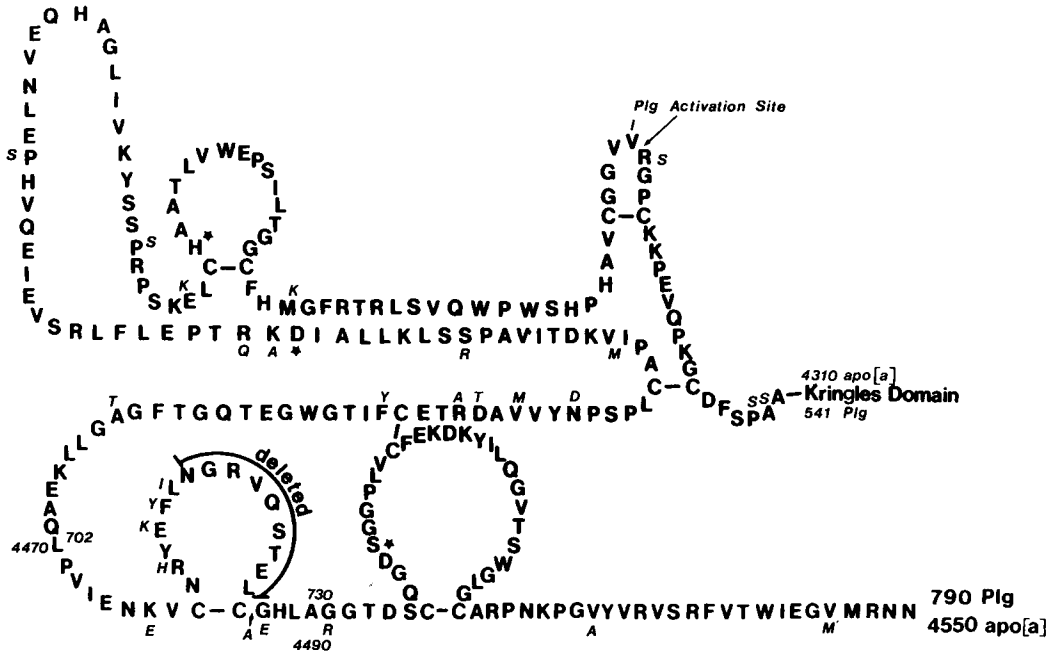

Fig. 2. Comparison of plasmin and the proposed serine protease domain of apo[a]. The amino acid sequence of the apo[a] protease domain is highly similar to that of plasmin. Illustrated in this figure are 28 substitutions (in lower case letters for apo[a]), one insertion, and nine deleted residues in apo[a]. The site of activation by tPA, urokinase, and streptokinase is indicated by the enclosed arrow. Amino acids of the catalytic triad are indicated by asterisks.

with risk of myocardial infarction.[75] Furthermore, a recent study has demonstrated that plasma clot lysis in vitro was enhanced by the addition of Lp[a].[76] The former studies[29] may reveal that the three-dimensional (structural) features of plasmin and apo[a] in this region are similar and conserved, but this has not led to similar function.

In the protease domain of apo[a] there are several amino acid substitutions, insertions, and deletions which suggest a uniquenss for its tertiary structure in a region separate from the plasmin activation site. It is generally accepted that any type of amino acid substitution is significant when it creates or exposes to the surface an arginine-containing polypeptide stretch that is accessible to cleavage by a serine protease. Apo[a] contains a region where multiple changes have occurred. Eight substitutions, 9 deletions, and 1 insertion are apparent between positions 4470 and 4492 when comparing the protease domains of apo[a] and plasmin. One noteworthy substitution occurs at position 4491, where arginine replaces glycine. The new sequence, -Leu-Ala-Arg-Gly-Thr_{4493}-, is comprised of the same amino acids (i.e., small amino acids such as Ala, Gly, and Thr) as those present at the activation cleavage sites of Factor IX. This site is located in the activation peptide at position 145 and contains the sequence, -Leu-$Thr-Arg_{145}$-Ala-Glu-.[77] Factor IX is cleaved by activated-Factor XI at this site and at a second site at the end of the activation peptide.[77] Prothrombin is cleaved by activated Factor X at a site composed of -Glu-Gly-Arg_{271}-Thr-Ala-,[77] which is also similar to the site on apo[a]. Furthermore, this sequence is similar to the site, -Ser-Ala-Arg-Gly-His-, on the β-chain of fibrinogen that is subject to cleavage by α-thrombin.[78] Hence, the alteration of arginine content and location may well control its activation as a protease and its suceptibility or resistance to endogenous proteolysis, thereby significantly affecting the lifetime and metabolic fate of Lp[a].

CONCLUSIONS

Apo[a] is an apoprotein unique to Lp[a], but is similar to plasminogen, and possesses many features which are common to adhesion molecules, and other proteins involved in thrombosis. The apo[a] amino acid sequence inferred by the cDNA sequence[19] has revealed many intriguing features which have led to an intensified effort to demonstrate a pathophysiologic role for Lp[a]. Some investigators view apo[a] as strictly a homologue of plasminogen containing a "nonfunctional" serine protease. The 11 different kringle types contained in the only cloned apo[a] polymorph (LPaK1 through LPaK11) are of great interest because polymorphs of apo[a] can contain as many as 50 kringles which could perhaps be involved in binding to different ligands or receptors during normal hemostasis or in a pathogenic thrombotic process. Because the sequence homology between all but one of apo[a]'s

TABLE VIII. Amino Acid Sequence Comparison of the Putative Serine Protease Catalytic Triad Regions of Human and Rhesus Apo[a] Proteins to Known Serine Proteases and Human Hepatocyte Growth Factors*

PLM	EWVLTAAHC$_{603}$	TRKDIALLKLS$_{652}$	CQGDSGGPLVC$_{745}$
Apo[a] HU	EWVLTAAHC$_{4372}$	TQADIALLKLS$_{4421}$	CQGDSGGPLVC$_{4506}$
tPA HU	CWILSAAHC$_{323}$	YDNDIALLQLK$_{378}$	CQGDSGGPLVC$_{484}$
UK HU	CWVISATHC$_{205}$	HHNDIALLKIR$_{262}$	CQGDSGGPLVC$_{363}$
Apo[a] RH	EWVLTAACC$_{1233}$	IGADIALLKLS$_{1282}$	CQGDNGGPVVC$_{1376}$
HGF HU	SWVLTARQC$_{535}$	EGSDLVLMKLA$_{585}$	CEGDYGGPLVC$_{679}$
HGF2 HU	SWVLTARQC$_{530}$	EGSDLVLMKLA$_{579}$	CEGDYGGPLVC$_{674}$

*See abbreviations footnote.

kringles and PGK4 is relatively high, apo[a] would be expected to bind to lysine alone or on fibrin(ogen), which then would preclude normal fibrinolytic proteolysis by plasmin. Therefore, the principal role for Lp[a] that has been considered thus far has been a pathogenic one.

It is evident that subtle amino acid substitutions in the kringle structure influence binding to lysine/fibrin. Our initial comparison of predicted secondary structures for the different apo[a] kringles and PGK4 suggested that these small differences may influence the internal structures of the kringle and thereby influence function.[79] Apparently, apo[a] possesses only one true copy of plasminogen kringle 4, i.e., LPaK10. LPaK10 has the highest probability of binding to lysine/fibrin, but LPaK2, which is the most abundant kringle type in apo[a], is not considered to have this capacity to bind lysine. Furthermore, in addition to N-glycosylation sites present in apo[a] kringles but absent in PGK4, PGK4 contains two short segments of Lys-Lys at positions 20 and 21, and 85 and 86 (Table I) which are changed in apo[a] kringles 1 through 10 Lys-Lys sequences appear to be cleavage sites in fibrinolytic proteins, plasminogen (80,81) and urokinase (82). Apo[a] kringles 1 through 10 have at least two copies of the sequence Arg-Thr. LPaK4, LPaK8, and LPaK10 have Arg-Thr sequences in positions 20–21, and Thr-Arg at 85–86. All but LPaK8 and LPaK11 have the sequence Arg-Thr at positions 36 and 38 which is homologous to the Lys-Thr sequence in PGK4 (Table I).

A positive physiologic role for Lp[a] is suggested by several molecular features which have not been emphazied previously. The homology between Ser/Thr- and Pro-rich sequences in apo[a] and Thr-/Pro-rich sequences in platelet GPIb-α chain, along with the presence of an "RGD" adhesion motif, suggest that LP[a] may be involved in platelet function. In apo[a], these sequences appear to be more like the amino terminal segments of IgG (light chain) than the "hinge" regions in IgA. The subtle homology between these regions of apo[a] and intercellular adhesion molecules (ICAMs) and neural cell adhesion molecule (NCAM) suggests a role in cell–protein adhesion interactions.

One of the most interesting and enigmatic aspects of apo[a] is its serine protease domain. Unlike the cloned apo[a] molecule sequence of the rhesus monkey,[83] the cloned human apo[a] sequence[21] contains the catalytic triad typical of other serine proteases and appears to contain all of the structural elements essential for activation. Some investigators have obtained evidence that apo[a] in the intact Lp[a] lipoprotein exhibits proteolytic activity toward fibronectin and arginine-containing synthetic peptides.[73] The rhesus apo[a] serine protease domain has an incomplete serine protease catalytic triad; however, it also resembles the corresponding sequence contained by the human hepatocyte growth factors,[84,85] shown in Table VIII.

A possible mechanism for the physiologic role of lipoprotein [a], suggested by the kringle and Ser/Thr and Pro-rich features and arginine-containing sequences in the protease region of apo[a], is one in which Lp[a] binds to platelets and endothelial cells via a receptor and/or to a protein like von Willebrand Factor (vWF); the protease domain is then activated in situ by autolysis or by a specific factor such as activated-Factor XI, activated-Factor X, or thrombin. If the particle is internalized by an activated platelet or endothelial cell, the active apo[a] protease might then cleave itself and the apo B-100 moiety of Lp[a] resulting in delivery of its lipid content to the cell for injury repair.[5]

ACKNOWLEDGMENTS

The authors gratefully acknowledge the graphic arts assistance of Ms Susan M. Kelly. Supported in part by NIH Grant HL-32971 to J.D.M. and NIH Grant 2S07RR-05425-29 to J.G.Jr. Some of the sequence analysis was performed using facilities of the Molecular Biology Information Resource, Department of Cell Biology, Baylor College of Medicine.

REFERENCES

1. Kostner, G.M., Avogaro, P., Cazzolato, G., Marth, E., Bittolo-Bon, G., Qunici, G.B. Lipoprotein Lp(a) and the risk for myocardial infarction. Atherosclerosis 38:51–61, 1981.
2. Hoefler, G., Harnoncourt, F., Paschke, E., Mirtl, W., Pfeiffer, K.H., Kostner, G.M. Lipoprotein Lp(a) A risk factor for myocardial infarction. Arteriosclerosis 8(4):398–401, 1988.
3. Dahlen, G.H., Guyton, J.R., Attar, M., Farmer, J.A.,

Kautz, J.A., Gotto, A.M., Jr. Association of levels of lipoprotein Lp[a], plasma lipids, and other lipoproteins with coronary artery disease documented by angiography. Circulation 74(4):758–765, 1986.
4. Jurgens, G., Koltringer, P. Lipoprotein(a) in ischemic cerebrovascular disease: A new approach to the assessment of risk for stroke. Neurology 37:513–515, 1987.
5. Rath, M., Pauling, L. Hypothesis: Lipoprotein(a) is a surrogate for ascorbate. Proc. Natl. Acad. Sci. U.S.A. 87: 6204–6207, 1990.
6. Gaubatz, J.W., Heideman, C., Gotto, A.M., Jr., Morrisett, J.D., Dahlen, G.H. Human Plasma Lipoprotein [a] structural properties. J. Biol. Chem. 258(7):4582–4589, 1983.
7. Utermann, G., Weber, W. Protein composition of Lp[a] lipoprotein from human plasma. FEBS Lett. 154(2): 357–361, 1983.
8. Seman, L.J., Breckenridge, W. C. Isolation and partial characterization of apolipoprotein(a) from human lipoprotein (a). Biochem. Cell Biol. 64:999–1009, 1986.
9. Gaubatz, J.W., Chari, M.V., Nava, M.L., Guyton, J.R., Morrisett, J.D. Isolation and characterization of the two major apoproteins in human lipoprotein[a]. J. Lipid Res. 28:69–79, 1987.
10. Morrisett, J.D., Guyton, J.R., Gaubatz, J.W., Gotto, A. M., Jr. Lipoprotein(a): structure, metabolism and epidemiology. In: "Plasma Lipoproteins." Gotto, A.M., Jr. (ed.), Chapter 4. Amsterdam: Elsevier Science Publishers B.V., 1987: 129–152.
11. Gaubatz, J.W., Ghanem, K.I., Guevara, J., Jr., Nava, M. L., Patsch, W., Morrisett, J.D. Polymorphic forms of human apolipoprotein[a]: Inheritance and relationship of their molecular weights to plasma levels of lipoprotein[a]. J. Lipid Res. 31:603–613, 1990.
12. Morrisett, J.D., Yang, C-Y. Unpublished results.
13. Fless, G.M., ZumMallen, M.E., Scanu, A.M. Physicochemical properties of apolipoprotein(a) and lipoprotein(a-) derived from the dissociation of human plasma lipoprotein(a). J. Biol. Chem. 261(19):8712–8718, 1986.
14. Yang, C-Y., Chen, S-H., Gianturco, S.H., Bradley, W.A., Sparrow, J.T., Tanimura, M., Li, W-H., Sparrow, D.A., DeLoof, H., Rosseneu, M., Lee, F-S., Gu, Z-W., Gotto, A.M., Jr., Chan, L. Sequence, structure, receptor-binding domains and internal repeats of human apolipoprotein B-100. Nature (London) 323:738–742, 1986.
15. Yang, C-Y., Chan, L., Gotto, A.M., Jr. The complete structure of human apolipoprotein B-100 and its messenger RNA. In: "Plasma Lipoproteins." Gotto, A.M., Jr. (ed.), Chapter 2. Amsterdam: Elsevier Science Publishers B. V., 1987: 77–93.
16. Yang, C-Y., Gu, Z-W., Weng, S-a., Kim, T. W., Chen, S-H., Pownall, H.J., Sharp, P.M., Liu, S-W., Li, W-H., Gotto, A. M., Jr., Chan, L. Structure of apolipoprotein B-100 of human low density lipoproteins. Arteriosclerosis 9(1): 96–108, 1989.
17. Yang, C-Y., Kim, T.W., Weng, S-a., Lee, B., Yang, M., Gotto, A.M., Jr. Isolation and characterization of sulfhydryl and disulfide peptides of human apolipoprotein B-100. Proc. Natl. Acad. Sci. U.S.A. 87:5523–5527, 1990.
18. Kratzin, H., Armstrong, V.W., Niehaus, M., Hilschmann, N., Seidel, D. Structural Relationship of an apolipoprotein (a) phenotype (570 kDa) to plasminogen: Homologous kringle domains are linked by carbohydrate-rich regions. Biol. Chem. Hoppe-Seyler 368:1533–1544, 1987.
19. Koschinsky, M.L., Beisiegel, U., Henne-Bruns, D., Eaton, D.L., Lawn, R.M. Apolipoprotein(a) size heterogeneity is related to variable number of repeat sequences in its mRNA. Biochemistry 29:640–644, 1990.
20. Hixson, J.E., Britten, M.L., Manis, G.S., Rainwater, D.L. Apolipoprotein(a) (Apo(a)) glycoprotein isoforms result from size differences in apo(a) mRNA in baboons. J. Biol. Chem. 264(11):6013–6016, 1989.
21. McLean, J.W., Tomlinson, J.E., Kuang, W.-J., Eaton, D. L., Chen, E.Y., Fless, G.M., Scanu, A.M., Lawn, R.M. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature (London) 330:132–137, 1987.
22. Ruoslahti, E., Pierschbacher, M.D. New perspectives in cell adhesion: RGD and integrins. Science 238:491–497, 1987.
23. D'Souza, S.E., Ginsberg, M.H., Burke, T.A., Lam, S.C-T., Plow, E.F. Localization of an Arg-Gly-Asp recognition site within an integrin adhesion receptor. Science 242:91–93, 1988.
24. Review of protein sequences in Protein Identification Resource databank.
25. Gonzalez-Gronow, M., Edelberg, J.M., Pizzo, S. V. Further characterization of the cellular plasminogen binding site: Evidence that plasminogen 2 and lipoprotein a compete for the same site. Biochemistry 28:2374–2377, 1989.
26. Miles, L.A., Fless, G.M., Levin, E.G., Scanu, A.M., Plow, E. F. A potential basis for the thrombotic risks associated with lipoprotein(a). Nature (London) 339:301–303, 1989.
27. Hajjar, K.A., Gavish, D., Breslow, J.L., Nachman, R.L. Lipoprotein(a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. Nature (London) 339:303–305, 1989.
28. Harpel, P.C., Gordon, B.R., Parker, T.S. Plasmin catalyzes binding of lipoprotein(a) to immobilized fibrinogen and fibrin. Proc. Natl. Acad. Sci. U.S.A. 86:3847–3851, 1989.
29. Edelberg, J.M., Gonzalez-Gronow, M., Pizzo, S. V. Lipoprotein (a) inhibits streptokinase-mediated activation of human plasminogen. Biochemistry 28(6):2370–2373, 1989.
30. Loscalzo, J., Weinfeld, M., Fless, G.M., Scanu, A.M. Lipoprotein (a), fibrin binding, and plasminogen activation. Arteriosclerosis 10(2):240–245, 1990.
31. Claeys, H., Sottrup-Jensen, L., Zajdel, M., Petersen, T.E., Magnusson, S. Multiple gene duplication in the evolution of plasminogen. Five regions of sequence homology with the two internally homologous structures in prothrombin. FEBS Lett. 61(1):20–24, 1976.
32. Magnusson, S., Sottrup-Jensen, L., Petersen, T.E., Dudek-Wojciechowska, G., Claeys, H. Homologous "kringle" structures common to plasminogen and prothrombin. Substrate specificity of enzymes activating prothrombin and plasminogen. In: "Proteolysis and Physiological Regulation." Ribbons, D.W., Brew, K. (eds.). New York: Academic Press, 1976: 203–238.
33. Castellino, F.J., Ploplis, V.A., Powell, J.R., Strickland, D. K. The existence of independent domain structures in human lys_{77}-plasminogen. J. Biol. Chem. 256(10):4778–4782, 1981.
34. Novokhatny, V.V., Kudinov, S.A., Privalov, P.L. Domains in human plasminogen. J. Mol. Biol. 179:215–232, 1984.
35. Garber, A., Jones, P., Morrisett, J.D. Manuscript in preparation.
36. Farmer, J.A., Ballantyne, C.M., Frazier, O.H., Radovancevic, B., Payton-Ross, C., Patsch, W., Morrisett, J.D., Gotto, A.M., Jr., Young, J.B. Lipoprotein [a] and apolipoprotein changes after heart transplantation. J. Am. Coll. Cardiol., in press, 1991.
37. Cushing, G.L., Gaubatz, J.W., Nava, M.L., Burdick, B.J., Bocan, T.M.A., Guyton, J.R., Weilbaecher, D., DeBakey, M.E., Lawrie, G.M., Morrisett, J.D. Quantitation and localization of apolipoproteins [a] and B in coronary artery bypass vein grafts resected at re-operation. Arteriosclerosis 9(5):593–603, 1989.
38. Rath, M., Niendorf, A., Reblin, T., Dietel, M., Krebber, H.-J., Beisiegel, U. Detection and quantification of lipoprotein(a) in the arterial wall of 107 coronary bypass patients. Arteriosclerosis 9(5):579–592, 1989.
39. Lerch, P.G., Rickli, E.E., Lergier, W., Gillessen, D. Localization of individual lysine-binding regions in human plasminogen and investigations on their complex-forming properties. Eur. J. Biochem. 107:7–13, 1980.
40. Plow, E.F., Collen, D. Immunochemical characterization of a low affinity lysine binding site within plasminogen. J. Biol. Chem. 256(21):10864–10869, 1981.
41. Vali, Z., Patthy, L. Location of the intermediate and high affinity omega-aminocarboxylic acid-binding sites in human plasminogen. J. Biol. Chem. 257(4):2104–2110, 1982.
42. Trexler, M., Vali, Z., Patthy, L. Structure of the omega-aminocarboxylic acid-binding sites of human plasminogen. J. Biol. Chem. 257(13):7401–7406, 1982.
43. Petros, A.M., Ramesh, V., Llinas, M. ^{1}H NMR studies of aliphatic ligand binding to human plasminogen kringle 4. Biochemistry 28:1368–1376, 1989.
44. Mulichak, A.M., Tulinsky, A. Structure of lysine-fibrin binding subsite of human plasminogen kringle 4. Blood Coag. Fibrin., 1:673–679, 1990.
45. Tulinsky, A., Park, C.H., Mao, B., Llinas, M. Lysine/fibrin binding sites of kringles modeled after the structure of

kringle 1 of prothrombin. Proteins: Struc. Func. Genet. 3:85–96, 1988.
46. Park, C.H., Tulinsky, A. Three-dimensional structure of the kringle sequence: Structure of prothrombin fragment 1. Biochemistry 25(14):3977–3982, 1986.
47. Tulinsky, A., Park, C.H., Skrzypczak-Jankun, E. Structure of prothrombin fragment 1 refined at 2.8 A resolution. J. Mol. Biol. 202:885–901, 1988.
48. Chou, P.Y., Fasman, G.D. Empirical predictions of protein conformation. Annu. Rev. Biochem. 47:251–276, 1978.
49. Garnier, J., Osguthorpe, D.J., Robson, B. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. 120:97–120, 1978.
50. Taylor, W.R., Thornton, J.M. Recognition of super-secondary structure in proteins. J. Mol. Biol. 173:487–514, 1984.
51. Allen, R.A., Pepper, D.S. Isolation and properties of human vascular plasminogen activator. Thromb. Heamostasis. 45:43–50, 1981.
52. Ichinose, A., Takio, K., Fujikawa, K. Localization of the binding site of tissue-type plasminogen activator to fibrin. J. Clin. Invest. 78:163–169, 1986.
53. Cleary, S., Mulkerrin, M.G., Kelley, R.F. Purification and characterization of tissue plasminogen activator kringle-2 domain expressed in Escherichia coli. Biochemistry 28: 1884–1891, 1989.
54. Vali, Z., Patthy, L. The fibrin-binding site of human plasminogen. Arginines 32 and 34 are essential for fibrin affinity of the kringle 1 domain. J. Biol. Chem. 259(22): 13690–13694, 1984.
55. Castellino, F.J., De Serrano, V.S., Powell, J.R., Johnson, W.R., Beals, J.M. Examination of the secondary structure of the kringle 4 domain of human plasminogen. Arch. Biochem. Biophys. 247(2):312–320, 1986.
56. Castellino, F.J., Beals, J.M. The genetic relationships between the kringle domains of human plasminogen, prothrombin, tissue plasminogen activator, urokinase, and coagulation factor XII. J. Mol. Evol. 26:358–369, 1987.
57. Brockway, W.J., Castellino, F.J. Measurement of the binding of antifibrinolytic amino acids to various plasminogens. Arch. Biochem. Biophys. 151:194–199, 1972.
58. Sjoholm, I., Wiman, B., Wallen, P. Studies on the conformational changes of plasminogen induced during activation to plasmin and by 6-aminohexanoic acid. Eur. J. Biochem. 39:471–479, 1973.
59. Wallen, P., Wiman, B. Characterization of human plasminogen II. Separation and partial characterization of different molecular forms of human plasminogen. Biochim. Biophys. Acta 257:122–134, 1972.
60. Mangel, W., Lin, B., Ramakrishnan, V. Characterization of an extremely large, ligand-induced conformation change in plasminogen. Science 248:69–73, 1990.
61. Eaton, D.L., Fless, G.M., Kohr, W.J., McLean, J.W., Xu, Q-T., Miller, C.G., Lawn, R.M., Scanu, A.M. Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen. Proc. Natl. Acad. Sci. U.S.A. 84: 3224–3228, 1987.
62. Armstrong, V.W., Harrach, B., Robenek, H., Helmhold, M., Walli, A.K., Seidel, D. Heterogeneity of human lipoprotein Lp[a]: Cytochemical and biochemical studies on the interaction of two Lp[a] species with the LDL receptor. J. Lipid Res. 31:429–441, 1990.
63. Lopez, J.A., Chung, D.W., Fujikawa, K., Hagen, F.S., Papayannopoulou, T., Roth, G.J. Cloning of the alpha chain of human platelet glycoprotein Ib: A transmembrane protein with homology to leucine-rich $alpha_2$-glycoprotein. Proc. Natl. Acad. Sci. U.S.A. 84:5615–5619, 1987.
64. Frangione, B., Wolfenstein-Todel, C. Partial duplication in the "hinge" region of IgA_1 myeloma proteins. Proc. Natl. Acad. Sci. U.S.A. 69(12):3673–3676, 1972.
65. Giranda, V.L., Chapman, M.S., Rossman, M.G. Modeling of the human intercellular adhesion molecule-1, the human rhinovirus major group receptor. Proteins: Struc. Func. Genet. 7:227–233, 1990.
66. Day, E.D. (ed). The immune responses and their regulation. In: "Advances In Immunochemistry," 2nd ed., Chapter 12. New York: Wiley-Liss, 1990: 626–630.
67. Staunton, D.E., Marlin, S.D., Stratowa, C., Dustin, M.L., Springer, T.A. Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell 52:925–933, 1988.
68. Staunton, D.E., Dustin, M.L., Springer, T.A. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. Nature (London) 339:61–64, 1989.
69. Simmons, D. Makgoba, M.W., Seed, B. ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. Nature (London) 331:624–627, 1988.
70. Lottspeich, F., Kellermann, J., Henschen, A., Foertsch, B., Muller-Esterl, W. The amino acid sequence of the light chain of human high-molecular-mass kininogen. Eur. J. Biochem. 152:307–314, 1985.
71. Reed, J., Hull, W.E., von der Lieth, C.-W., Kubler, D., Suhai, S., Kinzel, V. Secondary structure of the Arg-Gly-Asp recognition site in proteins involved in cell-surface adhesion. Eur. J. Biochem. 178:141–154, 1988.
72. Jurgens, G., Marth, E., Kostner, G.M., Holasek, A. Investigation of the Lp(a) lipoprotein: Lipoprotein aggregating and enzymatic activity associated with the Lp(a) polypeptide. Artery 3(1):13–26, 1977.
73. Salonen, E.-M., Jauhiainen, M., Zardi, L., Vaheri, A., Ehnholm, C. Lipoprotein(a) binds to fibronectin and has serine proteinase activity capable of cleaving it. EMBO J. 8(13): 4035–4040, 1989.
74. Podlasek, S. J., McPherson, R.A. Streptokinase binds to lactate dehydrogenase subunit-M, which shares an epitope with plasminogen. Clin. Chem. 35(1):69–73, 1989.
75. Alessi, M.C., Parra, H.J., Joly, P., Vu-Dac, N., Bard, J.M., Fruchart, J.C., Juhan-Vague, I. The increased plasma Lp(a):B lipoprotein particle concentration in angina pectoris is not associated with hypofibrinolysis. Clin. Chim. Acta 188:119–128, 1990.
76. Mao, S.J.T., Tucci, M.A. Lipoprotein(a) enhances plasma clot lysis in vitro. FEBS lett 267(1):131–134, 1990.
77. Hedner, U., Davie, E.W. Introduction to hemostasis and the vitamin K-dependent coagulation factors. In: "The Metabolic Basis of Inherited Disease," Vol. II, 6th ed. Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D. (eds.), Chapter 84. New York: McGraw-Hill, 1989: 2116–2117.
78. Furlan, M. Structure of fibrinogen and fibrin. In: "Fibrinogen, Fibrin Stabilization, and Fibrinolysis. Clinical, Biochemical and Laboratory Aspects." Francis, J.L. (ed.), Chapter 1. New York: VCH, 1988: 17–64.
79. Morrisett, J.D., Gaubatz, J.W., Knapp, R.D., Guevara, J. G., Jr. Structural properties of apo(a): A major apoprotein of human lipoprotein(a). In: "Lipoprotein(a)." Scanu, A.M. (ed.), Chapter 4. San Diego: Academic Press, 1990: 53–74.
80. Castellino, F.J., Powell, J.R. Human plasminogen. Methods Enzymol. 80:365–378, 1981.
81. Robbins, K.C., Summaria, L., Wohl, R.C. Human plasmin. Methods Enzymol. 80:379–387, 1981.
82. Holmes, W.E. Pennica, D., Blaber, M., Rey, M.W., Guenzler, W.A., Steffens, G.J., Heyneker, H.L. Cloning and expression of the gene for pro-urokinase in *Escherichia coli*. Bio/Tech 3:923–929, 1985.
83. Tomlinson, J.E., McLean, J.W., Lawn, R.M. Rhesus monkey apolipoprotein(a). J. Biol. Chem. 264(10):5957–5965, 1989.
84. Miyazawa, K., Tsubouchi, H., Naka, D., Takahashi, K., Okigaki, M., Arakaki, N., Nakayama, H., Hirono, S., Sakiyama, O., Takhashi, K., Gohda, E., Diakuhara, Y., Kitamura, N. Molecular cloning and sequence analysis of cDNA for human hepatocyte growth factor. Biochem. Biophys. Res. Commun. 163:967–973, 1989.
85. Seki, T., Ihara, I., Sugimura, A., Shimonishi, M., Nishizawa, T., Asami, O., Hagiya, M., Nakamura, T., Shimizu, S. Isolation and expression of cDNA for different forms of hepatocyte growth factor from human leukocyte. Biochem. Biophys. Res. Commun. 172:321–327, 1990.