Comparative Catabolism of Prothrombin and Antithrombin in Normal and Alloxan-Diabetic Rabbits

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Previous studies have shown that alloxan-induced diabetes in rabbits effects a slower release of plasma proteins from the liver, a slower synthesis of ^{35}S-glycosaminoglycan in the extracellular matrix of the arterial wall, and a concurrent reduction in the fractional catabolic rates of several plasma proteins. In the present study, the catabolism of two hemostatic proteins, prothrombin and antithrombin, are compared in alloxan-induced diabetic rabbits (of 6 months' duration) and age-matched control rabbits. Differentially radiolabeled prothrombin and antithrombin were injected intravenously, and arterial blood was sampled over a 7-day period to measure the clearance from plasma. A three-compartment model was used to determine the fractional catabolic rate and compartmental distribution of the two proteins. As observed for other plasma proteins, the whole-body fractional catabolic rates (j_T) for prothrombin and antithrombin were significantly less in diabetic rabbits (prothrombin, 0.33 d^{-1}; antithrombin, 0.27 d^{-1}) than in control rabbits (prothrombin, 0.37 d^{-1}; antithrombin, 0.30 d^{-1}; $P < .001$ and $P < .005$, respectively). In absolute terms, the catabolism of antithrombin and prothrombin in diabetic rabbits was 5.1 and 6.2 mg · kg^{-1} · d^{-1}, respectively, equivalent to a molar ratio for antithrombin to prothrombin of 0.94. For the control rabbits, catabolism accounted for 6.3 mg · kg^{-1} · d^{-1} of antithrombin and 7.3 mg · kg^{-1} · d^{-1} of prothrombin, equivalent to a molar ratio of 1.01. The fractional distribution of these proteins was not significantly different within the intravascular and extravascular spaces in diabetic and control rabbits. The decreased catabolic rates observed for prothrombin and antithrombin in diabetic rabbits conform with results obtained previously for other plasma proteins, and probably reflect a generally decreased rate of plasma protein production by diabetic rabbit liver compared with control liver.

PREVIOUS STUDIES FROM our laboratory have shown that certain rabbit plasma proteins, namely fibrinogen,[1,2] albumin,[1,3] antithrombin-α,[1] and plasminogen (glycoforms I and II),[4] are catabolized more slowly in alloxan-induced diabetic rabbits than in normal rabbits of the same age. In keeping with these conclusions, we have recently shown that the rate of plasma protein (measured as protein-bound ^{3}H-leucine) secretion by the livers of alloxan-induced diabetic rabbits during perfusion is significantly less than that in age-matched normal rabbits, which confirms an observation reported previously in diabetic (BB/W) rats.[5] Furthermore, we compared the morphology of and ^{35}S-sulfate incorporation in vivo into proteoglycans of the extracellular matrix of the aorta wall in the normal and diabetic rabbit, and concluded that proteoglycans, and consequently glycosaminoglycans, are metabolized more slowly in the extracellular matrix in diabetic versus normal aorta.[6] These data indicate that the alloxan-induced diabetic rabbit may synthesize and catabolize macromolecules more slowly than the normal rabbit.

The present study tests this hypothesis by measuring the catabolic behavior of two proteins with opposing function in hemostasis, namely prothrombin and antithrombin (previously known as antithrombin III), in groups of diabetic and age-matched control rabbits. The results show that both proteins are catabolized significantly more slowly in the diabetic than in the normal rabbit.

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MATERIALS AND METHODS

Purification of Proteins

Prothrombin was purified from New Zealand White (NZW) rabbit plasma, anticoagulated with acid-citrate-dextrose (ACD),[17] using a combination of two published methods.[8,9] The full preparative method and the properties of rabbit prothrombin are reported in detail elsewhere.[10] Purified prothrombin (0.5 to 0.8 mg/mL) was snap-frozen in liquid N_2 and stored at −40°C. The protein appeared as a single band (M_r 72 kd relative to prestained standard proteins obtained from Novex, San Diego, CA) on polyacrylamide gel electrophoresis (PAGE) in 0.1% sodium dodecyl sulfate (SDS)[11] and using a nondenaturing PAGE system.[10] Activation of purified prothrombin by factor Xa in the presence of thromboplastin, Ca^{2+}, and factor V yielded 1.3 IU thrombin/μg (measured as plasma clotting activity[12] relative to a known thrombin standard).[10]

Antithrombin was purified from rabbit ACD plasma by heparin-Sepharose chromatography.[13] The principal glycoform, antithrombin-α, was used for all experiments described herein. Details of the purity and properties of rabbit antithrombin-α have been described previously.[1,13]

Radiolabeling Prothrombin and Antithrombin

The procedure using Iodogen-coated glass vials has been described previously.[14,15] In a screw-capped glass vial coated with 5 μg Iodogen, approximately 45 to 70 μg of either protein was reacted with 1 mCi of either ^{125}I or ^{131}I (ICN Biomedicals, Costa Mesa, CA) in 400 μL 0.1-mol/L Na phosphate, pH 7.4, for 2 minutes at room temperature. In the procedure for radiolabeling prothrombin,[10] ε-aminohexanoic acid (ε-AHA) was included in the reaction (final concentration, 25 mmol/L) to prevent aggregation of ^{131}I-prothrombin during iodination. Both proteins were dialyzed against 0.01 mmol/L sodium phosphate–buffered saline, pH 7.4, with ε-AHA (final concentration, 2.5 mmol/L) added to the dialysate for ^{131}I-prothrombin.

The properties of ^{131}I-prothrombin preparations were compared with those of unlabeled prothrombin using both SDS-PAGE and nondenatur-

ing PAGE methods.[10] Activation of ^{131}I-prothrombin, by human factor Xa in the presence of thromboplastin and Ca^{2+} (Simplastin; Organon Teknika, Durham, NC), to ^{131}I-thrombin was tested using SDS-PAGE as described previously.[10] The properties of ^{125}I-antithrombin and unlabeled antithrombin were compared before and after reaction with a known quantity of purified bovine thrombin, using SDS-PAGE as described previously.[16] After electrophoresis, all gels were fixed, dried, and exposed to Kodak XAR-5 film (Eastman Kodak, Rochester, NY).

Animal Studies

All animal experimentation protocols were approved by the Animal Research Ethics Board of McMaster University.

The procedure of Miller and Wilson,[17] modified only slightly,[1,2] was used to induce a chronic diabetic condition in NZW rabbits. The mean weight of the rabbits at the time of alloxan (or saline) treatment was 3.5 kg. After treatment, the diabetic and control rabbits were housed in the Central Animal Facility at McMaster University Health Sciences Centre under conditions approved by the Canadian Council for Animal Care. All rabbits were fed normal rabbit chow and given water ad libitum. Only alloxan-treated rabbits with a plasma glucose more than 15 mmol/L over at least a 6-month period were used for the experiments. Plasma glucose levels in blood samples taken at approximately 6-week intervals were measured by the Section of Laboratory Medicine, Chedoke-McMaster Hospitals (Hamilton, Ontario, Canada).

Blood samples (~2 mL) were taken from the ear artery of six diabetic and four age-matched control rabbits at 6 months after alloxan or saline treatment, into a known volume of ACD.[7] The blood samples were weighed, and the plasma fraction was recovered after centrifugation (10,000 × *g* for 3 minutes). Enzyme-linked immunosorbent assays (ELISAs) were used to measure prothrombin and antithrombin concentrations in both control and diabetic plasma. Six dilutions of each plasma sample were measured in duplicate relative to standard quantities of rabbit prothrombin or antithrombin. Final values were adjusted to correct for ACD content, assuming a hematocrit of 42% for rabbit blood.

The ELISA for rabbit antithrombin has been described previously.[16,18] Affinity-purified polyspecific anti-rabbit antithrombin (raised in a sheep) was used as the capture antibody, biotin-linked anti-rabbit antithrombin as the detection antibody, and then streptavidin-linked alkaline phosphatase (Amersham Canada, Oakville, Ontario) as the detecting enzyme.

The procedure for measuring rabbit plasma prothrombin is described briefly as follows. All incubations were performed at 37°C. An affinity-purified sample of polyspecific anti-rabbit prothrombin (raised in a sheep) was used as the capture antibody; the appropriate dilution of this antibody was placed in all wells of a 96-well plate and incubated for 1 hour. After rinsing the plate and blocking nonspecific binding sites of all the wells with 5% wt/vol bovine serum albumin (1 hour), dilutions of antigen were applied to appropriate wells and incubated (1 hour). The plate was then rinsed, and the primary detection antibody, affinity-purified anti-rabbit prothrombin (raised in a laying hen; immunoglobulin G-[IgG] isolated from the egg yolks[19]), was added to each well (1-hour incubation). The plate was rinsed, and alkaline phosphatase–linked anti-chicken IgG (Zymed, San Francisco, CA) was added (1-hour incubation). The plate was rinsed again, and a standard substrate (*p*-nitrophenyl phosphate) was added to each well. The plate was incubated at 37°C for 20 to 30 minutes, and absorbance was read at 405 nm using an automatic plate reader (Titertek Multiskan Plus Mark II; Labsystems, Helsinki, Finland).

Catabolism of ^{131}I-Prothrombin and ^{125}I-Antithrombin

The general procedure (intravenous injection, frequency and method of blood sampling, treatment, and radioactivity measurement of blood or plasma samples, correction for ACD content of blood, and correction for protein-bound radioactivity in plasma) has been described previously.[1,2] Only specific details will be given here.

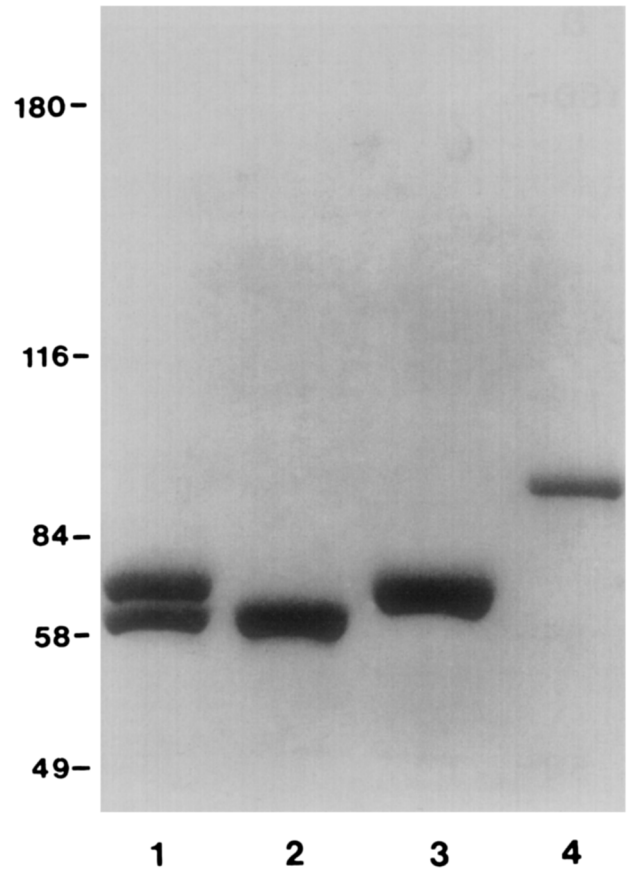

Fig 1. SDS-PAGE of the rabbit antithrombin glycoforms and rabbit prothrombin. The gel (7.5% polyacrylamide) was stained with Coomassie brilliant blue. Lane 1, a mixture of antithrombin-α and -β (3 μg each); lane 2, antithrombin-β (6 μg); lane 3, antithrombin-α (6 μg); lane 4, prothrombin (3 μg).

Five diabetic NZW rabbits (mean ± SD: weight, 3.8 ± 0.3 kg; plasma glucose, 19.4 ± 1.6 mmol/L) and five age-matched control NZW rabbits (5.1 ± 0.2 kg; 5.4 ± 0.3 mmol/L) were injected (ear vein) with both radiolabeled proteins, each in 1 mL sterile saline (dose for ^{125}I-antithrombin, ~10 μg/kg, 5 to 6 μCi/kg; for ^{131}I-prothrombin, 15 μg/kg, 8 to 10 μCi/kg). Blood sampling (23-gauge needle, ~1 to 2 mL blood collected into known volume of ACD) from an ear artery was started at 5 minutes after injection and repeated at defined intervals for 7 days.

Interpretation of Plasma Curves

The process of curve-peeling clearly exposed three exponentials for all plasma curves of prothrombin and antithrombin clearance. For this reason,[20] a three-compartment model was used to calculate the fractional catabolic rate and compartmental distribution of prothrombin and antithrombin in the diabetic and control rabbits. The preferred model was that reported by Carlson et al[21] to best fit the data for clearance of ^{125}I-antithrombin from the circulation of the rabbit; the three compartments described represent the intravascular space, noncirculating intravascular space (ie, the portion that is adsorbed reversibly to the vascular wall), and extravascular space. Using the curve-peeling

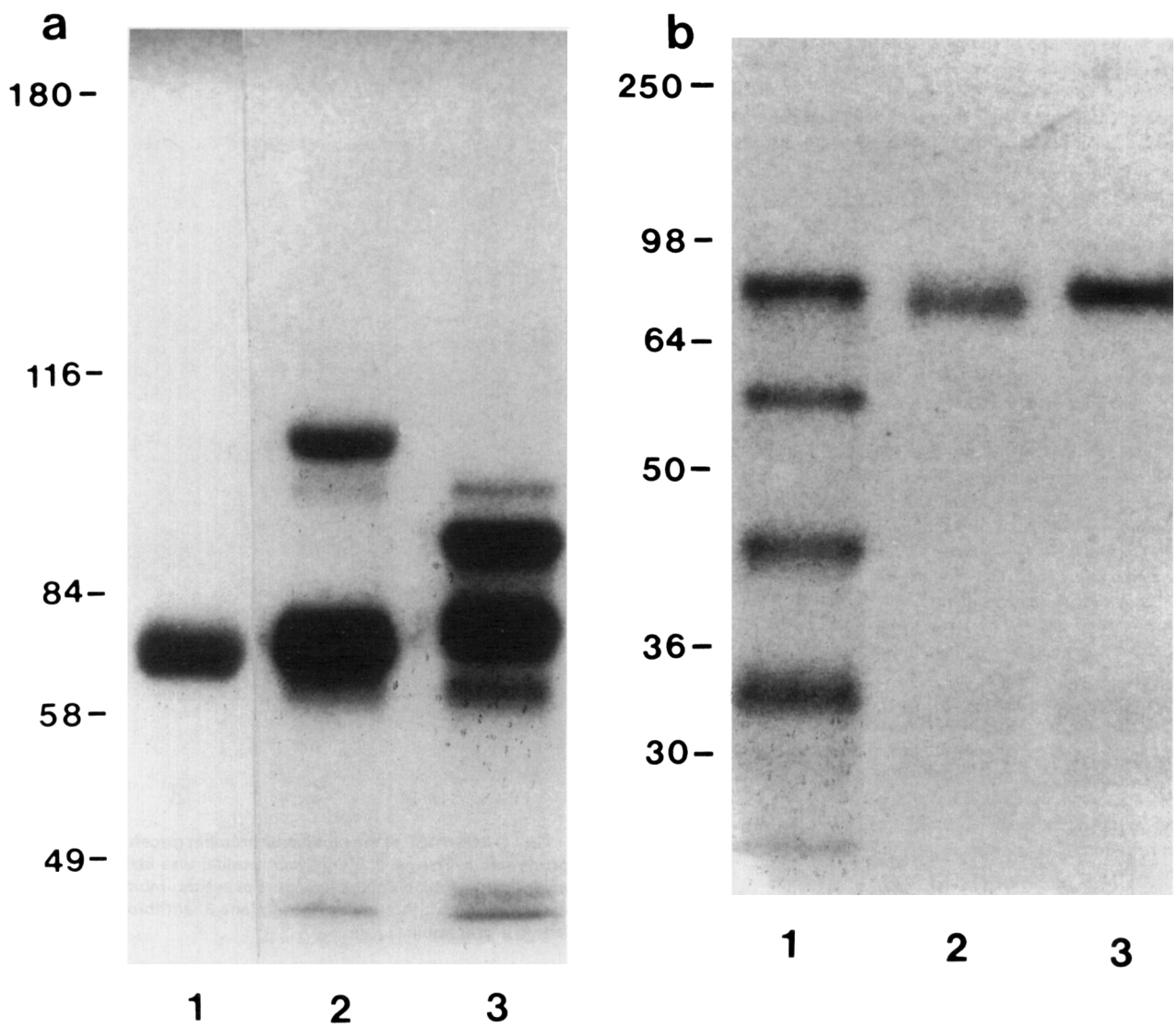

Fig 2. SDS-PAGE followed by autoradiography of (a) ^{125}I-antithrombin (before and after reaction with thrombin) and (b) ^{131}I-prothrombin (before and after reaction with factor Xa, Ca^{2+}, and phospholipid).[10] All incubations (10 to 20 μL) were for 15 minutes at 37°C. Incubations were stopped by addition of 5 μL aqueous solution containing 2% SDS-12.5% glycerol followed by heating at 100°C for 1 minute. Samples (10 μL) were then electrophoresed. (a) Lane 1, ^{125}I-antithrombin incubated with saline; lane 2, ^{125}I-antithrombin incubated with unlabeled thrombin (molar ratio for antithrombin to thrombin, 1,2) to yield thrombin-^{125}I-antithrombin complex at ~100 kd; lane 3, ^{125}I-antithrombin incubated with thrombin (molar ratio, 0.5); excess thrombin has partially degraded the thrombin-^{125}I-antithrombin complex. (b) Lane 1, ^{131}I-prothrombin incubated with factor Xa, thromboplastin, and Ca^{2+} to yield intermediates including ^{125}I-thrombin (~32 kd); lane 2, ^{131}I-prothrombin incubated with saline; lane 3, ^{131}I-prothrombin that had not been incubated. After the gels were fixed and dried, they were exposed to Kodak X-AR5 film for autoradiography.

technique and linear regression analysis, curve components were calculated to define three exponentials for each plasma curve, $^*A_p = C_1e^{-a_1t} + C_2e^{-a_2t} + C_3e^{-a_3t}$, where $^*A_p$ is the fraction of a radiolabeled protein remaining in the circulation at t days after injection; C_1, C_2, and C_3 are the fractional constants for the three compartments; and a_1, a_2, and a_3 are the respective rate constants of exchange between the compartments. The constants C and a were calculated for the plasma curve of each radiolabeled protein obtained from individual rabbits. From the C and a values of each plasma curve, fractional catabolic rates were calculated for the plasma compartment (j_3), the plasma plus noncirculating vascular wall compartment ($j_{3,5}$), and the total body (j_T). The j values were used to calculate the distribution of each protein within the plasma (A_p) and noncirculating vascular wall (A_w) and extravascular (A_e) compartments.[21]

Calculation of Data

Where appropriate, data are presented as the mean ±1 SD. Statistical significance was determined using the Student *t* test (two-tailed).

RESULTS

Properties of the Radiolabeled Proteins

Figure 1 shows typical SDS-PAGE results obtained with the preparations of unlabeled prothrombin and antithrombin-α used

for radiolabeling. Antithrombin-β is shown for comparison. Unreacted ^{125}I-antithrombin appeared as a single band of M_r 62 kd (Fig 2a, lane 1). After reaction with thrombin at a molar ratio for thrombin to antithrombin of 1.2, much of the ^{125}I-antithrombin reacted to yield thrombin-^{125}I-antithrombin complex (at M_r ~100 kd) and some post–complex ^{125}I-antithrombin (ie, antithrombin that has reacted with but separated from thrombin[13]; M_r ~70 kd), and a large proportion of unreacted ^{125}I-antithrombin (~62 kd). The molar ratio of the load in lane 3 was 2; the thrombin-^{125}I-antithrombin complex had been degraded by excess thrombin to a smaller-size complex (M_r ~86 kd) that, from immunoblotting,[13] was reactive with antibodies against antithrombin or against thrombin. Most of the radiolabeled product in lane 3 was the 70-kd post–complex antithrombin that was immunoreactive against anti-antithrombin antibodies but not against antithrombin antibodies.[13] A small quantity of unreacted ^{125}I-antithrombin was also observed. Freshly prepared rabbit ^{131}I-prothrombin was observed as a single band of approximately M_r 72 kd (Fig 2b, lane 3). Incubation for 15 minutes with saline caused essentially no change in the electrophoresis profile (lane 2). However, in the presence of factor Xa, thromboplastin, and Ca^{2+}, much of the ^{131}I-prothrombin was cleaved into three smaller fragments that on nonreducing SDS-PAGE, were observed at approximately 58 kd (equivalent to prothrombin lacking the F.1 fragment), 40 kd (prothrombin lacking fragments F.1 and F.2), and 32 kd (thrombin).

Plasma Clearance of ^{131}I-Prothrombin and ^{125}I-Antithrombin

The behavior of ^{131}I-prothrombin and ^{125}I-antithrombin (both as protein-bound radioactivity) in diabetic rabbits compared to control rabbits is shown in Fig 3a and b, respectively. The plasma clearance of both proteins in control rabbits was significantly faster than in diabetic rabbits.

Linear regression analysis was used to calculate a and C values for the three exponentials of each plasma curve representing prothrombin and antithrombin clearance in each rabbit. The mean R^2 values for the exponentials of the five prothrombin curves for control rabbits were calculated to be .991 ± .003, .998 ± .002, and .976 ± .007, and for diabetic rabbits, .991 ± .006, .996 ± .004, and .962 ± .019. The mean R^2 values for the exponentials of the five antithrombin curves for the control rabbits were .990 ± .003, .997 ± .003, and .967 ± .019, and for the diabetic rabbits, .986 ± .006, .998 ± .001, and .970 ± .018.

The fractional rates of catabolism of prothrombin and antithrombin within the vascular space (j_3) in diabetic rabbits were slower than in control rabbits (Table 1). This observation for prothrombin and antithrombin was further supported by the measurement of the whole-body catabolic rate (j_T), which was significantly lower in diabetic rabbits compared with age-matched controls. No significant differences in compartmental distribution were noted for either protein (Table 1).

Plasma Concentrations of Prothrombin and Antithrombin

The prothrombin concentration in plasma samples from four control and six diabetic rabbits was determined by ELISA to be 1.93 ± 0.07 μmol/L and 1.79 ± 0.20 μmol/L, respectively. Thus, the prothrombin concentration was not significantly different in diabetic plasma versus control plasma. These values compared well with a previously reported value of 1.8 μmol/L for normal rabbit plasma.[10] In the present report, a plasma concentration of 1.8 μmol/L (equivalent to a blood concentration of 1.04 μmol/L) was used for both control and diabetic rabbits.

The antithrombin concentration in the control plasma was 2.55 ± 0.12 μmol/L, and in the diabetic plasma, 2.58 ± 0.22 μmol/L. Thus, no significant difference was detected for the antithrombin concentration in control versus diabetic rabbit plasma. These values compared closely with rabbit plasma antithrombin content as measured by yield after heparin-Sepharose affinity chromatography.[13] After correction for the antithrombin loss during preparation and the ACD content of the plasma, Witmer and Hatton[13] calculated a value of 16.0 mg antithrombin-α and -β/100 mL plasma (ie, 2.54 μmol/L) in

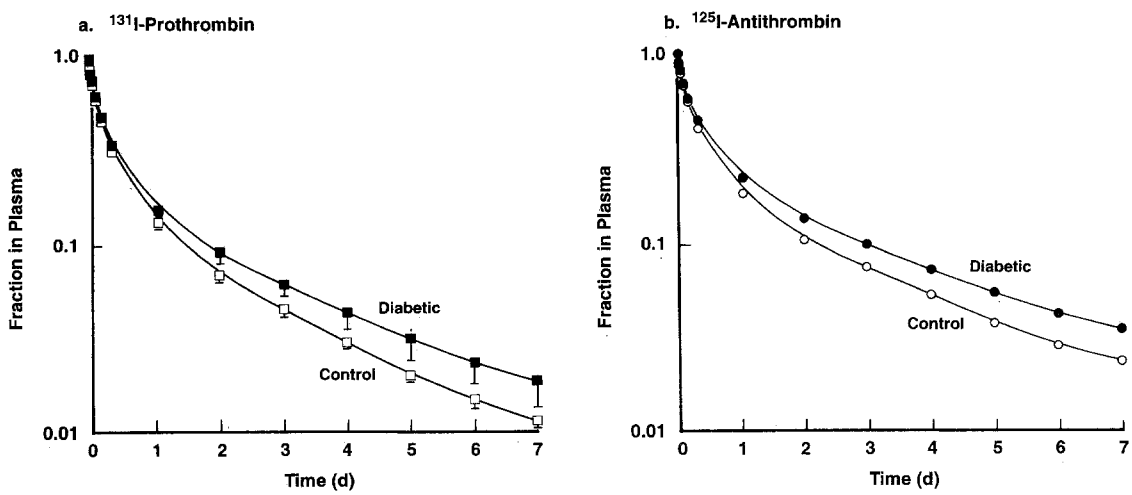

Fig 3. Mean plasma clearance curves for (a) ^{131}I-prothrombin and (b) ^{125}-I-antithrombin (as protein-bound radioactivity) from the circulation of 5 diabetic rabbits and 5 age-matched control rabbits. Error bars are shown only when they exceed the size of the symbol. Data calculated from plasma curves of individual rabbits were used to calculate the data in Table 1.

Table 1. Fractional Catabolic Rate and Compartmental Distribution of Rabbit Prothrombin and Antithrombin Measured in Diabetic and Control Rabbits

Protein	j_3 (d^{-1})	$j_{3.5}$ (d^{-1})	j_T (d^{-1})	A_p	A_w	A_e
Controls						
Prothrombin	1.70 ± 0.14	1.07 ± 0.17	0.37 ± 0.01	0.22 ± 0.02	0.14 ± 0.04	0.65 ± 0.06
Antithrombin	1.19 ± 0.07	0.86 ± 0.12	0.30 ± 0.01	0.25 ± 0.01	0.10 ± 0.05	0.65 ± 0.05
Diabetics						
Prothrombin	1.45 ± 0.19	0.97 ± 0.15	0.33 ± 0.04*	0.23 ± 0.02	0.11 ± 0.02	0.66 ± 0.02
Antithrombin	0.96 ± 0.05*	0.73 ± 0.09	0.27 ± 0.03†	0.28 ± 0.02	0.09 ± 0.03	0.64 ± 0.03

NOTE. Five diabetic rabbits and their age-matched controls were injected intravenously with ^{125}I-antithrombin-α and ^{131}I-prothrombin; blood samples were taken at intervals over 7 days after injection. Protein-bound radioactivity was determined from the total radioactivity of each plasma sample to obtain a plasma clearance curve (see Fig 3a and b). Data were calculated using a 3-compartment model,[21] after peeling individual plasma curves.

Abbreviations: j_3, $j_{3.5}$, and j_T, fractional rate of catabolism per day in the vascular, noncirculating vascular, and total body compartments, respectively; A_p, A_w, and A_e, fractional distribution of each protein in the intravascular, vessel-wall, and extravascular compartments, respectively.
*$P < .001$, †$P < .005$: diabetic *v* control.

plasma samples from five healthy rabbits. A plasma antithrombin concentration of 2.54 μmol/L, equivalent to a blood concentration of 1.47 μmol/L, was taken for both control and diabetic rabbits.

DISCUSSION

In these studies, the properties of ^{131}I-prothrombin and ^{125}I-antithrombin were used to gain new insight into the catabolism of prothrombin and antithrombin in vivo. Since the behavior of ^{125}I-antithrombin and ^{131}I-prothrombin was similar but not identical to the behavior of their respective unlabeled proteins in vitro, we assumed that the behavior in vivo would compare closely with that of endogenous antithrombin and prothrombin in the intravascular and extravascular circulation.

The catabolic rates for prothrombin and antithrombin in the diabetic rabbit were found to be lower than in the normal rabbit. A decreased rate of catabolism for other plasma proteins synthesized by the liver, eg, albumin[1,3,22] and fibrinogen,[1,2] has been reported previously in experimentally induced diabetic rats[22] and rabbits.[1-3] To maintain a constant plasma concentration of any protein, a decreased catabolic rate is consistent with a proportionately decreased rate of protein synthesis. In the drug-induced diabetic rat and rabbit, a decreased synthesis of albumin and other plasma proteins has been reported from liver perfusion studies ex vivo[4,23,24] and from a study in vivo.[25]

This observation may not be limited to the cells of the liver, as it is probable that most (if not all) macromolecular products synthesized by insulin-dependent cells are metabolized more slowly than their counterparts from normal cells. If blood insulin levels are decreased to the extent that glucose supply to peripheral cells is compromised, then adenosine triphosphate (ATP) production will be correspondingly threatened. This situation likely will jeopardize the turnover of all metabolites, but particularly the synthesis of biopolymers that are the products of energy-dependent multistep pathways, eg, proteins and polysaccharides. In short, ATP production in the diabetic cell becomes a rate-limiting factor in protein and polysaccharide synthesis. It follows that, in general, if synthesis rates are decreased, then catabolic rates will be decreased also to maintain homeostatic balance.

From the data in Table 1, the compartmental distribution of the two hemostatic proteins showed that prothrombin and antithrombin are in approximately similar molar concentrations in all three body compartments. Knowing that the blood concentrations of prothrombin (1.04 μmol/L) and antithrombin (1.47 μmol/L) found in healthy rabbits[10] are also found in diabetic rabbits, the mean molar ratio of the noncirculating fraction of antithrombin/prothrombin associated with the endothelium (A_w) is 0.93 for the vasculature of normal rabbits and 0.89 for diabetic rabbits (Table 2). These values indicate the presence of a relatively greater quantity of bound prothrombin than of antithrombin at the uninjured vascular wall of normal and diabetic rabbits. We emphasize that this observation refers to the vascular wall of the systemic circulation as a whole and does not assess the prothrombotic quality of the endothelium at any specific site of the vascular bed. These data also do not take into consideration the quantity of bound heparin cofactor II or any other thrombin inhibitor. However, we would note that the endothelial surface of the healthy vascular wall is recognized generally as nonthrombogenic,[26] whereas previous study from our laboratory has indicated that the aortic intima of the diabetic rabbit has relatively prothrombotic properties.[1]

Assuming a blood volume of 58 mL/kg, the data in Table 1 indicate that a 5-kg rabbit contains about 99.1 mg prothrombin and 105.3 mg antithrombin distributed among the three compartments. From the j_T values, approximately 36.4 mg prothrombin (equivalent to 7.3 mg · kg^{-1} · d^{-1}) and 31.5 mg antithrombin (or 6.3 mg · kg^{-1} · d^{-1}) are catabolized per day, equivalent to a molar ratio for antithrombin to prothrombin of 1.01. If we assume a similar blood volume per kilogram for the diabetic rabbit, then for a 3.8-kg rabbit, 23.6 mg prothrombin (equivalent to 6.2 mg · kg^{-1} · d^{-1}) and 19.4 mg antithrombin (or 5.1 mg · kg^{-1} · d^{-1}) are catabolized per day, equivalent to a cata-

Table 2. Molar Ratio of Antithrombin to Prothrombin in the Three Body Compartments (intravascular space, vascular wall, and extravascular space) of Diabetic and Control Rabbits

	Molar Ratio (antithrombin/prothrombin)		
Group	Intravascular	Vessel Wall	Extravascular
Control	1.43	0.93	1.23
Diabetic	1.41	0.89	1.11

NOTE. The molar ratio refers to the entire body and not to any specific organ or blood vessel. It is calculated from the fractional compartmental distribution (Table 1).

bolic molar ratio for antithrombin to prothrombin of 0.94. The finding that equimolar amounts of prothrombin and antithrombin are catabolized per day probably indicates that under normal (ie, nontraumatic) conditions antithrombin colocalizes with prothrombin and provides efficient protection against the possibly damaging activities of thrombin. Even after a balloon catheter–induced deendothelializing injury to the rabbit aorta, the molar ratio of antithrombin and prothrombin taken up by the healing aorta surface was observed to be approximately unity throughout the wound-healing process (at least 20 months).[27] Presumably, if an injury is extensive enough to cause a decrease of this molar ratio, then acute thrombosis could result with life-threatening consequences.

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REFERENCES

1. Witmer MR, Hadcock SJ, Peltier SL, et al: Altered levels of antithrombin and fibrinogen in the aortic wall of the alloxan-induced diabetic rabbit: Evidence of a prothrombotic state. J Lab Clin Med 119:221-230, 1992

2. Hatton MWC, Southward S, Ross-Ouellet B, et al: Catabolism of unglycated and naturally glycated forms of rabbit fibrinogen: Their interaction with the healthy and de-endothelialized aorta wall in normal and diabetic rabbits. J Lab Clin Med 121:588-597, 1993

3. Hatton MWC, Winocour PD, Richardson M: On glucose transport and non-enzymic glycation of proteins in vivo. J Theor Biol 161:481-490, 1993

4. Hatton MWC, Southward SMR, Ross-Ouellet B, et al: Comparative metabolism of plasminogen glycoforms I and II in the alloxan-diabetic rabbit. Am J Physiol 269:E1017-E1023, 1995

5. Jefferson LS, Liao WSL, Peavy DE, et al: Diabetes-induced alterations in liver protein synthesis. Changes in the relative abundance of mRNAs for albumin and other plasma proteins. J Biol Chem 258:1369-1375, 1983

6. Richardson M, Hadcock SJ, Hatton BD, et al: Proteoglycan alterations in the aortic intima-media of alloxan-diabetic rabbits: An ultrastructural and biochemical study. Exp Mol Pathol 62:145-159, 1995

7. Aster RH, Jandl JH: Platelet sequestration in man. I. Methods. J Clin Invest 43:843-854, 1964

8. Bajaj SP, Mann KG: Simultaneous purification of bovine prothrombin and factor X: Activation of prothrombin by trypsin-activated factor X. J Biol Chem 248:7729-7741, 1973

9. Modi GJ, Blajchman MA, Ofosu FA: The isolation of prothrombin, factor IX and factor X from factor IX concentrates. Thromb Res 36:537-547, 1984

10. Hatton MWC, Southward SMR, Serebrin SD, et al: Catabolism of rabbit prothrombin in rabbits: Uptake of prothrombin by the aorta wall before and after a de-endothelializing injury in vivo. J Lab Clin Med 126:521-529, 1995

11. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685, 1970

12. Hatton MWC: Studies on the fibrinogen-coagulant enzyme from *Aqkistrodon rhodostoma* venom: Isolation and some properties of the enzyme. Biochem J 131:799-807, 1973

13. Witmer MR, Hatton MWC: Antithrombin III-β associates more readily than antithrombin III-α with the uninjured and de-endothelialized rabbit aortic wall in vitro and in vivo. Arterioscler Thromb 11:530-539, 1991

14. Fraker PJ, Speck JC: Protein and cell membrane iodinations with a sparingly soluble chloramide, 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. Biochem Biophys Res Commun 80:849-857, 1978

15. Regoeczi E: Iodine-Labeled Plasma Proteins, vol 1. Boca Raton, FL, CRC, 1984, pp 49-53

16. Hatton MWC, Hoogendoorn H, Southward SMR, et al: Comparative metabolism and distribution of rabbit heparin cofactor-II and rabbit antithrombin in rabbits. Am J Physiol 272:E824-E831, 1997

17. Miller RA, Wilson RB: Atherosclerosis and myocardial ischemic lesions in alloxan-diabetic rabbits fed a low cholesterol diet. Arteriosclerosis 4:586-591, 1984

18. Sheffield WP, Castillo JE, Blajchman MA: Intracellular events determine the fate of antithrombin Utah. Blood 86:3461-3467, 1995

19. Polsen A, Coetzer T, Kruger J, et al: Improvements in the isolation of IgY from the yolks of eggs laid by immunized hens. Immunol Invest 14:323-328, 1985

20. Carson ER, Jones EA: Use of kinetic analysis and mathematical modeling in the study of metabolic pathways in vivo. N Engl J Med 300:1016-1027, 1979

21. Carlson TH, Atencio AC, Simon TL: In vivo behavior of radioiodinated rabbit antithrombin III. Demonstration of a noncirculating vascular compartment. J Clin Invest 74:191-199, 1984

22. Murtiashaw MH, Baynes JW, Thorpe SR: Albumin catabolism in diabetic rats. Arch Biochem Biophys 225:256-262, 1983

23. Marsh JB: Effects of fasting and alloxan diabetes on albumin synthesis by perfused rat liver. Am J Physiol 201:55-57, 1961

24. Jefferson LS, Liao WSL, Peavy DE, et al: Diabetes-induced alterations in liver protein synthesis: Changes in the relative abundance of mRNAs for albumin and other plasma proteins. J Biol Chem 258:1369-1375, 1983

25. Peavy DE, Taylor JM, Jefferson LS: Correlation of albumin production rates and albumin mRNA levels in livers of normal, diabetic, and insulin-treated diabetic rats. Proc Natl Acad Sci USA 75:5879-5883, 1978

26. Damus PS, Hicks M, Rosenberg RD: Anticoagulant action of heparin. Nature 246:355-357, 1973

27. Hatton MWC, Southward SMR, Ross-Ouellet B, et al: An increased uptake of prothrombin, antithrombin and fibrinogen by the rabbit balloon-deendothelialized aorta surface in vivo is maintained until reendothelialization is complete. Arterioscler Thromb Vasc Biol 16:1147-1155, 1996