eter with a Couette attachment, between shear rates of 290 and 1155 sec^{-1} for various time intervals at 4° and 37°C, and tested for loss in clottability. Results were not influenced significantly by temperature. It has been found that three enzyme systems tested in a similar way were not significantly affected by surface denaturation (*1*). The plasma was prepared by passage through an ion-exchange column where calcium was removed to prevent clotting during the test (*2*).

Clottability was measured by addition of thrombin (0.2 ml, 25 unit/ml) to plasma (0.5 ml) in a buffer (1.5 ml) containing 0.15*M* ammonium acetate, 0.1*M* 6-aminocaproic acid, and 17 m*M* Ca ion. The clot that formed after the sample was incubated for 3 hours at 37°C was washed and dissolved in 40 percent urea in 0.2*N* NaOH; its optical density at 280 nm ($O.D._{280}$) was read against a urea blank. The optical density of the sample was compared with that of plasma (*3*).

The results shown in Fig. 1 indicate that fibrinogen clottability is lost with shearing. The data on the relation between shear rate and time and loss of clottability are resolved in a single curve when the loss in clottability is plotted against log $\gamma\theta$ (see Fig. 2). When $(\gamma\theta)^1$ or $(\gamma\theta)$ is less than 10^5, there is no loss in clottability of fibrinogen. At $\gamma\theta$ of 5×10^8, there is a 50 percent loss. The shearing effect is the same at 4° as at 37°C.

Plasma was also recycled by a finger pump through a cylindrical tube (203 by 0.11 cm) (*1*). The mass average shear was calculated from Eq. 2, and the loss of clottability associated with this shear was taken from Fig. 2. The loss in clottability was also determined experimentally, and the calculated loss was compared with the experimental loss after the loss due to the pump was determined. The results are in agreement (see Table 1).

The mass average shear associated with human circulation may be estimated from the L/D of the various vessels and the volume of blood in the vessels. If we use Burton's values for L/D and the volume of blood (*4*), the mass average shear per second for a 60-kg man is about 1470 per second (Table 2 and Eq. 2). The greatest shear is calculated to occur in the pulmonary capillaries.

From Fig. 2, the shear for 50 percent loss in clottability is $\gamma\theta = 5 \times 10^8$. The $(\gamma\theta)^1$ per second is 1.47×10^3 (Table 2), and the time required in the circulation for this half-life to occur is $(5 \times 10^8)/(1.47 \times 10^3) = 3 \times 10^5$ seconds or about 4 days.

There have been several reports that 50 percent of the fibrinogen in the circulation is turned over every 4 to 5 days (for example, *5*). The cause of the loss in vivo is not known. Some investigators have attributed fibrinogen turnover to constant intravascular formation of fibrin.

In view of the agreement of the half-life of fibrinogen between reported values and our values obtained by shearing degradation of fibrinogen in vitro, our results strongly suggest that shearing is the chief contributor to fibrinogen degradation in the circulation. Although the relationships derived here apply strictly to a Newtonian fluid in laminar flow, they permit an approximation to the actual conditions associated with blood flow.

Thus, shearing may also be the mechanism for reported degradation of lipoproteins, transferrin, acid α-glycoprotein, albumin, and globulins which also occurs in the circulation (*5*).

Appendix

To obtain $(\gamma\theta)_{av} = (8/3)\ (L/R_w)$ the following substitutions are made in Eq. 1 before integrating: $Q = (\pi/8\mu)\ (P/L)\ (R_w^4)$, Poiseuille's equation; $\theta_r = L/v$; and $\gamma_r = PR/4\mu L$. Here P/L is the drop in pressure per unit length and μ is the viscosity.

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6. We thank Professors W. Hughes and F. Rothstein for advice and P. Leavis for the plasma. B.L.W. is supported by a Medical School fellowship. Supported in part by PHS grant 5 PO7 RR00346-02.

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Gibbon Fibrinopeptides: Identification of a Glycine-Serine Allelism at Position B-3

Abstract. *The fibrinopeptides A and B of the gibbon (an Asian ape) have been characterized and their relation to other primate types examined. An allelic situation was discovered at location B-3; two of the gibbons studied had both glycine and serine at that position, whereas four others were homozygous for glycine.*

In the course of a study on primate relationships based on molecular comparisons, we examined the fibrinopeptides of an individual gibbon (*Hylobates lar entelloides*). Gibbons are Asian apes, a group which also includes the siamang (great gibbon) and orangutan. Because of the small size and high value of these creatures, it was necessary to employ plasmapheresis to obtain enough blood plasma for this study without endangering the animal. Using previously described techniques (*1–4*), we were able to prepare fibrinogen from about 100 ml of plasma and to characterize the fibrinopeptides A and B (Fig. 1).

The gibbon peptides are closely related to other hominoid types, although the differences are numerous enough to emphasize how much more closely man is related to the African apes (chimpanzee) than to Asian apes, confirming previous immunochemical observations (*5*). The gibbon fibrinopeptide A differed from that of chimpanzee and human, whose fibrinopeptides are identical (*4*), in 2 of 16 positions. At position A-14 the gibbon has a threonine, just as do all the Old World monkeys examined to date; chimpanzees and humans have serine. At position A-10 the gibbon has a glutamic acid instead of the aspartic acid which exists in the other known primate structures. In the case of the fibrinopeptide B, there was one unambiguous amino acid difference between the gibbon and chimp-human structures, the latter having a phenylalanine at position B-5 where the gibbon has a leucine. Furthermore, an internal deletion of one residue has occurred on the gibbon line at position B-7 or B-8 (Fig. 2).

Table 1. Amino acid compositions of fibrinopeptides A and B from six individual gibbons. Results are expressed as the number of residues per mole of peptide (calculated as molar ratios of amino acids recovered). Determinations were made on a Spinco model 120B amino acid analyzer after total acid hydrolysis.

Residue	Fibrinopeptide A						Fibrinopeptide B					
	Blacky	Fang	Thor	Heidi	Whitey	Pop*	Blacky	Fang	Thor	Heidi	Whitey	Pop*
Aspartic acid	0.98	1.10	1.14	1.06	1.07	1.06	3.00	2.96	2.72	2.74	2.90	2.70
Threonine†	0.97	0.93	0.82	0.98	0.92	0.91						
Serine†							0.51	0.50				
Glutamic acid	3.24	3.11	3.22	3.14	3.09	3.09	2.11	2.10	2.11	2.16	2.10	2.16
Glycine	4.96	5.17	4.98	4.93	4.98	4.97	2.48	2.59	3.07	3.15	3.02	3.12
Alanine	2.04	1.92	1.92	1.91	1.94	1.92	1.01	0.99	1.09	0.99	1.00	1.01
Valine	0.93	0.79	0.92	0.92	0.92	1.02	0.91	0.90	0.93	0.91	0.96	0.96
Leucine	1.09	0.98	1.01	1.06	1.02	1.01	0.97	0.99	1.06	1.02	0.99	0.97
Phenylalanine	0.97	1.00	0.99	1.02	1.04	1.02	1.00	0.99	1.02	1.04	1.02	1.03
Arginine‡	0.82	0.92	0.92	1.07	1.07	0.95	1.01	(1)‡	0.99	0.90	0.96	1.08
Total residues	16.00	15.92	15.90	16.09	16.05	15.95	13.00	13.02‡	12.99	12.91	12.95	13.03

* This animal had previously been assigned to the subspecies. *H. lar agilis.* † These values have not been corrected for losses during acid hydrolysis. ‡ Basic residues were not measured quantitatively in all cases.

In addition to the amino acid replacements and deletion noted above, the individual examined was heterozygous at position B-3, and approximately half-molar amounts of both glycine and serine were found. As a result of the finding, five additional gibbons were subjected to plasmapheresis and their fibrinopeptides were isolated (*6*). As a further check, the original animal (Blacky) was also reexamined. In each case the total amino acid compositions of the fibrinopeptides A and B were determined (Table 1). In addition, the chymotryptic fragments were isolated and their compositions established (Table 2). A second animal (Fang) was found to have the same heterozygous condition at B-3; the four other specimens, including an individual (Pop) who has previously been classified in a subspecies (*H. lar agilis*) (*7*), were all homozygous for glycine at position B-3. In view of these observations, we propose that glycine be regarded as the wild type allele until a larger census can unequivocally establish the gene frequencies. The two heterozygote gibbons arrived from collectors in shipments several months apart, minimizing the possibility that they are siblings.

In the past, fibrinopeptides have been particularly useful in phylogenetic studies because of the wide *inter*specific variability exhibited by certain portions of the molecules (*8*). Recently we attempted to measure the amount of *intra*specific variation in the human

Table 2. Amino acid compositions of chymotryptic fragments from fibrinopeptides of six individual gibbons. Results are expressed as the number of residues per mole of peptide (calculated as molar ratios of amino acids recovered). Chymotrypsin splits the gibbon fibrinopeptide A into two octapeptides (A-Ch-1 and A-Ch-2) and the fibrinopeptide B into a decapeptide (B-Ch-1) and a tripeptide (B-Ch-2).

Residue	Blacky	Fang	Thor	Heidi	Whitey	Pop*	Blacky	Fang	Thor	Heidi	Whitey	Pop*
	A-Ch-1						*A-Ch-2*					
Aspartic acid	1.01	1.02	1.10	1.18	1.08	1.08						
Threonine†	0.91	0.93	0.94	0.89	0.94	0.89						
Serine†												
Glutamic acid	2.08	2.09	2.07	2.06	2.21	2.04	1.12	1.17	1.11	1.10	1.10	1.08
Glycine	2.03	2.06	2.14	2.07	2.00	2.00	3.23	3.23	3.18	3.21	3.21	3.12
Alanine	0.99	0.92	0.76	0.78	0.78	0.90	1.08	1.08	1.06	1.04	1.05	1.05
Valine							0.93	0.82	0.90	0.90	0.93	0.84
Leucine							0.82	0.80	0.81	0.74	0.75	0.92
Phenylalanine	0.97	0.99	0.95	1.03	0.99	1.09						
Arginine‡							0.84	0.90	0.94	(1)‡	(1)‡	(1)‡
Total residues	7.99	8.01	7.96	8.01	8.00	8.00	8.02	8.00	8.00	7.99‡	8.04‡	8.01‡
	B-Ch-1						*B-Ch-2*					
Aspartic acid	3.03	2.88	2.86	2.80	2.97	2.82						
Threonine†												
Serine†							0.45	0.38				
Glutamic acid	2.08	2.11	2.07	2.19	2.14	2.19						
Glycine	2.00	2.14	2.14	2.28	2.07	2.11	0.51	0.52	0.89	0.91	0.89	0.94
Alanine							1.04	1.11	1.11	1.09	0.98	1.06
Valine	0.94	0.89	0.98	0.78	0.89	0.88						
Leucine	0.97	0.92	0.94	0.93	0.93	0.92						
Phenylalanine	0.99	1.07	1.01	1.03	1.00	1.10						
Arginine‡							(1)‡	(1)‡	(1)‡	(1)‡	1.13	(1)‡
Total residues	10.01	10.01	10.00	10.01	10.00	10.02	3.00	3.01	3.00	3.00	3.00	3.00

* This animal had previously been assigned to the subspecies. *H. lar agilis.* † These values have not been corrected for losses during acid hydrolysis. ‡ Basic residues were not measured quantitatively in all cases.

fibrinopeptide population by searching for heterozygotes on an amino acid composition basis. We found none in a survey of 125 normal individuals (*9*).

However, a comparable amino acid allelism has been reported for human immunoglobulin kappa chains at position 191. Of ten normal humans examined, Terry *et al.* (*10*) reported that seven were homozygous for valine at that position, whereas three had both leucine and valine.

Not enough data are presently available to warrant speculation on whether or not these single amino acid replacement allelisms are undergoing Darwinian selection (*11*) or represent the "random fixation of neutral alleles" (*12*). It is interesting to note, however, that the glycine-serine allelism at position B-3 of gibbon fibrinopeptides represents both branches of the ancestral tree, the chimp-human type being serine, whereas the ancestral Old World monkey type is glycine (Fig. 2).

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	16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
GIBBON A	Ala–Asp–Thr–Gly–Glu (Gly, Glu) Phe (Leu, Ala, Glu, Gly, Gly, Gly, Val) Arg
HUMAN A	Ala–Asp–Ser*–Gly–Glu–Gly–Asp–Phe–Leu–Ala–Glu–Gly–Gly–Gly–Val–Arg
CHIMPANZEE A	Ala–Asp–Ser*–Gly–Glu (Gly, Asp) Phe (Leu, Ala, Glu, Gly, Gly, Gly, Val) Arg
DRILL A	(Ala, Asp, Thr, Gly, Asp, Gly, Asp, Phe)(Ile, Thr, Glu, Gly, Gly, Gly, Val) Arg
GREEN MONKEY A	Ala–Asp–Thr–Gly–Glu–Gly–Asp–Phe–Leu–Ala–Glu–Gly–Gly–Gly–Val–Arg
MACAQUE	Ala–Asp–Thr–Gly–Glu–Gly–Asp–Phe–Leu–Ala–Glu–Gly–Gly–Gly–Val–Arg
GIBBON B	PCA (Gly, Val, Asx, Asx, Asx, Glx) --- (Gly, Leu) Phe ($\frac{\text{Ser}}{\text{Gly}}$, Ala) Arg
HUMAN B	PCA–Gly–Val–Asn–Asp–Asn–Glu–Glu–Gly–Phe–Phe–Ser–Ala–Arg
CHIMPANZEE B	PCA (Gly, Val, Asn, Asp, Asn, Glu, Glu, Gly, Phe) Phe (Ser, Ala) Arg
DRILL B	PCA (Gly, Val, Asx, Gly)(Asx, Glx, Glx, Gly, Leu) Phe–Gly–Gly–Arg
GREEN MONKEY B	PCA (Gly, Val, Asx, Gly) Asn–Glu–Glu–Gly–Leu–Phe–Gly–Gly–Arg
MACAQUE B	(–––––––?–––––––) Asn–Glu–Glu–Ser–Pro–Phe–Ser–Gly–Arg

Fig. 1. Amino acid sequences of gibbon fibrinopeptides compared with previously reported primate fibrinopeptides: human (*3*), chimpanzee (*4*), drill (*2*), green monkey (*13*), and macaque (*13*). The deleted region (–––––––) in the macaque B peptide may be the result of an isolation artifact. In the cases of human and chimpanzee, the asterisk at A-14 signifies a fractionally phosphorylated serine residue. It has been reported (*14*) that the baboon fibrinopeptide A is identical to that of the green monkey and macaque. Abbreviations are: Ala, alanine; Asp, aspartic acid; Thr, threonine; Gly, glycine; Glu, glutamic acid; Phe, phenylalanine; Leu, leucine; Val, valine; Arg, arginine; Ser, serine; Ile, isoleucine; Asn, asparagine; Glx, glutamine or glutamic acid; Pro, proline; Asx, asparagine or aspartic acid; and PCA, pyrrolidone carboxylic acid.

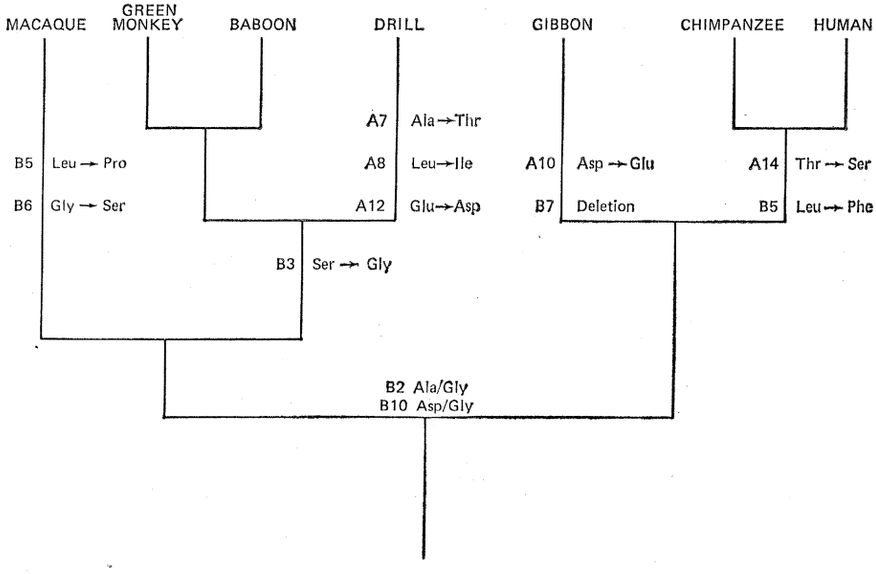

Fig. 2. A simple cladogram of amino acid changes in primate fibrinopeptides. The deletion noted on the gibbon line at B-7 could equally well be at B-8 (Fig. 1). The identity of the green monkey and baboon applies only to the fibrinopeptide A, as the baboon fibrinopeptide B has not yet been reported.

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