

Primary structures of human α -fetoprotein and its mRNA

(cDNA clones/three-domain structure/molecular evolution)

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ABSTRACT DNA complementary to human α -fetoprotein (AFP) mRNA was cloned in the plasmid pBR322. Analysis of three overlapping cDNA clones revealed most of the nucleotide sequence of AFP mRNA, and the remaining nucleotides at the 5' end of the mRNA were elucidated from a cloned genomic DNA fragment. The amino acid sequence was deduced from the nucleotide sequence, which revealed 19 amino acids in the signal sequence and 590 amino acids in mature AFP. There are 15 regularly spaced disulfide bridges, which generate a folding structure having three repeating domains. There is one potential N-glycosylation site, Asn-Phe-Thr, in the amino acid sequence. In comparison with mouse AFP, 66% of the amino acid sequence was conserved, with the highest identity (72%) in domain 3, followed by domain 2 (67%) and domain 1 (59%). In comparison with human albumin, a 39% conservation of primary structure was found. Again, the similarity was the highest in domain 3 and the lowest in domain 1. Human AFP and human albumin are similar in overall structure, but certain parts of the molecules differ significantly in their predicted secondary structure.

α -Fetoprotein (AFP) is a major serum protein (M_r , $\approx 70,000$) synthesized during fetal life (1–4). Reappearance of AFP in adult serum often signals pathological conditions, particularly hepatocarcinomas and teratocarcinomas (1–4). In contrast, albumin increases steadily during fetal and neonatal growth and shows no apparent changes in concentration associated with development of liver and germ cell tumors.

In the past several years, DNAs complementary to mouse (5, 6) and rat (7) AFP mRNAs and rat (8) and human (9, 10) albumin mRNAs have been cloned. Nucleotide sequences of these clones and the amino acid sequences deduced from them have provided valuable information on the structure of these proteins. However, the rat cDNA clones are not full-length and consequently, molecular comparisons have been limited to partial sequences of mouse and rat AFPs. Also, comparisons between AFP and albumin were possible only interspecifically because neither mouse albumin nor human AFP mRNA sequences were known in their entirety.

In this paper we report the complete nucleotide sequence of human AFP mRNA and the amino acid sequence of AFP. These data allow comparisons of the entire primary structures of AFPs interspecifically and AFP and albumin intraspecifically.

MATERIALS AND METHODS

The production of the cDNA clone, pHAF2, containing 841 nucleotides or about 40% of the human AFP mRNA sequence at the 3' end was described (11). By using the nick-translated insert of pHAF2 as a probe, a second clone, pHAF6, with an additional 320 nucleotides at the 5' end was obtained (Fig. 1). To clone the missing nucleotides a primer fragment was pre-

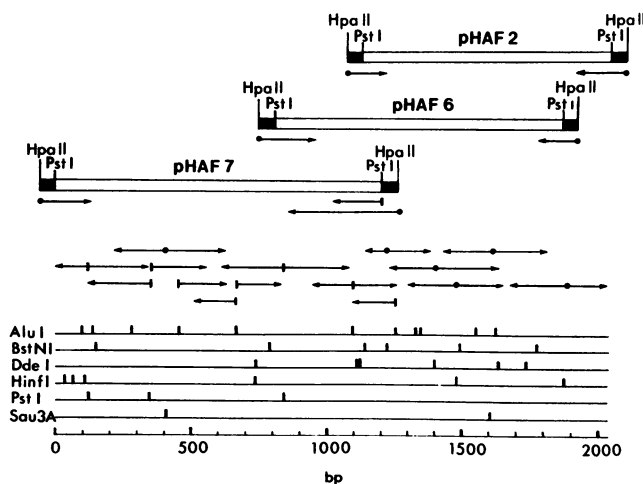


FIG. 1. Restriction endonuclease maps of human AFP cDNA clones and the sequence analysis strategies. Open bars and closed bars represent the human AFP cDNA inserts and the pBR322 DNA, respectively. Arrows with dots and vertical bars indicate the nucleotides determined by Maxam-Gilbert procedure (12) and dideoxy chain termination procedure (13), respectively.

pared from pHAF6 and extended toward the 5' end of the mRNA as follows. pHAF6 was digested with *Bst*NI and the 400-base-pair (bp) *Hpa* II/*Bst*NI fragment at the 5' end (see Fig. 1) was recovered. This was digested with *Escherichia coli* exonuclease III and annealed with partially purified AFP mRNA (11). cDNA copies were synthesized by using avian myeloblastosis virus reverse transcriptase. DNA synthesized was converted to double-stranded DNA and inserted into the *Pst* I site of plasmid pBR322 (11). *E. coli* LE392 transformants with the chimeric plasmid were screened for human AFP mRNA sequence by using the nick-translated *Pst* I/*Alu* I fragment prepared from the 5' region of pHAF6 as a probe (see Fig. 1). Nucleotide sequence analysis was done by the procedure of Maxam and Gilbert (12) and by the dideoxy chain termination procedure (13) using the phage M13 cloning-sequencing system (14).

RESULTS

Nucleotide Sequence of Human AFP mRNA. Analysis of three cDNA clones (Fig. 1) provided the major part of the nucleotide sequence of human AFP mRNA. The missing portion (mostly the 5' noncoding sequence) was determined by analysis of a cloned genomic DNA fragment (unpublished results). The nucleotide sequence of human AFP mRNA with a putative capping site (determined by S1 nuclease mapping) is presented in Fig. 2 together with the amino acid sequence deduced from it. The human AFP mRNA consists of 44 nucleotides in the 5'

noncoding region, 1,830 nucleotides in the coding region, and 155 nucleotides in the 3' noncoding region. A termination codon, TAA, was found next to GTT, a codon for valine, the known COOH-terminal amino acid residue of human AFP (4, 15). There are five additional termination codons in the 3' noncoding sequence, as has been observed with mouse AFP (5) and human

albumin mRNA (10). The characteristic poly(A)-addition signal, A-A-T-A-A-A, is located 14 nucleotides upstream from poly(A). Codons are used relatively randomly in the human AFP mRNA as compared to other human mRNAs (16).

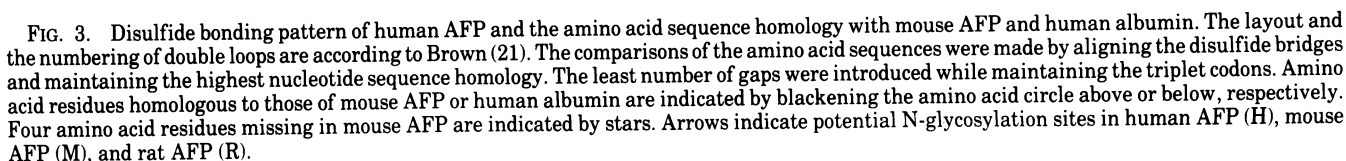
Protein Structure of Human AFP. The molecular weight of mature human AFP is calculated to be 66,300 without carbo-

	-19	-10	AT (2)
	met lys trp val glu ser ile phe leu ile phe leu leu asn phe thr glu ser arg		
ATTGTGCTTCCACCACTGCCAATAACAAATAACTAGCAACC	ATG AAG TGG GTG GAA TCA ATT TTT TTA ATT TTC CTA CTA AAT TTT ACT GAA TCC AGA		(101)
1	10	20	30
thr leu his arg asn glu tyr gly ile ala ser ile leu asp ser tyr gln cys thr ala glu ile ser leu ala asp leu ala thr ile			
ACA CTG CAT AGA AAT GAA TAT GGA ATA GCT TCC ATA TTG GAT TCT TAC CAA TGT ACT GCA GAG ATA AGT TTA GCT GAC CTG GCT ACC ATA			(191)
31	40	50	60
phe phe ala gln phe val gln glu ala thr tyr lys glu val ser lys met val lys asp ala leu thr ala ile glu lys pro thr gly			
TTT TTT GCC CAG TTT GTT CAA GAA GCC ACT TAC AAG GAA GTA AGC AAA ATG GTG AAA GAT GCA TTG ACT GCA ATT GAG AAA CCC ACT GGA			(281)
61	70	80	90
asp glu gln ser ser gly cys leu glu asn gln leu pro ala phe leu glu glu leu cys his glu lys glu ile leu glu lys tyr gly			
GAT GAA CAG TCT TCA GGG TGT TTA GAA AAC CAG CTA CCT GCC TTT CTG GAA GAA CTT TGC CAT GAG AAA GAA ATT TTG GAG AAG TAC GGA			(371)
91	100	110	120
his ser asp cys cys ser gln ser glu glu gly arg his asn cys phe leu ala his lys lys pro thr pro ala ser ile pro leu phe			
CAT TCA GAC TGC TGC AGC CAA AGT GAA GAG GGA AGA CAT AAC TGT TTT CTT GCA CAC AAA AAG CCC ACT CCA GCA TCG ATC CCA CTT TTC			(461)
121	130	140	150
gln val pro glu pro val thr ser cys glu ala tyr glu glu asp arg glu thr phe met asn lys phe ile tyr glu ile ala arg arg			
CAA GTT CCA GAA CCT GTC ACA AGC TGT GAA GCA TAT GAA GAA GAC AGG GAG ACA TTC ATG AAC AAA TTC ATT TAT GAG ATA GCA AGA AGG			(551)
151	160	170	180
his pro phe leu tyr ala pro thr ile leu leu trp ala ala arg tyr asp lys ile ile pro ser cys cys lys ala glu asn ala val			
CAT CCC TTC CTG TAT GCA CCT ACA ATT CTT CTT TGG GCT GCT CGC TAT GAC AAA ATA ATT CCA TCT TGC TGC AAA GCT GAA AAT GCA GTT			(641)
181	190	200	210
glu cys phe gln thr lys ala ala thr val thr lys glu leu arg glu ser ser leu leu asn gln his ala cys ala val met lys asn			
GAA TGC TTC CAA ACA AAG GCA GCA ACA GTT ACA AAA GAA TTA AGA GAA AGC AGC TTG TTA AAT CAA CAT GCA TGT GCA GTA ATG AAA AAT			(731)
211	220	230	240
phe gly thr arg thr phe gln ala ile thr val thr lys leu ser gln lys phe thr lys val asn phe thr glu ile gln lys leu val			
TTT GGG ACC CGA ACT TTC CAA GCC ATA ACT GTT ACT AAA CTG AGT CAG AAG TTT ACC AAA GTT AAT TTT ACT GAA ATC CAG AAA CTA GTC			(821)
241	250	260	270
leu asp val ala his val his glu his cys cys arg gly asp val leu asp cys leu gln asp gly glu lys ile met ser tyr ile cys			
CTG GAT GTG GCC CAT GTA CAT GAG CAC TGT TGC AGA GGA GAT GTG CTG GAT TGT CTG GAT GGG GAA AAA ATC ATG TCC TAC ATA TGT			(911)
271	280	290	300
ser gln gln asp thr leu ser asn lys ile thr glu cys cys lys leu thr thr leu glu arg gly gln cys ile ile his ala glu asn			
TCT CAA CAA GAC ACT CTG TCA AAC AAA ATA ACA GAA TGC TGC AAA CTG ACC ACG CTG GAA CGT GGT CAA TGT ATA ATT CAT GCA GAA AAT			(1001)
301	310	320	330
asp glu lys pro glu gly leu ser pro asn leu asn arg phe leu gly asp arg asp phe asn gln phe ser ser gly glu lys asn ile			
GAT GAA AAA CCT GAA GGT CTA TCT CCA AAT CTA AAC AGG TTT TTA GGA GAT AGA GAT TTT AAC CAA TTT TCT TCA GGG GAA AAA AAT ATC			(1091)
331	340	350	360
phe leu ala ser phe val his glu tyr ser arg arg his pro gln leu ala val ser val ile leu arg val ala lys gly tyr gln glu			
TTC TTG GCA AGT TTT GTT CAT GAA TAT TCA AGA AGA CAT CCT CAG CTT GCT TCA GTA ATT CTA AGA GTT GCT AAA GGA TAC CAG			(1181)
361	370	380	390
leu leu glu lys cys phe gln thr glu asn pro leu glu cys gln asp lys gly glu glu glu leu gln lys tyr ile gln glu ser gln			
TTA TTG GAG AAG TGT TTC CAG ACT GAA AAC CCT CTT GAA TGC CAA GAT AAA GGA GAA GAA TTA CAG AAA TAC ATC CAG GAG AGC CAA			(1271)
391	400	410	420
ala leu ala lys arg ser cys gly leu phe gln lys leu gly glu tyr tyr leu gln asn ala phe leu val ala tyr thr lys lys ala			
GCA TTG GCA AAG CGA AGC TGC GGC CTC TTC CAG AAA CTA GGA GAA TAT TAC TTA CAA AAT GCG TTT CTC GTT GCT TAC ACA AAG AAA			(1361)
421	430	440	450
pro gln leu thr ser ser glu leu met ala ile thr arg lys met ala ala thr ala ala thr cys cys gln leu ser glu asp lys leu			
CCC CAG CTG ACC TCG TCG GAG CTG ATG GCC ATC ACC AGA AAA ATG GCA GCC ACA GCA GCC ACT TGT TGC CAA CTC AGT GAG GAC AAA			(1451)
451	460	470	480
leu ala cys gly glu gly ala ala asp ile ile ile gly his leu cys ile arg his glu met thr pro val asn pro gly val gly gln			
TTG GCC TGT GGC GAG GGA GCG GCT GAC ATT ATT ATC GGA CAC TTA TGT ATC AGA CAT GAA ATG ACT CCA GTA AAC CCT GGT GTT GGC			(1541)
481	490	500	510
cys cys thr ser ser tyr ala asn arg arg pro cys phe ser ser leu val val asp glu thr tyr val pro pro ala phe ser asp			
TGC TGC ACT TCT TCA TAT GCC AAC AGG AGG CCA TGC TTC AGC AGC TTG GTG GTG GAT GAA ACA TAT GTC CCT CCT GCA TTC TCT GAT			(1631)
511	520	530	540
lys phe ile phe his lys asp leu cys gln ala gln gly val ala leu gln thr met lys gln glu phe leu ile asn leu val lys gln			
AAG TTC ATT TTC CAT AAG GAT CTG TGC CAA GCT CAG GGT GTA GCG CTG CAA ACG ATG AAG CAA GAG TTT CTC ATT AAC CTT GTG AAG CAA			(1721)
541	550	560	570
lys pro gln ile thr glu glu gln leu glu ala val ile ala asp phe ser gly leu leu glu lys cys cys gln gly gln glu gln glu			
AAG CCA CAA ATA ACA GAG GAA CAA CTT GAG GCT GTC ATT GCA GAT TTC TCA GGC CTG TTG GAG AAA TGC TGC CAA GGC CAG GAA CAG			(1811)
571	580	590	
val cys phe ala glu glu gly gln lys leu ile ser lys thr arg ala ala leu gly val ter			
GTC TGC TTT GCT GAA GAG GGA CAA AAA CTG ATT TCA AAA ACT CGT GCT TTT GGA GTT TAA ATTACTTCAGGGGAAGAGAAGACAAACAGTCT			(1908)
TTTATCGGTGTGAACCTTTTCTTTAATTTAACTGATTAAACACTTTTGTGAATTAATGAATGATAAAGACTTTTATGTGAGATTCTCTATCACAGAAATAAAATATCTCCAAA (2027)			
TG(A) 10 (2039)			

FIG. 2. Nucleotide sequence of human AFP mRNA and the amino acid sequence deduced from the nucleotide sequence. The first 55 nucleotides were determined from a genomic clone (unpublished data). Five potential termination (ter) codons in the 3' noncoding region are underlined.

quence has been observed; in fact, one of the two major amino acid sequences found by Aoyagi *et al.* (15) begins with threonine.

Fig. 3 shows a folding structure of human AFP constructed by the formation of 15 disulfide bridges between 30 out of 32 cysteine residues. These disulfide bridges are located at positions equivalent to those of human albumin, leading to a three-



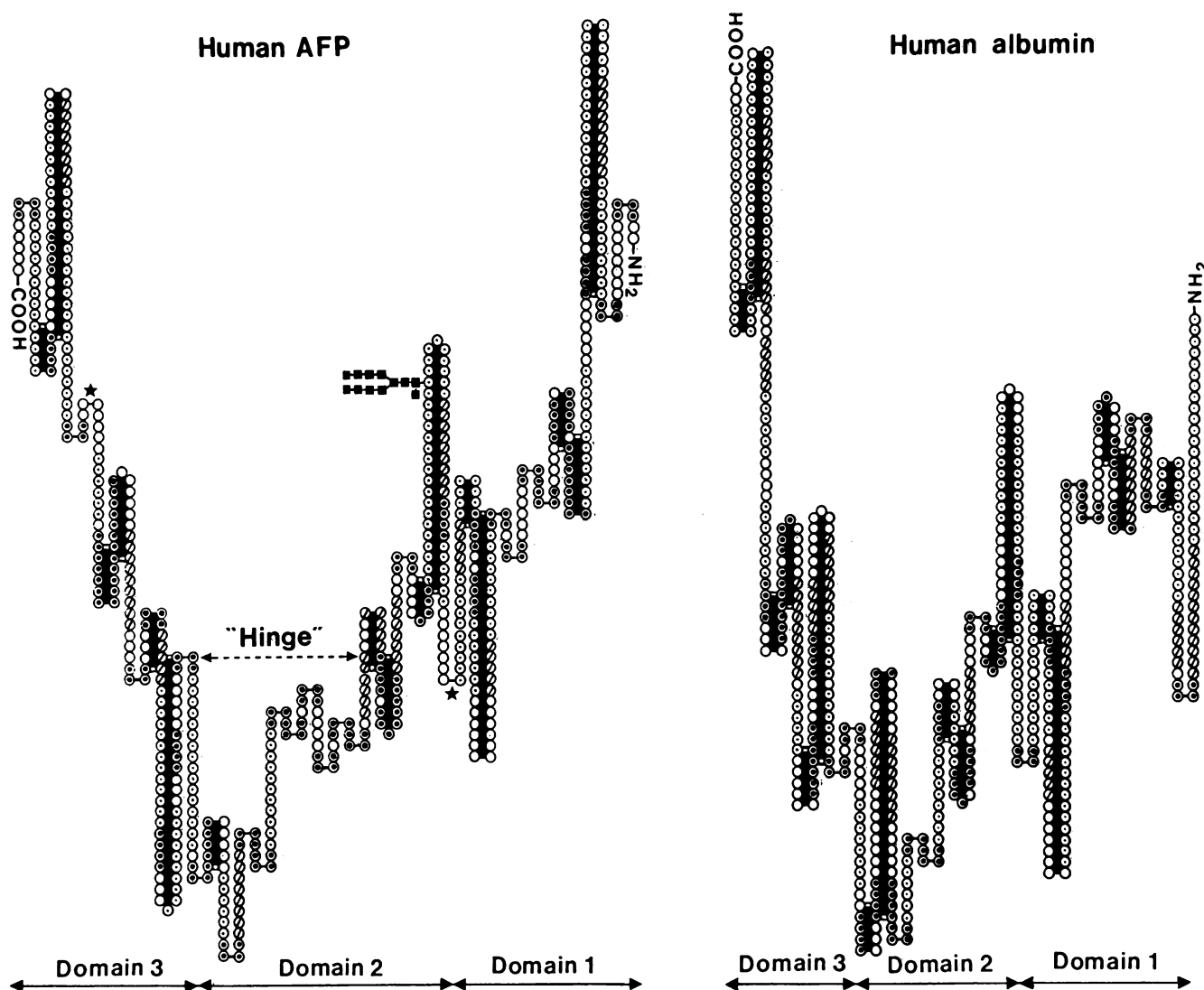


FIG. 4. Molecular configurations of human AFP (*Left*) and human albumin (*Right*) based on the predicted secondary structures. The amino acid sequence published by Dugaiczky *et al.* (10) was used for human albumin. The amino acid residues participating in the formation of α -helices, β -sheets, β -turns, and random coils are indicated by \bigcirc , \odot , \bullet , and \otimes , respectively. The loops formed by disulfide bonding are filled in black. Stars indicate extra turns introduced in human AFP at amino acid residues 195–198 and 504–507 where the probabilities of β -turn occurrence were higher ($\langle p_t \rangle = 0.56 \times 10^{-4}$ and $\langle p_t \rangle = 0.70 \times 10^{-4}$, respectively) than the average probability of β -turn occurrence ($\langle p_t \rangle = 0.55 \times 10^{-4}$) though lower than the cut-off value ($\langle p_t \rangle = 0.75 \times 10^{-4}$), and satisfied the following conditions: $\langle P_t \rangle > 1.00$ and $\langle P_t \rangle < \langle P_t \rangle > \langle P_\beta \rangle$ (see ref. 22). Black squares represent the carbohydrate residues attached to asparagine-232 in AFP. The first loop at the NH_2 terminus in AFP is formed assuming cysteines 18 and 67 participate in a disulfide linkage.

domain structure similar to that proposed for human albumin (21). It is to be noted, however, that human AFP has two disulfide bridges fewer than human albumin, contributing to the formation of a unique "hinged" structure (see Fig. 4 and *Discussion*).

A comparison of the amino acid sequences of human AFP and mouse AFP is shown in Fig. 3. The overall identity is 66% with the highest identity found in domain 3 (72%), followed by domain 2 (67%) and domain 1 (59%) (Table 1). Similar identities are found between human AFP and rat AFP (Table 1). All three AFPs share a long stretch of sequence in domain 3 (residues 510–564) with an 87% identity between human AFP and mouse or rat AFP and 95% between mouse and rat AFPs.

Comparisons of amino acid sequences between human AFP and human albumin (9, 10) are also shown in Fig. 3. The overall identity was 39% (Table 1) with, as in the case of AFP, the highest identity found in domain 3 (48%), followed by domain 2 (40%) and domain 1 (30%) (Table 1). The similarity at the NH_2 -terminal region is particularly low, showing only a 16% identity at residues 1–86.

A search for the sequences at which N-linked glycosylation occurs [Asn-X-Thr or Asn-X-Ser (23)] revealed that human AFP contains one potential site at residues 232–234 (Fig. 3).

The secondary structure of human AFP, as predicted from the amino acid sequence according to the procedure of Chou and Fasman (22), is shown in Fig. 4 *Left*. It is very similar to that of mouse AFP (data not shown) but significantly different from that of human albumin (Fig. 4 *Right*).

DISCUSSION

Human AFP is the second mammalian AFP whose primary structure has been elucidated in its entirety from cDNA clones. Mature human AFP contains 590 amino acids; mouse AFP contains 586 (5, 6). The four extra amino acid residues are found in domain 1. This domain has the least amino acid sequence homology and it may be speculated that species differences in AFP functions may be attributed to this domain. For instance, the binding of estrogen may occur at domain 1, because this activity is reported to be specific to mouse and rat AFPs (24,

Table 1. Nucleotide and amino acid sequence comparisons between human AFP and mouse AFP (MAFP), rat AFP (RAFP), and human albumin (HSA)

Region	Positions	Identical nucleotides, %			Positions	Identical amino acids, %		
		MAFP	RAFP	HSA		MAFP	RAFP	HSA
5' noncoding	1-44	57	—	36				
Signal peptide	45-101	63	—	60	-19 to -1	47	—	53
Domain 1	102-692	71	(72)	45	1 to 197	59	(59)	30
Domain 2	693-1,268	78	74	54	198 to 389	67	65	40
Domain 3	1,269-1,871	78	76	57	390 to 590	72	74	48
3' noncoding	1,872-2,029	64	57	48				
Overall								
(mature protein)		75	(74)	52		66	(67)	39

Figures in parentheses are calculated on the basis of the known partial sequence of rat AFP.

25). However, several groups (26, 27) have observed that human AFP also binds to estrogen. This controversy should be resolved once the estrogen-binding activity is correlated to a specific amino acid sequence(s).

In contrast to domain 1, domain 3 of human AFP exhibits a high degree of amino acid sequence homology to mouse AFP. In particular, the sequence covering amino acid residues 510-564 shows an 87% identity (Fig. 3). The significance of this homologous stretch is not known at present.

AFP is shown to have a high affinity to unsaturated fatty acids (28). On the other hand, there have been controversies over the binding of diethylstilbestrol to AFP (29, 30) and the immunosuppressive activity of AFP (18). Some of the contradicting results may be caused by ligands associated with AFP preparations. It is essential, therefore, to characterize these activities on the basis of the structure of AFP in order to establish the intrinsic physiological role of AFP.

Human AFP shows a 39% overall amino acid sequence identity to human albumin. It is noteworthy that the majority of the homologous amino acid residues are the same as those conserved in mouse AFP. This suggests that these amino acid residues are important in preserving basic structural features common to AFP and albumin.

The availability of the primary and secondary structures of human AFP allows certain predictions regarding the overall configuration of this molecule. For instance, the lack of a double disulfide bridge in the second half of domain 2 (residues 295-396) may result in the formation of a long polypeptide link ("hinge") between domains 2 and 3. (Fig. 4 *Left*). In contrast, human albumin contains a double disulfide bridge in this region, forming a long double loop (10, 21) (Fig. 4 *Right*). This, coupled with the difference in predicted secondary structures in other parts of the molecules, suggests that human AFP and human albumin have significantly different molecular configurations. Thus, although AFP and albumin are shown to be similar in various physical and chemical properties, they are clearly distinct from each other in the details of their molecular structures.

There is one potential site in human AFP at which N-glycosylation occurs, in agreement with chemical analysis (31). In mouse and rat AFPs, there are three potential N-glycosylation sites, one being at the same location as in human AFP (Fig. 3). The second site is found in domain 2 in both species, and the third is located in domain 3 in mouse AFP but in domain 1 in rat AFP. Conservation of the carbohydrate attachment sites in human, mouse, and rat AFPs suggests that this moiety may play an important role, possibly in catabolism of AFP molecules (32).

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