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A common ancestor for mammalian 3β -hydroxysteroid dehydrogenase and plant dihydroflavonol reductase

The enzymic complex 3β -hydroxysteroid dehydrogenase (EC 1.1.1.145)/ Δ^5 - Δ^4 isomerase (EC 5.3.3.1), hereafter called 3β -hydroxysteroid dehydrogenase, catalyses the oxidative conversion of pregnenolone to progesterone (Fig. 1), which can be converted to various other steroids that function as glucocorticoids, mineralocorticoids, progestins, androgens, and oestrogens (Luu-The *et al.*, 1989b). As such, this enzyme is at a key branching point in the synthesis of steroids with central roles in mammalian metabolism and reproductive function. Recently, the sequences of two other steroid dehydrogenases, 17β -hydroxysteroid hydrogenase (Peltoketo *et al.*, 1988; Luu-The *et al.*, 1989a) and corticosteroid 11β -hydroxy dehydrogenase (Agarwal *et al.*, 1989), that are important in steroid metabolism, were determined and found to show similarity to each other (Agarwal *et al.*, 1989; Baker, 1990), even though they modify different classes of steroids and act as different positions in the steroid nucleus. Evidently, gene duplication and its subsequent divergence led to two proteins that are important in the synthesis and degradation of oestrogens, androgens and corticosteroids. Thus, when the sequences of human and bovine 3β -hydroxysteroid dehydrogenase were determined (Luu-The *et al.*, 1989b; Zhao *et al.*, 1989), it came as a surprise that there was no sequence similarity to either 17β -hydroxysteroid dehydrogenase or 11β -hydroxysteroid dehydrogenase, even at the most distant level. Moreover, 3β -hydroxysteroid dehydrogenase showed no similarity to other dehydrogenases, cytochrome P-450 enzymes, steroid isomerases, steroid binding proteins, or any other protein sequence, over 10000 of which are now in the protein sequence database. This left the ancestry of 3β -hydroxysteroid dehydrogenase unelucidated.

Recently, we uncovered a clue to resolving this puzzle, after completing a search of an updated version of the GenBank database for sequences with similarity to 3β -hydroxysteroid dehydrogenase. This search revealed a novel similarity between 3β -hydroxysteroid dehydrogenase and barley A1 protein, an NADPH-dependent reductase that converts dihydroquercetin to *cis*-leucocyanidin (Fig. 1) in barley (Rohde *et al.*, 1987). Homologous dihydroflavonol reductases in maize (Schwarz-Sommer *et al.*, 1987) and snapdragons (Coen *et al.*, 1986) are used to form pigments from flavonoid precursors (Reddy *et al.*, 1987). The sequence similarity between bovine β -hydroxysteroid dehydrogenase and residues 3–232 of barley A1 protein is sufficiently strong to suggest that these mammalian mammalian and plant enzymes are derived from a common ancestor.

Fig. 2 shows the alignment of residues 8–236 of bovine 3β -hydroxysteroid dehydrogenase and residues 3–232 of barley A1 protein. There are 55 matches out of a possible 227 matches (24%) and 29 conservative replacements (13%), with five gaps added by the computer to maximize the alignment between the two proteins. The ALIGN (Dayhoff *et al.*, 1983) comparison score between these proteins is 10.25 standard deviations higher than that of 1000 comparisons of randomized sequences of these

Fig. 1. (a) Conversion of pregnenolone to progesterone catalysed by human 3β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 isomerase, and (b) conversion of dihydroquercetin to *cis*-leucocyanidin catalysed by barley A1 protein; the C-4 ketone is reduced to an alcohol

protein segments. The probability of getting such a score by chance is 6×10^{-25} . Bovine 3β -hydroxysteroid dehydrogenase contains 372 residues. Most, but not all, of the sequence of barley (331 residues) and maize (367 residues) A1 proteins have been determined. The full maize A1 protein sequence contains an additional 12 residues at its N-terminus and 48 residues at its C-terminus (Rohde *et al.*, 1987). Barley A1 protein is likely to be about the same length as maize A1. Thus, the region of similarity between bovine 3β -hydroxysteroid dehydrogenase and barley dihydroflavonol reductase extends over more than half of each protein, which may be due to this area binding the nucleotide cofactor that is important in both proteins' enzymic activity.

The similarity between 3β -hydroxysteroid dehydrogenase and dihydroflavonol reductase could be due to either convergent evolution or to divergence from a common ancestor. We favour the latter possibility for several reasons. First, the proteins' ALIGN comparison score of 10.25 standard deviations has probability of occurring by chance of 6×10^{-25} . An event with such low probability usually is interpreted as indicating that the two proteins are derived from a common ancestor. This interpretation is supported by additional evidence of similarity between 3β -hydroxysteroid dehydrogenase and A1 protein: these proteins have similar oxido-reductase activity and there are similarities in the structure of their substrates (Fig. 1). Moreover, as described below, there are interesting functional similarities between their substrates, flavonoids and steroids, which may shed light on the origins of intercellular communication in vertebrates (Baker, 1990).

Although it would not appear, at first glance, that the flavonoid precursors of plant pigments have any biological function in common with steroids in vertebrates, in fact, like steroids, there is evidence that flavonoids can regulate gene transcription in a variety of organisms. The best characterized examples are in bacteria that interact with plants (Djordjevic *et al.*, 1987; Firmin *et al.*, 1986; Long, 1989; Peters *et al.*, 1986; Stachel *et al.*, 1985). The dependence of flavonoid biological activity in intercellular communication between plants and rhizobia on flavonoid structure has similarities to that of steroids in vertebrates. Thus, the addition or removal of a hydroxyl group from a flavonoid, or the oxidation of a hydroxyl group to a ketone, can have important effects on a flavonoid's (i) biological potency and (ii) selectivity for different rhizobia (Firmin *et al.*, 1986). Different plant

Bovine 3̢-OH Barley Flavonol	8 3	V V V	T T T	G G G	G A S	G G G	F Y *	L I *	G A S	Q W	R L *	I V *	C K L	L L L	V L *	E H	E -	K R G	D Y	L T	Q V	E
Bovine 3̢-OH Barley Flavonol		I R	R A	V T	L V *	D R D	V T	F A D	P P P	E K K	V K T	R E L	E H	F L	S Q	K A L	Q E *	S G A	- K K	I E	K R *	L L
Bovine 3̢-OH Barley Flavonol		T H	L L L	E F K	G A S	D *	I L L	D E *	E E E	Q C T	L F D	K G S	A A A	C I	Q A G	G T C	S D	V V V	V V V	I F H	H T T	
Bovine 3̢-OH Barley Flavonol		A A A	S S S	V P F	I Y H	D V N	R N V	A K D	V P P	P K P	R E *	T A E	I L *	M D P	N V A	N V V	K N G	G T T	Q L T	L N V	L V *	
Bovine 3̢-OH Barley Flavonol		L L L	E R S	A C C	V K C	Q K A	A S A	S I *	V P K	V R V	F I I	H V V	T T S	S S *	I M A	E A *	V A *	G Y P	N N N	S G Y	K	
Bovine 3̢-OH Barley Flavonol		R P	E R	I T	I P	Q D *	D V V	G V V	R E *	E E E	H T W	E F S	S S S	A A A	W E V	S S C	P E K	Y N K	Y Q	S K	K W	K Y
Bovine 3̢-OH Barley Flavonol		L V *	A L S	E K K	A T K	V L *	L A E	G E E	N A A	G A W	A K W	L F A	K N K	G D N	G T G	L L L	Y E I	T C I	A I T	L I *	R N	
Bovine 3̢-OH Barley Flavonol		P P P	M T M	Y V *	I Y I	G G G	E P L	G L L	S Q P	P P P	F T L	L S N	A T S	Y M A	H E A	G A I	L L K	N - K	F I N	N N N	G G G	
Bovine 3̢-OH Barley Flavonol		I S	L S S	T S *	N T Y	H C A	K N F	F S C	R - F	V G V	N P W	V V V	Y V V	V V V	G K D	N V A	A V A	W A A	H A H	I I I	L L L	A A A

Fig. 2. Alignment of residues 8–236 of bovine 3 β -hydroxysteroid dehydrogenase and residues 3–232 of barley dihydroflavonol reductase

Identities are shown below the alignment; an asterisk denotes conservative replacements. Out of 227 possible matches there are 55 (24%) identities and 29 (13%) conservative replacements. An ALIGN analysis of these sequences, with a gap penalty of 10, yields a score that is 10.25 standard deviations higher than that obtained with 1000 comparisons of randomized sequences of these segments. The probability of getting such a score by chance is 6×10^{-25} . An ALIGN analysis of the maize A1 and bovine 3 β -hydroxysteroid dehydrogenase yields a score of 9.7 standard deviations ($P = 10^{-24}$). An ALIGN analysis of human 3 β -hydroxysteroid dehydrogenase with barley A1 protein yields a score of 9.85 standard deviations.

flavonoids have chemotactic activity towards specific rhizobia. Like steroids, flavonoid potency and target specificity can be controlled by enzymic modification of functional groups. Moreover, some flavonoids can inhibit gene transcription in *Rhizobium*, when in competition with an inducer (Djordjevic *et al.*, 1987; Firmin *et al.*, 1986), thus acting as antagonists, a property that is important in steroid biology. Interestingly, flavonoids bind to oestrogen receptors and have oestrogenic actions in mammals (Martin *et al.*, 1978). Moreover, the isoflavonoid quercetin has anti-oestrogen-like actions when binding to type II binding sites in rat uterus (Markaverich *et al.*, 1988). These parallels between flavonoids and steroids support the notion of a common ancestry for 3 β -hydroxysteroid dehydrogenase and dihydroflavonol reductase inferred from their sequence similarity.

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