Transferrin D_1: Identity in Australian Aborigines and American Negroes

Abstract. *Human transferrin D_1 obtained from an Australian aborigine was found to have the same substitution of glycine for aspartic acid in peptide 1C previously shown in transferrin D_1 from an American Negro. This finding is relevant to formation of distinct Australoid and African populations.*

The polymorphism of human plasma transferrin (Tf) has been well established, with some 18 electrophoretic variants described (*1*). The variant forms are inherited as simple codominant Mendelian traits, presumably reflecting the action of a series of alleles at one locus, although this has been verified for only a few forms.

The most common form of transferrin is designated Tf C and is present in all populations tested. Most of the variants are rare, and those which are not are limited to certain populations. Among the variants with appreciable frequencies in large populations are Tf D_1 (in Africans, American Negroes, Australian aborigines, Melanesians, and Micronesians) and Tf D_{Chi} (in Orientals and some American Indian tribes).

Because the designations of type are based entirely on electrophoretic mobility, possibly more than one primary structure (hence, more than one allele) is represented by a designation such as Tf D_1. This is possible particularly where the populations classified as possessing the same variant in high frequency are thought not to be closely related (for example, Africans and Australian aborigines). To ascertain whether the Tf D_1 from diverse sources might be different, we made a comparison of the peptide fingerprints and amino acid substitutions between Tf D_1 from an Australian aborigine and Tf D_1 from an American Negro. The latter Tf D_1 differs from Tf C in that a glycine residue is substituted for an aspartic acid residue (*2*).

A pint of blood was obtained from an aborigine previously classified as homozygous Tf D_1 (*3*). The separated plasma was shipped by air to Austin, Texas. Transferrin was isolated by the procedure of Sutton and Karp (*4*). Digestions with trypsin and α-chymotrypsin (*5*) were carried out for 3 hours at *p*H 8.0, 37°C, with 1 mg of enzyme per 100 mg of transferrin. The method of Ingram (*2*, *6*) was used for peptide mapping.

Individual peptides were isolated by prolonged paper electrophoresis (60 volt/cm, 70 minutes) and prolonged descending paper chromatography (48 hours) in the same buffers and solvents used for peptide mapping. The pure peptides were hydrolyzed in 6*N* HCl under reduced pressure at 110°C for 16 hours. The hydrolyzates were analyzed for amino acids on a Spinco automatic amino acid analyzer (model 120B, modified).

Inspection of the chymotryptic peptide maps of the Australian Tf D_1 revealed a pattern identical to that observed with Tf D_1 from an American Negro and differing from Tf C by one peptide. No difference was observed between maps of tryptic digests of the two sources of Tf D_1. The variant peptide was earlier designated 1C.

To verify that the same amino acid substitution was involved, the peptide was isolated and assayed for amino acid composition. Results (Table 1) were compared with earlier results on 1C(C) from Tf C and 1C(D_1) from Tf D_1 (*2*). The 1C peptide is apparently identical in the two sources of Tf D_1, and differs from 1C(C) by having an extra glycine in place of the one aspartic acid.

The existence of the same primary allele in both Africans and Australoids poses some interesting questions for human evolution and race formation. Possible answers are: (i) The mutations arose independently and have become widespread through selection both in Africa and Southeast Asia. The mutation rates for specific amino acid substitutions are not known but must be very low, perhaps on the order of 10^{-8} or less per gamete. The virtual absence of Tf D_1 in large Caucasian populations suggests that the mutation could not have occurred very often with similar selective advantage in those populations. At the present time, no difference in fitness for any human transferrin variants is known, although perhaps this negative evidence is not very meaningful. Ashton obtained evidence compatible with differences in fitness among bovine transferrin genotypes (*7*). (ii) A single mutation occurred. This might have happened before separation of the Australoid and African populations, although they are not considered to be closely related. Indeed, analysis of gene frequencies at other loci suggests that these two groups separated early in the diverging evolutionary lines antecedent to present populations (*8*). Introduction of the gene from one population to another by migration of individuals (as opposed to populations) would have had to be accompanied by parallel selection to raise the frequency so high.

Implicit in these hypotheses is the assumption that all or most Africans and Australians classified as Tf D_1 have the same structural genes as those tested in our very limited sample. In view of the fact that the aborigine was homozygous, two Tf D alleles were tested in reality. That both are the same variant is suggested by the absence of the Tf 1(C) peptide in digests of this sample. Similarly, a single peptide difference was found in one homozygous and one heterozygous American Negro, representing three Tf D alleles. Thus, the Tf D_1 observed probably constitutes a significant portion of, and possibly all of, the D variants in these populations.

While the present finding does not provide the final answer to the relationship of Australian aborigines to Afri-

Table 1. Amino acid analysis of the 1C peptide isolated from transferrin D_1 obtained from an Australian aborigine. Results from analysis of the 1C peptide from Tf C (Caucasian) and from Tf D_1 (American Negro) are also given (*3*). I and II: Results from independently isolated peptides. The amino acids not listed, if present, were in amounts too low to quantify.

Amino acid	Analyses of 1C (Australian aborigine)				Residues in 1C peptide (No.)		
	Micromoles		Mole fractions		1C (Australian aborigine)	1C(C) (Caucasian)	1C(D_1) (Negro)
	I	II	I	II			
Lysine	0.0275	0.0274	0.367	0.328	2+	2+	2+
Arginine	.0007	.0024	.009	.029			
Aspartic acid	.0011	.0015	.015	.018		1	
Serine	.0094	.0113	.125	.135	1	1	1
Glutamic acid	.0105	.0107	.140	.128	1	1	1
Glycine	.0172	.0205	.229	.245	2	1	2
Phenylalanine	.0086	.0098	.115	.117	1	1	1

cans, it emphasizes the necessity of analyzing genetic similarities as near to the level of primary gene structure as possible. In practice, this amounts to analysis of the primary polypeptide structure. In that blood group systems probably reflect metabolic events, functional identity is often not the result of allelic identity and may be misleading.

The only other variant which achieves relatively high frequency is Tf D_{Chi}, present particularly in populations of Asia adjacent to the areas containing Tf D_1 (*9*). Although the electrophoretic mobilities of Tf D_{Chi} and Tf D_1 are similar and may be difficult to distinguish (*10*), Tf D_{Chi} has been observed to carry a different amino acid substitution (*11*).

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References and Notes

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Photooxidation of Hydrocarbons in the Presence of Aliphatic Aldehydes

Abstract. *A new group of gas-phase reactions have been shown to contribute to the photooxidation of hydrocarbons. The photooxidation of aliphatic aldehydes at wavelengths below 3400 angstroms produces intermediates that react with olefinic and aromatic hydrocarbons. Although the photooxidation rates are slower than those induced by nitrogen oxide, the rates are significant, considering the interest in urban atmospheric reactions. These results may indicate that the control of nitrogen oxides alone may not effectively reduce photochemical air pollution.*

The work of A. J. Haagen-Smit and co-workers (*1*) proved the importance of photooxidation of hydrocarbons in the presence of nitrogen oxides in photochemical air pollution. Subsequent studies have been concerned with more detailed measurements of such systems (*2*, *3*). Leighton (*2*) has discussed a variety of substances of possible significance in primary photochemical processes. Aliphatic aldehydes were suggested as one of a number of substances possibly contributing to such processes. However, experimental work was insufficient to determine whether any primary process other than the photolysis of nitrogen dioxide was in fact significant in atmospheric photochemical reactions.

Several years ago, it was observed that the rates of photooxidation of mixtures of small amounts of aldehyde and nitrogen oxide (ppm) in air were higher than were the rates of the corresponding hydrocarbon and nitrogen oxide when radiation was below 3400 Å (*4*). Aliphatic aldehydes in air may react when irradiated with simulated solar radiation to form oxidants and phytotoxicants (*5*). The photooxidation of propionaldehyde in air (in the absence of nitrogen oxides) has been studied with simulated solar radiation and with sunlight (*6*, *7*). Significant rates of reaction were obtained, with ethylhydroperoxide and carbon monoxide as major products. A free-radical mechanism was suggested to explain the results (*6*).

We have now shown that aliphatic aldehydes, when photooxidized in air with radiation at shorter wavelengths than 3400 Å, will produce intermediate species that react with olefinic and aromatic hydrocarbons. These reactions (even at very low concentrations, that is, parts per million) proceed at significant rates in the absence of nitrogen oxides. These reactions have been investigated both with sunlight-fluorescent lamps (maximum intensity at 3100 Å) and with sunlight.

The reaction mixtures were prepared in plastic containers fabricated from FEP (fluorinated ethylene-propylene copolymer) film. These containers were placed between two parallel banks, each usually having 14 sunlight fluorescent lamps with maximum intensity at 3100 Å, and the mixtures were irradiated for periods ranging from 1 to 3 hours. The reaction mixture was diluted with air so that the final volume was 150 liters, and the temperature was maintained at 23° ± 1°C. Experiments in sunlight were conducted by placing the FEP containers on the roof of the laboratory building for 5 or 6 hours around midday. Experiments were always done on clear, sunny days, but overcast occasionally developed later while the experiment was in progress.

The rates of consumption of aldehyde and of olefin were determined with a gas chromatograph (equipped with a flame ionization detector), on a bis-2(2-methoxyethyl)adipate column and a silicon fluid (SF-96) column, respectively. Formaldehyde was determined by a modification of the chromotropic acid method (*8*). Hydrogen peroxide was analyzed by the titanium-8 quinolinol method (*9*). Total oxidant was measured by a modification of the ferrous thiocyanate method (*10*); alkyl hydroperoxide was determined by kinetic colorimetry with catalyzed potassium iodide reagent (*11*).

The reactant consumptions for mixtures of one of the three aldehydes—formaldehyde, acetaldehyde, or propionaldehyde—with ethylene, *trans*-2-butene, 2-methylbutene-1, 2,3-dimethylbutene-2, or 1,3,5-trimethylbenzene are listed in Table 1 (for our experiments in the laboratory). Consumption of the olefin or aromatic hydrocarbons occurred in all these experiments. The order of increasing percentage consumption (Table 1) was as follows: 2,3-dimethylbutene-2 > 2-methylbutene-1 ~ 1,3,5-trimethylbenzene > ethylene. The rate of reaction of the olefins accelerated with increasing reaction time, an