Biochemical and population genetics of S-adenosylhomocysteine hydrolase in NW Portugal

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INTRODUCTION

Human S-adenosylhomocysteine hydrolase (AHCY; EC 3.3.1.1) exhibits a genetic polymorphism first described by Bissbort et al. (1983) who demonstrated the occurrence of two common electrophoretic phenotypes (AHCY 1 and AHCY 2-1) determined by two codominant alleles (AHCY*1 and AHCY*2) at an autosomal locus.

More detailed studies on the electrophoretic patterns of AHCY, have shown convincing evidence of the presence of at least one freely reactive sulfhydryl group in the AHCY*1 gene product; Corbo et al. (1987) found a net increase in the anodal mobility of the AHCY 1 phenotype when haemolysates were submitted to prolonged storage or pretreated with negatively charged thiol reagents prior to electrophoretic analysis. Scozzari et al. (1987), on the other hand, also described similar changes in the behaviour of AHCY from stored samples and have shown that they could be reversed upon treatment with mercaptoethanol. In general such changes have been recognized as complicating factors in the correct identification of AHCY phenotypes since they lead to the confusion between the AHCY 1 and AHCY 2-1 electrophoretic patterns.

More recently we described an improved technique for AHCY typing by means of isoelectric focusing using haemolysates pretreated with dithiothreitol (Rocha et al., 1988). We also reported an heterogeneity in the modification of the AHCY 1 patterns of untreated samples, possibly due to the occurrence of sulfhydryl variants within the AHCY*1 electromorph.

In the present work we investigate this hypothesis studying the effects of pretreating haemolysates with dithiothreitol and iodoacetic acid on the separation of AHCY 1 and 2 allozymes by means of isoelectric focusing. The results obtained from the analysis of 248 unrelated individuals indicate that AHCY*2 gene product also has at least one freely reactive -SH group but do

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not support the occurrence of any sulfhydryl variants within AHCY*1 or AHCY*2 electromorphs.

MATERIAL AND METHODS

Blood samples from 248 unrelated individuals living in Porto district (N.W. Portugal), were collected by venous puncture and used after storage up to 7 years.
Haemolysates were prepared by sonication, treated with toluene and diluted 1:1 in solutions containing either dithiothreitol (20 mM) or iodoacetic acid (20 mM). The mixtures were incubated at 37°C for 30 minutes and left overnight at room temperature.
Separation of AHCY allozymes by isoelectric focusing and activity staining were performed according to Rocha et al. (1988).

RESULTS AND DISCUSSION

Biochemical genetics

In our sample only the AHCY 1 and AHCY 2-1 phenotypes were found. Figure 1 depicts a zymogram showing the differences between these phenotypes after pretreatement with dithiothreitol (DTT) or iodoacetic acid (IACH).
All the samples reduced with DTT and identified as AHCY 1 clearly showed a more anodal pattern when alkylated with IACH. This observation is in accordance with the findings of Corbo et al. (1987) and Scozzari et al. (1987) and confirm their suggestions on the occurrence of at least one freely reactive -SH group in the AHCY*1 gene product. Moreover, it seems to rule out the possibility of occurrence in our sample of sulfhydryl variants within the AHCY*1 electromorph.
Likewise, the AHCY 2-1 pattern presents an anodal displacement upon alkylation with iodoacetic acid indicating the presence of at least one free sulfhydryl group also in the AHCY*2 allozyme. Since this displacement was found in all 2-1 individuals, it also excludes heterogeneity in the sulfhydryl reactivity of the AHCY*2 gene products analysed.

The satisfactory distinction between the AHCY phenotypes obtained by means of isoelectric focusing after sample pretreatement with DTT or IACH under the conditions here described seems to remove some of the difficulties in working with the AHCY polymorphism at the protein level reported by

Arredondo-Vega et al.(1989) on the basis of an unsuccessful separation of AHCY variant allozymes by this kind of technique.

Population genetics

In Table 1 we present the observed distribution of AHCY phenotypes and the corresponding expected values according to Hardy-Weinberg formalism.
Table 2 shows some of the available data on AHCY gene frequencies.
The frequency of the AHCY*1 allele found in our sample is the highest reported so far. Unlike all other European populations no evidence for the occurrence of the AHCY*3 allele was found.

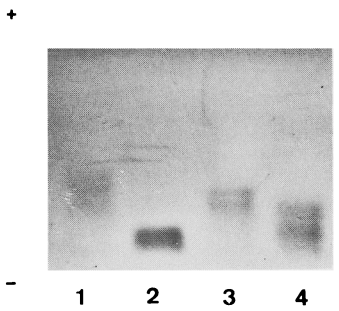

Fig. 1. AHCY phenotypes demonstrated by isoelectric focusing.
Lane 1: AHCY 2-1, pretreated with IACH
Lane 2: AHCY 1, pretreated with DTT
Lane 3: AHCY 1, pretreated with IACH
Lane 4: AHCY 2-1, pretreated with DTT

Table 1. Distribution of AHCY phenotypes in a sample from NW Portugal

	phenotypes		
Sample size	1	2-1	2
248	241 (241.10)	7 (6.85)	0 (0.05)

Table 2. AHCY gene frequencies (%) in various populations

Population	N	AHCY*2	AHCY*3	Reference
Japan	214	4.7	0.0	Akiyama et al. (1984)
SW Germany	114	4.0	0.0	Bissbort et al. (1983)
W Germany	647	2.5	0.5	Scheil and Borner (1985)
England	166	2.4	0.6	Arredondo-Vega et al.(1989)
Italy	386	2.3	0.9	Corbo et al. (1987)
Italy	374	2.4	0.7	Scozzari et al. (1987)
Sardinia	93	1.1	1.6	Scozzari et al. (1987)
NW Portugal	248	1.4	0.0	this work

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