SUBSTITUTION OF LYSINE FOR THREONINE AT POSITION 100 IN HUMAN CARBONIC ANHYDRASE Id MICHIGAN*

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Summary--The position of the amino acid substitution in the human red cell carbonic anhydrase I variant, CA Id Michigan, has been determined by sequence analysis of the altered tryptic peptide. The threonine to lysine substitution was found to be located at position 100, and is expressed as CA $I^{100\ Lys}$.

A genetically-determined electrophoretic variant of human red cell carbonic anhydrase I (CA I) [also referred to as CA B] was reported from a Negro woman and her daughter living in Detroit, Michigan and designated CA Id (1). This variant was subsequently characterized chemically by Shows (2) who demonstrated that a lysine residue has been substituted for a threonine residue in the mutant enzyme. However, the exact position of this substitution could not be determined at that time. Recently, the complete sequence of human CA I has been determined (3,4,5). It has thus become possible to locate the position of the Thr → Lys change by sequencing the peptide isolated from CA Id Michigan which contains the substitution. In the present communication, we demonstrate that the threonine residue at position 100 has been substituted for lysine in the variant enzyme.

METHODS

Both the normal (CA I) and variant (CA Id) enzymes were purified using DEAE-cellulose and DEAE-Sephadex column chromatography as previously des-

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scribed (6). The purity of the isolated components was confirmed using starch gel electrophoresis. Following purification, both the normal and variant forms of the enzyme were treated, in the presence of 0.05 M N-ethyl morpholine, pH 8.2, with a 10-fold excess of citraconic anhydride to block all of the lysine residues to tryptic cleavage (7). Excess reagent was removed by extensive dialysis against 0.02 M N-ethyl morpholine buffer, pH 8.2, after which both samples were treated with 2% trypsin for two hours at 37°. After the tryptic digestion was completed, the volume of each sample was reduced by using a stream of nitrogen gas and mild heat (50-55°); this procedure prevents the precipitation of the large tryptic peptides that are produced from the blocking of the lysine residues. The concentrated digests were subsequently fractionated on a Sephadex G-150 column equilibrated with 0.05 M N-ethyl morpholine.

A Beckman automatic sequencer was employed to sequence that region of the molecule containing the variant amino acid residue. A standard DMAA (N,N-dimethylallyamine) peptide program (Beckman 080570) was used without alteration except that butanedithiol was added to the butyl chloride. The converted hydantoin derivatives (1 N HCl, 80° for 10 min) were analyzed with standard gas chromatographic procedures (8). Concurrently, a portion of the thiazolanone derivative from each sequencer step was converted to the constituent amino acid by treating with 5 N HI at 140° for 24 hours and identified on an amino acid analyzer (9).

RESULTS

Isolation of 30 mg of CA I and 20 mg of CA Id was carried out using the above methods. Starch gel electrophoresis revealed no contamination of either of the purified forms. Each form was subsequently treated with citraconic anhydride followed by tryptic digestion. Figure 1 shows a typical elution profile of the lysine-blocked tryptic digests fractionated on Sephadex G-150.

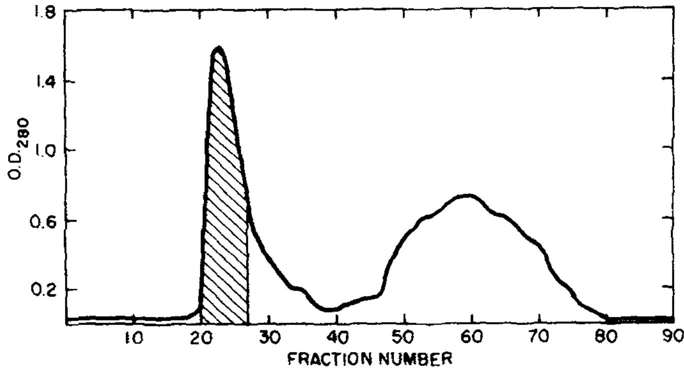

Figure 1. Elution profile of a lysine-blocked tryptic digest of CA I. A 1.5 x 90 cm Sephadex G-150 column equilibrated with 0.05 M N-ethyl morpholine was used. Approximately 1.5 ml fractions were collected. Both CA I and CA Id showed identical elution patterns.

Previous experience in this laboratory has shown that the large peak, centered at fraction 23 (Fig. 1), contains that portion of the molecule where, according to Shows (2), the amino acid substitution in CA Id is located. For this reason, the material was further purified using a 0.9 x 60 cm Sepharose 4B column equilibrated with 0.05 M N-ethyl morpholine, pH 8.2. The void volume peptide was partially sequenced to determine the exact nature of the presumed amino acid substitution. No contaminating peptides were observed by this procedure.

The gas chromatograph and amino acid analyzer results are presented in Table I. As can be seen, it is at step 11 that the Thr → Lys substitution occurs. The presence of lysine in CA Id was confirmed by amino acid analysis of the regenerated amino acid.

DISCUSSION

Based on the sequence of both the normal and variant peptides, it is clear that the Thr → Lys substitution in the variant molecule occurs at sequencer step 11. This site corresponds to residue 100 in the over-all sequence of human CA I as independently determined in three laboratories (3,4,5). Shows (2) reported that this amino acid substitution did not

TABLE I

Identification of the PTH derivatives from sequence analysis of the normal (CA I) and variant (CA Id) lysine-blocked tryptic peptides (Leu-90 to Arg-169/173*) isolated by Sepharose 4B column chromatography.

Position of residue in CA I sequence**	Sequencer step	Derivatives identified by gas chromatography		Derivatives identified with amino acid analyzer
		CA I	CA Id	CA Id
90	1	Leu	Leu	Leu
91	2	Phe	Phe	Phe
92	3	Gln	Gln	Glx
93	4	Phe	Phe	Phe
94	5	His	His	His
95	6	Phe	Phe	Phe
96	7	His	His	His
97	8	Trp	Trp	-
98	9	Gly	Gly	Gly
99	10	Ser	Ser	Ser
100	11	<u>Thr</u>	<u>Lys</u>	<u>Lys</u>
101	12	Asn	Asn	Asx
102	13	Glu	Glu	Glx
103	14	His	His	
104	15	Glu	Glu	
105	16	Ser	Ser	
106	17	Glu	Glu	
107	18	His	His	
108	19	Thr	-	
109	20	Val	-	

*This Arg is at position 169 in the sequence of Lin and Deutsch (4), and 173 in the sequences of Andersson *et al.* (5) and Laurent-Tabusse *et al.* (3).

**Residue numbers from published sequences of human CA I (3,4,5).

seem to effect either the CO_2 hydrase activity or the esterase activity of the mutant enzyme; however, the variant molecule did appear to be more thermolabile, and was found to be present in lower amounts, than the normal enzyme. In view of these findings, it is of interest that this region of

the molecule contains the two histidine residues, 94 and 96, which have been implicated as forming two of the three ligands to the single zinc atom at the active site (10). It also appears that position 100 is on the surface of the molecule. This is based on the three-dimensional structure of the other isozyme of human red cell carbonic anhydrase, CA II or CA C (10), in which His-95 (homologous to His-96 of CA I) is located at the C-terminus of β-structure δ, and the residue which occurs at position 99 (comparable to Thr-100 in CA I) is located on the surface of the molecule.

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