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CHEMICAL CHARACTERIZATION OF A NEW JAPANESE VARIANT OF CARBONIC ANHYDRASE I, CA $I_{NAGASAKI\ 1}$ (76 Arg → Gln)

炭酸脱水酵素の日本の新しい変異型，CA $I_{NAGASAKI\ 1}$ (76 Arg → Gln) の化学的性質について

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SUMMARY

A new fast inherited variant of carbonic anhydrase I (CA I), designated CA $I_{Nagasaki\ 1}$ (CA $I_{NGS\ 1}$), was discovered during a survey of hemolysates from 5,852 individuals from the cities of Hiroshima and Nagasaki in Japan. Analysis of the variant tryptic peptide from the CA $I_{NGS\ 1}$ variant indicated that a glutaminyl residue was substituted for an arginyl residue at position 76. Heat degradation studies showed that the CA $I_{NGS\ 1}$ variant was less stable than normal CA I. The CO_2 hydrase and esterase activities of the normal and variant CA I, as well as the relative amounts of the two enzymes in heterozygotes, were similar.

INTRODUCTION

Three isozymes of carbonic anhydrase, carbonic anhydrases I, II, and III (CA I, CA II, CA III), are known to occur in humans.[1,2] Knowledge of the tissue distribution of these isozymes is still incomplete; however, CA I appears to be found mainly in erythrocytes and gastrointestinal mucosa, CA II in erythrocytes and a wide variety of tissues, and CA III in skeletal muscle. The CA I and CA II isozymes appear to be products of two closely linked genes in mammals.[3-5]

要 約

広島・長崎の5,852人の溶血液の調査で CA $I_{Nagasaki\ 1}$ (CA $I_{NGS\ 1}$) と命名された炭酸脱水酵素 I (CA I) の新しい変異型を検出した．CA $I_{NGS\ 1}$ 変異型のトリプシン処理したペプタイドの分析で，76番目のアルギニン残基が，グルタミン残基に変化していることが確認された．熱変性試験では，CA $I_{NGS\ 1}$ 変異型は正常の CA I に比べて不安定であった．CO_2 水酸化活性とエステラーゼ活性は正常と同じであり，ヘテロ接合体では正常の酵素量と変異型の酵素量は同じであった．

緒 言

炭酸脱水酵素の三つのアイソザイム，炭酸脱水酵素 I，II，III (CA I，CA II，CA III)，が人類に認められている．[1,2] これらの酵素の組織分布についてはまだ完全には解明されていないが，CA I は大部分赤血球と胃腸粘膜に認められ，CA II は赤血球と広く組織にあり，CA III は骨格筋に認められている．CA I と CA II のアイソザイムは哺乳動物では連鎖した二つの遺伝子により作られているとされている．[3-5]

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The amino acid sequences of human CA I and CA II as well as their three-dimensional structures have been determined.[6-11]

To date, 15 electrophoretic variants of CA I, and 3 variants of CA II, have been reported after screening hemolysates from many different human populations.[12-14] Of these, the amino acid substitutions have been determined for five (and tentatively for two more) of the CA I variants, and one of the CA II variants.[12]

Reported here are the amino acid substitution, comparative enzyme activities, and thermostability of a new fast variant of CA I discovered in a Japanese family from Nagasaki, Japan. This variant is designated CA $I_{Nagasaki\ 1}$ (CA $I_{NGS\ 1}$) according to the nomenclature recommended for protein variants by Ferrell et al.[15] Previously, only one CA I variant (which moved cathodal to normal band electrophoretically), CA Ih Hiroshima (CA $I_{HIR\ 1}$) has been reported from the Japanese population.[16,17]

MATERIALS AND METHODS

Electrophoretic Techniques

The conditions for electrophoresis and staining used in this study were the same as those previously described.[12] For the screening procedures, the esterase activities of red cell carbonic anhydrases after electrophoresis, were detected using the fluorogenic substrates, 4-methyl umbelliferyl acetate for CA I and fluorescein diacetate for CA II.[18]

Purification of the Variant Carbonic Anhydrase

The normal and variant CA I isozymes were isolated from approximately 200 ml of whole blood which was pooled from the three individuals heterozygous for the CA $I_{NGS\ 1}$ variant. The two forms of CA I were initially separated by affinity chromatography on a sulfonamide-bound Sephadex column by the method of Osborne and Tashian.[19] Further purification was achieved by rechromatography on a DEAE-Sephadex column.[20] The concentration of the carbonic anhydrases was determined using a molar absorption value at 280 nm of 4.9×10^4 liter $mole^{-1}$ cm^{-1}.

Peptide Analysis

Tryptic digestion and peptide mapping of the carbonic anhydrases were carried out with the

人類のCA I，CA IIのアミノ酸配列とともにその3次構造が決定されている.[6-11]

今日までに15の電気泳動による変異型がCA Iで，CA IIでは三つが多くの人類集団の溶血液のスクリーニングで報告されている.[12-14] これらのうちアミノ酸置換の決定は，CA Iの五つの変異型(試案的なものは二つ追加)で，CA IIの一つの変異型で行われている.[12]

この報告では，長崎の日本人一家系で発見された新しいCA Iの速い変異型のアミノ酸置換，酵素活性の比較と熱安定性について述べる．この変異型はFerrell ら[15] の蛋白質変異型の命名法によって，CA $I_{Nagasaki\ 1}$ (CA $I_{NGS\ 1}$) と命名された．かって日本人集団においては，電気泳動上正常よりも陰極側に移動するただ一つの CA I変異型，CA Ih Hiroshima (CA $I_{HIR\ 1}$) が報告されている.[16,17]

材料及び方法

電気泳動法

この研究に用いた電気泳動条件と染色法は以前報告されたものと同じ方法である.[12] スクリーニングは，赤血球炭酸脱水酵素の電気泳動後のエステラーゼ活性には蛍光基質，CA Iに対しては 4-methyl umbelliferyl acetate，CA IIに対しては fluorescein diacetate を用いて判定した.[18]

変異型炭酸脱水酵素の精製

正常型と変異型の CA Iアイソザイムを，CA $I_{NGS\ 1}$ 変異型ヘテロ接合体をもつ3人から採血した約200mlの全血から分離した．二つの形の CA Iを初めに sulfonamide 結合 Sephadex カラムを用いたアフィニティークロマトグラフィーで，Osborne 及び Tashian[19] の方法に従って分離した．更に DEAE-Sephadex カラムを用いて再クロマトグラフィーを行い精製した.[20] 炭酸脱水酵素の濃度は280nm の分子吸光係数，4.9×10^4 liter $mole^{-1}$ cm^{-1}を用いて決定した．

ペプタイド分析

炭酸脱水酵素のトリプシン処理とペプタイドマップ

method of Tashian[21] and a modification of the method of Henderson.[11]

In order to locate the altered tryptic peptide of the CA $I_{NGS\ 1}$ variant, the latter method was used. The procedure is as follows: 20 mg of CA I was first adjusted, with rapid stirring, to pH 3.0 with 0.1 HCl, and after about 10 minutes adjusted to pH 10.6 with 0.2 M NaOH. Trypsin (trypsin to CA I ratio of 2:100) was then added and the mixture incubated at 37C for 16 hours. The pH was then adjusted to 5.0 with concentrated acetic acid, and after 2 hours the digest was centrifuged at 8,000 rpm for 30 minutes. Separation of the tryptic peptides in the pH 5.0 insoluble fraction (containing the variant peptide) was carried out by dissolving the fraction in 5.0 M guanadine-HCl buffer, pH 5.0, followed by chromatography on a Sephadex G-50 column equilibrated with the same buffer. The fractions containing the altered peptide were pooled, desalted, lyophilized, and dissolved in 10% acetic acid and rechromatographed on a Sephadex G-25 column equilibrated with 10% acetic acid.

One dimensional electrophoretic separation of tryptic peptides was carried out on chromatography paper (Whatman 3 MM) in a pyridine-acetate buffer, pH 6.4, for 70 minutes at 2,000 volts. The peptides were eluted from the paper with 25% acetic acid. Amino acid compositions of the eluted peptides were performed on an amino acid analyzer after hydrolysis for 21 hours in 6 N HCl at 100C.

Thermostability Studies

Heat denaturation studies were determined on the normal and variant CA I isozymes by utilizing the azosulfonamide binding procedure of Osborne and Tashian.[22]

Enzyme Assays

The CO_2 hydrase activities of the carbonic anhydrases were measured by the method of Nyman[23] which follows the rate of change in the ultraviolet absorbance of Veronal buffer at 276 nm. The esterase activity toward *p*-nitrophenyl acetate was measured by the method of Armstrong et al.[20]

RESULTS

The CA $I_{NGS\ 1}$ variant was discovered in a normal male member of a family from Nagasaki

Tashian の方法[21] と Henderson の方法[11] に準じて行った.

CA $I_{NGS\ 1}$の変化したトリプシン処理ペプタイドを決定するためには後者の方法を用いた. 方法は次のとおりである: 20mg の CA Iをまずかくはんしながら0.1 N HCl を用いてpH 3.0に合わせた. 約10分後0.2 M NaOH を加えて pH 10.6に合わせた. トリプシン(トリプシンと CA Iの比は 2 : 100)を加え, 37℃で16時間静置し, その後pH を5.0に濃酢酸を用いて合わせた. 2時間後その液を30分間8,000回転で遠心した. トリプシン処理ペプタイドの pH 5.0での不溶性分画(変異型ペプタイドを含む)の分離は, 5.0 M のグアニジン塩酸緩衝液pH 5.0に溶解し, Sephadex G-50カラムを用いて, 同上緩衝液でのクロマトグラフィーを行った. 変異ペプタイドを含む分画を集め, 脱塩し凍結乾燥を行った. 次に10%酢酸に溶解し, Sephadex G-25カラムを用いて同じ緩衝液を使用して再クロマトグラフィーを行った.

トリプシン処理ペプタイドをクロマトグラフィー用紙(Whatman 3 MM), ピリジン酢酸緩衝液pH 6.4を用いて2,000ボルト70分の一方向沪紙電気泳動で分離した. ペプタイドを25%酢酸を用いて沪紙より抽出した. 抽出したペプタイドのアミノ酸組成は 6 N HCl , 100℃, 21時間加水分解を行った後に, アミノ酸分析装置を用いて決定した.

熱安定性試験

正常型と変異型 CA Iアイソザイムの熱変性は, Osborne と Tashian[22] の azosulfonamide 結合法によって測定した.

酵素活性

炭酸脱水酵素の CO_2水酸化活性は276nmでの Veronal 緩衝液の紫外吸収の変化率を用いる Nyman[23] の方法で測定した. *p*-nitrophenyl acetate に対するエステラーゼ活性は Armstrong ら[20] の方法に従って行った.

結 果

CA $I_{NGS\ 1}$変異型が広島(3,267人), 長崎(2,585人)

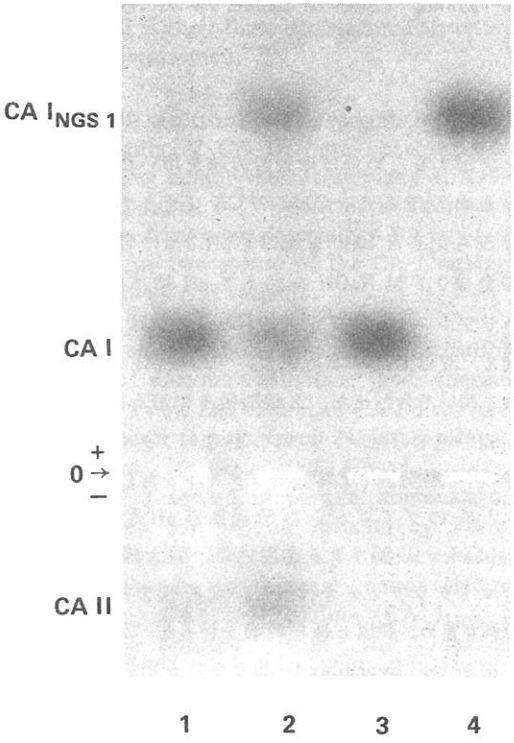


Figure 1. Protein patterns (Nigrosin stained) of normal CA I and CA $I_{NGS\ 1}$ after vertical starch-gel electrophoresis at 4C for 18 hours at 8 v/cm, pH 8.6. Hemoglobin removed by extraction of hemolysates with a mixture of 40% ethanol and chloroform (1:0.5 by vol.) at −10C. (1) normal CA I and CA II pattern, (2) CA $I_{NGS\ 1}$/CA I heterozygote pattern, (3) purified CA I, (4) purified CA $I_{NGS\ 1}$. Purified normal and variant enzymes from chromatographic separation shown in Figure 2.

図1．正常型CA IとCA $I_{NGS\ 1}$の4℃，18時間，8v/cm，pH 8.6での垂直澱分ゲル電気泳動後のニグロシン染色した蛋白像．溶血液に40%エタノールとクロロフォルム（1：0.5の割合）の混合物を−10℃で加え，ヘモグロビンを抽出した．(1)正常型CA IとCA II像，(2)CA $I_{NGS\ 1}$/CA Iヘテロ接合体像，(3)精製されたCA I，(4)精製されたCA $I_{NGS\ 1}$，図2のクロマトグラフィー分離より精製された正常型と変異型酵素．

in an examination of blood samples from 5,852 offspring of individuals exposed to atomic bomb radiation, and suitable controls, from the cities of Hiroshima (3,267) and Nagasaki (2,585). The variant was also found in the sister and father of the propositus. Since the variant was found in the father of the propositus, its presence is of course unrelated to the radiation history.

の被爆者及び対照群の子供5,852人の血液の検査で，長崎の一家族の正常な男性に発見された．変異型は発端者の姉と父親にも発見された．変異型が発端者の父親に発見されているので，変異型の存在と被爆歴とは無関係である．

The electrophoretic pattern of CA $I_{NGS\ 1}$ is shown in Figure 1. The anodal migration of this variant suggests that it is the result of a single additional negative charge. The similar intensities of the protein stains of the normal and variant CA I indicate that the levels are similar.

CA $I_{NGS\ 1}$の電気泳動パターンを図1に示した．陽極に移動しているこの変異型は一つの陰電価の増加を意味している．正常型と変異型のCA Iの蛋白染色の濃度はよく似ており，それらの量が同じぐらいであることを示している．

Isolation of the CA I Variant

After the initial separation of the normal and variant CA I by affinity chromatography, the two forms were further purified on DEAE-Sephadex columns (Figure 2). About 40 mg of normal CA I and 45 mg of CA $I_{NGS\ 1}$ were separated from 200 ml of whole blood. The purity of the separated forms of CA I can be seen in the electrophoretic patterns in Figure 1.

CA I変異型の分離

正常型と変異型CA Iのアフィニティークロマトグラフィーによる最初の分離後，DEAE-Sephadexカラムで更に二つの形を精製した（図2）．約40mgの正常型CA Iと45mgのCA $I_{NGS\ 1}$が200mlの全血より分離された．分離されたCA Iの濃度の純度は図1の電気泳動パターンのとおりである．

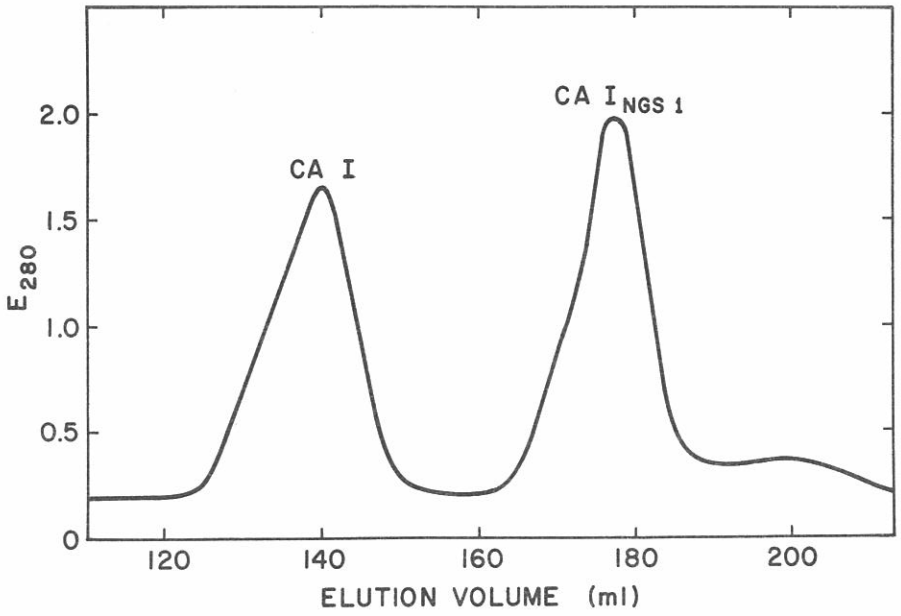


Figure 2. Chromatography of normal CA I and CA $I_{NGS\ 1}$ on DEAE Sephadex (1.5 × 90 cm column) eluted with 0-0.1 M NaCl gradient in 0.05 M Tris-HCl, pH 8.7.

図2．正常型 CA I と CA $I_{NGS\ 1}$ の DEAE-Sephadex （1.5×90 cm カラム）で 0-0.1M NaCl 濃度勾配0.05M Tris-HCl，pH 8.7緩衝液でのクロマトグラフィー．

Peptide Analysis

The tryptic peptide patterns of normal CA I and the CA $I_{NGS\ 1}$ variant are compared in Figure 3. As can be seen, one of the peptides (arrow) present in normal CA I is absent in the variant. This peptide, designated T-2, represents residues 77-80 (Ser-Val-Leu-Lys) in the normal sequence (Table 1). The absence of this peptide in CA $I_{NGS\ 1}$ suggests that Arg 76 has been substituted for an uncharged residue in the variant enzyme, and that the resultant tryptic peptide T-3 (positions 58-80) is located in the insoluble tryptic digest of CA $I_{NGS\ 1}$.

In order to locate the insoluble T-3 peptide, the insoluble tryptic digest of CA $I_{NGS\ 1}$ was dissolved in 5 M guanadine-HCl, pH 5.0, and the tryptic peptides were separated on a Sephadex G-50 column. The elution pattern is shown in Figure 4. The fractions known to contain the insoluble peptide T-1 (positions 58-76) in normal CA I were pooled, desalted, lyophilized, dissolved in 10% acetic acid, and rechromatographed on a G-25 column. The elution patterns from the normal and variant enzymes are shown in Figure 5. The fractions containing the

ペプタイド分析

正常型CA IとCA $I_{NGS\ 1}$変異型のトリプシン処理ペプタイドのパターンを図3で比較した．正常型CA Iでみられる一つのペプタイド(矢印)が変異型で欠損しているのが分かる．このT-2と書かれたペプタイドは，正常型のアミノ酸配列(表1)では77-80(Ser-Val-Leu-Lys)の残基である．CA $I_{NGS\ 1}$におけるこのペプタイドの欠損は，76番目のArgが変異型酵素では電価のない残基に置換されていることと，トリプシン処理ペプタイドT-3(58-80位)はCA $I_{NGS\ 1}$のトリプシン処理不溶性分画に存在することを示唆している．

不溶性T-3ペプタイドを決定するために，CA $I_{NGS\ 1}$のトリプシン処理不溶性分画を5Mグアニジン塩酸pH 5.0に溶解し，トリプシン処理ペプタイドをSephadex G-50カラムで分離した．溶出パターンは図4のとおりである．正常型CA Iの不溶性ペプタイドT-1(58-76位)を含んでいる分画を集め，脱塩し，凍結乾燥し，10%酢酸に溶解してG-25カラムで再クロマトグラフィーを行った．正常型及び変異型酵素の溶出パターンを図5に示す．CA Iの正常

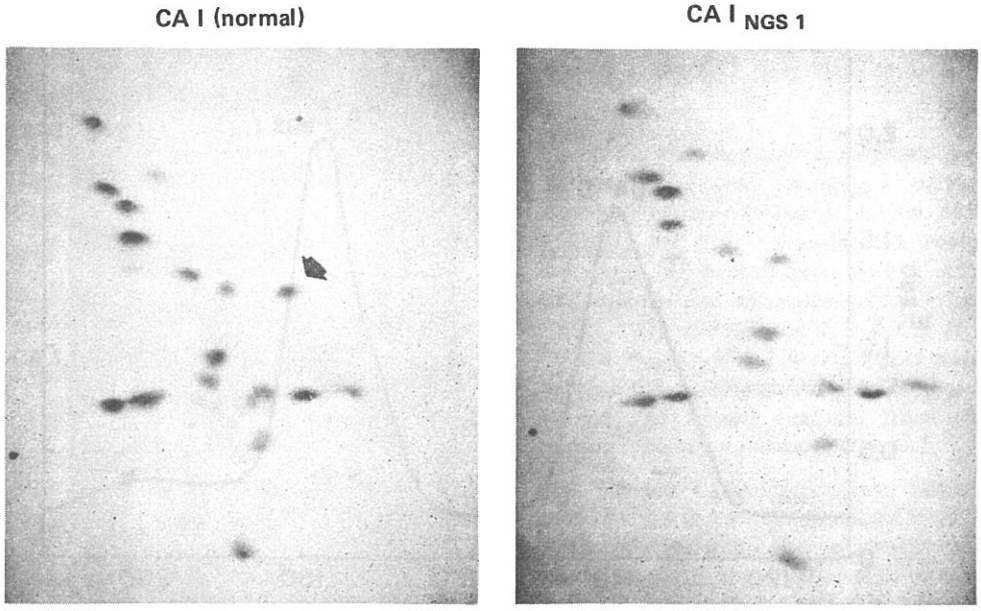
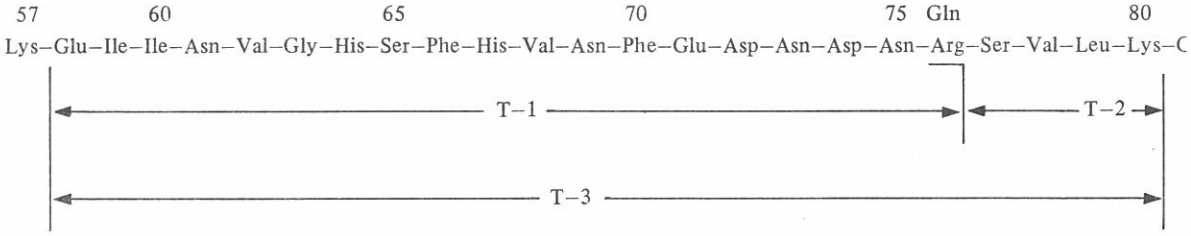


Figure 3. Tryptic peptide patterns of normal CA I and CA $I_{NGS\ 1}$. The arrow indicates peptide T-2 which is missing in CA $I_{NGS\ 1}$.
図3．正常型CA IとCA $I_{NGS\ 1}$のトリプシン処理ペプタイドのパターン．矢印はCA $I_{NGS\ 1}$で欠損しているペプタイドT-2である．

TABLE 1 PORTION OF CA I SEQUENCE SHOWING TRYPTIC PEPTIDES, T-1 AND T-2, OF NORMAL CA I AND T-3 OF CA $I_{NGS\ 1}$
表1　正常型CA Iのトリプシン処理ペプタイド，T-1，T-2とCA $I_{NGS\ 1}$のT-3のCA I配列の部位



CA I sequence from Andersson et al[6] and Lin & Deutsch[8]. Andersson ら[6] と Lin 及び Deutsch[8] の CA I 配列.

normal peptide T-1 from CA I and the variant peptide T-3 from CA $I_{NGS\ 1}$ were then lyophilized, resuspended in 10% acetic acid and subjected to paper electrophoresis. Since both peptides are negatively charged at pH 6.4, the anodally migrating bands were eluted from the paper with 25% acetic acid and hydrolyzed. The amino acid compositions of the two peptides are compared in Table 2. As can be seen, the variant peptide contains one additional serine, valine, leucine, lysine, and glutamic acid, and no arginine. Since only one additional negative

型ペプタイドT-1とCA $I_{NGS\ 1}$の変異型ペプタイドT-3を含む分画を凍結乾燥し，10%酢酸に再懸濁して沪紙電気泳動を行った．二つのペプタイドはpH 6.4で陰性に荷電するので，陽極側に移動したバンドを25%酢酸で沪紙から溶出し，加水分解を行った．二つのペプタイドのアミノ酸組成を表2で比較する．変異型ペプタイドはセリン，バリン，ロイシン，リジン，グルタミン酸が各々一つずつ多く，アルギニンはない．ただ一つの陰電荷が増加した状態が

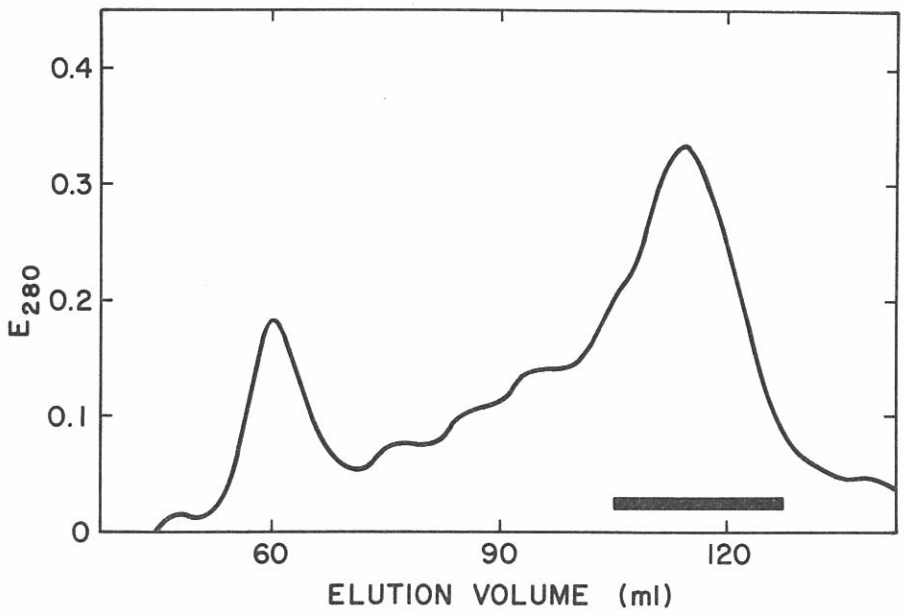


Figure 4. Chromatography of pH 5.0 insoluble fraction of CA $I_{NGS\ 1}$ tryptic digest on Sephadex G-50 (0.9 X 120 cm column) eluted with 5.0 M guanadine-HCl. Bar indicates fractions containing peptide T-3; this region in the elution of normal CA I contains peptide T-1.

図4． CA $I_{NGS\ 1}$のトリプシン処理，pH 5.0不溶性分画の Sephadex G-50 (0.9×120cmカラム)，5.0Mグアニジン塩酸で溶出したクロマトグラフィー．横棒はペプタイドT-3を含んでいる分画を示している．正常型CA Iの溶出でこの分画はペプタイドT-1を含んでいる．

TABLE 2 COMPARISON OF THE MOLAR RATIOS OF AMINO ACID RESIDUES IN TRYPTIC PEPTIDES, T-1 AND T-2, FROM CA I (NORMAL) AND T-3 FROM CA $I_{NGS\ 1}$

表2　トリプシン処理ペプタイド，CA I(正常型)のT-1，T-2とCA $I_{NGS\ 1}$のT-3とのアミノ酸残基のモル比の比較

Amino Acid	CA I (normal)		CA $I_{NGS\ 1}$
	T−1	T−2	T−3
Aspartic acid	6.36 (6)	–	6.07 (6)
Serine	0.99 (1)	0.53 (1)	1.87 (2)
Glutamic acid	2.25 (2)	–	2.81 (3)
Glycine	0.91 (1)	–	0.91 (1)
Valine	1.86 (2)	0.99 (1)	2.96 (3)
Isoleucine	1.87 (2)	–	2.31 (2)
Leucine	–	1.08 (1)	1.37 (1)
Phenylalanine	1.98 (2)	–	1.88 (2)
Histidine	1.98 (2)	–	1.75 (2)
Lysine	–	0.93 (1)	1.13 (1)
Arginine	0.99 (1)	–	–
Total Residues	19	4	23

Figure in parentheses is assumed number of residues. ( ) 内は残基の推定組成比．

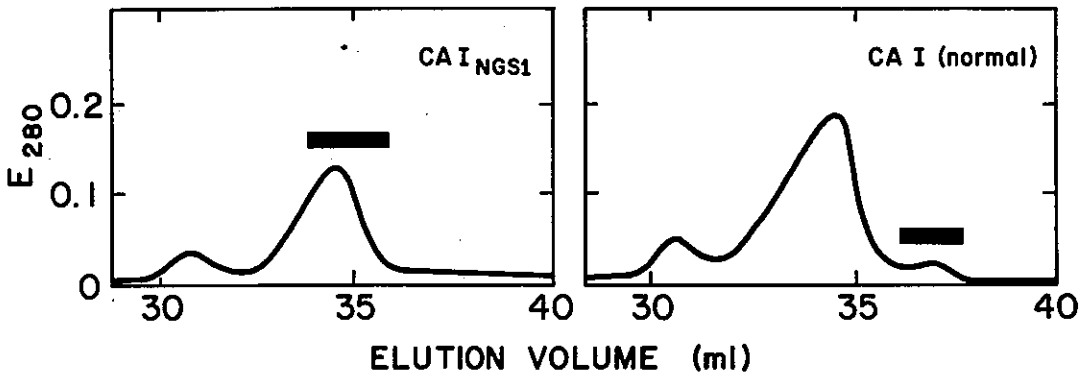


Figure 5. Chromatography of pooled fractions (indicated by bar in Figure 4) on Sephadex G-25 superfine (0.9 X 120 cm column) eluted with 10% acetic acid. Bars indicate fractions containing peptides T-3 from CA $I_{NGS\ 1}$ and T-1 from normal CA I.

図5．図4で横棒で示した分画の Sephadex G-25 superfine（0.9×120cmカラム）を用い，10%酢酸で溶出したクロマトグラフィー．横棒は CA $I_{NGS\ 1}$ のペプタイド T-3，正常型 CA I の T-1 を含んでいる分画を示している．

TABLE 3 ENZYME ACTIVITIES OF NORMAL CA I AND CA $I_{NGS\ 1}$
表3　正常型 CA I と CA $I_{NGS\ 1}$ の酵素活性

CA I Types	Esterase Activity (*p*-nitrophenyl acetate) (units [a]/mg)	CO_2 hydrase activity (units [b]/mg)
CA I (normal)	0.28	4.8
CA $I_{NGS\ 1}$	0.28	4.9

[a] *μmoles p-nitrophenol formed/minute*　b *Δ OD at 276 nm/sec*

charge is indicated by the migration pattern of CA $I_{NGS\ 1}$ (Figure 1), the most likely explanation is that a glutaminyl residue has substituted for an arginyl residue at position 76.

CA $I_{NGS\ 1}$（図1）の移動パターンから分かるので，最も適当な説明はグルタミン残基が76番目でアルギニン残基と置換していることである．

Thermostability

The CA $I_{NGS\ 1}$ variant was found to be less stable than normal CA I at 57C, pH 8.0. The comparative thermostability curves are given in Figure 6.

熱安定性

CA $I_{NGS\ 1}$ は正常な CA I に比べて57℃，pH 8.0で不安定であった．熱安定性の比較を図6に示す．

Enzyme Activities

The esterase activities and CO_2 hydrase activities of the normal and variant CA I are listed in Table 3. Under the conditions of the assays, the

酵素活性

正常型と変異型 CA I のエステラーゼ活性と CO_2 水酸化活性を表3に示す．この測定の条件下では，

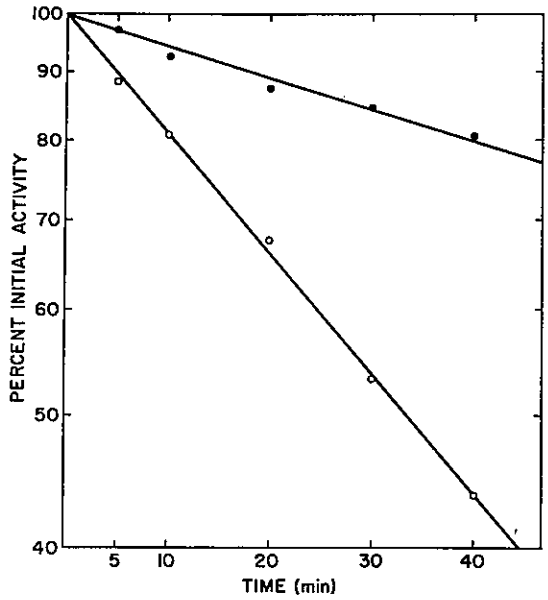


Figure 6. Heat degradation of normal CA I (•) and CA $I_{NGS\ 1}$ (○) at 57C in 10 mM Hepes buffer, pH 8.0.

図6．57℃，10mM Hepes 緩衝液，pH 8.0，での正常型 CA I（●）と CA $I_{NGS\ 1}$（○）の熱変性試験．

activities of CA I and CA $I_{NGS\ 1}$ were essentially the same.

CA IとCA $I_{NGS\ 1}$の活性は基本的には同じであった．

DISCUSSION

The residue at position 76 has now been sequenced in the CA I of orangutan, rhesus macaque, and ox.[1] In these species the residue is arginine as in human CA I. The homologous residue in the CA II of human, sheep, ox, and rabbit is lysine.[1]

In bovine CA III, the homologous residue is arginine as in CA I (Tashian, unpublished data). Thus, a positive charge has been maintained at this position in all three carbonic anhydrase isozymes of mammals. However, except for the lower thermostability, the substitution of glutamine for arginine at this position does not seem to have effected either the activities or quantitative levels of the variant enzyme. Based on the three-dimensional structures of human CA I and CA II residue 76 is located on the outside of the molecule and is not a part of any secondary structure.[7,10]

In an earlier survey of adults born prior to the A-bombs from Hiroshima (2,604) and Nagasaki (1,365), Ueda et al[24], found four unrelated individuals with the CA $I_{HIR\ 1}$ variant in the Hiroshima sample and none in the Nagasaki sample.

考　察

76番目の残基はオランウータン，赤毛猿，雄牛の CA Iで決定されている．[1] これらの動物の残基は人間の CA Iと同じくアルギニンである．人間，羊，雄牛，兎の CA IIに相応する残基はリジンである．[1]

牛 CA IIIに相応する残基は人間の CA Iと同じくアルギニンである（Tashian，未発表データ）．このように，すべての哺乳動物の三つの CA アイソザイムではこの位置は陽電荷をもっている．しかしながら熱安定性の低下を除けば，この部位でのグルタミンとアルギニンの置換は変異型酵素の活性や量には影響を与えていないようである．人類 CA Iと CA IIの3次構造に基づいて，76番目の残基は分子の外側で2次構造のどの部分にもあたらない．[7,10]

広島（2,604人）及び長崎（1,365人）の被爆以前に生まれている成人についての以前の調査で，上田ら[24]は広島で血縁関係のない4人の CA $I_{HIR\ 1}$変異型を認めたが長崎では認めなかった．

Of the 15 electrophoretic variants of CA I which have now been reported from various human populations, in only three (CA I 2 Australia, CA I 4 Australia, and CA I Parsi) have the frequencies of the variant alleles been found to be greater than 2% in some populations. Two of these, CA I 2 Australia and CA I 4 Australia[13] were found in Australian Aboriginals, and CA I Parsi was found in a Parsi population from Bombay, India.[14]

CA $I_{NGS\ 1}$ and CA I 4 Australia are the only fast variants of CA I that have been reported. The electrophoretic patterns of these fast variants appear to be similar. As yet, however, the CA I 4 Australia variant has not been characterized chemically.

CA Iについて15種の電気泳動変異型が様々な人間集団で報告されているが，ただ3種（CA I 2 Australia, CA I 4 Australia, CA I Parsi ）だけが幾つかの集団で2%以上の変異型遺伝子座の頻度を有している．これらのうち二つ，CA I 2 Australia と CA I 4 Australia[13] はオーストラリアの原住民で認められ，CA I Parsi[14] はインドのボンベイの Parsi 集団で認められた．

CA $I_{NGS\ 1}$ と CA I 4 Australia だけが今まで報告されている CA Iの変異型の中では速い変異型である．これらの速い変異型の電気泳動上のパターンは似ているが，CA I 4 Australia 変異型の化学的性質は分かっていない．

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