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Basis for the equilibrium constant in the interconversion of L-lysine and L-β-lysine by lysine 2,3-Aminomutase

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SUMMARY

L-β-Lysine and β-glutamate are produced by the actions of lysine 2,3-aminomutase and glutamate 2,3-aminomutase, respectively. The pK_a values compounds have been titrimetrically measured and are for L-β-lysine: $pK_1 = 3.25$ (carboxyl), $pK_2 = 9.30$ (β-aminium), and $pK_3 = 10.5$ (ε-aminium). For β-glutamate the values are $pK_1 = 3.13$ (carboxyl), $pK_2 = 3.73$ (carboxyl), and $pK_3 = 10.1$ (β-aminium). The equilibrium constants for reactions of 2,3-aminomutases favor the β-isomers. The pH- and temperature dependencies of K_{eq} have been measured for the reaction of lysine 2,3-aminomutase to determine the basis for preferential formation of β-lysine. The value of K_{eq} (8.5 at 37 °C) is independent of pH between pH 6 and pH 11; ruling out differences in pK -values as the basis for the equilibrium constant. The K_{eq} -value is temperature-dependent and ranges from 10.9 at 4 °C to 6.8 at 65 °C. The linear van't Hoff plot shows the reaction to be enthalpy-driven, with $\Delta H^\circ = -1.4$ kcal mol⁻¹ and $\Delta S^\circ = -0.25$ cal deg⁻¹ mol⁻¹. Exothermicity is attributed to the greater strength of the bond C β —N β in L-β-lysine than C α —N α in L-lysine, and this should hold for other amino acids.

Keywords

lysine 2,3-aminomutase; β-lysine, pK_a -values; β-glutamate, pK_a -values; K_{eq} of lysine/β-lysine, ΔH° of lysine/β-lysine, ΔS° of lysine/β-lysine

INTRODUCTION

β- Amino acids have been found to play important biological roles (1,2) as precursors in the biosynthesis of antibiotics, anticancer agents, neurotransmitters, and other high molecular weight polymers. For example, β-lysine is a precursor for the biosynthesis of antibiotics viomycin and streptothricin in *Streptomyces*(3,4).β-Lysine is also the intermediate for the complete catabolism of lysine to carbon dioxide, ammonia and water in *Clostridium subterminale* SB4 (5,6).

The transformation of L-lysine into L-β-lysine is catalyzed by lysine 2, 3-aminomutase in a reaction that involves migration of the α-amino group to the β-carbon, with the concomitant transfer of the 3-pro-*R* hydrogen in L-lysine to the 2-pro-*R* position of L-β-lysine (7,8). The enzyme incorporates a [4Fe–4S] cluster and requires *S*-adenosylmethionine and pyridoxal-5'-phosphate (PLP) as coenzymes. In the resting state of the enzyme, PLP bound as an internal aldimine with Lys 337 (9,10). Upon binding L-lysine, the internal PLP-aldimine undergoes

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transaldimination with the α -amino group to form an external PLP-aldimine of L-lysine, which undergoes transformation into the external PLP-aldimine of L- β -lysine. The migration of the amino group between C2 and C3 proceeds by a radical mechanism, in which the 3-pro-*R* hydrogen in L-lysine is abstracted by the 5'-deoxyadenosyl radical generated in the interaction of *S*-adenosylmethionine with the $[4\text{Fe}-4\text{S}]^{1+}$ cluster (11-14). The resultant 5'-deoxyadenosine is held in the active site, while the substrate-related radical rearranges to the β -lysyl radical, which then abstracts a hydrogen atom from the methyl group of 5'-deoxyadenosine to C2 of β -lysine and regenerates the 5'-deoxyadenosyl radical and subsequently *S*-adenosylmethionine.

In this paper the effects of temperature and pH on the equilibrium constant of the reaction of lysine 2, 3-aminomutase are investigated, and the apparent ionization constants of its reaction product, L- β -lysine, as well as of β -glutamic acid, are measured. The ionization properties of the amino groups in L-lysine and L- β -lysine are discussed with reference to the substrate binding and product release in catalysis.

MATERIALS AND METHODS

Materials

L-lysine, L-glutamic acid, β -glutamic acid, *S*-adenosylmethionine, pyridoxal 5'-phosphate (PLP), sodium hydrosulfite, 2-(*N*-morpholino) ethanesulfonic acid (MES), *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES), 4-(2-hydroxyethyl) piperazine-1-propanesulfonic acid (EPPS), cyclohexylaminoethanesulfonic acid (CHES), and 3-(cyclohexylamino)propanesulfonic acid (CAPS) are purchased from Sigma-Aldrich. Phenylisothiocyanate (PITC), triethylamine, pyridine were from Pierce. *S*-Adenosylmethionine was purified by chromatography through a 2×20 cm column of CM-cellulose eluted with 40 mM HCl. Purified *S*-adenosylmethionine was stored at -70°C . All other compounds were used directly without further purification.

Enzyme Preparation

Recombinant lysine 2, 3-aminomutase was expressed and purified under anaerobic conditions as described previously (15,16). Enzyme was activated and assayed using a radiochemical method as described (17,18).

Synthesis of L- β -Lysine

The synthesis of L- β -Lysine was carried out enzymatically using purified recombinant lysine 2, 3-aminomutase. The enzyme (26 μM) was activated by anaerobic incubation in 80 mM Epps buffer at pH 8.0 in the presence of 1.5 mM ammonium iron (II) sulfate, 0.6 mM PLP, and 16 mM L-cysteine for ~ 3.5 hrs. The synthetic reaction was carried out anaerobically in 180 mM Epps buffer at pH 8.0 and 21°C . The reaction was initiated by addition of L-lysine, sodium dithionite, *S*-adenosylmethionine, and the activated enzyme to Epps buffer in a sealed container. The final concentration of the reaction mixture contained 570 mM L-lysine, 8.0 mM sodium dithionite, 35 μM *S*-adenosylmethionine, and 3.5 μM enzyme. The progress of the reaction was monitored by separating L-lysine and L- β -lysine by paper electrophoresis and visualizing with ninhydrin staining. When equilibrium was attained, the reaction temperature was lowered to 4°C by moving the reaction vessel to a refrigerator for several hours. This was done to maximize yield after finding (Table 2) that low temperatures favored the transformation to L- β -lysine. The reaction was stopped by lowering the pH to ~ 4.5 with acetic acid and boiling for 5 min. Denatured protein was removed by filtration with two layers of Whatman # 1 paper. The filtrate solution was acidified to pH 2.7 with 2 N HCl and filtered with moist charcoal and filter aid (Celite) through Whatman #1 paper with suction. The purification of L- β -lysine from

L-lysine and other impurities was carried out using a procedure as described by Chirpich et al (19) with some minor modifications.

Measurement of Equilibrium Constants

The influence of pH on the equilibrium in the reaction of lysine 2, 3-aminomutase was studied at pH-values ranging from 6 to 11. The pH of the reaction was controlled by using buffer solutions made with MES ($pK_a = 6.15$), HEPES ($pK_a = 7.56$), EPPS ($pK_a = 8.0$), CHES ($pK_a = 9.4$), or CAPS ($pK_a = 10.4$). The pH of the reaction was made within ± 0.5 of pK_a -values of the buffering reagents. The enzyme was reductively activated as described (18). The reaction components were then added to the corresponding buffer solution of designated pH in the following order: L-lysine, sodium dithionite, *S*-adenosylmethionine, and activated enzyme and thoroughly mixed. The final concentrations of various reaction components after mixing were as follows: 200 mM buffer reagents at various pHs, 0.5 μ M enzyme, 30 mM L-lysine, 32 μ M *S*-adenosylmethionine, 6.0 mM sodium dithionite, except for the reactions at pH 6.0 and 6.5, in which the substrate L-lysine concentration was lowered to 10 mM and the enzyme concentration was increased to 3.0 μ M. This was to ensure that equilibrium would be reached despite the instability of enzyme at this lower pH.

The influence of temperature on the equilibrium was studied by carrying out the reactions at various temperatures ranging from 4°C to 65°C. The reaction temperature was controlled by incubating sealed reaction containers in a FisherScientific water bath with heating and refrigerating. When equilibrium was established, reactions were stopped by addition of perchloric acid to 0.2 M, and precipitated enzyme was removed by centrifugation. Aliquots of the supernatant containing L-lysine and L- β -lysine were derivatized with phenylisothiocyanate (PITC) and analyzed by HPLC. The relative amounts of reactant and product at equilibrium were determined by double integration of the PITC- L-lysine and PITC- L- β -lysine peaks and comparison with standard samples as described previously (19,20).

In both the temperature and pH dependent equilibrium studies, L- β -lysine was also used as the initial substrate to verify that the equilibrium of the reaction was indeed reached. The equilibrium constants were the same regardless of whether L-lysine or L- β -lysine was the initial substrate.

Titration Procedures and Calculation of Dissociation Constants

In the titration of L- β -lysine, two 20 mL solutions from 20 mM to 30 mM were prepared. The L- β -lysine solutions were titrated with 1.00 N HCl and 1.00 N NaOH, respectively, by addition of 20 μ l acid or base at a time at 25 °C. The pH of the solution was monitored using a Corning pH meter Model 430 and recorded after each addition of acid or base. Two 20 ml water blanks were also titrated. The volumes of acid or base used to titrate the amino acid solutions to each pH value were corrected by subtraction of the volumes of acid or base used to titrate the water blanks to the same pH values. Titration curves were constructed by plotting the pH values versus the corrected volumes of the acid and base used to titrate to the corresponding pH. The equivalence points in each titration were identified from the first and second derivatives of the titration curves (21).

The dissociation constant of the β -amino group of L- β -lysine, $K_{a\beta}$, was calculated by solving $K_{a\beta}$ from equation 1

$$\frac{V_{HCl}[HCl]}{V_{\beta Lys}[\beta Lys]} = \frac{K_{a\beta}}{[H^+] + K_{a\beta}} + \frac{K_{a\epsilon}}{[H^+] + K_{a\epsilon}} \quad (1)$$

where V_{HCl} was the volume of titrant, HCl, added to reach the equivalence point, $[HCl]$ was the molar concentration of the titrant, and $V_{\beta Lys}$ and $[\beta Lys]$ were the volume and molar concentration of the analyte, L- β -lysine, respectively. Inasmuch as V_{HCl} , $[HCl]$, $V_{\beta Lys}$, $[\beta Lys]$, and the pH ($[H^+]$) were all known at the equivalence point, and assuming the dissociation constant of the ϵ -amino group of L- β -lysine, $K_{a\epsilon}$, to be the same as that of L- α -lysine, 10.55 (22), the dissociation constant of the β -amino group, $K_{a\beta}$, and therefore $pK_{a\beta}$ ($pK_{a\beta} = -\log(K_{a\beta})$) could be calculated (please see supplementary material for the derivation of Eqn 1). The dissociation constant of the carboxyl group of β -lysine was determined from the half neutralization point between the two equivalence points as described by Harris, (21).

In the titration of β -glutamic acid, 20 mM β -glutamic acid hydrochloride and β -glutamic acid sodium salt were prepared by titrating 0.4 mmole of β -glutamic acid in ~ 15 ml of double distilled water with either 2 M HCl or NaOH until all the β -glutamic acid was dissolved. The volumes were then adjusted to 20 ml to make 20 mM solutions. The 20 mM β -glutamic acid solutions were then titrated with 1.00 M NaOH or 1.00 M HCl, respectively, as described above at 25 °C. The pH and the volumes of the added acid and base were recorded. The titration curves were corrected by subtracting with the curve obtained from titrating the same volume of de-ionized water. The two halves of the titration curve were then combined to make a complete titration curve. The pK_a -values of the carboxyl groups and that of the amino group of β -glutamic acid were determined by identifying the equivalence points from the first derivatives of the titration curve and using the volume and concentration of the titrant and analyte at the equivalence points to calculate the pH at the half neutralization points.

The pK_a values of the carboxyl group of β -lysine and the carboxyl and amino groups of β -glutamic acid can be determined from the inflection points in the titration curves (the half neutralization points) between the two equivalence points identified from the first and second derivatives of the titration curves.

RESULTS AND DISCUSSION

The Ionization of L- β -Lysine

Figure 1A shows the structure of L- β -lysine. It differs from L- α -lysine in that one methylene group separates the amino group from the carboxyl group. The acid-base titration curve of L- β -lysine is shown in Figure 2. The first derivatives of the titration curve (not shown) have been used to determine the equivalence points of the titration of the three ionizable groups. The pH and acid/base concentrations at the equivalence points were used to calculate the dissociation constants and pK_a values of the amino and carboxyl groups of L- β -lysine as described in Methods. The calculated pK_a values are shown in Table 1. The pK_a values of L-lysine, alanine, and β -alanine are also listed in Table 1 for comparison purposes.

With the amino group insulated from the carboxyl group by one methylene unit in L- β -lysine, the pK_2 -value of the β -amino group is 0.35 higher than for the α -amino group in L-lysine. In contrast, the carboxyl group of L- β -lysine is less acidic than in L-lysine, with a pK_1 -value more than one unit higher than in L- α -lysine. These differences result from a weaker electrostatic interaction between the two ionizing groups in L- β -lysine. The differences are comparable to the corresponding differences between the amino groups and carboxyl groups in L-alanine and β -alanine.

The Ionization of β -Glutamic Acid

Values of pK_a for the carboxyl groups and the amino group in β -glutamic acid were determined from acid-base titration data (Figure 3) by identifying the equivalence points from derivatives of the titration curve and the pH- values at the half neutralization points.

The measured pK_3 -value of the β -amino group is given in Table 1. From the titration curve, the dissociation constants of the two carboxyl groups can be deduced. While the carboxyl groups are chemically equivalent, they are not statistically equivalent, and the dissociation constants must differ by a factor of 4 (26-29). This is the statistical correction for a dicarboxylic acid in which the two carboxylic groups cannot interact ($\text{HOOC}-(\text{CH}_2)_n\text{-COOH}$). The two identical carboxyl groups can ionize in two different ways, so that the observed first dissociation constant is 2-times what it would be in the absence of a statistical correction. Because the fully ionized dicarboxylate ion ($^-\text{OOC}-(\text{CH}_2)_n\text{-COO}^-$) has two positions to which a proton can bind, the observed second dissociation constant is 1/2-times the value it would have in the absence of a statistical correction. Therefore the ratio $(2)/(1/2) = 4$ is the statistical correction, and this is the same as the ratio of dissociation constants because the carboxylic acid groups are identical, the only difference in the dissociation properties being the statistical factor.

The dissociation constants can be evaluated from the half-equivalence point, which is 3.47 in the titration curve, by use of a variant of eq. 1, in which $K_{\alpha\beta} = K$ and $K_{\alpha\epsilon} = 4K$ (or vice versa: see Supplementary Information for a derivation). When this is done, $K = 1.87 \times 10^{-4}$ and $4K = 7.47 \times 10^{-4}$, and the corresponding values of pK_1 and pK_2 are given in Table 1.

In general, dissociation constants of short-chain dicarboxylic acids differ by more than the statistical factor of 4 for electrostatic reasons. The statistical factor of 4 translates into a ΔpK_a of 0.6 for a dicarboxylic acid in which there is no electrostatic interaction between the carboxyl groups. β -Glutamic acid is a five-carbon acid, and the question arises whether there would be an electrostatic interaction between the carboxyl groups. There is such an interaction in glutaric acid, in which the value of ΔpK_a is 1.1. Therefore, the electrostatic contribution to ΔpK_a must be 0.5 for the simplest five-carbon dicarboxylic acid. The electrostatic component arises from the fact that the first ionization of glutaric acid generates a negative charge, and this constitutes an electrostatic barrier to the second ionization from the same molecule. Such a barrier does not exist in the ionization of β -glutamic acid because of the presence of the β -aminium group. Thus, the first ionization of β -glutamic acid (pK_1) does not lead to an anion but to a neutral zwitterion, which does not present an electrostatic barrier to the dissociation of the second carboxyl group (pK_2). Therefore, only the statistical inequivalence of the carboxyl groups in β -glutamic acid contributes to the difference of 0.6 between pK_1 and pK_2 .

Literature pK_a -values for L-glutamic acid are included in Table 1. Unlike L- glutamate, the carbon bearing the amino group in β -glutamic acid is insulated from both carboxyl groups by one methylene unit. This increases the pK_a -value of one carboxyl group by ~ 1 unit and decreases that of the other relative to L-glutamic acid. Differences in pK_a values between L-glutamic acid and β -glutamic acid arise mainly from differences in electrostatic interactions between the amino and carboxyl groups. The results for β -glutamic acid are also compatible with the pK_a values for aspartic acid, in that the value of pK_2 for β -glutamic acid is similar to that for the chemically similar β -carboxyl group of aspartic acid.

Certain homologs of lysine 2, 3-aminomutase can accept L-glutamic acid as the substrate and transform it into β -glutamic acid [30]. Knowledge of the pK_a values of β -glutamic acid may be of use in engineering more efficient enzymes that can accept these molecules as substrates and transform them into new chemical entities.

pH-Independence of K_{eq}

The equilibrium constants in the reaction of lysine 2,3- aminomutase were measured at 25°C by HPLC analyses of the PITC-derivatized reaction products. Values of K_{eq} were obtained at pH values ranging from 6 to 11 and are shown in Fig 4. The equilibrium constant is essentially pH-independent within the range from 6 to 11 under the experimental conditions employed.

However, the enzyme shows increased instability at pH values lower than 6.5, and a 10 to 15% increase in initial rate can be observed at slightly alkaline pH ($\text{pH } 9 \pm 0.5$).

In one hypothesis to explain the reported value of K_{eq} , the presumed higher basicity of the β -amino group in L- β -lysine relative to the α -amino group in L-lysine might explain the preference for L- β -lysine in neutral or weakly basic solutions. The results in Table 1 show that the β -amino group is indeed the more basic, but it is not basic enough to account for the equilibrium constant. The difference of 0.35 might explain an equilibrium constant of 2 but the value of 8.5 in Fig. 4. Furthermore, the value of K_{eq} is pH-independent throughout the pH-range in which both amino groups undergo ionization. All of the present results contradict the hypothesis, and it is disproven.

Influence of Temperature on the Equilibrium

The equilibrium constants of the reaction at temperatures ranging from 4°C to 65°C were measured by HPLC as described in Methods. The measured equilibrium constants at various temperatures using either L-lysine or L- β -lysine as the beginning substrate are included in Table II. The natural logarithms of the equilibrium constants, $\ln(K_{\text{eq}})$, were plotted against the reciprocals of the reaction temperatures in Kelvin ($1/T$) based on the *van't Hoff* equation (eq. 2):

$$\ln K = -\frac{\Delta_r H^\circ}{RT} + \frac{\Delta_r S^\circ}{R} \quad (2)$$

A straight line with a positive slope was obtained, as illustrated in Figure 5, which shows the influence of temperature on the equilibrium in the reaction of lysine 2, 3-aminomutase. From the line in Fig. 5, the standard enthalpy change of the reaction was calculated to be $-1.4 \text{ kcal mol}^{-1}$ from the slope ($-\Delta_r H^\circ/R$) of the line; the standard entropy change was calculated to be $-0.25 \text{ cal deg}^{-1} \text{ mol}^{-1}$ from the ordinate intercept ($\Delta_r S^\circ/R$).

The thermodynamic parameters suggest general conclusions: The reaction of lysine 2,3-aminomutase reaction is exothermic, so that the formation of L- β -lysine is favored at low temperatures (Table II). The reaction is driven mainly by the enthalpy change, and the transformation of L-lysine into L- β -lysine is associated with a very slight loss of entropy.

The thermodynamic parameters have some practical value. Conducting the reaction at 21°C rather than at 37°C increases the product yield by ~12%. Considering lysine 2, 3-aminomutase is a very fast enzyme (35 to 40 s^{-1} turnover) and very stable under anaerobic conditions. Lowering the reaction temperature from 37 °C to ambient temperature will not significantly increase costs but will increase the product yield.

The thermodynamic parameters may also explain the equilibrium constant and the preferential formation of L- β -lysine. The reaction is hardly affected by entropy, suggesting that solvation is not important in determining the position of equilibrium. The reaction is driven by the enthalpy change, suggesting that L- β -lysine is simply more stable than L-lysine. The small difference in stability may be localized to the C—N bond of L- β -lysine. As a rule, the greater the electronegativity difference between covalently bonded atoms, the stronger the covalent bond. The theoretical basis for the rule, first put forward in 1932 [30], is that the electrostatic component in a polar bond increases its strength. Adopting any scale of electronegativity, the electronegativity of nitrogen in the amino group will be the same in L-lysine and L- β -lysine. In contrast, the electronegativities of C2 in L-lysine and C3 in L- β -lysine will not be the same: carbon-2 in L-lysine will be partially positively charged, owing to electron withdrawal by the carboxylate group, whereas C3 in L- β -lysine will be insulated from this effect. Carboxylate groups are electron withdrawing as proven by the fact that the value of the α -carbon- pK_a of

acetate anion is 33 [31], and not >45 as in ethane. Therefore, C2 in L-lysine is more electronegative than C3 in L-β-lysine. For this reason, the difference in electronegativities between C3 and N(β) in L-β-lysine is greater than between C2 and N(α) in L-lysine, and the bond is stronger in L-β-lysine. A similar difference can be expected between L-glutamate and β-glutamate; accordingly, the value of K_{eq} for that transformation is 15 [32].

The foregoing explains the greater enthalpic stability of L-β-lysine relative to L-lysine and of β-glutamate relative to L-glutamate. The only other potential explanations would be solvation effects or differential internal hydrogen bonding. The small entropy change does not support an effect of solvation, and the possibilities for internal hydrogen bonding in an α-amino acid and its β-isomer are similar. Further experiments on bond strengths may clarify this issue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.
Structures of L-β-lysine and β-glutamate.

Fig 2. Acid-base titration curve of β -lysine at 25°C

The titration curve shown was obtained from the titration of 20 ml of 31 mM L- β -lysine with 1 M HCl and 1 M NaOH solutions. The titration began at 0 (zero) on the abscissa. To the right of 0 the titrant was NaOH, and to the left of 0 the titrant was HCl. The curve has been corrected by subtracting with a curve obtained from titrating 20 ml de-ionized H₂O blank with 1 M HCl and 1 M NaOH. The curve shows the changes of pH with increasing amount of 1 M HCl and 1 M NaOH added during the titration process. The first derivatives of the curve were used to determine the equivalence (end) points and half neutralization points of the titration.

Fig 3. Acid-base titration curve of β -glutamic acid

The titration curve shown was obtained from the titration of 20 ml of 20 mM L- β -glutamic acid with 1 M HCl and 1 M NaOH at 25°C. The titration began at 0 (zero) on the abscissa. To the right of 0 the titrant was NaOH, and to the left of 0 the titrant was HCl. The curve has been corrected by subtracting with a curve obtained from titrating 20 ml de-ionized H₂O blank with 1 M HCl and 1 M NaOH. The first derivatives of the curve were used to determine the equivalence (end) points and half neutralization points of the titration process.

Fig 4. The effects of pH on the equilibrium of lysine 2, 3-aminomutase

Equilibrium constants at various pH were measured by HPLC method. Open circles represent data when α -lysine was used as starting reactant; open squares represent data when β -lysine was used as the starting reactant. The data shows independence of LAM reaction equilibrium on pH within the experimental pH range.

Fig 5. The influence of temperature changes on the equilibrium of lysine 2, 3- aminomutase reaction Equilibrium constants at various temperatures were measured by HPLC method. The natural logarithm of the equilibrium constants at various temperatures were plotted against the reciprocals of the reaction temperatures (K) based on the the *van't Hoff* equation. The plot was derived from two sets of data using α -lysine and β -lysine as the starting material, respectively. The positive slope of the linear line indicates an exothermic reaction and more product-favored at lower temperatures.

Table 1The apparent pK_a values of β -lysine and β -glutamic acid at 25 °C

Amino acid	pK_1	pK_a pK_2	pK_3	pI
β -Lysine ^a	3.25	9.30	10.5	9.9
Lysine ^b	2.18	8.95	10.5	9.7
β -Glutamic acid, ^{ac}	3.13	3.73	10.1	3.5
Glutamic acid ^d	2.19	4.25	9.36	3.2
β -Alanine ^e	3.61	10.24	-	6.9
Alanine ^f	2.34	9.69	-	6.0
Aspartic acid ^f	2.09	3.86	9.82	3.0

^a Measured as described in Material and Methods. The average deviation from the mean in quadruplicate determinations of pK_a -values was ± 0.04 .

^b Measured by Greenstein et al. (22).

^c The half-equivalent pH of 3.47 in the ionization of the two carboxylic acid groups has been corrected for the statistical requirement that the dissociation constants differ by at least 4, that is the $\Delta pK_a = 0.6$ (26), as described in the text. Average deviation from the mean in duplicate determinations of pK_a -values was ± 0.02 .

^d Measured by Llopis and Ordonez (23).

^e Measured by Christensen, Oscarson and Izatt (24).

^f From Danishefsky (25).

Table 2

Effect of temperature on the equilibrium of lysine 2, 3-aminomutase reaction

Temperature (K)	Equilibrium Constant (K_{eq}) ^a	
	α -lysine as starting substrate	β -lysine as starting substrate
277	10.82	10.98
283	10.36	10.34
294	9.40	9.54
303	8.84	8.93
310	8.45	8.55
315	8.12	8.00
328	7.40	7.37
333	7.20	
338		6.84

^aEquilibrium constant is the ratio of [β -lysine]/[α -lysine] when reaction reaches equilibrium and is measured by HPLC method as described in Methods.