a corresponding outflow region in the Venus vortex at some lower layer, possibly close to the surface. An inflow region in the upper levels of the atmosphere can easily exist on Venus. The stratosphere (portions of the atmosphere above 38 km) is known to be very stable and stratified (at least in low latitudes). The bulk of the solar energy which maintains the circulation appears to be deposited in this layer (*6*). There is thus a possibility that an upper boundary layer exists on Venus as suggested by Goody and Robinson (*7*) but with the return circulation at the poles rather than at the antisolar point.

We have, at present, no evidence that large amounts of latent heat are released in the Venus atmosphere. Hence there is a major difference between the circulation on Venus and that of a tropical cyclone. The tropical cyclone is maintained dominantly by the release of latent heat in the atmosphere; the circumpolar vortex in the stratosphere of Venus is maintained through conversion of solar energy into sensible heat. The bright polar cloud at the center of the global vortex is a puzzle and invites further research.

The observations during the Mariner 10 flyby lasted only 8 days, and we must not draw too general conclusions from this very limited data set, particularly since ground-based observations show that the bright cloud appears and disappears occasionally in the polar regions. The cause of such long-term instabilities is still a mystery. Nevertheless, polar cloud or no polar cloud, the atmospheric circulation in polar and high latitudes is different from that at other latitudes and is important for a deeper understanding of the Venus atmosphere and its circulation.

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Isoleucine Epimerization in Peptides and Proteins: Kinetic Factors and Application to Fossil Proteins

Abstract. *The observed rate of isoleucine epimerization in peptides and proteins is dependent on (in addition to time and temperature) (i) the position of isoleucine in the peptide chain, (ii) the nature of adjacent amino acids, and (iii) the stability of the isoleucine peptide bonds. The relative rate is: NH_2-terminal > COOH-terminal ≫ interior ≥ free amino acid. The gradual hydrolysis of peptides and proteins to the more slowly epimerizing free amino acid causes a decrease in the apparent first-order rate constant with time. These results explain the isoleucine kinetics observed in fossil shells.*

Amino acid racemization-epimerization reactions are being used increasingly as a geochronological tool to date fossil bones, shells, and deep-sea foraminiferal deposits (*1*). Efforts to develop an accurate dating technique based on the kinetics of amino acid racemization-epimerization have been somewhat more successful with bone than with carbonate shell. In bone, diagenetic racemization reactions apparently follow reversible first-order kinetics approximately to equilibrium (*2*), while nonlinear kinetics prevail for the racemization-epimerization reactions in mollusk shells and foraminifera (*3–5*). The nonlinear kinetics in carbonates may be a result of the different racemization-epimerization rates experienced by amino acids in various fractions (free amino acids, small and large peptides, and proteins) formed by slow hydrolysis of the original skeletal protein over geologic time. If diagenetic racemization-epimerization reactions are ever to be useful in dating carbonate fossils, however, a better understanding of the factors responsible for the nonlinear kinetics is necessary. In our experiments we focused attention on the interconversion of L-isoleucine and D-alloisoleucine (alle), an epimerization reaction which has been studied in fossils (*1*).

Generally, isoleucine is epimerized to different degrees in the various amino acid fractions of fossil shells and foraminifera, with free isoleucine being more extensively epimerized than the protein- or peptide-bound isoleucine (*4–6*). These relations in specimens of modern *Mercenaria*, heated to simulate geologic aging, are illustrated in Fig. 1. The conclusion usually drawn from these observations is that, in fossils, isoleucine epimerizes faster in the free state than in the bound state. The greater degree of epimerization of free isoleucine in foraminifera and other carbonate fossils has been attributed to a concerted process of hydrolysis and epimerization (*4*) or to catalysis by metal ions (*5*). We now present evidence that the extensive epimerization of isoleucine does not occur in the free state. Instead, isoleucine preferentially epimerizes at the terminal position of a peptide or protein chain, with a markedly lower rate of epimerization occurring at both interior and free positions. Subsequent hydrolysis of the terminal, more extensively epimerized, isoleucine leads to an enrichment of alloisoleucine relative to isoleucine in the free fraction. We suggest that this mechanism offers a better explanation both for the observed epimerization relationships in fossils and for the nonlinear kinetics.

To investigate the kinetics of isoleucine epimerization we have conducted, at elevated temperatures, kinetic studies of various dipeptides, tripeptides, and proteins containing isoleucine. Samples (1 ml) of 0.1 percent aqueous solutions (pH = 8.0) were sealed under nitrogen in Pyrex tubes and heated at 152°C in a sand bath. At the end of the heating period, a portion of the samples was hydrolyzed (6*N* HCl at 105°C for 24 hours). During the heating some hydrolysis occurred. The ratios of alloisoleucine to isoleucine of both the free fraction and the total hydrolyzed (free plus bound) fraction were measured on an automated amino acid analyzer.

Initial epimerization rates of isoleucine are different for each dipeptide and tripeptide and are up to an order of magnitude greater than the epimerization rate of free isoleucine in aqueous solution; but they are about the same as ini-

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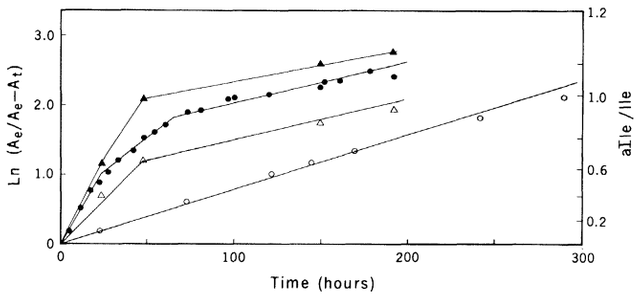
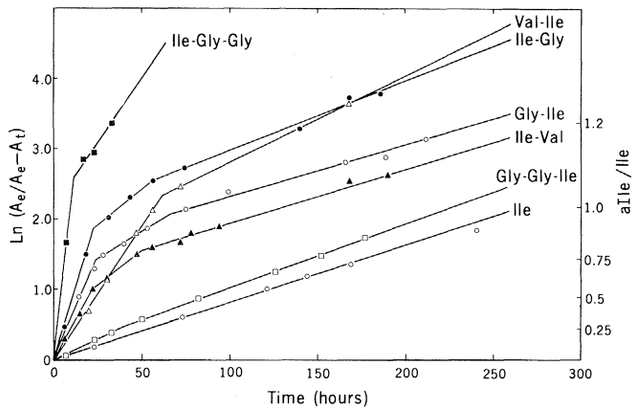


Fig. 1 (above). Kinetics of epimerization of isoleucine in different fractions of heated *Mercenaria* compared to free isoleucine in aqueous solution (*p*H 8.0) at 152°C. Closed triangles, free fraction in *Mercenaria*; closed circles, total fraction in *Mercenaria*; open triangles, bound fraction in *Mercenaria*; open circles, free isoleucine in aqueous solution. Fig. 2 (right). Kinetics of epimerization of isoleucine in different dipeptides and tripeptides at 152°C.



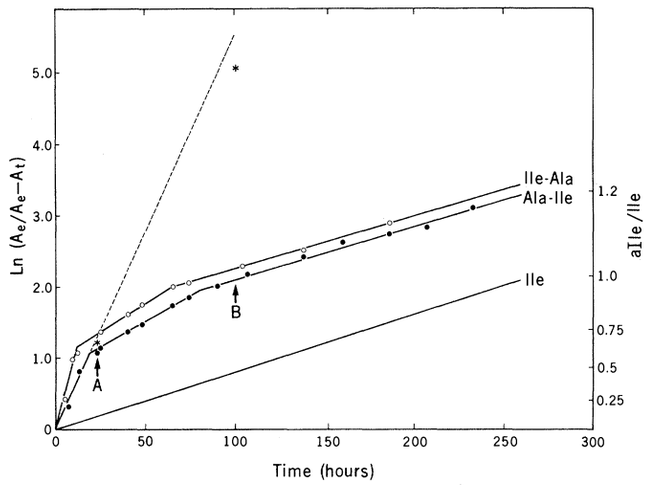
tial rate constants obtained for isoleucine in shells and foraminifera (Table 1) (Fig. 2). These results demonstrate that the presence of adjacent amino acid residues has a strong effect on the epimerization rate and that the rate for bound isoleucine is significantly enhanced as compared to that of the free amino acid rate. In addition, the rate is influenced by the nature of the adjacent amino acids and the position of isoleucine in the peptide chain. For each pair of dipeptides the rate of epimerization for NH_2-terminal isoleucine is slightly greater than that for COOH-terminal isoleucine. In addition, the ratio of alloisoleucine to isoleucine in the free fraction of heated dipeptides is always considerably less than that in the total (free + bound) fraction (Table 1). This is in contrast to the general observation that calcareous fossils contain a much higher ratio in the free fraction compared to that in the total or bound fractions. Thus, the enhanced epimerization rates in these experiments cannot be attributed to preferential epimerization of free isoleucine. Instead, in our experiments, it is the peptide fraction that is preferentially epimerizing.

Besides initial rate constants, there are additional similarities between the results of our experiments and the isoleucine kinetics observed in fossils. One is that the overall rate of epimerization does not completely follow the reversible first-order rate law that free isoleucine obeys, even though isoleucine is present in these experiments in the simplest bound form, that of dipeptides. Depending on the dipeptide, isoleucine follows reversible first-order kinetics to alloisoleucine to isoleucine ratios ranging between about 0.5 and 1.0 (Fig. 2). In various carbonate samples isoleucine epimerization is reported to follow comparable reversible first-order kinetics to alloisoleucine/isoleucine ratios ranging between about 0.3 and 0.9 (*3*–*5*). Another similarity is in the shape of the entire kinetic curve. Natural samples usually have two linear portions to the kinetic curve, separated by a transition zone, with the second linear portion having an order of magnitude lower rate constant than the first linear portion. The dipeptides also show a decrease of an order of magnitude in the apparent rate constant for the second linear portion; in all cases, this lower apparent rate constant is approximately equal to that for free isoleucine in aqueous solution. Indeed, as the results from a pair of dipeptides illustrate, the apparent rate constant for the second linear portion is governed entirely by the interconversion of free isoleucine which, at this stage of the reaction, comprises a significant fraction of the total isoleucine (Fig. 3). While the overall rate of epimerization based on total isoleucine (free + bound) follows reversible first-order kinetics only to an alloisoleucine/isoleucine ratio of about 0.6, the ratio in the bound fraction appears to follow first-order kinetics to equilibrium, which it reaches in a relatively short time compared to either the free or total fractions. With the bound fraction essentially at the equilibrium ratio, any subsequent changes in the alloisoleucine/isoleucine ratio of the total fraction result solely from epimerization of free isoleucine, and the rate constant for this portion of the reaction is approximately that of free isoleucine. Evidently, isoleucine not only epimerizes faster when it is in the bound state, but deviation from first-order kinetics and a decrease in the apparent rate constant occur when a significant fraction of the isoleucine is hydrolyzed to the free state. This interpre-

Table 1. Epimerization and hydrolysis constants of isoleucine in various peptides and proteins. Abbreviations: Ile, isoleucine; Gly, glycine; Ala, alanine; Val, valine, Tyr, tyrosine; Phe, phenylalanine.

Sample	Isoleucine		Alloiosleucine/ isoleucine	
	Initial $K_{epim} \times 10^{-2}$	$K_{hyd} \times 10^{-3}$	Total	Free*
Free isoleucine	0.45			
Ile-Gly	4.44	4.60	1.116	0.765 (74)
Gly-Ile	3.0			
Ile-Ala	5.30			
Ala-Ile	2.77	3.9	1.004	0.582 (102)
Ile-Val	2.51	0.98	0.890	0.681 (78)
Val-Ile	2.09			
Ile-Tyr	3.40	1.70	0.929	0.837 (73)
Tyr-Ile	0.38	2.51	0.570	0.381 (160)
Ile-Phe	3.57	1.19	1.051	1.051 (160)
Phe-Ile	0.36	5.42	0.503	0.446 (160)
Ile-Gly-Gly	14.5	2.51	1.151	0.669 (23)
Gly-Gly-Ile	0.57	68.8	0.158	0.097 (23)
Albumin	0.64	2.52	0.461	1.142 (72)
Eledoisin	1.38	1.49	1.00	0.95 (92)
Insulin	0.96	0.49	0.831	0.932 (99)
Melittin	3.01	4.27	0.821	0.754 (70)
Oxytocin	1.02	9.62	0.427	0.47 (48)
Mercenaria	2.33	14.3	0.459	0.635 (24)

*The time of heating in hours is shown in parentheses.



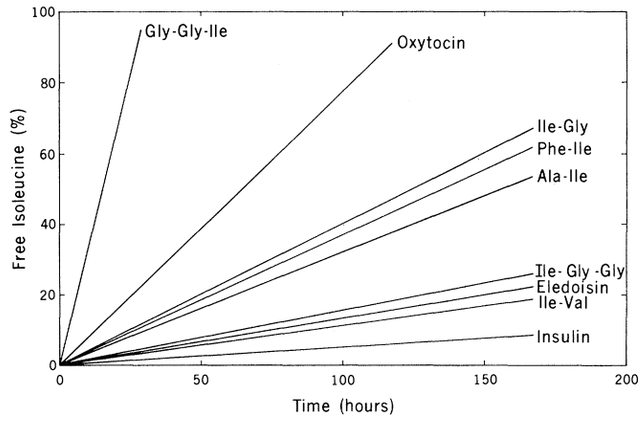


Fig. 3 (left). Kinetics of epimerization of isoleucine in the dipeptide pair Ile-Ala and Ala-Ile at 152°C. (A and B) Samples taken for analysis of the ratio of alloisoleucine to isoleunine in free and bound fractions. The ratios in the bound fraction are plotted on the dashed line. Fig. 4 (right). Rate of production of free isoleucine from different peptides and proteins during heating experiments at 152°C.

tation is further supported by the results obtained from a pair of tripeptides.

The epimerization rates of two dipeptide-tripeptide pairs with NH_2-terminal and COOH-terminal isoleucine are shown in Fig. 2. The curves indicate the effect of one additional amino acid on the rate of isoleucine epimerization. In one pair, the NH_2-terminal isoleucine tripeptide has a greater initial and overall rate than the NH_2-terminal isoleucine dipeptide. Figure 4 shows the relative production of free isoleucine from the different peptides and proteins. It is clear from the smaller amount of isoleucine released that the Ile-Gly bond in the tripeptide is more stable than that in the dipeptide. Apparently, the relatively rapid rate of hydrolysis of the Ile-Gly bond in the dipeptide results in a more rapid increase in free isoleucine; consequently there is a slower initial rate of epimerization, and deviation from first-order kinetics occurs at a lower alloisoleucine/isoleucine ratio. In contrast, the rate of epimerization of the COOH-terminal isoleucine in the dipeptide Gly-Ile is greater than that in the tripeptide Gly-Gly-Ile. In this case isoleucine is almost completely released from the tripeptide in a short time of heating. Thus, the observed slow rate of isoleucine epimerization for this tripeptide, about equal to the rate of epimerization of free isoleucine, is mainly a consequence of the rapid rate of hydrolysis of isoleucine to the free state. The lability of the glycine-isoleucine bond in the tripeptide Gly-Gly-Ile is not surprising since the middle Gly unit no longer possesses an amino terminal position which normally increases the stability of the adjacent peptide bond (*7*).

These observations indicate that the hydrolytic reaction also has an important role in the overall epimerization rate. The rate of release of free isoleucine is dependent on the strength of the peptide bond, and the lability of a peptide bond to hydrolysis, as for epimerization, is determined by the nature of adjacent amino acid residues (*7*). In the calcified protein of a mollusk shell, peptide bonds formed by alanine, serine, and glycine residues are very labile (*8*). Dipeptides containing COOH-terminal valine. leucine, or isoleucine are not as resistant to hydrolysis as those containing these amino acids as NH_2-terminal residues (*7*). In our study, the observation that the kinetic curves of NH_2-terminal isoleucine dipeptides tend to "break" at slightly higher alloisoleucine/isoleucine ratios than those of the COOH-terminal isoleucine is consistent with the relative strength of the respective peptide bonds (Figs. 2 and 3).

The rate of epimerization of isoleucine located in interior positions of a peptide or protein is more difficult to deduce because isoleucine may be partially hydrolyzed to the terminal position during heating. The best approximation to the kinetics of interior epimerization is obtained for proteins or peptides in which isoleucine forms stable peptide bonds with both adjacent amino acid residues. The kinetics of isoleucine epimerization obtained for a number of proteins and larger peptides suggest that the rate is much slower in interior positions compared to the rate in terminal positions (Table 1). The initial rate constants obtained for albumin (bovine), insulin (bovine), and oxytocin (containing three, one, and one residues of isoleucine, respectively) are smaller than those obtained for most of the dipeptides, with the rate for albumin approximately equal to that for free isoleucine. The greater K_{epim} (epimerization constant) for melittin and eledoisin indicates that in these compounds isoleucine may have been converted relatively rapidly to the terminal positions, which epimerize faster. The very low release rate of free isoleucine from the larger molecules, particularly insulin and albumin, and the low epimerization rate of isoleucine in these proteins suggest that interior isoleucine epimerizes at a much slower rate than terminal isoleucine. The slower rate of epimerization of interior isoleucine may be due to the lesser stability of the intermediate carbanion in the internal configuration or to the restriction in rearrangement of the intermediate carbanion structure as a consequence of intromolecular hydrogen bonding. The overall relative rate of isoleucine epimerization is, therefore,

$$NH_2\text{-terminal} > \text{COOH-terminal} \gg \text{interior} \geq \text{free}$$

Although di- and tripeptides represent relatively simple systems compared to calcified proteins, the similarities in their respective kinetics suggest that identical reactions are occurring. On the basis of the results of our heating experiments, we interpret the isoleucine epimerization relationships and kinetic curves in samples such as foraminifera and mollusk shells as follows. (i) Initially, most or all of the isoleucines are in interior positions. Through partial hydrolysis, and at a rate depending on the amino acid sequence, some of the interior isoleucine will be hydrolyzed to terminal positions where it will preferentially epimerize. (ii) Because only one peptide bond must be hydrolyzed, terminal isoleucine (alloisoleucine) will be more readily released to the free state than interior isoleucine. The free fraction derived by hydrolysis of the preferentially epimerized terminal isoleucine will have an alloisoleucine/

isoleucine ratio greater than that of the bound fraction, which consists largely of slower epimerizing interior isoleucine. (iii) After hydrolysis, free isoleucine epimerizes at the much slower, free amino acid rate. Consequently, as the protein hydrolyzes to free amino acids, the overall rate of epimerization will decrease, and the slope of the second linear segment of the kinetic curve, which is approximately parallel to the rate curve of free isoleucine, will be governed largely by the rate of epimerization of free isoleucine.

The alloisoleucine/isoleucine ratios of the free fractions of heated dipeptides and proteins provide additional evidence for this interpretation. With the exception of two polypeptides (eledoisin and melittin), the ratio in the free fraction of heated polypeptide and proteins (and fossil proteins) is greater than that of the bound fraction: for di- and tripeptides and two polypeptides, this relation is reversed (Table 1). In the case of proteins, only a small portion of the total bound isoleucine will be in the preferentially epimerizing terminal position; overall, therefore, the ratio in the total bound fraction (terminal and interior) will be relatively low. Because the free isoleucine of these samples is derived from preferential hydrolysis of the terminal, highly epimerized amino acid, the ratio in the free fraction will exceed that in the bound fraction. However, in the case of dipeptides, or any polypeptides having isoleucine rapidly hydrolyzed to the terminal position, the bound isoleucine occurs only in terminal positions and the ratio in the bound fraction will therefore be relatively high. The ratio in free amino acids derived from the dipeptides will be less than that in the bound fraction because the first additions to the free amino acid fraction will be from dipeptides with initially low ratios of alloisoleucine to isoleucine, and these additions will subsequently epimerize very slowly compared to the epimerization rate of the remaining terminally bound isoleucine.

In conclusion, epimerization-racemization of amino acids is not only time- and temperature-dependent, but, as our study demonstrates, the rate of epimerization of isoleucine is also dependent on the position of isoleucine in the peptide chain and on the strength of the isoleucine peptide bonds. Because the position of isoleucine changes from internal to terminal to free during protein hydrolysis, and because K_{epim} of isoleucine is different for each position, the rate of epimerization changes during the course of the reaction; the gradual buildup of slowly epimerizing free isoleucine results in a decrease in the apparent first-order rate constant. Furthermore, inasmuch as the amino acid sequence of calcified protein is unique for each species, the shape of the isoleucine epimerization curves for fossils will be species-dependent; variable rates of isoleucine epimerization have already been demonstrated for different species of foraminifera and mollusk shells (*9*). In order to use kinetics of amino acid epimerization-racemization for dating purposes, it is necessary, therefore, to establish independently the kinetics for each matrix or species of interest; kinetics obtained for one species may not be applicable to other species.

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Inland Ice Sheet Thinning Due to Holocene Warmth

Abstract. *The climatic warming of 10,000 years ago is now affecting the central portions of ice sheets, causing ice-flow acceleration. This process explains the present-day thinning of the ice sheet in West Antarctica. Former ice sheets must have also responded to climatic warming with a delay of thousands of years. This lag in response is important in the climatic interpretation of glacial deposits and of changes in ice volume obtained from deep-sea cores.*

The response of large ice sheets to climatic change is important to understanding the dynamics of the present-day ice sheets as well as to understanding the volumetric changes of ice during glaciations. A number of mechanisms have been proposed to account for ice sheet changes (*1*), but one of the simplest has escaped close attention in the literature.

The temperature of an ice sheet is generally somewhat less than freezing for most of the thickness. A climatic change in temperature at the surface is slowly propagated into the ice mass with the ice motion and by conduction. Because the creep of ice is temperature-sensitive, a climatic warming must cause an ice-flow acceleration some time later than the climatic change.

It is possible to calculate the lag time from climatic warming to ice acceleration and the amount of acceleration. I have done this for the present-day West Antarctic ice sheet, and the slow thinning of the central portion (*2*) can be explained in this way as a result of the Holocene warmth. The mechanism is also applicable to the former Laurentide and Scandinavian ice sheets, and the rapid expansions of those ice sheets, especially at the end of the glaciation, could have been responses to climatic warming.

The change in the heat content of a particle of ice in an ice sheet is described by

$$C_v \frac{d\theta}{dt} = \vec{\nabla} \cdot K\vec{\nabla}\theta + \text{heat} \qquad (1)$$

where θ represents temperature, t is time, K is the conductivity, C_v is the heat capacity, and $\vec{\nabla}$ is the gradient operator. Heat is released as a result of deformation of the ice as it flows. In the central portion of an ice sheet, heat conduction is almost entirely in the vertical direction, z, and horizontal advection and variations in conductivity and capacity are not very important. Applying these simplifications, Eq. 1 becomes

$$C_v \frac{\partial\theta}{\partial t} = K \frac{\partial^2\theta}{\partial z^2} - C_v \frac{\partial\theta}{\partial z} w + \text{heat}$$

The vertical velocity of a particle in the ice sheet, $|w|$, is about 0.2 m year^{-1} at the top surface of a typical ice sheet and decreases to near zero at the bed.

Let the steady-state temperature before a climatic change be described by θ_0 and the temperature after a climatic change by $\theta = \theta_0 + \theta_1$. Thus, θ_1 repre-

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