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Interlaboratory comparison of amino acid enantiomeric ratios in Pleistocene fossils

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ABSTRACT

It has been nearly three decades since the last systematic interlaboratory comparison of amino acid racemization (AAR) measurements among active laboratories. The advent of new methods and improved instrumentation for existing techniques requires that these comparisons be conducted more frequently than has occurred. The present study represents a first step in this process. Five homogeneous liquid samples were distributed to six participating laboratories that use one or more of the following analytical methods: Ion-exchange liquid chromatography (IEx), Reverse-phase liquid chromatography (RP), or Gas chromatography (GC). The five samples have been used in previous formal or informal interlaboratory comparisons: three are Pleistocene mollusk samples, two are Pleistocene eggshell samples. Use of homogeneous liquids eliminated variables involved in the majority of the sample preparative steps (sample cleaning, hydrolysis, desalting), so any observed variability between laboratories can be attributed to instrumental factors or possible small effects associated with the hydration procedures employed prior to instrumental analysis. Although most results indicate good agreement (within 10%) for all amino acid D/L values, there are some notable exceptions for certain amino acids or certain samples. For the five amino acids that are most commonly used in geochronological applications (Asx, Glx, Leu, Val, and A/I), inter-method comparisons reported here provide quantitative regressions that can be used when results from one method are compared with those from another.

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1. Introduction

Amino acid racemization (AAR) is a common tool in Quaternary stratigraphy and geochronology, with applications in a variety of marine, terrestrial, and coastal settings. In the early years (~1965–1975) of AAR, the dominant method of analysis was ion-exchange liquid chromatography (IEx), based on the classic methods reviewed by Hare (1969). Under proper conditions, IEx resolves D-alloisoleucine from L-isoleucine, the former being produced by the epimerization of the latter. As the number of AAR laboratories increased, both IEx and then gas chromatographic methods (GC) were used, often both methods being used in the same laboratories. GC methods permitted measurement of multiple D/L values, but usually required larger samples and longer, more complicated sample preparation schemes than IEx. Instrumental improvements, new GC columns (Frank et al., 1977), and refinements in sample preparation schemes (e.g., Goodfriend, 1991) contributed to the development of GC procedures during the 1980's and 1990's. In 1998, Kaufman and Manley (1998) introduced reverse phase liquid chromatography (RP) as a new tool for the analyses of D/L values in fossils. RP combines the simplicity of analysis and sensitivity inherent to IEx with the ability of GC to obtain D/L values for multiple amino acids. These characteristics have led to RP being the most commonly employed method among the currently active labs in the US, Europe, and Australia, although GC and IEx continue to be used.

During the period in which these methods have been developed and applied, several efforts at interlaboratory comparisons have been made. Some of these efforts have been rather informal, involving a few laboratories; others have been more formal, attempting to include all active laboratories and representing all of the then-available analytical schemes (e.g., Kvenvolden, 1980). Wehmiller (1984) published the last such interlaboratory comparison, including results obtained by eleven laboratories for three homogeneous powdered Pleistocene mollusk samples with varying extents of racemization. The powdered samples (identified as ILC-A, ILC-B, and ILC-C) prepared for that study continue to be used, although other "within-lab" reference materials are also in use. Most AAR publications since 1984 include results for some or all of the ILC samples.

The present study was initiated to assess the comparability of D/L results from the three common analytical methods (IEx, GC, and RP). Although many AAR studies rely heavily on the use of Asx and Glx D/L values (Kaufman et al., 2008), there are many examples where other

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amino acids such as leucine (Leu), valine (Val) and alloisoleucine/isoleucine (A/I) are more useful (Miller et al., 1997, 2005; Meijer and Cleveringa, 2009; Hearty and Kaufman, 2009; Murray-Wallace et al., 2010; Wehmiller et al., 2010; Penkman et al., 2011). Because A/I values are the only D-L ratio obtained by IEx, results for this amino acid are historically important in AAR literature. Any comparisons between GC or IEx A/I values and those from newer methods will require unambiguous conversion factors, factors that can only be obtained using reliable interlab- or inter-method comparison samples or rigorously defined standards. Some laboratories that are using both RP and IEx report similarities between D/L Val by RP and A/I by IEx, and because Val can be determined by GC and RP (at least by most RP labs), it appears that Val may provide a basis for comparison of results between the three methods (G.H. Miller, personal communication, 2010; Bakeman, 2006).

The current study is a first step in the effort to link results obtained by the newer RP methods with those obtained by the older IEx and GC methods. Although the results permit statistical analyses of differences (or similarities) between different laboratories' results, it is not possible to determine which results are "correct" because none of the samples used here has been standardized against known analytical reference material. The present study involves five samples that were distributed as homogeneous liquids (dried for shipment), thereby avoiding the effects of any differences among the actual sample preparation schemes of the participating laboratories. In principle, the results presented here represent only analytical differences between laboratories, although slight differences in the rehydration procedures for chromatographic analysis could introduce some variability as well. Comparisons of the total sample preparation schemes of the different labs are found in the proficiency test study by Powell et al. (2013). The study by Wehmiller (1984) included results for both homogenous liquid samples and "whole method" samples.

2. Procedures

The six laboratories that participated in this study are listed in Table 1. Other laboratories that have been active in the past were invited to participate, but schedules or instrumental issues prevented their involvement.

Three powdered Pleistocene mollusk samples (ILC-A, ILC-B, and ILC-C of Wehmiller, 1984) and two Pleistocene eggshell (*Genyornis*) samples (here referred to as ILC-G and ILC-R) were prepared in quantities sufficient to allow distribution to the six participating laboratories. ILC-A is a powder of late Pleistocene *Saxidomus* from Cape Blanco, Oregon, USA; ILC-B and ILC-C are powders of *Mercenaria* from late and early Pleistocene sites, respectively, in South Carolina, USA. Collectively the three ILC samples span a nearly full range of D/L values (~0.20–~0.95). The two *Genyornis* samples are from sites in Australia, one with intermediate D/L values (ILC-G) and one that is essentially racemic (ILC-R). The mollusk powder samples were prepared at the University of Delaware, the *Genyornis* samples at the University of Colorado. Sufficient quantities of all the samples were hydrolyzed using "normal" conditions (22 h, 110 °C at Colorado, 22 h, 105 °C at Delaware). If hydrolysis occurred in separate containers, the hydrolyzates were later combined and then separated into individual vials for drying prior to distribution. The dried residues of the hydrolyzates each represented approximately 0.1 g of original sample material, sufficient for multiple chromatograms (some laboratories reported results from more than 20 chromatograms). Because of concerns about the possible effects of storage at room temperature for lengthy periods during shipment, vials of the three ILC samples were stored at Delaware for nearly three weeks prior to analysis to test for this possible effect. No detectable differences between stored samples and those analyzed immediately after preparation were noted.

Table 1
Participating laboratories and methods employed.

Laboratory	Chromatographic method(s)[a]
University of Colorado (US)	RP and IEx
Northern Arizona University (US)	RP and IEx
University of Delaware (US)	GC
University of York (UK)	RP
University of Wollongong (AUS)	RP
University of Madrid (ESP)	RP

[a] Abbreviations: RP Reverse-phase liquid chromatography; IEx Ion-exchange liquid chromatography; GC Gas chromatography.

Participants were asked to report data as they would normally present results for publication; typical instrumental operating procedures can be found in representative publications from those laboratories. All data reports included mean D/L values and standard deviations, along with the number of chromatograms. Because GC procedures involved preparation of a derivative that can be stored for weeks or even months, the number of chromatograms obtained by GC represents multiple injections of the same derivative unless separate derivatives were made from the same starting solution (not the case for the present exercise). For RP and IEx, the rehydrated residue was, in some cases, split into separate aliquots from which one or more chromatograms were obtained. The results presented here simply represent the mean and standard deviation from all the chromatograms reported by each laboratory, regardless of how the samples were split prior to derivatization or injection. No instrumental blanks were involved in this study, as they were not considered necessary given the relatively high concentrations of amino acids in the analyzed samples.

3. Results

Results are presented in Table 2. Data for each sample were reported by each laboratory as means, standard deviations (SD), coefficients of variation (CV), and number of chromatograms. Laboratories are identified only with letters and the method of analysis (the University of Delaware laboratory is identified because it is the only one reporting GC values). In some cases the number of chromatograms is not identical for all amino acids because of baseline interference.

4. Discussion

With few exceptions, CV values are generally less than 4% for all amino acids except serine (which is not used for chronological purposes). CV values are usually lower for the most extensively racemized samples (ILC-R, ILC-C) and for those amino acids that are usually present in higher relative concentrations. In addition, because of the overall higher concentration of amino acids in the eggshell samples (and the overall "cleanliness" of these samples), chromatographic resolution is quite good and CV values for these two samples are generally lower than those for the mollusk samples that have smaller amino acid concentrations. Beyond these generalizations, CV values do vary from one laboratory to another, even when the same (RP) method is used, indicating instrumental (column) vulnerability to issues such as peak interference or peak distortion.

4.1. Comparison of all results from all laboratories

Fig. 1a–e present the data from Table 2 in the form of "spider diagrams" (see, for example, Wehmiller et al., 2010). These figures show the mean D/L values for each amino acid.

Table 3 summarizes statistics for the data presented in Fig. 1a–e. Table 3 focuses on those amino acids (Asx, Glx, A/I, Leu, and Val) that

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