RADIOCARBON, Vol 52, Nr 4, 2010, p 1657–1659

DEVELOPMENT OF RADIOCARBON DATING METHODS FOR MODERN BONE COLLAGENIZATION

K J Kim[1,2] • W Hong[1] • J H Park[1] • H J Woo[1] • G Hodgins[3] • A J T Jull[3]

ABSTRACT. The relationship between temperature and time required for collagenization using modern bone samples was investigated. Gelatinized samples of bone collagen were filtered to selectively collect different molecular weight fractions. The results of this study suggest that heating to 70 °C for a duration of 12 hr provides the optimal conditions for gelatinization.

INTRODUCTION

One of the primary objectives of this research was to develop an effective pretreatment method for the optimization of radiocarbon dating of degraded bone samples. Understanding the characteristics of bone samples associated with high degradation is important for both archaeological and anthropological studies. Studies often conflict due to the fact that many bone samples are difficult to date. The main reason is that the existing method for bone pretreatment is not designed for severely degraded bones. It is known that environmental factors that lead to the degradation of collagens—including pH, hydrology of the matrix, oxygenation, temperature, and changes brought by soil flora and fauna (Henderson 1987)—are especially enhanced in underground environments. For example, samples from caves are associated with a relatively slow process in protein hydrolysis to peptides, which then break down into amino acids. However, in a burial environment, spontaneous rearrangement of the inorganic crystalline matrix weakens the protein-mineral bone and leaves the bone susceptible to dissolution by the action of internal and external agents. The changes that would occur during diagenesis include random cross-linking, humification of parts of the molecule, attachment of exogenous humic materials, and hydrolysis with preferential loss of some amino acids (Hedges and van Klinken 1992).

In this study, we aim to determine the relationship between temperature and time required for collagenization using modern bone samples. The best gelatinization condition is then applied to accomplish trial experiments with degraded bone samples using Centriprep™ filters, which allow us to selectively collect molecular weight fractions.

METHODS

Study with Modern Cow Bone

We pretreated modern cow bone samples at the Korea Institute of Geoscience and Mineral Resources (KIGAM) by varying time settings and temperature conditions for collagenization. For this step, we adapted the procedures of 2 accelerator mass spectrometry (AMS) laboratories—Oxford University (Jacobi et al. 2006) and the University of California, Irvine (Beaumont et al. 2010). In order to characterize the property of collagenization of bone samples, the relationship between temperature and time of collagenization was investigated using 200 mg of modern cow bone sample.

Modern cow bone samples were cleaned and ground to powder for acid-base-acid treatment. A powdered bone sample was added to 40 mL 0.5M HCl solution in a test tube and placed in a water bath shaker at 20 °C for 30 min at 50 rpm, and subsequently washed to be neutralized. This first acid treatment removes materials such as carbonates and fulvic acids from the bone samples. In the sec-

[1]Korea Institute of Geoscience and Mineral Resources, 92 Gwahang-no, Yuseong-gu, Daejeon 305-350, Korea.
[2]Corresponding author. Email: kjkim@kigam.re.kr.
[3]NSF Arizona AMS Laboratory, University of Arizona, 1118 E. Fourth St., Tucson, Arizona 85721, USA.

ond step, 40 mL of 0.1M NaOH solution was added to the test tube and placed at 20 °C for 60 min in the shaker and then the sample was washed to neutral pH. This step removes unwanted humic acid. In the third step, a second acid treatment, 40 mL of 0.5M HCl, was added to the sample in a water bath for 30 min; the sample was then washed to a pH of 3.

A number of other AMS laboratories rely on overnight sample preparation for each step with lower temperature settings of 70 °C for usually well over 12 hr. In our study, we gelatinized modern bone samples at 65, 70, and 75 °C for durations of 9, 12, or 15 hr to complete the collagenization process of the degraded bone samples.

Using a 1.2-µm glass filter, the remaining bone collagen was removed from the solution of each sample. As a final step, the filtered solution for each sample was separated using Centriprep ultrafilters with molecular weight fractions of 3, 10, 30, and 50 kD. Each collected collagen sample was then freeze-dried and weighed. Examples of collagen extracted from the cow bone are shown in Figure 1.

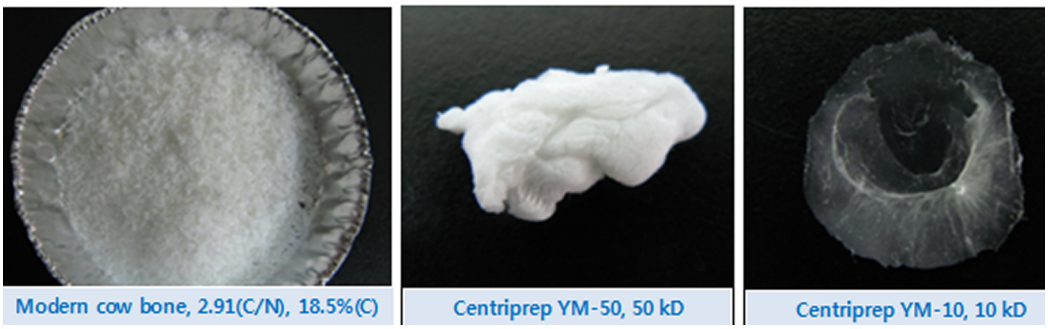

Figure 1 Photos from this study of a powdered modern cow bone sample (left) and collagen extracted from the modern cow bone sample (center and right).

RESULTS AND DISCUSSION

Time and Temperature for Collagenization Using a Modern Bone Sample

The results of our study show that at 70 °C, the filtered amounts were much lower than those at 65 °C and after only 9 hr. This suggests that the collagenization process is much more completely accomplished this way than at 65 °C (Figure 2). The 8 collagen samples were separated with 4 Centriprep filters of different molecular sizes of 3, 10, 30, and 50 kD. Most samples had a significant, large amount of collagen remaining in the filter of 50 kD except the sample that was pretreated at 65 °C for a duration of 15 hr. In the case of the sample treated at 65 °C for a duration of 15 hr for gelatinization, the largest collagen remained in the filter of a 10-kD molecular cutoff. For most samples, the total recovered collagen fractions for all but those smaller than 10 kD were about 8.6–12.4%, for initial samples of 200 mg bone. The C/N ratios of the cleaned raw modern cow bone and collagen of the modern cow bone were found to be 2.91 and 3.72, respectively. Also, the carbon content of a modern bone was found to be 18.5% (Kim et al. 2010).

In the case of the sample treated at 75 °C, residue amounts after 9 hr and 12 hr of heating were significantly lower than the samples treated at 65 and 70 °C. It has been suggested that treating bones at higher temperatures for more than 12 hr of heating for gelatinization may break down the collagen into smaller molecules (Brown et al. 1988; Beaumont et al. 2010).

https://doi.org/10.1017/S0033822200056381 Published online by Cambridge University Press

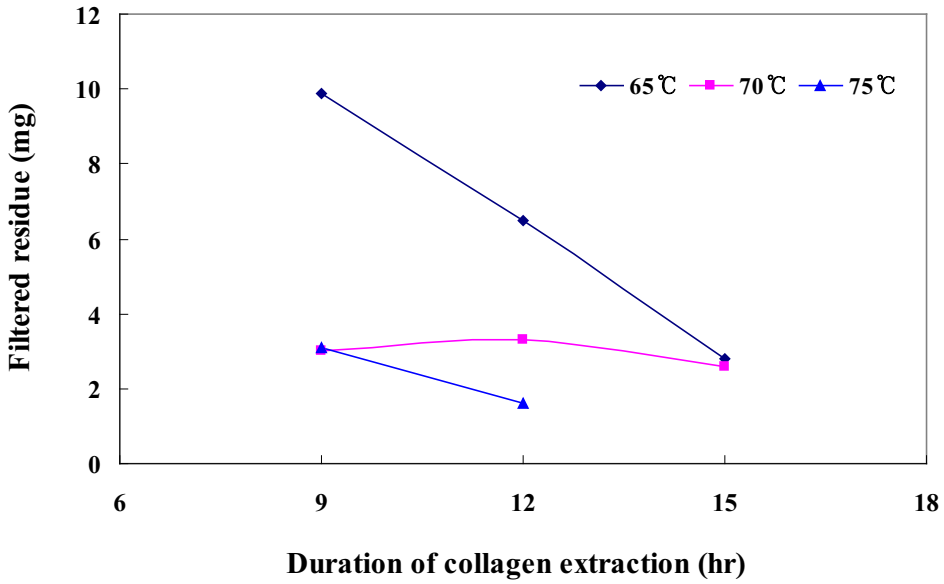

Figure 2 The amount of residue filtered by a 1.2-µm glass filter is listed with respect to the time duration for gelatinization. At 70 °C, the residue amount filtered was not changed as time increased. However, this should be reconfirmed.

CONCLUSIONS

As a result of these investigations, we determined that heating at 70 °C for a duration of 12 hr provides the optimal condition for gelatinization and for further experimentation to extract collagen from various degraded-bone and extracted-collagen samples. We report further on the studies as applied to archaeological samples in a separate paper in this issue (Kim et al. 2010).

ACKNOWLEDGMENTS

We would like to thank the staff at the KIGAM and Arizona AMS laboratories for their assistance, and John Southon (University of California, Irvine) for discussions. This work has been supported by the planning project (08-530270) of the Korea Institute of Geoscience and Mineral Resources. The work conducted at Arizona was supported in part by NSF grant EAR06-22305.

REFERENCES

Beaumont W, Beverly R, Southon J, Taylor RE. 2010. Bone preparation at the KCCAMS laboratory. *Nuclear Instruments and Methods in Physics Research B* 268(7–8):906–9.

Brown TA, Nelson DE, Vogel JS, Southon JR. 1988. Improved collagen extraction by modified Longin method. *Radiocarbon* 30(2):171–7.

Hedges REM, van Klinken GJ. 1992. A review of current approaches in the pretreatment of bone for radiocarbon dating by AMS. *Radiocarbon* 34(2):279–91.

Henderson J. 1987. Factors determining the state of preservation of human remains. In: Boddington A, Garland AN, Janaway RC, editors. *Death, Decay, and Reconstruction*. Manchester: Manchester University Press. p 43–54.

Jacobi RM, Higham TFG, Bronk Ramsey C. 2006. AMS radiocarbon dating of Middle and Upper Palaeolithic bone in the British Isles: improved reliability using ultrafiltration. *Journal of Quaternary Science* 21(5): 557–73.

Kim KJ, Jull AJT, Kim JY, Lee YJ, Woo HJ. 2010. Radiocarbon dating of the Mansuri Paleolithic site, Cheongwon, Korea. *Radiocarbon* 52(4):1545–51.