Int J Leg Med (1994) 107:152–155

ORIGINAL ARTICLE

Cadja Lassen · Susanne Hummel · Bernd Herrmann

Comparison of DNA extraction and amplification from ancient human bone and mummified soft tissue

Received: 17 February 1994 / Received in revised form: 9 June 1994

Abstract South american precolumbian male mummies were employed as source material for a comparative investigation of bone and soft tissues by DNA analysis. The suitability of the DNA extracts from both sources was tested and evaluated by their effectiveness as target DNA in PCR amplifications. The results suggest that skeletal material should be given preference over soft tissues for PCR analysis if the material is severely degraded. This seems to be independent of the specific anatomical origin of the samples.

Key words Ancient DNA · Bone · Soft tissue
Automated DNA extraction · PCR · Sex determination

Zusammenfassung Für eine vergleichende Untersuchung von Knochen und Weichgewebe als Quellenmaterial für DNA-Analysen wurden südamerikanische, präkolumbische, männliche Mumien verwendet. Die Eignung der DNA-Extrakte beider Quellen wurde über ihre Effektivität als Ziel-DNA in PCR-Amplifikationen bewertet. Die Ergebnisse zeigen, daß bei Verwendung stark degradierten Materials Knochen den Weichgeweben für PCR-Analysen vorzuziehen sind. Dies scheint unabhängig von der spezifischen anatomischen Herkunft der Proben zu sein.

Schlüsselwörter alte DNA · Knochen · Weichgewebe
automatisierte DNA-Extraktion · PCR
Geschlechtsbestimmung

Introduction

A wide variety of ancient DNA (aDNA) extracted from degraded biological material is available for molecular biological analysis (Herrmann and Hummel 1993; Hummel et al. 1993). Therefore a comparison of different tissues with regards to their suitability for DNA extraction and amplification becomes a focus of interest. First approaches to comparative studies of different source materials have been reported by Thuesen and Engberg (1990), Nielsen et al. (1993) and Cooper (1993) but an evaluation of the tests is lacking. Here, we report on the extraction of aDNA from bones and soft tissues of 2 south american mummified precolumbian male individuals and compare their suitability for PCR analysis. PCR as a crucial experiment was chosen since the general suitability of a DNA extract as a target in an enzymatic amplification procedure is most informative for aDNA work. In our experience the DNA yield with portions of human and microbacterial origin (Hummel 1992; Jeffreys et al. 1992; Herrmann and Hummel 1993) seems to have no immediate influence on amplification success. Contemporary modern human bones and soft tissues from male and female individuals were included as positive and negative control samples. Bones and soft tissues from mummified individuals require different preparation and purification since aDNA extracts from soft tissues contain more inhibiting substances due to degradation during the mummification process (Cooper 1992). Inhibition can result from either reducing sugars (Pääbo 1990) or an excess of co-extracted microbacterial DNA (Jeffreys et al. 1992). Reducing sugars are indicated by a blue fluorescence in ethidium bromide stained gels. Their inhibitory quality was empirically investigated by an additional purification and PCR amplification.

Materials and methods

Preparation of bone samples

A total of 45 bone samples were collected from different skeletal elements (femur, tibia, humerus, ulna, cranium, vertebra, rib, pelvis) of both individuals. The outer surfaces of the bones were exposed to UV light (254 nm) for 5 min to inactivate possible contaminating modern DNA (Cone and Fairfax 1993). After powdering the samples in a mixer mill (Retsch Typ MM2) 0.5 g was

C. Lassen (✉) · S. Hummel · B. Herrmann
Institut für Anthropologie, Georg-August-Universität Göttingen, Bürgerstrasse 50, D-37073 Göttingen, Germany

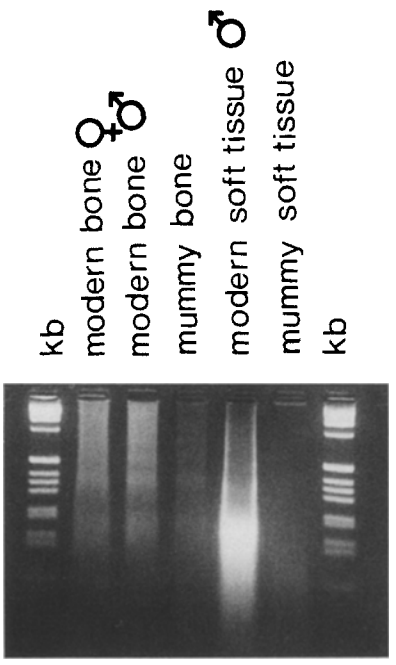

Fig. 1 The DNA extracts (10 µl) were run on a 3% agarose gel and stained with ethidium bromide. The 1KB DNA-Ladder (Gibco BRL) was utilized as molecular weight standard. High molecular weight DNA could be extracted from all modern control samples and on a smaller scale from bone of the mummified individuals. In contrast aDNA from mummified soft tissues only exhibits low molecular weight DNA fragments. Additionally, in these samples, the orange fluorescence of the DNA under UV light (254 nm) was overlapped by blue fluorescence which indicates inhibiting impurities. The DNA extracts from the ancient samples will also contain portions of microorganism origin

mixed with 1 ml of 0.5 *M* EDTA (pH 8.1), shaken constantly for 45 hours at room temperature and centrifuged for 5 min in a Eppendorf bench top centrifuge 5415 C at 3000 rpm (≙ 750 × *g*). DNA extraction was carried out with the supernatants in an automated nucleic acids extractor (Gene Pure Typ 341 A, Applied Biosystems) starting with a proteinase K (0.93 mg, 39 U/mg) digestion at 56° C for 1 hour. A standard phenol and chloroform extraction was carried out, followed by mixing the samples with a silica powder (glass milk, Dianova). This produces a binding of DNA to the glass beads (Poinar 1994) in the presence of isopropanol and sodium acetate (2.0 *M*, pH 4.5). In the last phase of the automated extraction procedure the DNA/glass milk samples were collected on filtration membranes (Applied Biosystems) and washed with ethanol. Finally the DNA was manually eluted from the silica beads with sterile water (Ampuwa) and run on a 3% agarose gel (Serva) (Fig. 1) (for preparation of bone samples cf. Hummel et al. 1993).

Preparation of soft tissues

Mummified soft tissue samples ($n = 17$) of no further determinable histological quality were collected from various anatomical regions (hip, upper and lower extremities) of both individuals. Samples of 0.5 g tissue powder were mixed with 2 ml buffer consisting of 10 m*M* Tris-HCL, pH 8.0, 2 m*M* EDTA, 20 mg DTT, 0.1% SDS and 1.0 mg proteinase K (39 U/mg). After 27 hours constant shaking at 37° C the soft tissue solutions were heated to 94° C for 10 min to inactivate proteinase K. Thermic denaturation ensures reproducible uniformity within the samples in preparation independent of their number. After centrifugation (5 min, 3000 rpm ≙ 750 × *g*) the supernatants were transferred to the automated nucleic acids extractor. Following phenol and chloroform extraction and concentration by glass milk, the extracts (Fig. 1) from soft tissues still revealed inhibiting contaminations. Therefore a further purification step applying an additional filtration system (Magic PCR-Preps Purification System, Promega) (Meizei 1991) was performed on the extracts prior to PCR.

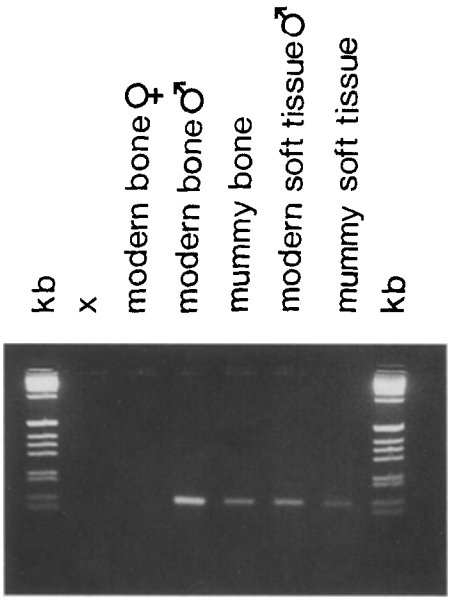

Fig. 2 Amplification products are seen in all male modern controls while no products are detectable in the no template control (x) and the modern female bone sample. Y-chromosomal products can also be identified in both types of mummy samples. Typically the products of the bone samples are more distinct, specific and effective. The 1KB DNA-Ladder (Gibco BRL) was used as molecular weight standard

PCR

The amplification product is a 154 bp sequence on a human Y-specific 3.4 Kb repeat sequence (Kogan et al. 1987). By amplification of this sequence a molecular biological sex determination is carried out (Muellener et al. 1989; Hummel 1992; Hummel et al. 1992; Hummel and Herrmann 1993). Two-step PCR (Cha and Thilly 1993) consisting of a denaturation and a combined anneal/extension temperature was carried out in 50 µl reaction mixes containing 10 m*M* Tris-HCL (pH 8.0), 50 m*M* KCL, 1.5 m*M* $MgCl_2$, 220 µ*M* each dNTP (Boehringer), 2.8 p*M* each primer, 35 µl sterile water (Ampuwa) and 2% of the aDNA extracts. Before adding the aDNA and the Taq DNA polymerase the mixture was exposed to UV light (254 nm) for 5 min. The PCR reaction started with 5 min denaturation at 94° C. During this time 2.5 U of Taq DNA polymerase (Promega) was added. Cycling parameters consisted of 40 cycles with 94° C for 1 min and 60° C for 30 sec in a Thermal Cycler (Perkin Elmer Cetus). The samples were run on a 3% agarose gel and stained with ethidium bromide (Fig. 2).

Results

After the application of appropriate extraction and purification procedures, the bone samples of the investigated mummified individuals regularly contained more DNA than the soft tissue samples. This was qualitatively de-

Table 1 Bone and soft tissue samples as source material for PCR amplification

		Mummy I	Mummy II	Total
Bone	n[a]	24	21	45
	PCR[b]	20	12	32
	in %	83.3	57.1	71.1
Soft tissue	n[a]	15	2	17
	PCR[b]	7	1	8
	in %	46.6	50.0	47.1

[a] Number of samples
[b] Number of samples with successful amplification

ducible from the gel electrophoresis results (Fig. 1) and quantitatively by determination of the DNA yield of single samples by UV spectrophotometry (Jouan S 750). Thereby DNA yields of about 70 ng/µl could be measured for the bone samples and less than 2 ng/µl for the soft tissue samples. It has to be taken into account that portions of this yields will be of microorganism origin. In addition the extracts from the bone samples revealed comparatively more high molecular weight DNA. Moreover they contained less inhibiting impurities and thus required less purification before performing PCR reactions. No differences concerning DNA quality could be detected within the different types and origins of bone samples. A total of 32 out of 45 ancient bone samples (= 71%) and 8 out of 17 ancient soft tissue samples (= 47%) could be amplified successfully (Table 1), whilst all positive and negative controls of contemporary bone and soft tissue samples confirmed the correctness of the results (Fig. 2).

Discussion

Current literature reports only either soft tissues or hard tissues as a source material for mainly population genetic studies in museum and archaeological contexts (e.g. Pääbo 1991; Ellegren 1993; Hagelberg 1993; Kurosaki et al. 1993) and historic or forensic identification case studies (Hagelberg et al. 1991; Hochmeister et al. 1991; Jeffreys et al. 1992; Gill et al. 1994). In contrast this study experimentally investigated the direct comparison of aDNA extracts derived from soft and hard tissues from the same individual with regard to their suitability for PCR analysis.

The results show that both bone and soft tissue samples are suitable for aDNA analysis, but skeletal remains should be given preference when source materials are severely degraded. This was concluded by the fact that amplifications with DNA extracts from bone samples were more successful than those from soft tissue samples. This empirical way of evaluating the samples is supported by data such as the DNA yields, and the qualitative estimations of DNA degradation and impurity content, especially as the soft tissue samples needed more purification handling in order to reveal the achieved results.

Automated DNA extraction of degraded samples with a GenePure 341 A is recommended, since it enables a reduction in the risk of contamination with contemporary DNA by laboratory handling to a minimum. In addition automated extraction permits exact reproducibility of extraction parameters (Hummel 1992; Hummel and Herrmann 1993).

This work was supported by the Bundesministerium für Forschung und Technologie.

References

Cha RS, Thilly WG (1993) Specificity, efficiency, and fidelity of PCR. PCR Methods Appl 3:18–29

Cone RW, Fairfax MR (1993) Protocol for ultraviolet irradiation of surfaces to reduce PCR contamination. PCR Methods Appl 3:15–17

Cooper A (1992) Removal of colourings, inhibitors of PCR, and the carrier effect of PCR contamination from ancient DNA samples. Ancient DNA Newsletter 1:31–32

Cooper A (1993) DNA from museum specimens. In: Herrmann B, Hummel S (eds) Ancient DNA. Recovery and analysis of genetic material from paleontological, archaeological, museum, medical, and forensic specimens. Springer, New York Berlin Heidelberg, pp 149–166

Ellegren H (1993) Genomic DNA from museum bird feathers. In: Herrmann B, Hummel S (eds) Ancient DNA. Recovery and analysis of genetic material from paleontological, archaeological, museum, medical, and forensic specimens. Springer, New York Berlin Heidelberg, pp 211–217

Gill P, Ivanov PL, Kimpton C, Piercy R, Benson N, Tully G, Evett I, Hagelberg E, Sullivan K (1994) Identification of the remains of Romanov family by DNA analysis. Nature Genet 6:130–135

Hagelberg E, Gray IC, Jeffreys AJ (1991) Identification of the skeletal remains of a murder victim by DNA analysis. Nature 352:427–429

Hagelberg E (1993) Mitochondrial DNA from ancient bones. In: Herrmann B, Hummel S (eds) Ancient DNA. Recovery and analysis of genetic material from paleontological, archaeological, museum, medical, and forensic specimens. Springer, New York Berlin Heidelberg, pp 195–204

Herrmann B, Hummel S (eds) (1993) Ancient DNA, Recovery and analysis of genetic material from paleontological, archaeological, museum, medical, and forensic specimens. Springer, New York Berlin Heidelberg

Hochmeister MN, Budowle B, Borer UV, Eggmann UT, Comey CT, Dirnhofer R (1991) Typing of DNA extracted from compact bone from human remains. J Forensic Sci 36:1649–1661

Hummel S (1992) Nachweis spezifisch Y-chromosomaler DNA-Sequenzen aus menschlichem bodengelagerten Skelettmaterial unter Anwendung der Polymerase Chain Reaction. Dissertation, Universität Göttingen

Hummel S, Nordsiek G, Herrmann B (1992) Improved efficiency in amplification of ancient DNA and its sequence analysis. Naturwissenschaften 79:359–360

Hummel S, Herrmann B (1993) Y-Chromosomal DNA from ancient bones. In: Herrmann B, Hummel S (eds) Ancient DNA. Recovery and analysis of genetic material from paleontological, archaeological, museum, medical, and forensic specimens. Springer, New York Berlin Heidelberg, pp 205–210

Hummel S, Herrmann B, Institut für den Wissenschaftlichen Film (1993) Molekularbiologische Analyse alter DNA (Film-Verleihnr. C1854). Göttingen 1993

Jeffreys AJ, Maxine JA, Hagelberg E, Sonnberg A (1992) Identification of the skeletal remains of Josef Mengele by DNA analysis. Forensic Sci Int 56:65–76

Kogan SC, Doherty M, Gitschier J (1987) An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences. N Engl J Med 317:985–990

Kurosaki K, Matsushita T, Ueda S (1993) Individual DNA identification from ancient human remains. Am J Hum Genet 53: 638–643

Meizei LM (1991) Direct purification of PCR product from amplification reaction. Promega Notes 34: 1–4

Muellener D, Rudin O, Pauli U, Zink P (1989) Geschlechtsbestimmung an menschlichen Mikroblutspuren und Einzelhaaren mit Hilfe gezielter DNA Amplifikation durch die Polymerase-Chain-Reaction. Arch Kriminol 184: 5–6

Nielsen H, Endberg J, Thuesen I (1993) DNA from arctic human burials. In: Herrmann B, Hummel S (eds) Ancient DNA. Recovery and analysis of genetic material from paleontological, archaeological, museum, medical, and forensic specimens. Springer, New York Berlin Heidelberg, pp 122–140

Pääbo S (1990) Amplifying ancient DNA. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols. Academic Press, San Diego, pp 159–166

Pääbo S (1991) Amplifying DNA from archaeological remains. A meeting report. PCR Methods Appl 1: 107–110

Poinar H (1994) Glass milk, a method for extracting DNA from fossil material. Ancient DNA Newsletter 2: 12–13

Thuesen I, Engberg J (1990) Recovery and analysis of human genetic material from mummified tissue and bone. J Archaelog Sci 17: 679–689