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X-RAYING ANCIENT BONE
A destructive method in connection with DNA analysis

The damaging effects of ionizing radiation on DNA has been known for a long time, but in archaeology, methods using X-rays are often considered to be non-destructive. We have tried to visualize the danger in exposing organic archaeological material to X-rays by extracting and amplifying DNA from radiated pig bone samples. The more the sample had been exposed to X-rays, the harder it was to amplify DNA from it. If DNA analysis is to be used on archaeological material, samples should be removed before X-raying.

Introduction

During the last few decades we have seen a tremendous increase in the use of X-rays in different analyses of archaeological material, producing an extensive range of methods in which X-rays are used in various ways. Most frequently however, X-rays are used for the radiography of bones: age of death can be determined by studying X-ray photographs of limb shafts, epiphyses and tooth-roots; diseases and sometimes causes of death can also be diagnosed off X-ray photographs; even mummies and small graves can be examined off X-ray photographs prior to excavation. These methods are considered to be non-destructive ways of studying archaeological material, a statement which may be true for morphological data but certainly not for molecular data.

In 1927, H. J. Muller (1927) published an article where he argued that X-rays affect the genome. He had noticed a high frequency of mutagenes among fruit flies after radiation, and in 1944, Avery et al. (1944) published their experiment where they used pneumococces to prove that the genome consists of DNA. Thus the damaging effects of radiation on DNA have been known for a long time. Even though it is well known that ionizing radiation affects the DNA molecule, archaeological material which might yield samples for DNA analysis is often exposed to X-rays.

Damage

X-ray is an ionizing radiation. It can be compared with ordinary light, but it possesses more energy. If the molecule is hit directly by an X-ray the result will probably be a rupture of the DNA chain, but the molecule will also suffer from indirect radiation-induced damage. If a H_2O molecule is ionized, one of the end products will be a OH radical which is highly reactive. This OH radical will attack the bases of the DNA chain and modify them (Téoule 1987). In a living cell this is a minor problem since the DNA helix is not that vulnerable if it is intact and there are anyway efficient repair systems. Ancient DNA does not benefit from the protection that modern DNA enjoys, and there are no enzymes engaged in repairing a post-mortem DNA molecule.

Material and methods

We performed an experiment to test our apprehensions. We used pig bone, divided into three parts for the analysis, whereby the first sample (1) was not radiated at all, the second (2) was radiated at 65 kV for 10 mAs, and the third (3) was radiated at 150 kV for 1000 mAs. The radiation distance was 1 m and the equipment used for radiation was an Andrex 155 BW with a beryllium filter. After radiation the samples were powdered with a coarse file and 2 g was used for DNA extractions. Extraction followed Boom et al. (1990) with a few modifications, where 5 ml L6 buffer was added to 2 g bone powder, the tube was vortexed and incubated at 60°C for 3 h with sporadical agitation. After incubation the supernatant was separated from the bone powder by centrifugation and transferred to a tube which contained 1 ml L2 buffer and 20 μl silica suspension. The tube was slowly agitated for 15 min, centrifuged at 3000 rpm for 2 min and the

supernatant was discarded. The silica pellet was resuspended in 1 ml L2 buffer and transferred to an eppendorf tube. The tube was centrifuged at 3000 rpm for 2 min and the supernatant was discarded. 1 ml 70% ethanol was added to the tube, the pellet was resuspended, the tube was centrifuged at 3000 rpm for 2 min and the supernatant was discarded. This procedure was repeated but the final pellet was dried in a vacuum drier. DNA was extracted from the silica by adding 65 μl H_2O to the tube and incubating it at 56°C for 15 min. The tube was centrifuged at 3000 rpm for 2 min and 50 μl of the supernatant was transferred to a new eppendorf tube. 65 μl H_2O was added to the tube with silica, the tube was vortexed to resuspend the pellet and it was incubated at 56°C for 5 min. After the final incubation the tube was centrifuged at 12,000 rpm for 3 min and 65 μl of the supernatant was transferred to the tube which already contained 50 μl. The tube was centrifuged at 12,000 rpm and 100 μl was transferred to a new tube, this final centrifugation was carried out to expel of the final traces of silica. The final volume was concentrated to 70 μl in a vacuum drier.

30 μl of each sample was run on a 2% agaros gel at 45 V for 30 min to visualize the amount of the DNA. The result can be seen in fig. 1. Spectrophotometrical values confirm the result; sample 1 contains 70 μg/ml, sample 2 contains 13.9 μg/ml and sample 3 contains 5.9 μg/ml.

We also carried out PCR reactions on mtDNA 12S and 16S to see if the extracted DNA was of a quality good enough to give PCR products. We used the primers L1091, H1479, 16H1 and 16L1. For each reaction, 5 μl of extracted DNA and 45 μl of a master mix for a total volume of 50 μl were used. Two master mixes were prepared from a Perkin Elmer Cetus Gene Amp PCR reagent Kit, one for each set of primers. For each master mix, 139.5 μl distilled H_2O, 22.5 μl 10×buffer, 13.5 μl 10 mM dNTP, 4.5 μl of each 10 μM primer, 18 μl 25 mM $MgCl_2$, 1.1 μl 5 u/μl Taq DNA polymerase were used. The following cycling profile was used: 97°C for 2 min, 1 cycle; 94°C for 30 s, 45°C for 30 s, 72°C for 1 min, 30 cycles; 72°C for 8 min, 1 cycle. 10 μl of each PCR reaction were run on a 1.5% agarose gel at 45 V for 30 min. The result can be seen in fig. 2.

Results

The bones we used were modern and fresh. The first time we carried out the experiment they were only a few days old, and the second time they were about 10 days old. We always kept the bones in the freezer prior to analysis, so the quality of the DNA is better by far than the quality of ancient DNA. This is necessary since we are using a rough method to measure the amount of the DNA. We are also aware of the problem that our fresh bones contain more H_2O than ancient bones usually do, which results in a greater number of OH radicals that could cause damage, but we still think that our results are relevant. Sample 1 indicates how much DNA the sample contained before it was radiated, samples 2 and 3 indicate how degraded the DNA gets from X-rays (fig. 1). Sample 2 received what could be described as a normal dose of radiation for an archaeological material and sample 3 received a very large dose. It is also clear that sample 2 has been degraded by the X-ray treatment while sample 3 has been so affected that no DNA can be made visible to the eye on a minigel, and 30 rounds of PCR cannot amplify mtDNA (fig. 2).

Conclusion

We conclude that archaeological samples that might be used for DNA extractions must be kept away from X-ray analysis. Also, if possible, the samples should be kept frozen prior to DNA analysis. However, more experiments are needed to get a full evaluation of ancient DNA and radiation. It is however clear that X-ray is destructive and precaution should be taken regarding this "non-destructive" method.

Acknowledgements

We thank Lars Hagman, Inspector, Technical Department, SAS, Stockholm, for excellent help with the radiation of the samples. We also thank the Laboratory of Molecular Systematics, Museum of Natural History, Stockholm, for letting us use their equipment and chemicals.

Fig. 1. Extracted DNA from modern pig bone. The first sample is unradiated, the second sample is moderatly radiated, the third sample is heavily radiated.

Fig. 2. PCR reactions on the radiated samples. Both 12S and 16S have been amplified from the unradiated sample; the band indicating the products from 12S are somewhat fainter on the moderatly radiated sample than on the unradiated sample but 16S still gives a strong signal; 12S could not be amplified at all from the heavily radiated sample and the band indicating 16S is not as strong as the other bands.

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