Borate, an Effective Mummification Agent in Pharaonic Egypt

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Mummification salts and mummified bones were assayed for borate. In two samples of mummification salt one from Deir el-Bahari (26th Dynasty, 700–600 BC) and salt from the embalming material of Tutankhamen (18th Dynasty, 1370–1335 BC) 3.9 and 2.1 μMol borate/gram were found, respectively. Six mummified bone fragments from the Old Kingdom contained up to 1.2 μMol/gram. It is suggested that borate containing salt was used during mummification. The reactivity of borate on bone alkaline phosphatase, which is known to survive mummification for more than 4000 years, was examined. Borate forms high relative molecular mass adducts of this enzyme being temperature resistant and functionally distinct.

Introduction

In Pharaonic Egypt the deceased were dehydrated using mummification salt and then pre-treated with many different resins, wood tar or even bitumen of fossil origin [1–6]. In this study emphasis is placed on the mineral components that are examined for their possible suitability to stabilize both structure and function of mummified enzymes.

Mummification salt used for dehydration of tissues frequently contains sodium ions, carbonate, hydrogen carbonate, chloride and sulphate. The possible presence of borate was of special interest both historically and biochemically. The ancient Egyptians were reported as users of borax in metallurgy, medicine and mummification, but none of this can be substantiated [7]. There were many different types of embalming the deceased, depending on how much one wished to spend. Usually the abdomen was emptied and treated with ‘natron’ which was the name given to the impure soda (sodium carbonate and bicarbonate) found in the dry lake deposits in Egypt. It was used, together with common salt (NaCl) and gypsum (CaSO₄), in cleansing and food-preservation as well as in embalming, and its use can be traced to the Tasian period of Egyptian civilization in the fifth millennium B.C. [8–9]. However, no one has suggested or detected the presence of borax in these Egyptian lake deposits, and perhaps the reason of this confusion arises from the later Arabic classification of minerals, which included natron as ‘borax’ [7].

Known as sal sedativum, borates were used in the eighteenth century to soothe the skin. Even before this time, borates were used as cleansing agents. Arab physicians used them as early as 875 A.D. for internal medication. Thus, salts from grave findings that were identified as mummification salts were examined for their possible content of borate. Likewise the borate content of mummified bones from different excavation sites was analysed.

Results and Discussion

Four salt samples two from the Ägyptologisches Museum Berlin, one from Deir el-Bahari (26th Dynasty, 700–600 BC) and one from the embalming material of Tutankhamen (18th Dynasty, 1370–1335 B.C.) were assayed for borate, respectively. While no borate was detected in the Berlin samples distinct concentrations of 3.9 ± 0.1 μMol borate per gram in the Deir el-Bahari sample and 2.1 ± 0.2 μMol borate per gram in the salt probe from the embalming material of Tutankhamen were noticed (Table 1).

In six of the examined bone fragments of the Old Kingdom the borate content was assayed up to 1.2 μMol borate per gram being close to that found in the mummification salts. It must be em-
Table 1. Borate content of mummification salts and mummified bone samples of Pharaonic Egypt derived from different excavation sites.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Historical period</th>
<th>Excavation site</th>
<th>Borate (µMol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salts</td>
<td>26th Dynasty</td>
<td>Deir el-Bahari</td>
<td>3.90 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>18th Dynasty</td>
<td>Luxor, Valley of</td>
<td>2.10 ± 0.20</td>
</tr>
<tr>
<td>Bones:</td>
<td>Perneba</td>
<td>Saqquara</td>
<td>0.80 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Old Kingdom</td>
<td>Giza</td>
<td>0.95 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>W9260b</td>
<td>Old Kingdom</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>W9261b</td>
<td>Old Kingdom</td>
<td>1.15 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>W9262b</td>
<td>Old Kingdom</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>W9263b</td>
<td>Old Kingdom</td>
<td>0.60 ± 0.05</td>
</tr>
</tbody>
</table>

*a Metropolitan Museum New York; b Kunsthistorisches and Naturhistorisches Museum Wien; c embalming material of Tutankhamen [6].

 emphasized that the usual borate content of contemporary autopsy is far below the detection limit. It can be concluded that borate containing salt had been used in the different mummification processes.

The question arose whether or not this tetrahydroxyboron anion may act as an inorganic stabilizing compound on alkaline phosphatase being richly abundant in bones. This enzyme was chosen as it was shown to survive mummification for more than 4000 years [3–4]. Its glycoprotein nature suggests that the carbohydrate residues are good candidates to form borate complexes in the presence of the $\text{B(OH)}_4^{-}$ anion usually for protection of 1,2- and 1,3-diols [10–11]. Furthermore, intermolecular borate complexes may lead to oligomerization of this glycoprotein.

Bone alkaline phosphatase was gel-filtrated in Tris acetate buffer and in borate buffer. In Tris-buffer the enzyme migrated in its usual dimeric form at a relative molecular mass of 200000 Dalton while in the presence of borate there is convincing evidence that due to cross linking between the enzyme molecules oligomeric adducts are formed. Alkaline phosphatase oligomers with a relative molecular mass near 400000 Dalton were observed (Fig. 1).

The resistance towards thermal vibrations of the enzyme protein molecule when chelated to borate was further examined (Fig. 2). After heating to 45 °C the time dependent decline of phosphatase activity was less pronounced when 2mM borate were present.

To summarize it can be concluded that borate has a significant stabilizing effect on both the molecular architecture and the catalytic function of bone alkaline phosphatase. The observed stabilizing effect of borate in Figs. 1 and 2 may be attrib-
uted to borate bridging between the carbohydrate residues of alkaline phosphatase (Fig. 3).

It appears rather unlikely that the ancient Egyptians actually knew that they were applying borate-containing salts. They might have realized that certain mummification salts were more suitable for conservation. Nevertheless, it should be emphasized that boric acid was widely used in medicine owing to its strong bactericidal and fungicidal reactivity. The search for borate containing mummification salts and borate enriched mummified bone samples will be continued.

**Experimental Section**

**Materials**

Bone alkaline phosphatase (Lot 418, 0.5 Units/mg) was obtained from CALZYME, San Luis Obispo, CA, USA. A Superdex-200 prep grade HiLoad™ 16/60 gel filtration column was obtained from Amersham Pharma Biotech LAB, Uppsala, Sweden. Carminic acid was purchased from Fluka, Buchs/Switzerland. All other reagents, like boric acid, were of analytical grade quality from Merck, Darmstadt/Germany.

**Borate in mummified bones and mummification salts**

Usually some 40 mg of mummification salt were dissolved in 0.5–1.0 ml of 6 M HCl and centrifuged. Ancient and contemporary bone fragments (400–600 mg) were weighed into a boron-free quartz crucible and heated for 1 h at 100 °C and then at 650 °C for another 4 hours. The sample was allowed to cool to 20 °C and 4–6 ml of 6 M HCl were pipetted into the crucible. The liquid was mixed and centrifuged. Acidified borate solutions were quantified using carminic acid [12]. 0.1 ml of the solution was transferred into a boron-free plastic tube and 0.4 ml of 18 M H₂SO₄ were added, followed by 0.5 ml of 0.025 % (w/v) carminic acid diluted in 18 M H₂SO₄. The tube was covered to exclude atmospheric moisture, shaken and allowed to stand for at least 5 min at room temperature to develop the colour. The absorbance of the solution was measured on a Hitachi U-2000 spectrophotometer in a 1 cm quartz cell at 575 nm against a blank sample. The borate content was determined using a standard curve derived from known quantities of borate (1–8 µMol/ml) employing the above assay.

**Relative molecular mass (Mₐ) determination by gel filtration**

10.8 mg of alkaline bone phosphatase (0.5 Units/mg; Lot 418; CALZYME, San Luis Obispo, CA, USA) were gel filtrated on a HiLoad Superdex-200 prep grade column (16 × 60 mm, Amersham Pharmacia Biotech) with a relative molecular mass (Mₐ) range between 10000–600000 Dalton. The column was previously equilibrated with 25 mM Tris-acetate (pH 8.0). Elution was performed using the same buffer. Two ml fractions were collected and assayed for enzymic activity. The active enzyme fractions at Mₐ = 200 kDa were collected and divided into two identical portions. The first sample was re-chromatographed in the same 25 mM Tris-acetate (pH 8.0) buffer. The second portion was gel filtrated through a Superdex-200 column.

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Fig. 3. Suggested borate bridging of alkaline phosphatase.
previously equilibrated with 25 mM boric acid–NaOH (pH 8.0). Either elution of alkaline phosphatase was performed using the very same buffer.

**Alkaline phosphatase activity**

The alkaline phosphatase activity assay in aqueous solution was based on the increase of \( p \)-nitrophenol formation as a result of \( p \)-nitrophenyl phosphate hydrolysis catalysed by alkaline phosphatase. One ml of the assay volume contained 20–100 µl enzyme solution in elution buffer and 900–980 µl substrate buffer (1.8 mM \( p \)-nitrophenyl phosphate, 0.5 mM magnesium acetate, 1 M diethanolamine, pH 9.6). The rate of inhibition was expressed as the percentage of the inhibited control where sodium tetraborate was omitted.

**Heat stability in the presence of sodium tetraborate**

Alkaline bone phosphatase (0.16 µg protein) was pre-incubated together with 10 µg of sodium tetraborate in 20 µl Tris buffer (20 mM Tris acetate, pH 7.4, 2 mM magnesium acetate, 0.1 % (v/v) Triton X-100) at 45 °C for 1–5 hours. The mixture was diluted 1/50 with substrate buffer (1.8 mM \( p \)-nitrophenyl phosphate, 0.5 mM magnesium acetate, 1 M diethanolamine, pH 9.6). The rate of inhibition was expressed as the percentage of the inhibited control where sodium tetraborate was omitted.

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