THE COMPOSITION OF FOSSIL OYSTER SHELL PROTEINS*

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Abstract.—Analyses of the protein residues recovered from fossil oyster shells of ages from the Pleistocene through the Cretaceous show substantially the same amino acids as are present in modern proteins. The amount of these residues declines sharply to the Oligocene, after which it proceeds more slowly. These older proteins contain relatively less aspartic acid and glycine than do their younger counterparts.

More or less intact proteins have now been recovered and analyzed from a number of fossil bones1, 2 and shells3 many millions of years old. These proteins contain the same amino acids as those derived from modern specimens but usually in rather different proportions. This brings to the fore the question of whether or not the composition of the ancient proteins originally was different from that of their modern counterparts. Before giving an affirmative answer it is necessary to be sure that contaminating proteins have not been introduced and to decide if the observed differences could be attributed to a slow denaturation of the original material. Careful inspection can minimize the danger from contaminations and we believe that help in choosing between the remaining alternatives can come through analyses of the proteins from a series of fossils of an animal species that has changed little with time. Such proteins are provided by invertebrate shells and with this in mind we have analyzed the proteins from a series of oyster shells from the Pleistocene to the Cretaceous.

Experimental.—The fossils whose analyses are recorded here are the following:

<table>
<thead>
<tr>
<th>Fossil</th>
<th>Age</th>
<th>Specimen number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crassostrea virginica, Horry Co., S.C.</td>
<td>Pleistocene</td>
<td>1 6932</td>
</tr>
<tr>
<td>Ostrea sculpurata, Myrtle Beach, S.C.</td>
<td>Pliocene</td>
<td>1 6933</td>
</tr>
<tr>
<td>Ostrea ventilabum, Klein-Spauwen, Belgium</td>
<td>Oligocene</td>
<td>1 6935</td>
</tr>
<tr>
<td>Ostrea johnsoni, Jackson, Ala.</td>
<td>Eocene</td>
<td>1 6937</td>
</tr>
<tr>
<td>Ostrea compressirostra, Potomac Creek, Va.</td>
<td>Paleocene</td>
<td>1 6938</td>
</tr>
<tr>
<td>Gryphaea convexa, Cream Ridge, N.J.</td>
<td>Cretaceous</td>
<td>1 6941</td>
</tr>
</tbody>
</table>

After being thoroughly cleaned by a scrubbing with distilled water and being ground to remove surface layers, 20–50 gram samples were powdered and dissolved in 2 N HCl. The insoluble residues, washed with distilled water, were hydrolyzed by heating with 6 N HCl for 24 hr at 100°C in a closed tube under nitrogen. The resulting solutions were evaporated to dryness at 60°C under reduced pressure, taken up in small quantities of distilled water and redried under the same conditions. These dried residues were then dissolved in 1 ml of sample-diluting buffer for column chromatography. Appropriate volumes, determined by preliminary ninhydrin tests, were analyzed in the semi-automatic chromatograph employed in this laboratory. The resulting curves were analyzed in the usual fashion, absolute amounts of the various amino acids being obtained by reference to parallel analyses of a standard mixture of acids.

Results.—The quantities of the various amino acids constituting these fossil
proteins, in terms of micrograms per gram of fossil shell, are listed in Table 1. The same analyses expressed in terms of residues per 100 (residue per cent) are given in Table 2. All the amino acids customarily found from present-day proteins except hydroxyproline were present though methionine sulfoxides and hydroxylysine occurred only in traces. Two minor, as yet unidentified, peaks have appeared on certain charts.

### Table 1. Quantities of amino acids in fossil oyster shell proteins (micrograms per gram).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Pleistocene</th>
<th>Pliocene</th>
<th>Oligocene</th>
<th>Eocene</th>
<th>Paleocene</th>
<th>Cretaceous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met-s</td>
<td>I 6932</td>
<td>I 6933</td>
<td>I 6935</td>
<td>I 6937</td>
<td>I 6938</td>
<td>I 6941</td>
</tr>
<tr>
<td>Hypro</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Asp</td>
<td>397.703</td>
<td>15.799</td>
<td>2.209</td>
<td>0.865</td>
<td>1.823</td>
<td>1.344</td>
</tr>
<tr>
<td>Thr</td>
<td>18.175</td>
<td>1.441</td>
<td>0.977</td>
<td>0.274</td>
<td>0.476</td>
<td>1.024</td>
</tr>
<tr>
<td>Ser</td>
<td>103.860</td>
<td>4.120</td>
<td>1.692</td>
<td>1.167</td>
<td>2.091</td>
<td>1.577</td>
</tr>
<tr>
<td>Glut</td>
<td>87.171</td>
<td>2.267</td>
<td>3.221</td>
<td>1.486</td>
<td>2.913</td>
<td>2.515</td>
</tr>
<tr>
<td>Pro</td>
<td>33.320</td>
<td>1.786</td>
<td>0.599</td>
<td>0.334</td>
<td>0.886</td>
<td>0.599</td>
</tr>
<tr>
<td>Gly</td>
<td>260.955</td>
<td>9.553</td>
<td>2.388</td>
<td>1.134</td>
<td>1.998</td>
<td>2.170</td>
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<tr>
<td>Ala</td>
<td>36.264</td>
<td>1.835</td>
<td>1.060</td>
<td>0.356</td>
<td>1.042</td>
<td>0.775</td>
</tr>
<tr>
<td>Val</td>
<td>35.500</td>
<td>2.719</td>
<td>0.984</td>
<td>0.457</td>
<td>1.102</td>
<td>0.926</td>
</tr>
<tr>
<td>Cys (1/2)</td>
<td>29.500</td>
<td>2.000</td>
<td>0.061</td>
<td>0.133</td>
<td>0.230</td>
<td>0.206</td>
</tr>
<tr>
<td>Meth</td>
<td>8.221</td>
<td>0.507</td>
<td>0.015</td>
<td>0.134</td>
<td>0.164</td>
<td>0.075</td>
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<tr>
<td>Ileu</td>
<td>23.511</td>
<td>2.165</td>
<td>0.708</td>
<td>0.394</td>
<td>0.682</td>
<td>0.551</td>
</tr>
<tr>
<td>Leu</td>
<td>39.504</td>
<td>4.080</td>
<td>1.378</td>
<td>0.774</td>
<td>1.496</td>
<td>1.207</td>
</tr>
<tr>
<td>Tyr</td>
<td>43.234</td>
<td>2.845</td>
<td>1.069</td>
<td>0.544</td>
<td>0.544</td>
<td>0.652</td>
</tr>
<tr>
<td>Phe</td>
<td>24.169</td>
<td>2.164</td>
<td>0.677</td>
<td>0.363</td>
<td>0.743</td>
<td>0.529</td>
</tr>
<tr>
<td>Hylys</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Tr</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Orn</td>
<td>1.309</td>
<td>0.225</td>
<td>0.053</td>
<td>0.013</td>
<td>0.040</td>
<td>0.053</td>
</tr>
<tr>
<td>His</td>
<td>0.618</td>
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<td>0.377</td>
<td>0.252</td>
<td>0.398</td>
<td>0.315</td>
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<tr>
<td>Lys</td>
<td>39.244</td>
<td>3.508</td>
<td>1.425</td>
<td>0.639</td>
<td>1.114</td>
<td>1.151</td>
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<tr>
<td>Arg</td>
<td>43.968</td>
<td>3.397</td>
<td>1.254</td>
<td>0.897</td>
<td>1.368</td>
<td>1.045</td>
</tr>
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</table>

### Table 2. Amino acid per cent composition of fossil oyster shell proteins.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Pleistocene</th>
<th>Pliocene</th>
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<th>Cretaceous</th>
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<tr>
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<td>I 6941</td>
</tr>
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<td>Hypro</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Asp</td>
<td>27.52</td>
<td>21.26</td>
<td>10.13</td>
<td>8.16</td>
<td>8.72</td>
<td>7.31</td>
</tr>
<tr>
<td>Thr</td>
<td>1.41</td>
<td>2.17</td>
<td>5.01</td>
<td>2.89</td>
<td>2.54</td>
<td>6.23</td>
</tr>
<tr>
<td>Ser</td>
<td>9.10</td>
<td>7.02</td>
<td>9.83</td>
<td>13.93</td>
<td>12.66</td>
<td>10.86</td>
</tr>
<tr>
<td>Glut</td>
<td>5.46</td>
<td>10.07</td>
<td>13.37</td>
<td>12.67</td>
<td>12.60</td>
<td>12.38</td>
</tr>
<tr>
<td>Pro</td>
<td>2.59</td>
<td>2.79</td>
<td>3.17</td>
<td>3.64</td>
<td>4.90</td>
<td>3.77</td>
</tr>
<tr>
<td>Gly</td>
<td>32.01</td>
<td>23.50</td>
<td>19.41</td>
<td>18.95</td>
<td>16.92</td>
<td>20.93</td>
</tr>
<tr>
<td>Ala</td>
<td>3.75</td>
<td>3.69</td>
<td>7.27</td>
<td>5.02</td>
<td>7.44</td>
<td>6.30</td>
</tr>
<tr>
<td>Val</td>
<td>2.79</td>
<td>4.16</td>
<td>5.13</td>
<td>4.89</td>
<td>5.98</td>
<td>5.72</td>
</tr>
<tr>
<td>Cys (1/2)</td>
<td>2.24</td>
<td>2.96</td>
<td>0.31</td>
<td>1.38</td>
<td>1.21</td>
<td>1.23</td>
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<tr>
<td>Meth</td>
<td>0.51</td>
<td>0.61</td>
<td>0.06</td>
<td>1.13</td>
<td>0.70</td>
<td>0.36</td>
</tr>
<tr>
<td>Ileu</td>
<td>1.65</td>
<td>2.96</td>
<td>3.30</td>
<td>3.76</td>
<td>3.31</td>
<td>3.04</td>
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<tr>
<td>Leu</td>
<td>2.77</td>
<td>5.57</td>
<td>6.41</td>
<td>7.40</td>
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<td>6.66</td>
</tr>
<tr>
<td>Tyr</td>
<td>2.20</td>
<td>2.81</td>
<td>3.60</td>
<td>2.51</td>
<td>1.91</td>
<td>2.61</td>
</tr>
<tr>
<td>Phe</td>
<td>1.35</td>
<td>2.35</td>
<td>2.50</td>
<td>2.76</td>
<td>2.86</td>
<td>2.32</td>
</tr>
<tr>
<td>Hylys</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Tr</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Orn</td>
<td>0.09</td>
<td>0.30</td>
<td>0.24</td>
<td>0.13</td>
<td>0.19</td>
<td>0.29</td>
</tr>
<tr>
<td>His</td>
<td>0.26</td>
<td>0.86</td>
<td>1.10</td>
<td>1.51</td>
<td>1.21</td>
<td>1.09</td>
</tr>
<tr>
<td>Lys</td>
<td>1.98</td>
<td>3.44</td>
<td>4.76</td>
<td>4.39</td>
<td>3.88</td>
<td>4.56</td>
</tr>
<tr>
<td>Arg</td>
<td>2.32</td>
<td>3.49</td>
<td>4.40</td>
<td>4.89</td>
<td>5.73</td>
<td>4.34</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.01</td>
<td>100.00</td>
<td>100.01</td>
<td>100.01</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Discussion.—Except for the Eocene fossil which is the least well preserved, the amount of recovered protein declines with increasing age of the specimen. This decrease, at first very rapid, is, however, minimal for the Oligocene and more ancient fossils; thus the Pleistocene sample contains almost 20 times as much protein as the Pliocene and this in turn has preserved about three times as much as those that are older. The proportions of the amino acids also change during the period of rapid protein loss. As Table 2 shows, the relative amounts of aspartic acid and glycine are drastically reduced in the Oligocene and older samples compared to the Pleistocene, with the Pliocene protein intermediate between these extremes. Decrease in these two acids is compensated by an increase in the relative amounts of most but not all the others.

To the extent that they are comparable, our results are in substantial accord with certain paper chromatographic measurements on recent and fossil scallop shells\(^4\) and with recently published\(^5\) column chromatographic analyses of prismatic proteins from modern and Eocene Pinna shells. There are significant differences in the relative amounts of several of the amino acids in the fossil proteins from these different sub-orders of invertebrates which can only be understood after many more analyses have been made. Nevertheless they all agree in showing that the relative amounts of aspartic acid and glycine are greatly reduced in older fossil proteins compared to those from Recent or Pleistocene shells.

Our analyses do not permit a final conclusion as to whether or not the concholins of ancient oysters were different from those of more recent species but they do show that if such changes occurred it was in the period between the Oligocene and the Pleistocene. If, as from some standpoints seems more probable, the observed differences in composition are attributed to a progressive denaturation of proteins that initially were very similar, the following remarks are pertinent. The early denaturation that reduces the relative amounts of aspartic acid and glycine results in a proteidic residue of remarkable stability since it changes little in the long interval between the Oligocene and the Cretaceous. Examination of modern shells\(^6\) has indicated that protein from prismatic shell may have more than one component and that these may disappear at different rates from a fossil. In any event as Akiyama\(^4\) has already pointed out, aspartic acid and glycine are among the amino acids considered\(^5\) chemically to be most stable. Clearly a denaturation such as that envisaged here cannot be one in which the least stable amino acid components are lost first.

We are indebted to Mrs. Jerri Thomsen Staudring for technical aid in performing this work.

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