Simultaneous binding of calcium and bicarbonate by conchiolin of oyster shells[1]

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Summary. Conchiolin of oyster shells selectively binds Ca^{2+}. Bicarbonate is also bound but can be displaced by phosphate and sulfate. Conchiolin can be extracted to yield individual proteins with very high apparent molecular weights.

Organic macromolecules in mollusc shells can be separated into an insoluble and a water-soluble fraction after removal of the inorganic material by EDTA extraction. Some soluble high molecular weight components have been demonstrated to have Ca^{2+}-binding properties[2-4].

So far no reports have appeared on the affinities for Ca^{2+} and HCO_3^{1-} of the insoluble fraction, which is the classical conchiolin. This material has been isolated from shells of freshly collected oysters (cultivated in the Bay of Arcachon (France)). The shells used did not exhibit any jelly formation or chambering, as described in an accompanying paper[5]. The isolation procedures included rigorous dialysis steps (Amicon filter systems) in order to remove even traces of EDTA[6]. Studies of the binding of Ca^{2+} and also HCO_3^{1-} by conchiolin were carried out by equilibrium dialysis[7]. The initial pH of suspensions of purified conchiolin in deionized water is 8.5. Binding studies were done at room temperature for 18 h. Scatchard plots[8] of the results revealed more than 1 type of binding site for calcium. Competing ions did not suppress the selective binding of Ca^{2+}, even when present in excess of 5×10^5.

Treatment of conchiolin by denaturating agents had different effects on the extent of Ca^{2+}-binding (table 1). Reagents splitting hydrogen bonds connecting polypeptides of an antiparallel β-sheet configuration[9,10] lowered the extent of Ca^{2+}-binding. Heating of conchiolin in aqueous suspensions (100 °C, 10 min) increased Ca-binding, probably by exposing charged groups interacting with water molecules and calcium. Heating of conchiolin in 2% SDS-solution showed no effect when the product was compared with the original conchiolin. This may be due to the fact that additional charged groups are exposed by heating, so that calcium-binding capacity is increased, but at the same time hydrogen linkages are disrupted by SDS, which has the opposite effect.

Accumulation of HCO_3^{1-} by conchiolin was tested by equilibrium dialysis. The experimental conditions were the same as described for Ca^{2+}-binding studies. The results obtained gave evidence for more than one type of binding site for HCO_3^{1-}. However, in contrast to Ca^{2+}-binding by conchiolin, HCO_3^{1-} was not selectively bound. An excess of PO_4^{3-} (5×10^5) eliminated bicarbonate binding completely. Also an excess of SO_4^{2-} reduced the bicarbonate affinity of conchiolin drastically.

Simultaneous binding of both calcium and bicarbonate by conchiolin could be an important factor in $CaCO_3$ formation. The pH of conchiolin suspensions is in the range which is required for $CaCO_3$ crystallization[11,12]. The attraction of Ca^{2+} by conchiolin at pH 8.5 can possibly be explained by chelate formation. At least 2 soluble high molecular weight sulfated glycoproteins (500 kdaltons), obtained by extraction from shells of *C. gigas* (IP 2.5), fractionation on Bio-Gel A-5m and electrophoresis (agarose gels) show selective binding of calcium but no affinity to bicarbonate. A comparable fraction isolated from *C. virginia* has been shown to reduce $CaCO_3$ crystallization[13].

Conchiolin of *C. gigas* can be further extracted by various agents, e.g. borate, SDS, and NaOH to yield individual proteins with very high apparent mol. wts ($\triangleq 6 \times 10^6$ daltons). Some solubilized protein fractions obtained so far have lost their Ca^{2+}- and HCO_3^{1-}-binding properties. Recombination of these solubilized proteins, however, partly restores their affinity for calcium and bicarbonate. Proteins extracted from conchiolin vary clearly in their amino acid composition (table 2) as do similar fractions obtained from *Acropora formosa*[14]. This raises the question whether the term 'insoluble' matrix is still tenable or should be changed, although there is an insoluble residue which is probably chitin[2]. These findings confirm earlier indications that conchiolin is a refractory mixture of proteins and polysaccharides and show that the total amino acid analyses of conchiolin may be of limited value in the determination of any role conchiolin may have in mineralization[2]. Each isolated protein fraction from conchiolin exhibits a tendency towards self-aggregation, resulting in the ready formation of insoluble material.

The observation that phosphate prevents binding of bicarbonate to conchiolin is consistent with earlier reports that naturally occuring phosphate and sulfate compounds inhibit the formation of calcium carbonate crystals[15]. The oyster mantle which secretes the shell may remove interfering ions from the region of calcification[15]. It has also been reported that conchiolin of oysters shows some ability to

Table 1. Effects of denaturation of conchiolin on Ca^{2+}-binding*

Untreated conchiolin	100
Conchiolin + 8 M urea	57
Conchiolin + 6 M guanidinium chloride	4
Conchiolin + 2% SDS	58
Conchiolin heated in aqueous suspension	125
Conchiolin heated in 2% SDS suspension	98

*Experiments carried out under same optimum binding conditions. Data given relative to untreated conchiolin (100).

Table 2. Amino acid composition of conchiolin extracts from oyster shells

	Extracts with Borate	SDS	NaOH	Insoluble residue
Aspartic acid	25.9	15.4	15.9	10.1
Threonine	4.8	4.5	6.9	3.8
Serine	16.1	8.1	12.5	8.2
Glutamic acid	11.2	11.0	13.6	10.3
Proline	–	5.7	–	7.8
Glycine	32.4	15.0	22.2	14.7
Alanine	9.4	8.0	28.7	21.3
½ Cystine	–	–	–	–
Valine	–	5.7	–	7.2
Methionine	–	0.3	–	–
Isoleucine	–	4.2	–	4.0
Leucine	–	7.4	–	5.3
Tyrosine	–	1.2	–	–
Phenylalanine	–	3.9	–	2.6
Lysine	–	4.0	–	4.1
Histidine	–	0.5	–	–
Arginine	–	3.7	–	–
Tryptophan	–	–	–	–

Data given in moles-%, calculated without ammonia. –, Not detectable.

induce the formation of calcite[15]. The simultaneous binding of calcium and bicarbonate by conchiolin could help to explain the specific formation in oyster shells of $CaCO_3$ rather than other insoluble Ca-components.

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Soluble matrix components in malformed oyster shells

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Summary. Soluble proteins isolated from normal and malformed oyster shells have been partly characterized indicating remarkable differences in chemical composition and Ca^{2+}-binding properties.

Malformation of the shell of the oyster *Crassostrea gigas* associated with the production of a jelly-like substance by the mantle tissue has frequently been observed during recent years. A very high percentage of the cultivated oysters in the Bay of Arcachon, France, exhibit this phenomenon which is also known from other parts of the littoral, as well as in Great Britain. From spring through the summer oysters secrete jelly which is rapidly covered by a thin crystalline layer (paper shell). This substance, enclosed in a cavity, decomposes completely, forming H_2S. The repitition of this phenomen leads to a superposition of cavities with the appearance of a lamellar structure of the shell which is fragile (chambering). This anomaly is accompanied by a considerable thickening of the valves which can modify remarkably the external morphology of the oyster[2,3].

So far no study has been published on changes in the distribution and composition of organic matrix components involved in molecular mechanisms of shell malformation. We report here on the results of analyses of water-soluble macromolecules in normal and malformed shells of *C. gigas* collected in oyster beds in the Bay of Arcachon. Malformed shells were divided into solid sections and extremely thin layers (paper shells) covering deposits of jelly in shell depressions (fig. 1). Details of the extraction procedures have been described previously[4]. Thoroughly dialyzed (Amicon filter system) substances exceeding a mol.wt of 30 Kdaltons were subjected to gel filtration (Bio-Gel A-15m). In essence 2 peaks (fig. 2) were obtained from extracts of normal shells, solid sections of malformed shells and paper shells. Peak I of each kind of extract had an apparent molecular size of $\triangleq 5 \times 10^6$ daltons, as judged by gel filtration. However, the proportion of peak I was highest in normal shells and lowest in 'paper shells'. The quantities of peak II obtained from each starting material were about the same, with an estimated apparent molecular

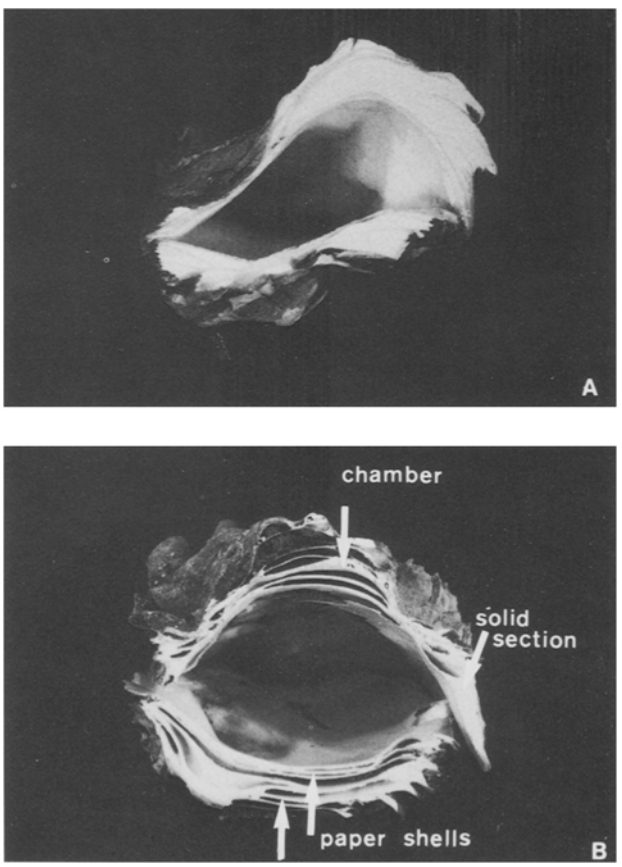

Figure 1. *A* Normal shells *(C. gigas); B* malformed shells *(C. gigas)* with chambers, paper shells and solid sections.