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

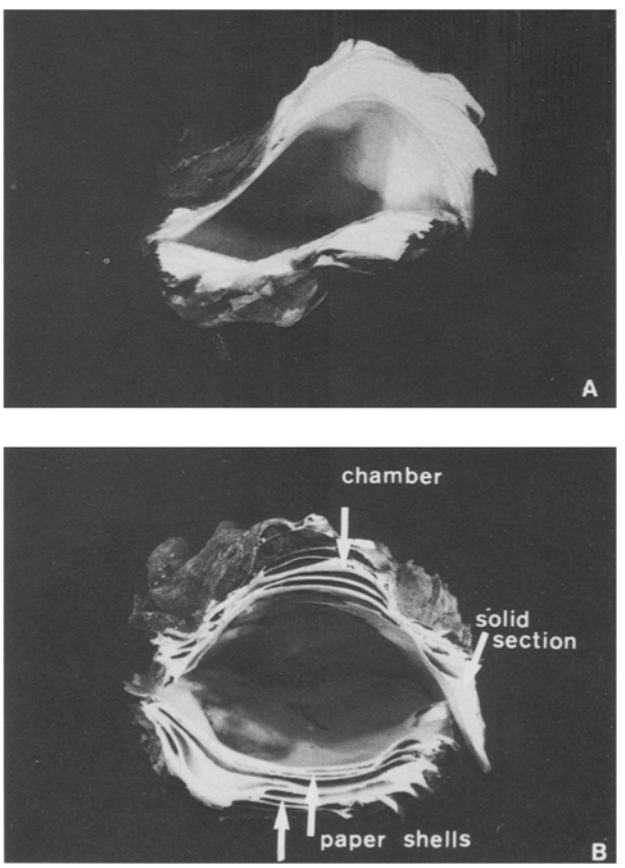

Figure 1. *A* Normal shells *(C. gigas); B* malformed shells *(C. gigas)* with chambers, paper shells and solid sections.

Amino acid composition of soluble proteins from oyster shells

	Peak I			Peak II			Jelly
	NS	CS	PS	NS	CS	PS	main peak
Aspartic acid	35.5	35.7	33.4	19.7	39.2	36.4	3.7
Threonine	1.6	+	2.7	2.4	2.5	2.9	27.9
Serine	17.4	21.3	26.4	9.7	18.1	15.7	2.7
Glutamic acid	5.4	5.5	6.0	9.8	8.5	8.7	15.4
Proline	+	6.5	+	3.6	+	+	10.9
Glycine	34.7	29.9	23.2	43.6	27.0	25.8	2.0
Alanine	2.1	+	2.0	3.7	2.9	1.7	6.8
½ Cystine	+	+	+	+	+	+	−
Valine	+	+	1.9	1.4	+	2.0	+
Methionine	+	+	+	+	+	+	+
Isoleucine	+	+	+	+	+	2.0	2.1
Leucine	+	+	+	+	+	+	2.1
Tyrosine	3.3	+	2.0	+	+	2.1	+
Phenylalanine	+	+	+	+	+	1.2	+
Lysine	+	+	2.0	1.9	1.6	2.6	8.5
Histidine	+	+	+	4.1	+	+	+
Arginine	+	+	+	+	+	+	+
Tryptophan	−	−	−	−	−	−	−
Galactosamine	−	−	−	−	−	−	20.6

Data given in moles%, calculated without ammonia. NS, normal shells; CS, compact sections of malformed shells; PS, paper shells of malformed shells; +, traces; −, not detectable.

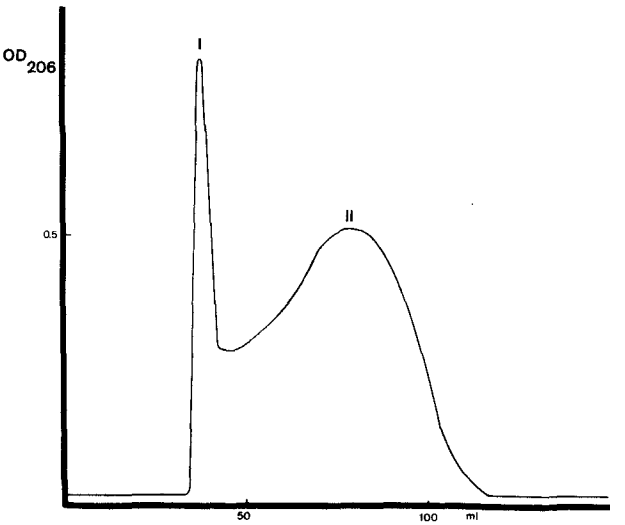

Figure 2. Fractionation on Bio-Gel A 15m of soluble shell extracts. 1 ml of concentrated shell extract (= 2 mg protein) was layered on a 50 × 1.5 cm column and eluted with 0.05 M NH_4HCO_3 solution. 1.5 ml fractions were collected, peaks pooled, lyophilized and rechromatographed.

size in the range of ≙ 5×20^5 daltons. Ion exchange chromatography and electrophoresis (agarose gels) of peak I did not result in further fractionation, indicating that this peak is relatively pure. Immunological tests also gave evidence for the homogeniety of peak I. Staining was possible only by Alcian Blue; Coomassie Blue S-250 alone gave negative results. The material in peak I extracted from normal shells had no binding capacity comparable with data recently reported[6]. Peaks II from normal shells and compact sections had a much higher affinity for Ca than did peak II from 'paper shells' (≙ 50%). Jelly produced by *C. gigas* had no calcium-binding properties at all. Calcium-binding properties of high molecular weight glycoproteins from mollusc shells have been reviewed recently[8,9].

The amino acid composition of peaks I and II as well as of jelly exhibited certain remarkable differences (table). Purified protein isolated from jelly differs in particular from soluble shell proteins in its very high content of threonine. It can also be concluded that the protein from peak I of normal shells is not the same as that from peak I of malformed shells although each of them was homogeneous as judged by the criteria described above. The same can be concluded from the data for peak II. These extremely high molecular weight substances may be involved in the molecular mechanisms of shell formation.

Several roles for the soluble matrix components have been suggested. In solution, the soluble matrix may reduce the rate, or delay the onset of the precipitation of calcium carbonate from solutions sufficiently supersaturated to precipitate spontaneously[9]. A soluble Ca-binding glycoprotein from *C. virginia,* probably comparable with peak II of normal *C. gigas* shells, has inhibiting effects on $CaCO_3$ crystallization[6] as has peak II, isolated from malformed shells (compact sections and paper shells). Peak I of malformed shells (both compact and paper shells) may also delay crystal formation. Both components (peaks I and II) together could act on shell formation by reducing shell thickness, whereas deposited jelly, although supersaturated with Ca^{2+}, does not show any $CaCO_3$ crystallization.

The reasons for malformed shells and jelly production could be due to different posttranslational modifications of soluble shell proteins or to the expression of different genes. The latter is certainly true since jelly proteins could not be detected in any tissues of normal animals *(C. gigas)* by 2D-electrophoresis[7]. Since the phenomenon of malformed shells and jelly production disappears when animals are transfered from the Bay of Arcachon to coastal waters in Bretagne (France), the causative agents must be exogenous, and must disturb the machinery of protein biosynthesis. Recent studies have shown that antifouling paints containing organo-tin components could be one cause of the disturbance of the calcification mechanisms in *C. gigas*[2]. On the other hand malformed oyster shells provide an excellent opportunity for investigating normal biological calcification processes in oysters.

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