Changes in the Composition of Acid Extracts of Purified *Echinus* Nuclei during Cleavage Stages and from Early Embryos

By MARGERY G. ORD and L. A. STOCKEN

Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K.

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Previous work with acid extracts from the sea-urchin *Echinus esculentus* during early cleavage stages (Ord & Stocken, 1970) had been carried out with crude nuclei extracted with 0.25 M-hydrochloric acid and had shown that the extract contained a complex mixture of basic and acidic components. Loeb, Fansler, Williams & Mazia (1969) have described a simple method for obtaining sea-urchin nuclei in good yield, and Sadgopal & Bonner (1970a,b) have reported that with metaphase nuclei 0.1 M-sulphuric acid rather than 0.25 M-hydrochloric acid decreased the complexity of the protein components extracted. It is also known (Bäckström, 1965; Ord & Stocken, 1968) that the basic proteins present in cleavage stages in sea-urchins have some differences from those present after gastrulation. We therefore decided to repeat some of our earlier experiments with purified nuclei and 0.1 M-sulphuric acid as extractant.

Methods. Mature eggs were obtained from potassium chloride-treated gonads and washed and fertilized at 10–11°C as previously described (Ord & Stocken, 1970). The fertilized eggs were examined 0–3 h after fertilization or were stirred gently at 11–14°C and sampled 28–46 h after fertilization, i.e. during gastrulation. Hatching occurred at about 26 h. Nuclei were obtained after sedimentation through M-sucrose (Loeb *et al.* 1969) except that the wash with M-glucose was omitted. The nuclei were stored at –15°C until analysis. The nuclear pellet was then washed three times with 1 mM-hydrochloric acid or 0.5 mM-sulphuric acid and extracted once or twice with 0.25 M-hydrochloric acid or 0.1 M-sulphuric acid. The extracts were clarified by centrifuging for 15 min at 100 000g and analysed as reported previously (Ord & Stocken, 1970). Preparative electrophoresis of extracts from 43 h embryos was performed as described by Buckingham & Stocken (1970).

Results and discussion. Amino acid analyses (I–IV; Table 1) of acid extracts from purified *Echinus* nuclei show only traces of the diffusible low-molecular-weight methylhistidine-containing basic protein found in the crude nuclear preparations used previously. This indicates that the methylated peptide (Ord & Stocken, 1970) is probably not a nuclear component but might come from cortical material contaminating our earlier

preparations, and be related to the contractile protein present in the cortex and studied by Sakai (1967).

The acidic protein found in 0.25 M-hydrochloric acid extracts of cleavage-stage crude nuclei, which just entered the 15% polyacrylamide gel (Ord & Stocken, 1968), was again seen on electrophoresis of 0.25 M-hydrochloric acid extracts from purified cleavage-stage nuclei and, to a smaller extent, in extracts from embryos. It was not detected in 0.1 M-sulphuric acid extracts of purified nuclei from either cleavage stages or 43 h embryos. The hydrochloric acid extracts were also of a lowered basicity (II and III, and VI and VII; Table 1) so that 0.1 M-sulphuric acid extracts were used for the rest of this work.

The thiol+disulphide content of these extracts decreased from about 150 nmol/mg of protein from 0–3 h after fertilization to 77 and 61 nmol/mg for 30 h and 46 h embryos respectively. As in our earlier experiments, the extracts had a higher proportion of thiol to disulphide at furrow (3 h) than in mature unfertilized eggs; extracts from the embryos were completely reduced.

The difference in thiol content is also shown by the amino acid analyses (V and VI; Table 1); the cysteine+cysteic acid contents from 30–46 h embryos are about half those in cleavage stages. The extracts from the embryos had a lower content of acidic residues and a relatively higher arginine content.

Analytical gel electrophoresis of both embryo and cleavage-stage extracts gave patterns similar to those reported previously (see Plate 1 in Ord & Stocken, 1968) except for the decrease in amount of acidic component just entering the gels. The two slowest of the major components were more prominent in cleavage stages than in embryos, with the third band, which migrated in a position similar to that of histone F1, becoming increasingly evident through gastrulation. The fourth band showed high [¹⁴C]N-ethylmaleimide binding and also ran to an extent allowing its tentative identification as histone F3. Ahead of this was a protein that could be obtained relatively homogeneous after preparative electrophoresis and that had an amino acid composition similar to that of histone F2a2 (Johns, 1967). The fastest major component was prominent only in extracts from embryos.

Table 1. *Amino acid analyses of acid extracts from Echinus nuclei*

Samples I-IV and VII were obtained from nuclei washed three times with 1 mM-HCl or 0.5 mM-H₂SO₄ and extracted once with 0.25 M-HCl or 0.1 M-H₂SO₄. Where indicated the extracts were dialysed overnight against two 100 vol. lots of water at 4°C. Samples V and VI were obtained from nuclei washed three times with 0.5 mM-H₂SO₄ and extracted twice with 0.1 M-H₂SO₄. These supernatants were pooled before analysis. No significant difference was found between the composition at cleavage-stage extracts from 0 to 3 h after fertilization or between 30 h- and 46 h-embryo extracts; results from all cleavage stages and from the embryos were therefore averaged and are expressed as mol/100 mol of total amino acids.

	Amino acid composition (mol/100 mol of total amino acids)									
	Cleavage stages					Embryos				
	I		II		III	IV		V	VI	VII
	Dialysed 0.25 M- HCl	Undialysed 0.25 M- HCl	Undialysed 0.1 M- H ₂ SO ₄	Undialysed 0.1 M- H ₂ SO ₄	Undialysed 0.1 M- H ₂ SO ₄	Dialysed 0.1 M- H ₂ SO ₄	Undialysed 0.1 M- H ₂ SO ₄	Undialysed 0.1 M- H ₂ SO ₄	Undialysed 0.1 M- H ₂ SO ₄	Undialysed 0.25 M- HCl
CySO ₃ H	0.15	1.9	1.4	0.1	0.8	0.3	Trace			
Asp	10.5	10.3	9.5	9.9	9.4	7.2	8.5			
MeHSO ₄	1.4	1.8	—	—	0.5	0.5	Trace			
Thr	5.5	5.2	5.1	4.6	5.3	5.6	5.4			
Ser	7.2	6.8	5.8	6.5	5.7	5.5	6.3			
Glu	11.7	10.9	11.1	9.9	10.7	9.5	10.5			
Pro	5.5	6.6	4.0	4.2	3.9	4.0	4.0			
Gly	9.5	9.5	8.9	8.7	8.9	8.9	8.6			
Ala	6.7	8.6	8.2	8.5	8.5	10.4	10.4			
Cys	0.9	Trace	—	1.6	1.1	0.5	Trace			
Val	5.3	5.4	6.1	5.9	6.5	6.1	5.6			
Met	0.5	Trace	1.1	1.5	1.5	1.0	1.0			
Ile	3.6	3.7	4.4	4.2	4.7	4.5	4.0			
Leu	6.3	6.3	6.9	6.9	7.0	7.8	7.5			
Tyr	2.3	1.6	1.8	2.5	1.9	2.4	2.2			
Phe	2.7	2.6	3.1	3.0	3.0	2.6	2.6			
His	2.4	2.1	2.5	2.6	2.2	1.7	1.8			
MeHis	Nil	Nil	Nil	Nil	0.1	Trace	0.2			
Lys	10.6	5.4	12.0	11.8	12.7	12.7	11.7			
MeLys	Trace	4.0	Trace	Nil	Nil	Nil	Nil			
Arg	7.4	7.3	8.1	7.7	7.1	8.8	7.1			
MeArg	Nil	Trace	Nil	Nil	Nil	Nil	Nil			
B	20.4	18.8	22.6	22.1	22.1	23.2	20.8			
A	22.2	21.2	20.6	19.8	20.1	16.7	19.0			
B/A	0.9	0.9	1.1	1.1	1.1	1.4	1.1			
Lys/Arg	1.4	1.3	1.5	1.5	1.8	1.4	1.7			

The results are consistent with there being a change in relative proportions of different histones during differentiation, the sulphur-containing proteins detectable in cleavage declining and the amount of histone F1 increasing through gastrulation. Changes in histone complement are well known during the maturation of some fish sperm (Marushige & Dixon, 1969) and are seen also in the subclasses of histone F1 during development of the mammary gland (Stellwagen & Cole, 1969).

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