J. Univ. Kuwait (Sci.) **7**, 1980

Localisation of hydroxyproline in the vegetative cellular slime mould *Dictyostelium discoideum*

MAYSOON S. YOUNIS,* JOHN ASHWORTH AND HADI AL-RAYESS†

Department of Biology, Essex University, Colchester, U.K.

ABSTRACT

Two types of hydroxyproline, free and combined, are present in *Dictyostelium discoideum*. Free hydroxyproline is present in the vacuole and constitutes more than 95% of the total hydroxyproline. This is a characteristic of the vegetative cells since it has been released during the preaggregation period. Combined hydroxyproline constitutes less than 5% of the total hydroxyproline. This remains constant during the differentiation process and is the only form of hydroxyproline in the fruiting body.

INTRODUCTION

Hydroxyproline has been found in the vegetative myxamoebae and in the differentiated cells of the cellular slime mould, *Dictyostelium discoideum* (Younis *et al.* 1979). During starvation, the cellular slime mould undergoes several changes during morphogenesis. Such changes include leakage of metabolites from the cells (Lee 1972). Since hydroxyproline can be detected in cells without their prior hydrolysis (Younis *et al.* 1979), it must be present in the cell in the free state and it is most likely, therefore, to be present in the vacuoles of the myxamoebae.

Several types of vacuoles have been identified and localised in the vegetative myxamoebae and in the differentiated cells. In the vegetative myxamoebae, three types of vacuoles have been identified. The first type refers to large irregularly shaped homogeneous vacuoles of low electron density that most likely correspond to the contractile vacuoles (water expulsion vesicles) of George & Hohl (1972) or the osmoregulatory vacuoles of Loomis (1975). The second type of vacuole is the food vacuole characterised by its content of bacteria at various stages of digestion (Hohl 1965). The third type is an electron-dense prespore vacuole. The prespore cell of *D. discoideum* is characterised by the synthesis of specific substances and the formation of these specific vacuoles (Maeda & Takeuchi 1969; Ikeda & Takeuchi 1971; Killick & Wright 1974). These vacuoles are specific to the prespore cell and were entirely absent from the prestalk cells; the contents of their vacuoles were identified as an acid mucopolysaccharide (Takeuchi 1972). Another type of vacuole which appears at the late

* Present address: Institute of Medical Technology, Bab Al-Muadam, Baghdad, Iraq.
† Present address: Department of Plant Protection, College of Agriculture, Abu-Ghraib, Baghdad, Iraq.

morphogenesis period and is distinguishable in the spores, appears to be electron-transparent and has the appearance of being compressed (Cotter *et al.* 1969). These vacuoles disappear during spore swelling and are possibly the prespore vacoules, food vacuoles or osmoregulatory vacuoles (Cotter *et al.* 1969). The hydroxyproline in the vegetative myxamoebae was in higher concentration than in the fruiting body (Younis *et al.* 1979). Thus, it is most likely that hydroxyproline is in one of the vacuoles of the vegetative cells.

MATERIALS AND METHODS

Growth and differentiation of the slime mould

D. discoideum cells of strain Ax-2 were grown axenically as described by Watts & Ashworth (1970).

Extraction of free hydroxyproline

Extracts of free hydroxyproline were made from vegetative cells as well as differentiated cells in the 'finger' and 'slug' states, grown either in the presence or absence of glucose. Samples were adjusted to 1×10^8 cells/cc, sonicated for a total of 1 min in 15 sec bursts. 2·1 cc of 11·4 N HCl were added to 2 cc of suspension containing 1×10^8 cells/cc making the final concentration 6 N. The samples were left to stand for 1 h to allow the protein to precipitate. The residue was separated by spinning at 3000 rpm for 15 min in a glass centrifuge tube at 4°C. The supernatants were dried in a vacuum desiccator at 60–70°C overnight, then 0·5 cc of distilled water was added to each sample. Hydroxyproline was determined in both the supernatant and the residue (protein) by the method of Blumenkrantz & Asboe-Hansen (1973). In samples containing low concentrations of hydroxyproline (less than 3·6 μg per 10^8 cells, for which the optical density was less than 0·1), concentrated samples at a cell density of 1×10^9 cells/cc were taken and the results were corrected to 1×10^8 cells/cc.

Calculation of the number of broken cells and the amount of hydroxyproline in liquid media

Vegetative myxamoebae were suspended in 250 cc of either distilled water or salt solution (0·4% NaCl solution was used for no-glucose grown cells and 0·7% NaCl solution was used for glucose grown cells) at a cell density of 1×10^7 cells/cc. The resulting suspensions were magnetically stirred and the cell densities were determined by periodic microscopic examination. Samples at cell density of 1×10^8 cells/cc were collected at 0, 0·5, 1, 1·5, 2, 2·5, 3 and 4 hr, then harvested for hydroxyproline determination. Experiments were carried out at 4°C.

Determination of hydroxyproline uptake by D. discoideum

D. discoideum cells were grown axenically in protease peptone yeast extract medium in the presence or absence of glucose as described by Watts & Ashworth (1970). The growth procedure of Watts & Ashworth (1970) was modified by adding 2·25 μg/cc, 9·0 μg/cc, 18·0 μg/cc, 655 μg/cc and 1311 μg/cc of hydroxyproline to the growth media. Then myxamoebae were harvested during the exponential phase of growth at 4×10^6

myxamoebae per cc, washed as described by Weeks & Ashworth (1972) and assayed for hydroxyproline.

RESULTS AND DISCUSSION

Many biochemical and physiological events will accompany starvation in the cellular slime mould. On solid media, the starved amoebae start to differentiate, and it has been shown that this organism loses about 50% of its dry weight during morphogenesis from vegetative cells to mature sorocarp and there is a breakdown of cell constituents (White & Sussman 1961; Sussman & Sussman 1969). The major decrease was reported in the protein, carbohydrate and RNA content of cell (Gregg *et al.* 1954; Gregg & Bronsweig 1956; Wright & Anderson 1960; White & Sussman 1963; Sussman & Sussman 1969; Mizukami & Iwabuchi 1970; Hames 1972, Hames & Ashworth 1974; Long and Coe 1974).

As shown in Tables 1 and 2, the addition of small amounts of hydroxyproline to the growth medium has no effect on the cellular hydroxyproline content. Uptake of amino acids is believed to be by passive diffusion (Lee 1972), whereas high molecular weight materials are taken up by the active process of pinocytosis. Tables 1 and 2 also reveal that the addition of high concentrations of hydroxyproline to the growth medium causes an increase in the cellular hydroxyproline content. This is in agreement with the suggestion of Lee (1972) that amino acid uptake is by passive diffusion.

When suspended in water, myxamoebae must excrete water rapidly in order to prevent cell lysis. The rate of efflux of water necessary to maintain cellular integrity will be proportional to the difference in osmotic pressure between the cell and the suspension medium. Previous results (Hames 1972) have shown that 0·7% NaCl is iso-osmotic

Table 1. The concentration of hydroxyproline (hyp.) in *D. discoideum* grown in the presence of glucose with or without the addition of different concentrations of chemical hydroxyproline to the medium. Total hydroxyproline in the cells, supernatants and the residue is indicated

Mean replicates	Concentration of chemical hyp. in media μg/cc	Total hyp. in hydrolysed cells in μg/10^8 cells	Concentration of hyp. in supernatants (vacuoles)		Concentration of hyp. in residue (protein)	
			μg/10^8 cells	as % of total hyp.	μg/10^8 cells	as % of total hyp.
	0	29·0	28·2	97·3	0·8	2·8
	0	28·6	27·5	96·1	0·9	3·2
Mean	0	28·8	27·9	96·7	0·9	3·0
	2·25	30·0	29·0	96·7	0·8	2·6
	4·50	29·0	27·7	95·5	1·1	3·7
	9·00	31·0	29·9	96·5	0·9	2·9
	18·00	28·0	27·0	96·4	0·8	2·8
Mean		29·5	28·4	96·3	0·9	3·0
	655	59·9	58·0	96·8	0·9	0·9
	1311	87·6	85·8	98·2	1·1	1·8

Table 2. The concentration of hydroxyproline (hyp.) in no-glucose grown cells of *D. discoideum*, with or without the addition of different concentrations of chemical hydroxyproline to the growth media. Total hydroxyproline of the cells, supernatants and the residue is indicated

Mean replicates	Concentration of chemical hyp. in media μg/cc	Total hyp. in hydrolysed cells in $\mu g/10^8$ cells	Concentration of hyp. in supernatants (vacuoles)		Concentration of hyp. in residue (protein)	
			$\mu g/10^8$ cells	as % of total hyp.	$\mu g/10^8$ cells	as % of total hyp.
	0	20·8	19·7	94·9	1·0	4·6
	0	20·0	19·0	95·0	0·8	4·0
Mean	0	20·4	19·4	94·9	0·9	4·3
	2·25	20·3	19·5	96·0	0·8	4·0
	4·50	21·1	20·2	95·6	0·7	3·4
	9·00	20·7	19·1	92·4	1·2	5·6
	18·00	20·7	19·6	94·7	0·9	4·3
Mean		20·6	19·6	25·1	0·9	4·3
	655	43·4	41·9	96·6	0·9	2·0
	1311	73·3	72·0	98·2	0·9	1·3

with glucose grown cells and 0·5% NaCl is iso-osmotic with no-glucose grown cells. As seen in Figs 1 and 2 and Tables 3 and 4, suspension of glucose grown cells in 0·7% NaCl leads to little or no loss of hydroxyproline, whereas suspension of both of these cells in water leads to a rapid loss of hydroxyproline. This is in agreement with the hypothesis that hydroxyproline is present in the vacuole.

Table 3. Number of broken cells and the concentration of hydroxyproline (hyp.) in the vegetative cells of the cellular slime mould *D. discoideum* grown in glucose media then transferred into no-nutrient liquid culture of water or of 0·7% NaCl for 4 hr

Time in hr	Number of broken cells		Concentration of hyp. $\mu g/10^8$ cells	
	Water	0·7% NaCl	Water	0·7% NaCl
0	$1{\cdot}0\times10^7$	$1{\cdot}0\times10^7$	28·1	29·1
0·5	$8{\cdot}0\times10^6$	$1{\cdot}0\times10^7$	25·3	29·7
1	$8{\cdot}0\times10^6$	$9{\cdot}5\times10^6$	19·4	29·0
1·5	$6{\cdot}5\times10^6$	$9{\cdot}0\times10^6$	12·0	27·3
2	$5{\cdot}6\times10^6$	$8{\cdot}6\times10^6$	8·7	28·2
2·5	$3{\cdot}2\times10^6$	$8{\cdot}5\times10^6$	5·3	28·9
3	$2{\cdot}0\times10^6$	$8{\cdot}5\times10^6$	3·3	27·0
4	$1{\cdot}4\times10^6$	$8{\cdot}2\times10^6$	1·2	26·5

Table 4. Number of unbroken cells and the concentration of hydroxyproline (hyp.) in the vegetative cells of the cellular slime mould *D. discoideum* grown in no-glucose media then transferred into no-nutrient liquid culture of water or 0·4% NaCl for 4 hr

Time in hr	Number of unbroken cells		Concentration of hyp. $\mu g/10^8$ cells	
	Water	0·4% NaCl	Water	0·4% NaCl
0	$1{\cdot}0 \times 10^7$	$1{\cdot}0 \times 10^7$	20·2	21·7
0·5	$8{\cdot}2 \times 10^6$	$1{\cdot}0 \times 10^7$	17·1	19·5
1	$8{\cdot}2 \times 10^6$	$1{\cdot}0 \times 10^7$	15·1	19·3
1·5	$7{\cdot}5 \times 10^6$	$9{\cdot}0 \times 10^6$	9·1	19·6
2	$4{\cdot}3 \times 10^6$	$9{\cdot}5 \times 10^6$	8·5	18·2
2·5	$3{\cdot}2 \times 10^6$	$9{\cdot}5 \times 10^6$	6·3	18·1
3	$2{\cdot}4 \times 10^6$	$9{\cdot}3 \times 10^6$	3·7	17·7
4	$1{\cdot}0 \times 10^6$	$8{\cdot}2 \times 10^6$	1·5	17·5

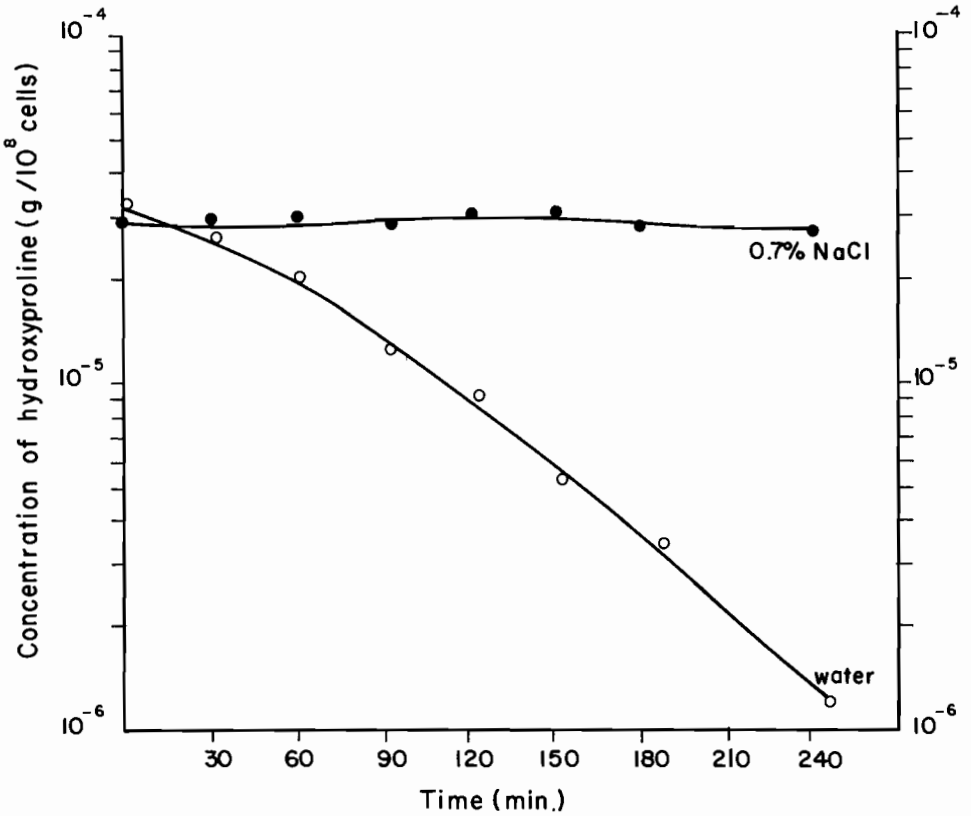

Fig. 1. The concentration of hydroxyproline in the vegetative cells of the cellular slime mould *D. discoideum* grown in glucose media then transferred into no-nutrient liquid culture of water or 0·7% NaCl for 4 hr.

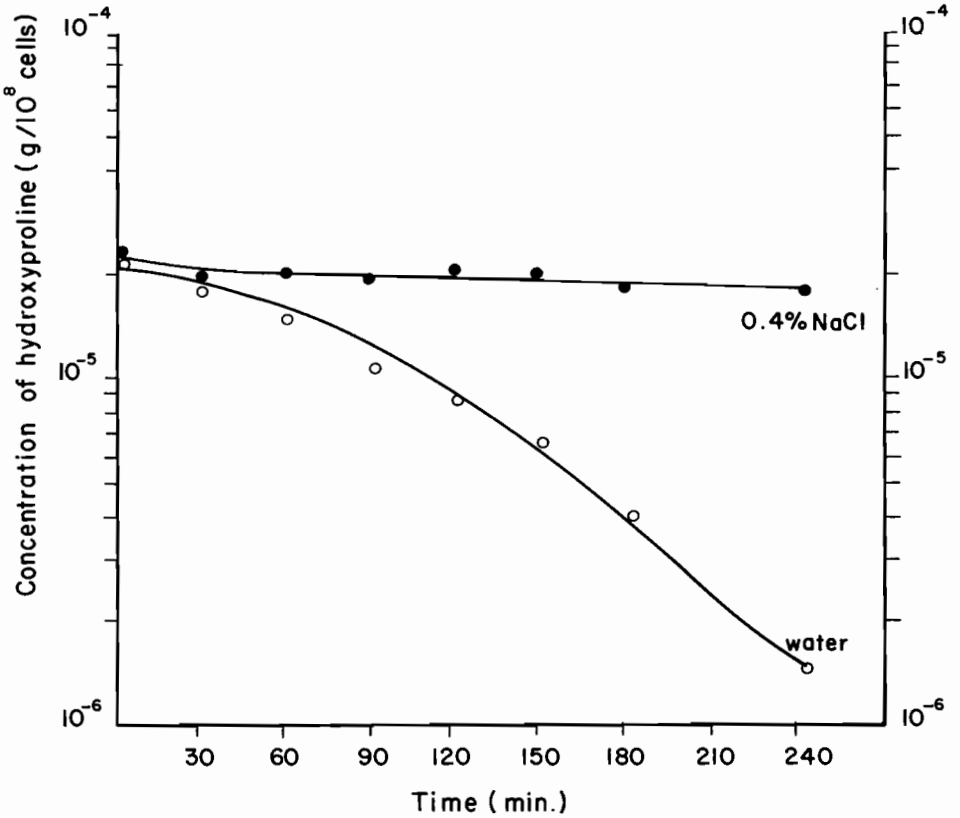

Fig. 2. The concentration of hydroxyproline in the vegetative cells of the cellular slime mould *D. discoideum* grown in no-glucose media then transferred into no-nutrient liquid culture of water or 0·4% NaCl for 4 hr.

However, we have found it impossible to eliminate all the hydroxyproline from the cells by this procedure. Therefore, the remaining cell hydroxyproline (3% of the total) must be present somewhere in the cell but elsewhere than in the vacuoles. This remaining hydroxyproline seems to be present in the form of a protein that is not degraded during morphogenesis. The results of Younis *et al.* (1979) indicate that cells of all stages during the life cycle contain 3–5% of the total vegetative hydroxyproline in this combined form.

The forms in which hydroxyproline occurs in the life cycle of the cellular slime mould can be divided into:

(a) Free hydroxyproline, comprising more than 95% of the total hydroxyproline, present in free form (Tables 1 and 2) and located in the vacuole (Tables 3 and 4). This free hydroxyproline is a characteristic component of vegetative cells since it is released during the pre-aggregation period (Younis *et al.* 1979).

(b) Combined hydroxyproline, comprising less than 5% of the total hydroxyproline, present in the protein (Tables 1 and 2) and undergoing no changes during the differentiation of vegetative cells to the mature sorocarp. This 3–5% of hydroxyproline was detected in the protein of the vegetative cells (Tables 1 and 2) and was the only remaining hydroxyproline in the fruiting body (Younis *et al.* 1979).

REFERENCES

Blumenkrantz, N. & Asboe-Hansen, G. 1973. A quick and specific assay for hydroxyproline. Analyt. Biochem. **55:** 288–91.

Cotter, D.A., Miura-Santo, L.Y. & Hohl, H.R. 1969. Ultrastructural changes during germination of *Dictyostelium discoideum* spores. J. Bacteriol. **100:** 1020–6.

George, R.P. & Hohl, H.R. 1972. Ultrastructural development of stalk producing cells in *Dictyostelium discoideum*, a cellular slime mould. J. Gen. Microbiol. **70:** 477–89.

Gregg, J.H. & Bronsweig, R.D. 1956. Biochemical events accompanying stalk formation in the slime mould *Dictyostelium discoideum*. J. Cell. Comp. Physiol. **48:** 293–300.

Gregg, J.H., Hackney, A.L. & Krivanek, J.O. 1954. Nitrogen metabolism of the slime mould *Dictyostelium discoideum* during growth and morphogenesis. Biol. Bull. **107:** 226–35.

Hames, B.D. 1972. The metabolic control of development in the cellular slime mould *Dictyostelium discoideum*. Unpublished Ph.D. thesis, Leicester University, U.K.

Hames, B.D. & Ashworth, J.M. 1974. The metabolism of macromolecules during the differentiation of myxamoebae of the cellular slime mould *Dictyostelium discoideum* containing different amounts of glycogen. Biochem. J. **142:** 301–16.

Hohl, H.R. 1965. Nature and development of membrane system in food vacuoles of cellular slime moulds predatory upon bacteria. J. Bacteriol. **90:** 755–65.

Ikeda, T. & Takeuchi, I. 1971. Isolation and characterisation of prespore specific structure of the cellular slime mould *Dictyostelium discoideum*. Development, Growth and Differentiation **13:** 221–9.

Killick, K.A. & Wright, B. 1974. Regulation of enzyme activity during differentiation in *Dictyostelium discoideum*. A. Rev. Microbiol. **28:** 139–65.

Lee, K.C. 1972. Permeability of *Dictyostelium discoideum* towards amino acids and inulin; a possible relationship between initiation of differentiation and loss of 'Pool' metabolites. J. Gen. Microbiol. **72:** 457–71.

Long, B.H. & Coe, E.L. 1974. Changes in neutral lipid constituents during differentiation of the cellular slime mould *Dictyostelium discoideum*. J. Biol. Chem. **249:** 521–9.

Loomis, W.F. 1974. *Dictyostelium discoideum*, a development system. Academic Press.

Maeda, Y. & Takeuchi, I. 1969. Cell differentiation and fine structures in the development of the cellular slime mould. Development, Growth and Differentiation **11:** 232–45.

Mizukami, Y. & Iwabuchi, M. 1970. Effects of actinomycin D and cycloheximide on morphogenesis and synthesis of RNA and protein in the cellular slime mould *Dictyostelium discoideum*. Exp. Cell Res. **63:** 317–24.

Sussman, M. & Sussman, R.R. 1969. Patterns of RNA synthesis and of enzyme accumulation and disappearance during cellular slime mould cytodifferentiation. Symp. Soc. Gen. Microbiol. **19:** 403–35.

Takeuchi, I. 1972. Differentiation and dedifferentiation in cellular slime mould. Aspect of Cell. Molec. Physiol. **1:** 217–36.

Watts, D.J. & Ashworth, J.M. 1970. Growth of myxamoebae of the cellular slime mould *Dictyostelium discoideum* in axenic culture. Biochem. J. **119:** 171–4.

Weeks, G. & Ashworth, J.M. 1972. Glycogen synthesis and the control of glycogen synthesis in the cellular slime mould *Dictyostelium discoideum* during growth (myxamoebal phase). Biochem. J. **126:** 617–26.

White, G.J. & Sussman, M. 1961. Metabolism of major cell constituents during slime mould morphogenesis. Biochim. et Biophys. Acta **53:** 285–93.

White, G.J. & Sussman, M. 1963. Polysaccharides involved in slime mould development. I. Water-soluble glucose polymers. Biochim. et Biophys. Acta **74:** 173–8.

Wright, B.E. & Anderson, M.L. 1960. Protein and amino acid turnover during differentiation in the slime mould. Biochim. et Biophys. Acta **43:** 62–6.

Younis, M.S., Ashworth, J.M. & Al-Rayess, H. 1979. Hydroxyproline in the life cycle of *Dictyostelium discoideum*. J. Univ. Kuwait (Sci.) **6:** 109–14.

(Received 15 April 1978)

موقع الهيدروكسي برولين في الخلايا الخضرية للفطريات الهلامية من نوع ديكتيوستيليوم ديسكويديوم

ميسون سليمان يونس* وجون اشوورث وهادي الريس**
قسم علوم الحياة بجامعة اسكس ، كولتشستر ، المملكة المتحدة

خلاصة

في هذا البحث ، ظهر ان الدكتيوستيليوم ديسكويديوم يحتوي على نوعين من الهيدروكسي برولين : الهيدروكسي برولين الحر الموجود في الفجوات ، والذي يشكل أكثر من ٩٥٪ من المجموع الكلي للهيدروكسي برولين الخلوى ، ويتحرر خلال المرحلة السابقة على مرحلة التجمع . والهيدروكسي برولين المتحد الذي يؤلف أقل من ٥٪ من المجموع الكلي للهيدروكسي برولين الخلوى ، وتبقى نسبته ثابتة خلال مرحلة التميز ، ويعتبر الشكل الوحيد للهيدروكسي برولين الموجود في الجسم الثمرى .

* العنوان الحالي : المعهد الطبي الفني ، باب المعظم ، بغداد ، العراق .
** العنوان الحالي : قسم وقاية النبات بكلية الزراعة ، أبو غريب ، بغداد ، العراق .