SPECIFIC WATER SOLUBILISATION OF CHOLESTEROL BY LIGHT GROWN Euglena gracilis

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Summary

Free and bound (esterified and water soluble) sterol fractions from light grown (green) and dark grown (white) Euglena gracilis are compared. Bound sterols constitute a high proportion of the total sterols of green and white E.gracilis, but with green E.gracilis alone there is a high specific binding of cholesterol predominantly in a water soluble form. The possibility of two distinct water soluble forms of sterols is considered.

A water soluble form of ergosterol has been shown to occur in yeast.[1] Further, yeast extract is capable of binding, in a water soluble form, exogenous ergosterol and cholesterol.[1,2] These water soluble forms are considered to be non-covalent saccharide complexes which can be destroyed by treatment with alkaline pyrogallol, silica gel chromatography, and solution in DMSO.

During our study of the metabolism of sterols in light

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grown (green) and dark grown (white) Euglena gracilis* we have found a form of sterols which is refractory to extraction with organic solvents, is soluble in water, and is extractable after treatment with alkaline pyrogallol. This water soluble sterol fraction accounts for a large part of the total sterols in E.gracilis. The composition of this water soluble form of sterols from white E.gracilis is similar to that of the free sterols. However, green E.gracilis shows a remarkable specificity for solubilisation of cholesterol.

Materials and Methods

E.gracilis Z was grown in a mineral medium containing agents and vitamins[4,5] into which air and CO_2 (5%) were passed. The dark grown culture had acetate added as carbon source. Light grown cultures were given 14 hours of light each day. Cells from both cultures of E.gracilis were harvested by centrifugation at the end of their logarithmic growth periods (7 days), washed, and treated in the above medium for 1 hour with similar amounts of 1-^{14}C-sodium acetate (2.10^9 dpm). Labelled cells from both cultures were lyophilised and thoroughly extracted with acetone and chloroform-methanol (2:1). The cell debris was extensively extracted with boiling water and the extract treated at room temperature with 2N-aqueous methanolic hydrochloric acid for 4 days. The acid solution was extracted with petroleum ether (bp 40-60°), neutralised, and concentrated, then refluxed for 2 hours in an alkaline aqueous methanolic pyrogallol solution.[2] The alkaline solution was extracted with petroleum ether.

Sterols from the above extracts were isolated by repeated

* Contrary to previous reports[3] ergosterol was hardly detectable in our extracts of E. gracilis.

preparative thin layer chromatography on silica gel monitored by radioscanning. A fraction containing sterol esters (fatty acids) was isolated, saponified and the sterols similarly separated. Further analysis was carried out by gas chromatography on 1% OV-17 at 270° and 1% XE-60 at 240° using tritium labelled acetates. Identification of cholesterol was confirmed by mass spectrometry.

Results and discussion

Some results of the above experiments are summarised in Table 1. From the Table it appears that a large proportion of the sterols in both green and white E.gracilis is present in

Table 1. Relative amounts and ^{14}C activities of sterol fractions from E. gracilis.

Sterols	Green		White	
	% of total*	^{14}C** dpm	% of total*	^{14}C** dpm
FREE	57	2.10^5	10	1.10^5
BOUND :				
Esterified	3	5.10^2	46	4.10^5
Water soluble - extracted after acid treatment	3	2.10^2	17	7.10^3
Water soluble - extracted after pyrogallol treatment	37	3.10^2	27	2.10^3

* Determined from gas chromatographic peak areas.

** Determined by liquid scintillation counting.

bound forms. In fact, bound sterols are much more important than free sterols in white E. gracilis. In all the fractions examined the sterol composition of white E. gracilis was very different from that of green E. gracilis. The sterol compositions of the bound forms from white E. gracilis were similar, and similar to the composition of the free sterol fraction. In each of the fractions cholesterol amounted to about 15%. Surprisingly this was not the case in green E. gracilis where cholesterol, which was only 1% of the free sterols, constituted more than 80% of the bound sterols. The most part of this cholesterol was found in the water soluble fraction after alkaline pyrogallol treatment.

It was found that the water soluble "sterol complex" from yeast extract was partially decomposed by acid treatment.[2] In E. gracilis, the relatively large amounts and higher specific activities of the sterols from the acid treated water extracts suggest that two different water soluble forms may be present.

These results underline the differences in sterol metabolism of white and green E. gracilis. The significance of these results, especially the remarkably specific water solubilisation of cholestero in green E. gracilis, is under investigation. To our knowledge such a high specificity in a bound sterol form has not previously been reported with the possible exception of esterified sterols in maize.[6]

More detailed results on sterol metabolism of white and green E. gracilis will be published shortly.

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