

It must be recognised, however, that the kinetics of acid-hydrolysis of these fragments of alginate are probably exceptionally simple in requiring only two rate-constants for their description, a fact which may be connected with their intramolecular, autocatalytic mechanism of hydrolysis.⁷ In general, significant differences can be expected between the rates of hydrolysis of internal and terminal linkages in the chains,¹⁰ and in some cases the rate of hydrolysis of a given linkage may also depend upon the identities of both the units that it adjoins.

Work is in progress on an expanded theoretical treatment which takes account of such differences, and which should extend the possible range of materials to which the method can be applied.

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Isolation of ³²P-Labelled Phosphorylthreonine from Ehrlich Mouse-ascites Tumour Cells Suspended in an Isotonic Medium Containing ³²P-Labelled Adenosine Triphosphate

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It was reported in a previous paper¹ that intact Ehrlich tumour ascites cells are capable of forming extracellular ATP in an isotonic medium containing the necessary substrates and cofactors. This supports the view that the enzymes concerned are located at the surface of the cells. The newly formed ATP cannot penetrate into the interior of the cells.¹ During efforts to find out possible functions for the *de novo* formed ATP in enzyme reactions at the cell surface, [³²P]phosphorylserine was isolated from the tumour cells.² Maximum labelling of phosphorylserine took place after relatively short incubation times. It is well known that the serine residue of the active center of several enzymes can be phosphorylated.³ It has also been possible to isolate phosphorylserine from such enzymes.³

The present paper describes the isolation of phosphorylthreonine from Ehrlich tumour ascites cells incubated with [³²P]ATP under similar conditions. The details of the incubation procedure have been described previously.^{1,2} The acid hydrolysate of the cells was chromatographed on a Dowex 50 column.⁴ A labelled peak was observed in the expected position for phosphorylthreonine.^{5,6} This labelled fraction was further purified by a second chromatography on Dowex 50. Fig. 1 illustrates the parallelism between the radioactivity and the ninhydrin reaction from a typical experiment. Further identification was obtained by high voltage paper electrophoresis using 0.1 N pyridine-acetic acid buffer as solvent, pH 5.0. A radioautogram was made of the electropherogram. As is seen from Fig. 2 one single ninhydrin spot was found on the electropherogram which had the same location as the radioactive spot.

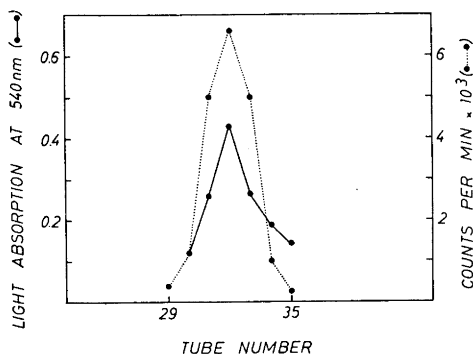


Fig. 1. Dowex 50 rechromatogram of acid hydrolysate of tumour cells incubated with [$\gamma^{32}\text{P}$]ATP. Column dimensions: 1.2×35 cm. Elution was performed with 0.01 M hydrochloric acid. ---, radioactivity; —, unlabelled phosphorylthreonine.

Table 1 illustrates the specific activity of phosphorylthreonine isolated from the tumour cells after different incubation periods. It is seen that maximum ^{32}P -labelled phosphorylthreonine was obtained with 30 sec incubation of the tumour cell suspension with the incomplete system¹ containing [$\gamma^{32}\text{P}$]ATP and unlabelled orthophosphate.² These data give evidence for an intermediate binding of the terminal phosphoryl group of ATP to phosphorylthreonine of a membrane protein of the tumour cells. The reasons are the following. The labelled ATP cannot penetrate the membrane to the cell interior.¹ Furthermore, [^{32}P]phosphorylthreonine could not

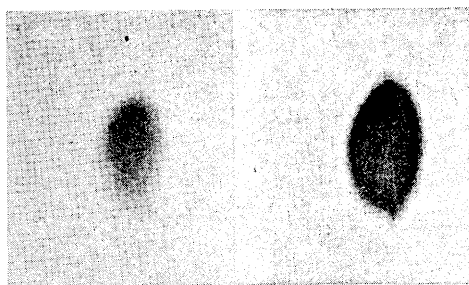


Fig. 2. Radioautogram (left) and electropherogram (right) of the peak in Fig. 1 (for details, see text).

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Table 1. Ehrlich mouse-ascites tumour cells incubated for various times at 37°C with [$\gamma^{32}\text{P}$]ATP in an isotonic medium. The medium contained 1×10^{-3} M orthophosphate. The incubations were terminated with perchloric acid and ^{32}P -labelled phosphorylthreonine (ThrP) was isolated from the acid insoluble material as given in the text. The figures denote the phosphoryl incorporation into phosphorylthreonine in $\mu\text{moles} \times 10^{-6}$ per mg dry weight (Schneider protein).

	5 sec ThrP	30 sec ThrP	60 sec ThrP	5 min ThrP
[^{32}P]ATP + incomplete system	0.16	0.49	0.36	0.28
incomplete system (^{32}P -labelled orthophosphate)	<0.01	<0.01	<0.01	0.01

be isolated from control experiments using (^{32}P)orthophosphate instead of [$\gamma^{32}\text{P}$]ATP in the incubation medium. A comparison with data for phosphorylserine isolated under the same conditions² shows that on an average the amount of phosphorylserine was 5 times that of phosphorylthreonine. The time for maximum incorporation seems to be the same for phosphorylthreonine and phosphorylserine (30 sec).

It is difficult from the present results to state whether one or several surface enzymes are reacting with ATP in the surrounding medium.

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