Rapid incorporation of [^{14}C]-glucose into a glucan-peptide complex of the rat diaphragm *in vitro*

By incubation of rat diaphragm in a medium containing [^{14}C]glucose it has been shown that ^{14}C is distributed into glycogen and CO_2 (ref. 1), as well as into lactic acid, hexose phosphates and an oligosaccharide fraction[2]. In the present work rapid distribution of ^{14}C from [^{14}C]glucose in the medium into two new compounds of rat diaphragm have been observed. One of these compounds has been identified as a glucan–peptide complex.

The experiments were performed as follows: 4 hemidiaphragms were preincubated for 15 min at 20° in phosphate–saline medium without addition of glucose, in order to remove free extracellular glucose. The diaphragms were then transferred to a vessel containing 2 ml phosphate-saline medium. The gas phase was oxygen; the incubation technique has been described earlier[3]. To each vessel was added 10 μC of [^{14}C]glucose (uniformly labelled) which was diluted to a specific activity of 3.6 μC/mg. Incubation was performed for 3 min at 30°. The diaphragms were then rapidly transferred to a stainless-steel mortar, frozen in liquid air, and ground. Extraction was performed with 0.6 N $HClO_4$, followed by centrigufation and neutralization of the supernatant. The supernatant was subjected to paper chromatography in different solvents and to electrophoresis on paper at different pH values. The radioactive zones were detected and quantitated with a paper-strip scanner, Radio Papierchromatograph Frieseke & Hoepfner, GMBH.

Fig. 1 represents the result of a typical radio paper chromatogram with butanol–acetic acid as the solvent. In addition to radioactive glucose and lactic acid two other radioactive compounds appear on the chromatogram, compound A and compound B. Compound A has been characterized as a complex between glucose and a peptide by the following observations. Compound A behaves as a single component in 5 different chromatographic systems. These include butanol–acetic acid–water (R_F, 0.03), phenol–water (R_F, 0.04), acetone–acetic acid–water (R_F, 0.70), picric acid–tert. butanol–water (R_F, 0.42), and butanol–ethanol–NH_3–water (R_F, 0.03). Also by electrophoresis at pH's 4.2 and 10.7, compound A migrates as a single component, negatively charged.

In further experiments compound A was purified on a preparative scale by the following procedure: (a) paper electrophoresis at pH 4.7, identification by autoradiography, and elution from the paper; (b) dialysis of the compound for 18 h against distilled water; compound A was found in the dialysate, while compound B was retained within the dialysis bag; (c) after concentration, the solution containing compound A was subjected to paper chromatography with butanol–acetic acid as the solvent. By this procedure compound A was separated from other radioactive material and from other compounds which gave ultraviolet absorption or positive staining reactions for phosphorus or ninhydrin. In particular, complete separation from hexose phosphates was obtained.

Compound A has been characterized as follows: (1) it possesses a net negative charge at pH's 4.2 and 10.7; (2) it gives a positive ninhydrin reaction; (3) it gives a positive reaction for carbohydrate on paper by spraying with anilin hydrogen phthalate[4], in solution a positive reaction for hexoses was obtained with the anthrone method[5]; (4) it is stable in 1 N HCl for 3 h at 110°. By hydrolysis in 2 N HCl for 8 h

at 110° compound A is split, and two main components are formed which chromatographically are identified as glucose and levulinic acid. Compound A is destroyed by treatment with 0.2 *N* NaOH for 4 h at 110°. (5) After complete hydrolysis of compound A in 6 *N* HCl for 16 h at 110° ten amino acids were detected by two-dimensional paper chromatography. (6) The presence of radioactive glucose in compound A was demonstrated chromatographically on a hydrolysed sample of the compound. No indication of other hexoses nor of glucosamine has been obtained. (7) Compound A gave no characteristic ultraviolet absorbtion at wavelengths between 240 and 300 mμ. No phosphorus was detected in a sample of compound A after wet ashing.

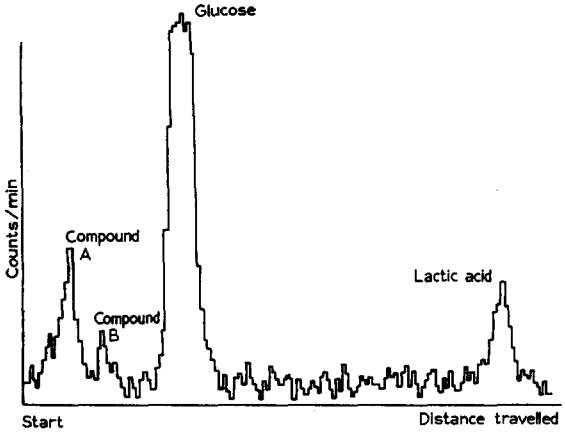

Fig. 1. Radio paper chromatogram of rat-diaphragm-muscle extract. Incubation of rat diaphragm in saline-phosphate medium containing 10 μC [^{14}C]glucose. Incubation time 3 min at 30°. The diaphragms were extracted in 0.6 *N* $HClO_4$. Paper chromatography of the supernatant in butanol–acetic acid–water (4:1:5).

According to these results compound A has been identified as a glucan–peptide or a glucan–polypeptide complex. The possible significance of this complex as a carrier in the "membrane transport" of glucose is under investigation. Recently similar hexose–protein complexes have been isolated from the membrane of yeast cells[6]. In this connection it seems of considerable interest that insulin added *in vitro* accelerates the incorporation of [^{14}C]glucose into compound A of rat diaphragm. An insulin effect of the magnitude of 200 % has been observed.

A complete report of this work will soon be published.

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