ALGAL PROTEIN HYDROLYSATE: ITS SUITABILITY AS A SOURCE OF PROTEIN PRECURSORS

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A high incorporation of radioactivity is observed into the protein fraction of rat pancreas pieces and isolated microsomes, when incubated with ^{14}C-algal protein hydrolysate. With isotope dilution of the amino acids, it can be shown that the incorporation found must be ascribed partly (pancreas pieces) or completely (isolated microsomes) to the labeled impurities, indicating that commercially available ^{14}C-algal protein hydrolysate as such is unfit as a source of protein precursors.

This explains the unusual behaviour of the incorporation into microsomes, viz. independency of energy and heat denaturation and RNAase insensitivity. The significance of similar observations reported by other authors with in vitro experiments with ^{14}C-algal protein hydrolysate is discussed.

Materials and methods

One rat pancreas is cut into pieces of approx. 20 mg each, washed, and incubated for 10 min. at 37°C in 3 ml Krebs-improved Ringer III medium (Krebs, 1950) with 2.5 µC ^{14}C-algal protein hydrolysate (0.2 mC/mg, The Radiochemical Centre, Amersham). The reaction is stopped by adding an equal volume of 10% trichloroacetic acid (TCA) and the precipitate treated according to Mans

and Novelli (1961). The protein is plated and the radioactivity is measured at infinitely thin layer with a Geiger-Müller counter (efficiency 5%).

Preparation and labeling of microsomes

One rat pancreas is minced and homogenized (Poort and Sangster, 1963) using a slitwidth of 0.15 mm in 12 ml sucrose medium (Hultin et al., 1961). The homogenate is centrifuged at 12000 x g for 6 min. and the microsomal pellet obtained at 150.000 x g for 90 min. The microsomal fraction is incubated at 37°C for 10 min. in 3 ml medium containing 3 µmoles ATP, 30 µmoles phosphoenolpyruvate, 50 µg pyruvate kinase, 2.5 µC ^{14}C-protein hydrolysate or DL-(^{14}C)leucine (36.6 mC/mmole, The Radiochemical Centre, Amersham) and the pH 5 enzyme obtained from the final supernatant according to Hoagland et al. (1956). After cooling, adding of 2 ml medium with cold amino acids and centrifugation at 105.000 x g for 30 min., the microsomal pellet is treated with TCA as mentioned before.

Results and discussion

When pancreas pieces are incubated with ^{14}C-protein hydrolysate, a high incorporation of radioactivity appears into the hot TCA insoluble fraction of the pancreas. It turned out, however, (Table I) that isotope dilution of the amino acids by means of a

TABLE I. Effect of isotope dilution on the incorporation of ^{14}C-protein hydrolysate into pancreas pieces

incubation system	counts/min/mg protein
complete	610
+ 100-fold excess cold amino acids	104

great excess of cold amino acids gives a substantial but not a complete reduction of the incorporation. This implies that the remainder must be attributed to the labeled impurities. At a similar conclusion arrived Craddock and Dalgliesh (1957), who attributed the high incorporation found with non-purified hydrolysate to labeled impurities, which were thought to be peptide-like in character.

A much greater influence of the impurities on the outcome of the experiments is observed in our results obtained with isolated microsomes. That most of the labeling of isolated microsomes is caused by the labeled impurities of ^{14}C-protein hydrolysate is demonstrated with isotope dilution and by the fact that ^{14}C-leucine is scarcely incorporated under the same conditions. A typical experiment is seen in Table II.

We think that our results with a cell-free system using ^{14}C-protein hydrolysate as a protein precursor source can shed new light on some results from the literature that have been difficult to explain. App and Jagendorf (1963), incubating ribosomes from spinach chloroplasts with ^{14}C-protein hydrolysate observed an energy independent incorporation, which moreover was RNAase insensitive, so that they were reluctant to identify the observed incorporation as protein synthesis. Burr and Finamore (1963) found

TABLE II. Incorporation of the amino acids of ^{14}C-protein hydrolysate into pancreas microsomes.

incubation system	counts/min/mg protein
complete	90
+ 100-fold excess cold amino acids	102
+ 2.5 µC ^{14}C-leucine*)	8

*) instead of ^{14}C-protein hydrolysate

on incubation of a 15.000 x g supernatant fraction from ovarian eggs of Rana pipiens an incorporation which was insensitive to heat denaturation of the cell-free system. According to these authors the observed incorporation is directly related to protein synthesis as is suggested by the close association of ^{14}C-activity with peptides derived from the proteins of the eggs. Abraham and Bhargava (1963) found that after the incubation of washed buffalo spermatozoa the incorporated radioactivity cannot be released by a 90 min. chase and they conclude that a synthesis of new proteins must take place, although Martin and Brachet (1959) found no incorporation at all in spermatozoa.

It became clear to us that the above mentioned results can be explained when the labeled impurities of ^{14}C-protein hydrolysate obscure the picture by such a close association with the proteins that even a treatment with hot TCA is not sufficient to remove the impurities from the resulting protein fraction. After incubation of isolated rat pancreas microsomes with protein hydrolysate we find an "incorporation" which is energy independent, RNAase insensitive, and not altered by heat denaturation of the system (Table III).

TABLE III. Influence of several factors on the labeling of pancreas microsomes by ^{14}C-protein hydrolysate.

incubation system	counts/min/mg protein
complete	90
- ATP, PEP, pyruvate kinase	103
+ RNAase (16 µg/ml)	95
heat denaturation*)	92

*) the microsomes and pH 5 enzyme were heated during 5 min. at 100°C before incubation.

When the microsomes after incubation with ^{14}C-protein hydrolysate are extracted with saline and the obtained extract is subjected to electrophoresis on agarose, we find that the radioactivity is closely associated with electrophoretically distinguishable proteins (Fig. 1). It makes no difference in the distribution of the

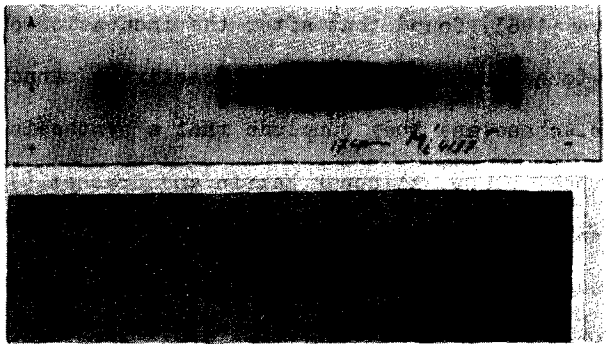

Fig. 1 (A) Electropherogram of a saline extract of rat pancreas microsomes, incubated with ^{14}C-algal protein hydrolysate. Electrophoresis on a microscope slide, covered with 2 ml 1% agarose in veronal buffer (pH 8.6, μ = 0.033), 30 min., 20 m.amp. (approx. 20 V/cm), stained with Amido Black B. (B) Kodak No-screen X-ray film after exposure to radiation of labeled proteins present in the electropherogram.

radioactivity whether or not an excess of cold amino acids had been added. This points towards a tight binding of radioactive impurities onto the proteins, which can erroneously be considered to be the result of an incorporation connected with protein synthesis. When we extract the pancreas pieces in the same manner and apply also electrophoresis to the extract, it appears that in this case the labeled impurities are not bound to the proteins present in the electropherogram , although the TCA treated proteins, as has been demonstrated before, contain labeled impurities. We must conclude that in our incubation system with pancreas pieces and probably with other cellular systems which are labeled in vitro with unpurified ^{14}C-algal protein hydrolysate one must be cautious

in interpreting the observations; control experiments including isotope dilution of ^{14}C-amino acids are indispensable. Furthermore it is evident from our experiments that in a microsomal system ^{14}C-algal protein hydrolysate cannot be used as a source of protein precursors.

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