Preparation of ^{32}P and ^{35}S labelled Coenzyme A*

Coenzyme A (CoA) has been implicated as a key compound in a large number of biochemical reactions[2]. Such biochemical reactions can be traced with great advantage if labelled CoA is available — particularly in cases[1] where the coenzyme reacts in concentrations not detectable with known color reagents on paper-chromatograms of the reaction mixtures. The facts that one molecule of CoA contains 3 atoms of phosphorus and one atom of sulfur and that commercially available CoA is obtained from micro-organisms suggest the possibility that it may be conveniently prepared labelled with ^{32}P and/or ^{35}S, two very cheap isotopes, through the micro-organisms. This has been achieved with yeast cells grown on a medium containing ^{32}P and ^{35}S labelled nutrients among others.

The medium used for the growth of the yeast cells had the following composition: Malt extract 15.0 g; Bactopeptone 0.78 g; Maltose 2.75 g; Dextrose 2.75 g; Glycerol 2.35 g; K_2HPO_4 1.00 g; NH_4Cl 1.00 g; $MgSO_4$ 0.25 g; water to 1000 ml; pH adjusted to 4.8. 150 ml of this medium was inoculated with several loopfuls of a young culture of *Saccharomyces cerevisiae* and grown for 24 hours at room temperature. Approximately 25 ml of this culture was added to 150 ml of the same medium in Erlenmeyer flasks containing 1 mc each of ^{32}P (as phosphates) and ^{35}S (as sulphate). Another set of flasks containing ^{35}S alone was also similarly inoculated. The flasks were shaken for aeration on a shaker for approximately 24 hours and the contents centrifuged. The supernatant was sterilized, reinoculated and grown for another 24 hours on the shaker to make most use of the radioactive tracers. The cells were then harvested, centrifuged and washed several times and lyophilized. The dry powder was scraped off the side of the flasks used for lyophilization and stored in a desiccator.

The method of extraction of the CoA from the lyophilized powder was essentially that of STADTMAN AND KORNBERG[3] modified for a micro scale operation. Some 300 mg of the lyophilized yeast powder was added to 2 ml of boiling water in a Pyrex centrifuge tube, boiled for 5 minutes, small chips of cracked ice added and the contents centrifuged in an International centrifuge at 2000 r.p.m. for 45 minutes. ^{35}S labelled CoA was also extracted into hot water in the same way.

Aliquots of the supernatant were then streaked along a line 4 cm away from the longer edge of Whatman's #3 filter paper as shown in Fig. 1, and chromatographed in the ascending way in 95% ethanol-Na-acetate-acetic acid-water (pH 3.7) in the ratio 1:1. A known sample of CoA (Pabst Laboratories) was spotted near the shorter edge of the paper for comparison. After development the paper was dried and exposed to Kodak "No-screen" X-Ray film contained in a Kodak exposure holder made of thick cardboard pieces. Another exposure holder containing an unexposed film was placed on top of it and the films developed after two weeks.

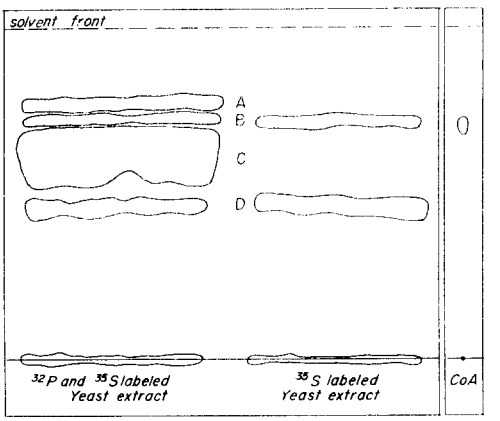

Fig. 1. Paper chromatograms of yeast extracts and of a known sample of CoA, using 95% ethanol-sodium acetate-acetic acid as solvent. The spots on the paper at left were detected by X-ray film, the spot at right was detected by nitroprusside test.

The left side of the chromatogram shown in Fig. 1 exhibits the labelled compounds present in the extracts of yeast cells grown on the medium containing ^{32}P and ^{35}S labelled compounds. Bands A and C correspond to very highly labelled compounds, probably phosphorylated sugars. Bands B on both sides of the chromatogram have the same R_F (0.73) as that of the known CoA sample detected by the nitroprusside reagent of TOENNIES AND KOLB[4]. Further confirmation of the suggestion that band B is due to CoA, is obtained from an examination of the film contained in the adjoining exposure holder separated from the paper chromatogram by two thick layers of cardboard pieces. If band B corresponds to a compound which contains both S and P then the left side of the film should show a band due to the fast (1.7 MeV) beta particles emitted by ^{32}P but no band on the right side as the soft (0.167 MeV) beta particles from ^{35}S would be absorbed by the cardboard layers. This is exactly what was found.

A comparison of the two sides of the film in contact with the chromatogram also revealed that in the case of band B on the left, both sides of the film exhibit darkening while that on the right had only one side darkened — the side in direct contact with the chromatogram. ^{14}C

and ^{32}P labelled compounds can also be distinguished in this way as ^{14}C is also a soft beta-emitter (0.155 MeV) and darkens only one side of the film.

The region of the paper chromatogram corresponding to band B on the radiogram was cut out, and eluted with water. The eluate contained fairly pure CoA of high specific activity. Biological experiments can be performed by the addition of carrier CoA.

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The enzymic hydrolysis of *iso*maltose. Its localization by means of paper electrophoresis

The assay for limit dextrinase as reported by BACK and co-workers[1] is long and tedious and any simplification of the method would be advantageous. An ideal substrate would be a simple carbohydrate in which hydrolysis could be measured by an increase in reducing power. STINSON[2] recently reported that *iso*maltose (6-O-α-D-glucopyranosyl-D-glucose) is rapidly hydrolyzed by partially purified limit dextrinase isolated from the culture medium of *Aspergillus niger* NRRL 330, and suggests that this disaccharide might serve as a substrate for limit dextrinase. Though the chemical structure of limit dextrin is not well known, it is recognized that this material does possess D-glucosidic linkages in the 1,6-position[3]. In addition, TSUCHIYA *et al.*[4] reported that *iso*maltose was hydrolyzed by a culture filtrate of *Aspergillus niger*. This note presents a brief report on the localization of the area on an "ionogram" which is responsible for the hydrolysis of *iso*maltose.

Filter-paper electrophoresis experiments[5] were performed on exhaustively dialysed culture filtrates of *Aspergillus niger* P.R.L. 558. The electrophoretic investigations were carried out in acetate buffer (pH 3.5, ionic strength 0.05) on a crude sample containing approximately 7.5 mg of protein. After the completion of a run, the paper strip was cut into 1 cm sections and extracted with 5 ml of pH 4.9 acetate buffer. Protein and enzymic activity were determined for each section.

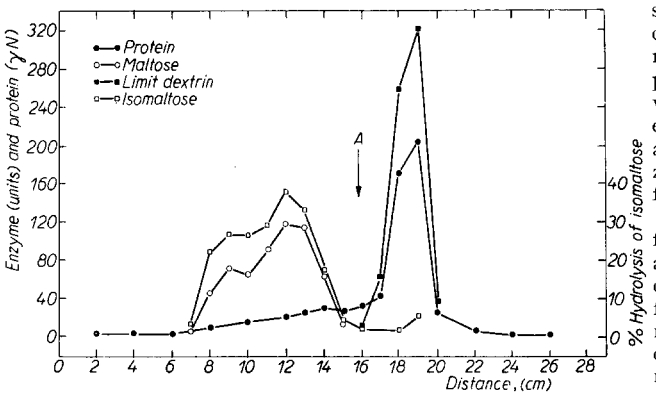

Fig. 1. A plot of the protein and enzymic zones on a paper electrophoresis showing the protein area (●——●) and the zones in which the hydrolysis of maltose (○——○), *iso*maltose (□——□), and limit dextrin (■——■) occurred.

Fig. 1 presents the data for a typical experiment. The arrow "A" represents the point of application after correction for electroosmotic effects. The major fraction is negatively charged at pH 3.5 while the minor ones carry a positive charge. The main protein component contains the limit dextrinase activity with a trace of maltase. From the figure it can be seen that the major portion of the maltase is found in the minor fraction. Though not shown in the figure, all the invertase activity was found in the minor protein component as well.