On the Biological Activity and Amino Acid Composition of Secretin

ERIK JORPES and
VIKTOR MUTT

Chemistry Department II, Karolinska Institutet, Stockholm, Sweden

We recently reported on secretin preparations having a strength of 7500 clinical units or 150 000 Hammarsten cat units (HCU) per mg. [1,2] They behaved in paper and starch gel electrophoresis and in two-dimensional paper chromatography as an almost homogeneous protein. Before making final conclusions about the purity of the product, it was considered desirable to check its homogeneity in other solvent and buffer systems and its behaviour in the Craig counter-current analysis. This has now been done and a fraction with a still higher activity, at least 2×10^4 clinical units or 4×10^5 HCU per mg has been isolated. One mequiv. of alkali will be secreted in response to 0.250 μg of secretin. The dose necessary for the clinical secretin test in man would be about 4 micrograms.

The starting material for this purification was the secretin-pancreozymin-cholecystokinin concentrate described by Jorpes and Mutt in 1957 [3]. This was purified essentially as described by Mutt in 1959 [4], *i.e.* separation of the secretin from the bulk of the impurities by extraction into methanol followed by chromatography of the methanol soluble material on carboxymethyl cellulose at pH 8.

60 mg of the purified material with an activity of 7×10^4 HCU per mg was subjected to a 60-transfer counter-current distribution in the system 0.1 M phosphate buffer/*n*-butanol, pH 7.0, each phase 10 ml. That secretin can be distributed in this system was demonstrated several years ago in this laboratory by Anders Vestermark.

The activity was found in tubes 18—30 and the bulk of the proteinaceous impurities in the first four tubes.

The active fractions were combined and 15 volumes of water added. The activity was adsorbed on alginic acid (0.5 g dry weight), eluted with 10 ml of 0.2 M HCl and the chloride exchanged for acetate on a column of DEAE-Sephadex in acetate form. After lyophilization the material weighed 3 mg and assayed at about 4×10^5 HCU per mg, which means that 1 mg could stimulate the secretion of 40 litres of 0.1 N bicarbonate solution.

A preliminary quantitative amino acid analysis according to the Spackman, Stein and Moore technique of 1958 was carried out by Mr. Börje Lindqvist at the Central Laboratory of Mjölkcentralen, Stockholm (Head: Prof. Torsten Storgårds).

Hydrolysis in 6 N HCl in evacuated sealed tubes during 67 h at 105°C. Not the slightest discoloration occurred. The following peaks came out, calc. as μmoles/mg lyophilized substance (Fig. 1): Lys 0.05,

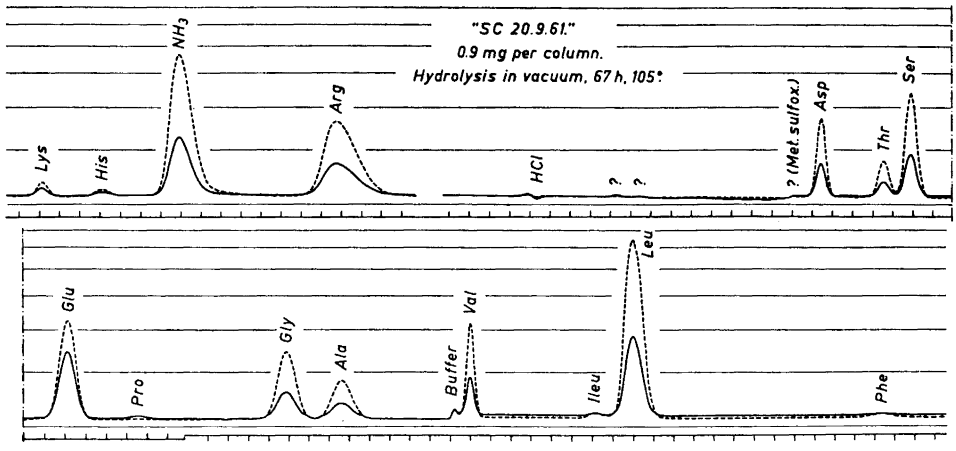

Fig 1.

His 0.03, NH_4 1.11 (3), Arg 0.96 (3), Asp 0.31 (1), Thr 0.16 (1?), Ser 0.52 (2), Glu 0.70 (2?), Gly 0.51 (2), Ala 0.29 (1), Val 0.27 (1) and Leu 1.56 (5).

This preliminary analysis seems to indicate that there are five moles of leucine, three moles of arginine and two moles each of glutamic acid and glycine per one mole of aspartic, acid, alanine and valine, and that proline, cystine, methionine, isoleucine, tyrosin and phenylalanine are absent. Tryptophane is known to be absent in preparations still containing isoleucine, tyrosin and a considerable amount of lysine [5].

At an early stage J. Sjöqvist, Lund, found that the content of leucine increased and that tyrosin disappeared during the purification process. Likewise Swan's reaction for cystine turned negative as found by A. Henschen of this laboratory. The traces of lysine and histidine still present indicate that a slight further purification could be achieved, possibly with a higher number of transfers in the counter-current distribution.

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Acid Phosphatases in *Escherichia coli*

BENGT v. HOFSTEN and JERKER PORATH

Institute of Biochemistry, University of Uppsala, Sweden

A cell-free extract of *Escherichia coli* will catalyze the hydrolysis of various monophosphate esters in a sodium acetate buffer at pH 4—5 [1-3]. We have found that the activity of this acid phosphatase is remarkably stable at low temperatures over a very wide pH-range. Thus no loss in enzymatic activity measured against *p*-nitrophenyl phosphate (Sigma 104) was encountered after 24 h at 4° in 0.2 M Na_2CO_3, pH about 11, or in 1 M acetic acid, pH 2.4, in the absence of salt. Repeated freezing and thawing of a crude cell extract was found to cause considerable losses of the phosphatase activity, and only 20—40 % could be recovered after lyophilization. However, solutions could be stored for several months at —10° in the presence of 60 % sucrose without detectable losses.

The interesting stability properties of the phosphatase activity prompted us to attempt its purification with the aid of gel filtration in acetic acid and zone electrophoresis. Sonicated cell-free extracts were prepared from cells grown in a succinate-inorganic salts medium essentially as described earlier by one of us [3]. The phosphatase activity was determined at a 0.01 M substrate concentration in 0.1 M sodium acetate buffer, pH 4.7. Gel filtration experiments [4] were performed on Sephadex G 75 (Pharmacia, Uppsala), and zone electrophoresis was carried out on vertical columns [5].

When an extract of *E. coli* was dialyzed against 0.1 M pyridinium acetate, pH 5, a heavy precipitate containing approximately 20 % of the total acid phosphatase activity was formed. If the supernatant solution was filtered through a column of Sephadex G75, in equilibrium with 1 M acetic acid, an interesting elution pattern was obtained. All the acid phosphatase activity was located in the region containing proteins, which pressumably have a lower molecular weight than those in the main, slightly turbid protein peak. Fig. 1 illustrates a gel filtration experiment in which 12 ml of solution was fractionated on a 22 × 500 mm Sephadex column