Free Amino Acids in Brewing Materials

L. Ljungdahl and E. Sandegren

AB Stockholms Bryggerier, Stockholm, Sweden

The free amino acids occurring in a sixrow barley (Stella) and in malt, wort and beer prepared from this barley have been quantitatively determined by means of chromatography on Dowex 50 [1].

The amino acids were determined in water extracts of barley and malt samples previously boiled in alcohol to inactivate the proteolytic enzymes. Barley contains very small amounts of free amino acids. Alanine, aspartic acid, asparagine and glutamic acid occur, however, in quite considerable quantities. Proline and γ-aminobutyric acid are also present in rather large amounts. During the malting there was a general increase of the free amino acids, but most remarkable was the increase of free proline.

The samples of wort, hopped wort, beer and beer refermented after addition of glucose, were treated with alcohol to precipitate proteins and carbohydrates before the determinations of the amino acids. During the mashing process there was a general increase of the free amino acids, which indicates that a considerable peptidase action occurred during the mashing. Arginine, leucine, lysine, phenylalanine and valine showed the greatest increase. When the wort was boiled with hops, there was an increase of some of the amino acids, particularly of aspartic acid, threonine and valine. There was a remarkable decrease, however, of arginine, histidine, lysine, glutamic acid and also of tyrosine during the boiling. A possible explanation for this decrease is that the boiling involves a reaction of amino acids and carbohydrates, with the formation of melanoidines. The fermentations were performed with bottom yeast *(Saccharomyces Carlsbergensis)*. During the first fermentation all of the amino acids decreased except γ-aminobutyric acid which increased. During the refermentation all amino acids decreased, but proline, γ-aminobutyric acid, glycine and alanine only to a rather small extent. In the chromatograms at least 10 unknown substances could be demonstrated, several of which are probably peptides. The results obtained in the fermentation experiments generally agree well with those of Barton-Wright [2] obtained with microbiological methods for the determination of the amino acids. The excretion of glutamic acid and the assimilation of glycine by the yeast found by Barton-Wright, however, could not be demonstrated in our experiments.

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2. Barton-Wright, E. C. *European Brewery Convention* **1949** 19.

The Incorporation Rate of Phosphorus into Phosphoproteins from Different Organs

Gunnar Ågren, Carl-Henric de Verdier and John Glomset

Institute of Medical Chemistry, University of Uppsala, Uppsala, Sweden

Earlier reported work [1] has been continued and the incorporation rate of phosphorus into phosphoproteins of rat brain, heart, intestinal mucosa, kidney, liver and spleen has been determined. The previously described procedure [1] has been followed which means that the specific activities have been determined in the phosphoserine and phosphopeptide fractions isolated on a Dowex 50 column after acid hydrolysis of the Schneider protein residues obtained from the different organs. In Table 1 the rate of P^{32}-incorporation in the liver phosphoserine fraction is illustrated.

Table 1. Specific activity of the phosphoserine fraction from rat liver at different times after the injection of 1 μC P^{32} per g body weight.

Time in hours	Specific activity in *cpm* per μg P
0.5	107
1	836
2	929
6	827
24	102
30	84

The highest value for the specific activity seems to be obtained in the liver. The phosphoserine and phosphopeptide fractions obtained from Dowex 50 columns are not homogeneous. Gradient elution from Dowex 1 in formate form [2] has enabled us to separate each of these fractions into several subfractions. Phosphoserine dominates the phosphoserine fraction, but otherwise each phosphopeptide from Dowex 50 seems to consist of several peptides with different amino acid composi-

tions. Further details of procedures and figures will be published in the near future.

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2. Busch, H., Hurlbert, R. B. and Potter, V. R. *J. Biol. Chem.* **196**, 717 (1952).

The Use of Ion-exchange Resin in the Purification of Hyaluronidase

Bertil Högberg

Research Department, AB Leo, Hälsingborg, Sweden

It has been shown that high molecular weight polyanions produce a complete reversible inhibition of hyaluronidase even in small amounts [1-4]. This led to the idea that a cation exchanger of a weakly acid type could be used for the isolation and purification of hyaluronidase.

Decapsulated ground bull testes were extracted with equal volumes of 0.1 *M* acetic acid. Extracts fractionated with $(NH_4)_2SO_4$. Fraction obtained between 30 and 70 % saturation was retained. An aqueous solution of this fraction was dialysed salt free. In order to denature proteins of high molecular weight the solution was vigourously shaken at pH 6 at room temperature for 15 minutes with 1/2 parts of chloroform without loss of activity. After centrifugation the upper aqueous layer was separated and dialysed against distilled water.

The hyaluronidase activity was determined as described by Diczfalusy *et al.*[3] The crude fraction had an activity of 700 VRU per mg N. The resin used was Amberlite XE-64 as the ammonium salt.

In a typical experiment 200 ml of crude hyaluronidase with a potency of 3 500 VRU per ml was run through a 1 cm × 11 cm Amberlite XE-64 column (2 g) at room temperature. Rate of flow: 1 ml per min. A gold-coloured impurity passed into the effluent. About 95 % of the activity was adsorbed on the resin. The resin was rinsed with 200 ml of water, 150 ml 0.1 *M* ammonium acetate, and 25 ml 0.1 *M* ammonia, respectively. The hyaluronidase was then eluated with minimal volumes of a 0.1 *M* KCl-HCl-buffer, pH 1.5.

On a nitrogen basis the eluate had en activity about 100 times that of the original material. The purified hyaluronidase fractions, thus obtained, are homogeneous in electrophoretic analyses. The protein impurity not adsorbed has neither activating nor inhibitory effects on purified hyaluronidase.

The method described showed that bacterial hyaluronidase from different origin cannot be adsorbed. These phenomena make it possible to separate testes hyaluronidase from bacterial hyaluronidase.

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On the Action of the Intestinal Flora on Conjugated Bile Acids

Arne Norman

Department of Physiological Chemistry, University of Lund, Lund, Sweden

Recently Bergström and Norman [1] have investigated the labelled products in bile and feces after injection of cholesterol-4-^{14}C in the rat. This compound is transformed and excreted into the bile to more than 90 % as taurocholic acid. However, only a minor part of the labelled product in feces consists of taurocholic acid. Most of the conjugates had been split and the cholic acid further modified. This is in accord with a number of older observations that the bile acids present in bile cannot be found in feces.

To obtain some information regarding the role played by the microorganisms in the biological splitting of the conjugated bile acids and the further modifications of the bile acid molecules, antibiotics were given to rats and the fecal bile acids were analyzed.

100 mg of terramycin and 250 mg of "sulftalyl"* was given by stomach-tube twice a day to rats. If the decrease in the total aerobic count was satisfactory, the rats were given 3—3.5 mg of cholic acid-24-^{14}C on the third day after administration of antibiotics. The feces were collected daily for five days. No increase in the amount of viable bacteria was found during this period. The labelled bile acid products were extracted and fractioned by reversed partition chromatography as described by Bergström and Norman [1].

Intraperitoneally administered cholic acid-24-^{14}C is excreted in the bile almost totally

* Pharmacia.