Amino Acid Analysis on Prorennin and Rennin

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In a previous paper[1] the purification and fractionation of prorennin by chromatography on columns of DEAE-cellulose was described. Further it was shown that the transformation of prorennin into rennin probably consisted in the splitting off of a minor part of the molecule.

Prorennin-B was prepared as described earlier. Aliquots were taken for nitrogen determination and amino acid analysis. Determination of amino acids was carried out by means of the Spinco-Beckman amino acid analyzer after 22, 48, and 76 h of hydrolysis with glass distilled, *ca.* 6 N HCl in vacuum-sealed tubes at 110°C.

The prorennin-B was transformed into rennin by being kept at pH 2 and 23°C for 5 min. The activation mixture was then chromatographed on a column of DEAE-cellulose. Of the nitrogen *ca.* 15 % was recovered in a front peak of peptides split off during the activation, *ca.* 10 % was found in an enzyme fraction corresponding to C-rennin, and 64 % was recovered in the peak of B-rennin. The fractions were analyzed for amino acid composition as described above.

Minimum molecular weights of prorennin and rennin were estimated on the basis of the molar ratios of certain amino acid residues found in the two proteins.

A molecule of prorennin-B of the estimated weight 36 200 appears to contain Lys_{13} His_5 Arg_7 Asp_{33} Thr_{21} Ser_{31} Glu_{36} Pro_{14} Gly_{29} Ala_{15} Val_{23} Met_7 $Ileu_{19}$ Leu_{26} Tyr_{18} Phe_{16}. B-rennin appears to have a molecular weight of 31 100 and to contain Lys_8 His_4 Arg_5 Asp_{31} Thr_{18} Ser_{27} Glu_{29} Pro_{12} Gly_{25} Ala_{13} Val_{21} Met_7 $Ileu_{15}$ Leu_{19} Tyr_{15} Phe_{14}. The contents of cystine and tryptophan are more uncertain. Preliminary results indicate three cystine bridges and two tryptophan residues in each of the two molecules. Agreement was found between the difference of the formulas suggested above and the molar ratios of amino acids in the mixture of peptides released during the activation of prorennin. No cystine and only traces of methionine were observed in the peptides. The high contents of lysine in the peptides split off during the activation are consistent with the previous observation that the isoelectric point of prorennin is higher than that of rennin. The proposed molecular weights for prorennin and rennin are consistent with tentative values calculated from ultracentrifuge studies. The sedimentation coefficients of prorennin and rennin found were $s_{20,w} = 3.5 \times 10^{-13}$ and 3.2×10^{-13}, respectively. The diffusion coefficient for rennin was estimated at 9×10^{-7}. From the sedimentation patterns that of prorennin appears to be of the same order.

Full details will appear in the *Compt. Rend. Trav. Lab. Carlsberg.*

1. Foltmann, B. *Compt. Rend. Trav. Lab. Carlsberg* **32** (1962) 425.

Differentiation of Free and Protein Bound Sulfonamide with the Aid of Sephadex G-25

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Dextran gels have found wide application in the separation of macromolecules from smaller molecules by gel filtration in column operation[1]. For the study of the extent of protein binding of loosely bound substances this technique is unsuitable as the equilibrium is displaced toward the unbound form during the passage through the column. This effect can be minimized by batch operation. Dry Sephadex powder is added to the protein solution. During the swelling of the Sephadex particles water and small molecules are taken up and protein molecules excluded. After separation of the gel particles by filtration with suction their content of sulfonamide is estimated by a standard procedure[2]. Procedure for serum: To 1.0 ml fresh serum in a test tube add 50 mg Sephadex G-25 Medium. Adjust pH by equilibrating for a few minutes with a gas containing 40 mm Hg CO_2. Stopper and keep on a water bath at 38°C for 45 min with occasional swirling. Filter on a small Büchner funnel. Transfer the Sephadex beads to another tube and proceed with the sulfonamide estimation. Proteinfree sulfonamide solutions were used as standards. The amount of protein trapped by Sephadex in this procedure was quite small corresponding to 7.2 ± 3.4 µl ($\pm 2s$) solution. No sulfonamide adsorption effects were noted. This method is discussed in relation to ultrafiltration and dialysis methods. The use of Sephadex for estimating protein binding of other substances is discussed.

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1. Flodin, P. *Dissertation*, Uppsala 1962.
2. Bratton, P. H. and Marshall, Jr., E. K. *J. Biol. Chem.* **128** (1939) 537.