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Giraffe pantheatic ribonuclease

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SUMMARY

Comparative studies of proteins, may not only provide information on the evolution, but also on the structure function relationship of these proteins. Here the isolation and purification of the ribonuclease from giraffe pancreas is reported. The pure pancreatic ribonuclease was studied as to its primary structure and glycoprotein nature. Giraffe pancreatic ribonuclease was also used in enzyme kinetic studies.

These studies are part of a project, with the ultimate goal to investigate the evolution of the enzyme pancreatic ribonuclease. The systematic comparative study of pancreatic ribonucleases however has a drawback, namely the uneven distribution of this enzyme within the mammals. To date the primary structure of the pancreatic ribonucleases from 21 species is known.

Part of the sequence studies on giraffe ribonuclease were performed with the use of automated machines. The usefullness of these machines in the sequence studies of pancreatic ribonuclease is discussed, together with the change in strategy and tactics necessary in the amino acid sequence studies of larger proteins if these machines are applied.

Giraffe pancreatic ribonuclease was isolated by ammonium sulfate precipitation, purification was obtained by affinity chromatography. The obtained protein was very pure as judged by different techniques. It was not possible to purify giraffe pancreatic ribonucleases by chromatography on cationexchangers.

The tryptic digests of the aminoethylated protein and a thermolysin digest of the oxidized protein were used to solve the complete amino acid sequence. Giraffe pancreatic ribonuclease consists of three components with the same amino acid sequence but different amounts of carbohydrate. The sugar residues in these carbohydrate chains were determined. Enzym kinetic studies show some differences in the kinetic parameters of giraffe and bovine ribonuclease. These data were compared with data from the literature.

The eleven differences found between the primary structures of giraffe and bovine pancreatic ribonuclease are discussed. Two of these substitutions are very interesting in respect with the results of studies with synthetic N- and C-terminal

peptides of bovine ribonuclease.

It is shown that homologeous proteins like the pancreatic ribonucleases may vary remarkably in the presence or absence and the amount of carbohydrate. From this, the conclusion was drawn that physicochemical properties of proteins may not be used in comparative studies, if possibly a carbohydrate chain is present.

The ribonuclease from okapi pancreas was isolated and purified in the same way as giraffe pancreatic ribonuclease. Unfortunately the complete amino acid sequence of this protein could not be solved, since only a small amount of okapi pancreatic ribonuclease was obtained.

ABBREVIATIONS

$A_{280\ nm}$, etc.	- absorbance at 280 nm etc.
DNS-, dansyl-	- dimethylamino-naphtalene-sulphonyl-
PITC	- phenylisothiocyanate
PTC-	- phenylthiocarbamyl-
PTH-	- phenylthiohydantoin-
Tris	- tris(hydroxymethyl)-aminomethane

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