Smith-Degraded Cartilage Proteoglycan as an Acceptor for Xylosyl Transfer

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The first reaction in the biosynthesis of the polysaccharide chains of the chondroitin sulphate proteoglycan is the transfer of xylose from UDP-xylose to serine hydroxyl groups of a peptide acceptor. Xylose transfer to endogenous acceptors has been shown in several tissue systems (Grebner, Hall & Neufeld, 1966; Robinson, Telser & Dorfman, 1966) and, more recently, exogenous acceptors have become available, including the tripeptide Ser-Gly-Gly and peptides from the carbohydrate–protein linkage region of the cartilage proteoglycan (Baker & Rodén, 1970). A far better acceptor has been isolated by Smith degradation of the intact proteoglycan in the following way. Proteoglycan from bovine nasal cartilage (Hascall & Sajdera, 1969) was oxidized with 0.05M-sodium periodate at pH 5.0 in 0.25M-sodium perchlorate for 3 days (Scott, 1968). After dialysis the product was reduced with borohydride, and after acidification to pH 1.0 the solution was kept at room temperature for 3 days. Little or no peptide-bond cleavage occurred under these conditions. The protein core of the proteoglycan was recovered in the void volume from a column of Sephadex G-200, and was free of chondroitin sulphate, although keratan sulphate chains remained.

The Smith-degraded proteoglycan was a substrate for xylosyl transfer from UDP-[^{14}C]xylose in the chick-embryo-cartilage system of Robinson *et al.* (1966). This exogenous acceptor has been of particular value in the purification (Stoolmiller, 1971) of the soluble fraction of the xylosyltransferase that constitutes 80–90% of the total activity of a cartilage homogenate.

In a survey of the substrate specificity of the particulate xylosyltransferase (sedimenting between 10000*g* and 100000*g*), many proteins were tested and found to be inactive as acceptors. However, ribonuclease B stimulated incorporation into endogenous as well as exogenous acceptor three- to four-fold, but was not itself an acceptor. The stimulatory effect was due to solubilization of xylosyltransferase, and after repeated treatments of the 100000*g* pellet with ribonuclease the amount of solubilized enzyme approached that present in the soluble fraction of the homogenate.

When chromatographed on Sephadex G-200 the ribonuclease-solubilized enzyme emerged at an effluent volume corresponding to a molecular weight of approx. 120000.

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An Evaluation of the Enzymes Involved in Glutamine Metabolism by the Kidney

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There are two major pathways by which the amide and amino groups of glutamine are liberated. Deamidation by the glutaminase I enzyme yields glutamate and ammonia (Carter & Greenstein, 1947). Transamination of the α-amino group with a keto acid yields 2-oxoglutaramate, which is deamidated by ω-amidase to form 2-oxoglutarate and ammonia (Meister, 1953). Glutaminase I is associated with the mitochondria and is activated by bivalent inorganic anions and organic acids (O'Donovan & Lotspeich, 1966). In characterizing the nature of glutaminase I from rat kidney, we have obtained evidence to indicate that there may be more than one form of this enzyme existing within the mitochondrion.

Rat kidney mitochondria were prepared by differential centrifugation. After washing in a sucrose medium, the mitochondrial equivalent of 1g of kidney tissue was diluted to 0.25ml with tris buffer, pH 8.0, and was sonicated for 2min at 4°C. The disrupted mitochondria were then centrifuged at 110000*g* for 30min. The supernatant and resuspended pellet were incubated with glutamine, and the ammonia liberated from glutamine during incubation was determined.

The addition of sodium deoxycholate (0.1%) to the mitochondrial suspension before sonication decreased the ammonia production from glutamine by the pellet and the supernatant. When 0.17M-sodium phosphate was present in the incubation medium the glutaminase activities of the supernatant and the pellet were increased. However,