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- $[^{14}\text{C}]$ 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 10000g and 100000g), 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 100000g 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-oxoglutarate, 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 110000g 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,

the enzyme activity of the supernatant was raised threefold more than that of the pellet. The further addition of deoxycholate decreased the phosphate activation of glutaminase. This decrease was greater for the pellet enzyme than for the supernatant enzyme.

The supernatant and pellet enzymes were then preincubated at elevated temperatures for various times. Preincubation at 60°C for 10 min almost completely destroyed the glutaminase activity of the pellet, but only slightly decreased the enzyme activity of the supernatant. In the presence of phosphate similar heat treatment almost completely destroyed the enzyme activity of both supernatant and pellet. These results indicate that the enzyme which is released in the supernatant is different from that which remains in the pellet.

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Mechanisms of Ammoniogenesis in Human Kidney

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It has been demonstrated that glutamine is the major and direct source of renal ammonia production in man (Owen & Robinson, 1963). Two distinct metabolic pathways of glutamine metabolism have been demonstrated in the kidney of the rat, guinea pig and dog. The predominantly intramitochondrial glutaminase I isoenzymes that hydrolyse glutamine to ammonia and glutamate and the subsequent deamidation of glutamate to ammonia and 2-oxoglutarate constitute the major metabolic route in the rat (Goldstein, 1967). However, the extramitochondrial glutamine aminotransferase- ω -amidase pathway (glutaminase II) has been shown to play an important role in the dog (Bourke, Frindt, Rubio-Paez & Schreiner, 1971). In human kidney glutaminase I activity has been demonstrated (Mattenheimer & De Bruin, 1964), but values obtained have ranged widely owing to enzyme instability in autopsy specimens. We obtained healthy portions of renal cortex from patients undergoing nephrectomy. Washed kidney mitochondria were incubated with glutamine (70 mM) in tris buffer, pH 8. Significant deamidation of glutamine occurred. Ammonia production increased by 75% in the presence of maleate and

fivefold in the presence of phosphate. This suggests that, as has been demonstrated in the rat, there are two isoenzymes of glutaminase I in man.

Previously there has been no direct evidence for a glutaminase II pathway in human kidney. We investigated this pathway using substituted glutamine, ω -N[¹⁴C]-methyl-L-glutamine. In contrast with glutamine, this compound on deamidation yields methylamine. Initial studies indicated that this compound was not metabolized by human kidney glutaminase I. The demonstration by Meister (1954) that it was deamidated by rat liver glutaminase II suggested its application for investigating the glutaminase II pathway in human kidney slices. Slices were incubated in Krebs-Ringer phosphate medium, pH 7.4, at 37°C under continuous bubbling with O₂+CO₂ (95:5) with added glutamine or ω -N[¹⁴C]-methylglutamine (70 mM). Significant quantities of either ammonia or methylamine production were demonstrated. In the presence of pyruvate (39 mM), a keto acid utilized in the glutaminase II pathway, methylamine production increased by over 50%, as did ammonia production. Under these circumstances methylamine production amounted to 10% of ammonia production, indicating a functionally important glutaminase II pathway in human kidney.

Malonate, which leads to accumulation of tissue 2-oxoglutarate, decreased ammoniogenesis from glutamine. High concentrations of fluorocitrate and pyruvate, which also inhibit the rate of utilization of 2-oxoglutarate, similarly depressed ammoniogenesis, supporting the postulate that 2-oxoglutarate utilization affects the activity of glutaminase I. Inhibition of methylamine production was also demonstrated under these circumstances, suggesting a similar control by 2-oxoglutarate on glutaminase II activity.

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The Permeability of Mitochondria to Carnitine and Acetylcarnitine

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Three distinct spaces may be operationally defined in mitochondria that have been sedimented into a pellet. Polymers such as dextran (mol.wt.