SHELL GLAND CATECHOL OXIDASE FROM RAJA ERINACEA

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An enzymic activity capable of oxidizing polyphenols and catechols to quinones has been demonstrated in tanning capsules and shell glands of several elasmobranchs. Brown reported that upon incubation in a solution of tyrosine, sections of skate (Raja spp.) egg capsule turned brown (Quart. J. microscop. Sci. 96, 483-488, 1955). The solution also turned brown and both reactions could be prevented by the addition of potassium cyanide. Polyphenol oxidase was demonstrated histochemically in shell glands of Scyliorhinus canicula by incubating sections of fixed glands with catechol (Threadgold, J. Histochem. Cytochem. 5, 159-166, 1957). Krishnan showed that both capsular material and sections of frozen glands from Chyloscyllium griseum oxidized catechol (Biol. Bull. 117, 298-307, 1959) and Rusaouën found that fresh sections of Scyliorhinus canicula shell glands oxidized both dihydroxyphenylalanine and pyrocatechol (J. exp. mar. Biol. Ecol. 23, 267-283, 1976). The enzyme is thought to catalyze the initial event in the tanning of selachian egg capsules thereby playing a pivotal role in the development of capsular material properties.

We have biochemically identified a catechol oxidase in extracts of tanning capsules from Raja erinacea and showed that it oxidized a variety of o-dihydroxyphenols to their corresponding o-quinones (Koob and Cox, The Bulletin 24, 78-80, 1984). In this report we describe the extraction and activation of this enzyme from shell glands of the little skate.

Materials and Methods

Shell glands from spawning little skates were minced over ice and then homogenized with a glass-glass homogenizer in 45 ml of 0.05M NaH_2PO_4, 1.0M NaCl, pH 7.4. Homogenates were centrifuged at 35,000g at 4°C for 30 min. The supernatant, which appeared cloudy and slightly pink, was assayed directly for catechol oxidase activity.

Catechol oxidase activity was measured by incubating diluted aliquots of the extract with 4-methylcatechol in 0.5M NaCl, 0.025M NaH_2PO_4, pH 7.4 at ambient temperature as previously described (Koob and Cox, op. cit.). Absorbance at 400 nm was monitored over the incubation period and compared to controls which had been boiled for one minute prior to incubation. In some experiments chymotrypsin (40-100 ug) was added and allowed to preincubate for periods up to 20 minutes.

Results

Shell gland extracts oxidized 4-methylcatechol and this activity was destroyed by heating to 100°C. Oxidation rates increased with increasing amounts of extract. Oxidation did not commence immediately upon addition of the substrate, but occurred only after a delay the duration of which depended on the amount of extract. These results suggested that an activation process was necessary for oxidation and that this process was

initiated by substrate addition.

Chymotrypsin was added to shell gland extracts in order to determine whether proteolytic cleavage resulted in activation of the catechol oxidase. Figure 1 shows that chymotrypsin significantly reduced the activation time following substrate addition. Incubates containing 40 ug of chymotrypsin began oxidizing 4-methylcatechol 1.75 minutes earlier than corresponding control incubates. Furthermore, initial rates of oxidation by chymotrypsin treated samples were significantly higher than control samples.

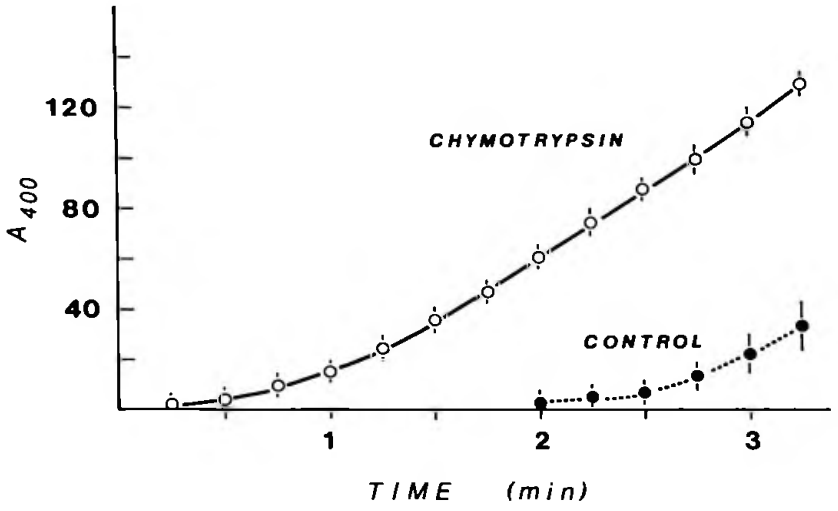

Figure 1. Initial rates of oxidation of 4-methylcatechol by shell gland extracts with and without added chymotrypsin (40ug).

As indicated in Figure 1, control incubates generated active catechol oxidase several minutes after the addition of substrate. This observation suggested that shell gland extracts were capable of autoactivation. Support for this suggestion was obtained by assessing oxidation rates of fully activated control and chymotrypsin treated incubates (Figure 2). Rates of oxidation in control incubates were not different from those of incubates containing 40, 60, 80 or 100 ug of chymotrypsin.

Discussion

These measurements suggest that Raja erinacea egg capsule catechol oxidase is synthesized in the shell gland as a zymogen which can be activated by proteolytic cleavage. They also suggest that the shell gland produces a native activator of procatechol oxidase which can be extracted along with the oxidase. It is unclear how activation affects the procatechol oxidase; however, since chymotrypsin mimics activation, it would appear the native activator is a proteolytic enzyme. Further studies will be necessary in order to delineate the nature of the activation process.

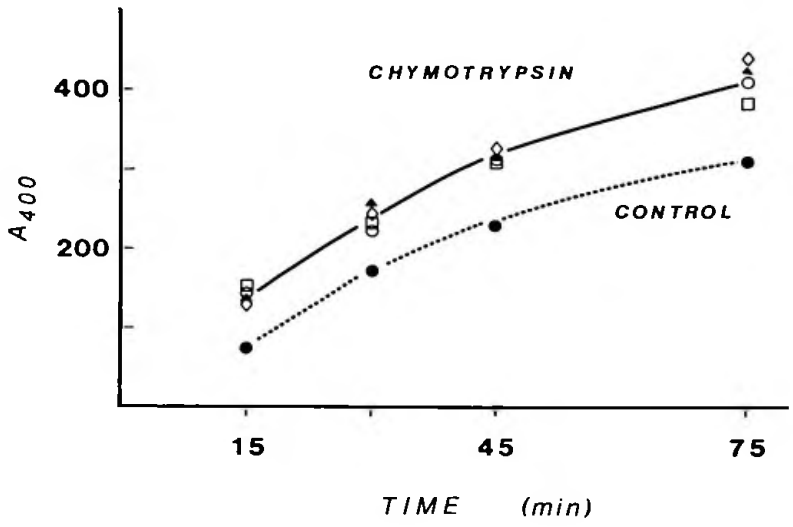

Figure 2. Maximal oxidation rates of 4-methylcatechol by shell gland extracts without additions or with 40 (◇-◇), 60 (▲-▲), 80 (○-○) or 100 ug (□-□) of chymotrypsin.

These observations also suggest that activation of shell gland procatechol oxidase requires the presence of the native substrate and that the substrate may initiate the activation process. This interdependence of proenzyme-activator-substrate for egg capsule tanning could provide a well controlled system for capsule formation. Capsule precursors are packaged in granules which coalesce following secretion. This type of activation system would ensure that the procatechol oxidase-activator complex remain inactive until it mixes with the substrate during capsule formation. Future experiments will determine if substrate activation of the procatechol oxidase activator occurs in this system. This work was supported by a grant from the Lucille Markey Charitable Trust and M.D.I.B.L. to T.J.K. and D.L.C.