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Ionic Effects on α_2-Macroglobulin Binding of Cationic Aspartate Aminotransferase

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Boyde and Latner (1962) reported that up to three zones of aspartate aminotransferase activity could be demonstrated by starch-gel electrophoresis of serum. One of these zones was coincident with the "slow α_2" band, and is now known to be due to binding of the cationic (mitochondrial) isoenzyme by α_2-macroglobulin (Boyde, 1967). Certain other proteins are known to be bound by α_2-macroglobulin, viz. trypsin, chymotrypsin (Haverback *et al.*, 1962, Schulze *et al.*, 1963, Mehl *et al.*, 1964, Ganrot 1966a, 1966b), papain (Potter *et al.*, 1960), plasminogen (Steinbuch *et al.*, 1965), thrombin (Lanchantin *et al.*, 1966), and certain non-specific hydrolytic enzyme activities of plasma (Lawrence *et al.*, 1960, Lawrence, 1964). In none of these publications is there clear evidence that binding occurs in the conditions of temperature, pH, and electrolyte concentrations which obtain in the plasma in life.

In the case of cationic aspartate aminotransferase, binding has only been demonstrated in dilute borate buffers (Boyde, 1967). Since macroglobulin is a glycoprotein (Schönenberger *et al.*, 1958, Demaille *et al.*, 1966), it would be expected that complexation with borate would occur, leading to an increased net negative charge on the molecule, and hence to the formation of a more stable complex than in the absence of borate, or even to the formation of a complex *de novo*. This paper describes briefly the experiments undertaken to establish whether binding of cationic aminotransferase, trypsin and chymotrypsin is a physiological phenomenon.

A preparation of cationic isoenzyme from human liver (Boyde, 1968) was added to human serum in the proportion of 100 or 200 mu. to 4 ml. serum. (One unit (u.) is taken as the quantity of enzyme which is able to convert 1 μmole per minute of aspartate to oxaloacetate under the assay conditions given by Boyde (1968)). After ultracentrifugation at 45,700 g. (Spinco Model L, SW 39L head, 24,000 rev./min.) for 17 hours at a rotor temperature of 37°C, the whole of the added activity was recovered in the sediment. There was no such sedimentation of cationic isoenzyme in water, 0.9% saline, 10% bovine albumin, 0.2% gamma macroglobulin, or serum preparations lacking α_2-macroglobulin; but addition of purified α_2-macroglobulin to these media resulted in sedimentation as for whole serum. Anionic isoenzyme (Boyde, 1968) was not sedimented under any of the conditions cited above.

Serum which had been previously dialysed against distilled water showed a somewhat lower capacity to sediment cationic aspartate aminotransferase than did whole serum, and the addition of sodium chloride caused a further sharp reduction in the proportion of cationic isoenzyme appearing in the sediment (55% throughout the range from 25 to 400mM NaCl). The contrast between these results and those with whole serum (containing approximately 100 m-equiv./l. chloride and 140 m-equiv./l. sodium ion) indicated that there was a dialysable factor in serum capable of potentiating binding. An ultrafiltrate of serum was prepared. This proved capable of restoring the binding capacity of dialysed serum to that of whole serum at the same dilution, and at the same sodium chloride concentration. A portion of serum ultrafiltrate was acidified, extracted with ether, the etheral extract evaporated to dryness and the residue redissolved in water. This preparation contained almost the whole of the binding-potentiator activity of the original serum ultrafiltrate.

Mixtures were prepared of dialysed serum with various concentrations of sodium phosphate buffer, pH 7.4, and sodium borate buffer, pH 8.4, and these were subjected to ultracentrifugation in admixture with cationic isoenzyme. In the case of phosphate, there was a progressive fall in the proportion of cationic isoenzyme sedimented as the ionic concentration was increased. With borate buffers there was at first an increase in the proportion sedimented, the maximum being at 25–30mM borate, and thereafter a progressive reduction.

Experiments were also done with trypsin (1 mg.) dissolved in 4 ml. normal or dialysed serum. Over 80% of the recovered activity was found in the sediment after ultracentrifugation. When both cationic aspartate aminotransferase (100 mu.) and trypsin (2 mg.) were ultracentrifuged in 4 ml. normal serum, the sediment contained over 80% of the recovered trypsin activity and none of the aminotransferase. In similar experiments with chymotrypsin, about 70% of the recovered chymotrypsin activity was found in the sediment: the proportion was not significantly changed by the addition of cationic aspartate aminotransferase, but a small proportion (approximately 10%) of the aminotransferase was sedimented.

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These results presumably indicate that both trypsin and chymotrypsin are more avidly bound by α_2-macroglobulin than is cationic aspartate aminotransferase under physiological conditions, and that probably trypsin is more avidly bound than chymotrypsin. Similar results were obtained by electrophoretic experiments (25mM borate, pH 8.4, 4.5% polyacrylamide gel) in which the addition of sufficient trypsin, chymotrypsin, or papain to mixtures of serum with cationic isoenzyme displaced the latter from combination with α_2-macroglobulin so that it appeared in the position of free cationic isoenzyme.

The relative affinity of trypsin and papain for α_2-macroglobulin is not known.

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