above are in agreement with observations on the inhibition of collagen fibril formation caused by the œstrogens[3] and on the decreased collagen synthesis in female rats as compared with males[4].

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Glutamic–Aspartic Transaminase of Pig Heart Muscle

THE glutamic–aspartic transaminase of pig heart muscle has been more than 98 per cent resolved into apoenzyme and co-factor by carrying out a differential heat inactivation in phosphate buffer in the course of purification of the enzyme. The apoenzyme has been purified 64-fold, by relatively simple and reproducible techniques. The highest purification factor previously reported[1] for the apoenzyme is 26. The specific activity of the purified apoenzyme compares favourably with that of an electrophoretically homogeneous preparation of the holoenzyme[2]. Cammarata and Cohen's suggestion[3] that purification of the apoenzyme necessarily results in partial inactivation has been disproved, since it is possible to account for all the enzyme activity among the various protein fractions obtained at each step in the purification.

The initial rate of reaction between aspartate and α-ketoglutarate is linearly dependent on the concentration of the purified transaminase only up to 0·004 mgm. protein c.c.$^{-1}$. Above this level the dependence of reaction-rate on enzyme concentration deviates markedly from a linear relation. For the reverse reaction, the dependence of the reaction-rate on enzyme concentration is linear up to 0·01 mgm. protein c.c.$^{-1}$. This phenomenon has been reported for crude enzyme preparations[3,4]; but its consequences have not been considered in any kinetic studies reported hitherto. The following evidence does not support the suggestion[4] that the non-linear dependence of reaction-rate on enzyme concentration is due to the existence in solution of an equilibrium between active and inactive forms of the enzyme. First, the initial rate of reaction is unaffected by reagents known to break down protein aggregates, for example, benzene, ionic detergents and high salt concentrations. Second, when the same low concentration of pyridoxal-5′-phosphate (4γ c.c.$^{-1}$) is used for reactivation of a concentrated and dilute solution of the apoenzyme, equivalent amounts of enzyme taken from the two preincubation mixtures give the same initial rate of transamination on addition to the substrates. This shows that if aggregates are formed at high protein concentrations the aggregation is such that the sites occupied by the co-factor are not masked. Third, the proposed equilibrium between active and inactive forms of the enzyme must be established very rapidly since the observed initial rates of reaction depend only on the enzyme concentration in the reaction mixture and not on the concentration of the enzyme during the period of preincubation with the co-factor.

The variation of the rate of reaction with enzyme concentration is independent of the purity of the enzyme and is unaffected by the addition of a boiled extract of pig heart muscle. It is therefore unlikely that the non-linear dependence of reaction-rate on enzyme concentration is due to the presence of an inhibitor in the original protein extract.

Bonavita and Scardi[5] concluded that the co-factor was attached to the enzyme through the 4-formyl group and that this group was not free to take part in Schiff's base formation with the substrates as suggested by Snell and co-workers[6]. This conclusion was based on the absence of inhibition of transamination by cyanide ions when added, in excess, to the reactivated enzyme followed by 12 hr. dialysis before assay, whereas inhibition did occur when the cyanide ions were preincubated with the co-factor before reactivation of the apoenzyme. In the present work, using cyanide ions and pyridoxal-5′-phosphate in equimolar concentrations, the same degree of inhibition (75 per cent) was observed both when the cyanide ions were added to the reactivated enzyme and when the co factor was incubated with cyanide prior to reactivation of the apoenzyme.

The equilibrium constants for the reaction between the apoenzyme and the two forms of the co-factor have been measured under conditions in which there can be no shift in the equilibrium during the assay of the holoenzyme concentration. Previous determinations of the equilibrium constants[1,7] have been unsatisfactory because such precautions have not been taken. It appears that the true equilibrium constant for pyridoxal-5′-phosphate is some ten times greater than that for pyridoxamine-5′-phosphate.

Braunstein[8] has suggested that the equilibrium constant for the overall transamination reaction depends on the purity of the transaminase preparation used. The equilibrium constant has been determined using a highly purified enzyme solution, and the mean value of K, from some twenty determinations, is 6·50, in close agreement with the value of 6·74 found by Krebs[9] using a crude extract of horse heart muscle.

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