INFLUENCE OF AN S-(α,β-DICARBOXYETHYL) CYSTEINE RESIDUE ON THE RESOLUTION OF PIG-HEART ASPARTATE AMINOTRANSFERASE *

A. Ballio, G. Marino and V. Scardi

Laboratorio di Chimica delle Sostanze Naturali
Istituto Chimico dell'Università di Napoli, Naples, Italy

Received October 10, 1966

As reported by Scardi et al. (1963), pig-heart aspartate aminotransferase (EC 2.6.1.1) prepared according to Jenkins et al. (1959) is not resolved into coenzyme and apoenzyme when heated 1 min at 60°C in the presence of 20% ammonium sulfate. These conditions, on the contrary, had been previously successfully used to resolve aspartate aminotransferase preparations obtained by the procedure of O'Kane and Gunsalus (1947). The different behavior was then ascribed to the fact that during the purification method of Jenkins et al. (1959) the enzyme is totally converted into the phosphopyridoxal form, in which the coenzyme is tightly bound to the apoenzyme through an azomethine linkage between its 4-formyl group and an ε-amino group of a lysine residue of the apoenzyme (Hughes et al., 1962). As the phosphopyridoxamine form of the aspartate aminotransferase, where such a linkage is obviously absent, is resolved under very mild conditions (30 min at 30°C in 0.5 M KH_2PO_4, pH 4.75), a quite simple resolution procedure was developed for the Jenkins et al. enzyme preparation; this involved a quantitative conversion of the phosphopyridoxal form into the phosphopyridoxamine form by preincubation with a slight excess of an amino acid substrate (Scardi et al., 1963).
In the course of a chemical and physico-chemical study of ox-heart

* Supported by a grant of C.N.R., Rome, Italy.

aspartate aminotransferase, recently prepared in a state of high purity (Marino et al., 1965, 1966 a), it has been observed that the phosphopyridoxal form of this enzyme preparation can be almost completely resolved by heat treatment at 62°C in the presence of 20% ammonium sulfate; the phosphopyridoxal form of the pig-heart aspartate aminotransferase is again unaffected by this stronger treatment. In the latter case, as a consequence of the addition of maleate during the purification procedure, one thiol group (over a total of 6 present in the molecule, on a basis of a m.w. of 55.000) is converted into the corresponding S-(α , β -dicarboxyethyl) derivative (Turano et al., 1964). The procedure used for the preparation of the ox-heart aspartate aminotransferase, in contrast, affords an enzyme in which all thiol groups (5 for a m.w. of 48.000) are free (Marino et al., 1966 b). It was therefore postulated that the modification caused by maleate to pig-heart aspartate aminotransferase might have been responsible for the failure in resolving it; to test this hypothesis, pig-heart aspartate aminotransferase purified in the presence of succinate instead of maleate was prepared (Turano et al., 1964) and tested for resolvability. As shown in Fig. 1 the enzyme preparation, which is in the phosphopyridoxal form, can now be resolved under the conditions used for the same form of the ox-heart aspartate aminotransferase; when treated with maleate according to Turano et al. (1964) it loses this property almost entirely (the resolved portion may be the consequence of an incomplete reaction with maleate).

These results firmly demonstrate that the resolution of pig-heart aspartate aminotransferase is prevented by the formation of an S-(α , β -dicarboxyethyl) derivative. Therefore, the observed stability to resolution of pig-heart aspartate aminotransferase prepared according to Jenkins et al. (1959) is not a characteristic of the enzyme but the consequence of a structural modification of a thiol group, which obviously plays a specific role in the stability of the coenzyme-apoenzyme linkage. Some evidences (e.g. solvent dependancy of the Schiff bases stability) suggest that this linkage is protected against hydrolysis by the hydrophobic character of the surrounding region. Such a region is probably made more unaccessible to water by a local conformational change following the introduction of the

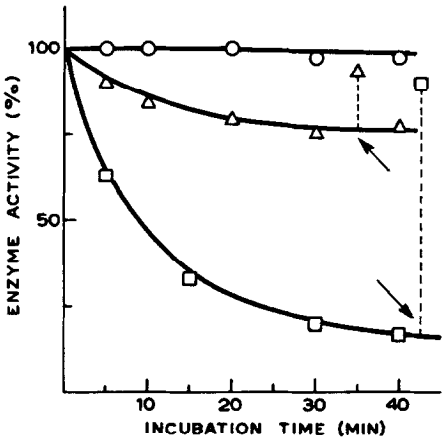

Figure 1. Action of 20% ammonium sulfate on the phosphopyridoxal form of pig-heart aspartate aminotransferase prepared in three different ways : (○) according to Jenkins et al. (1959), (□) same as above except that succinate was used instead of maleate, and (△) by treating this latter with maleate according to Turano et al. (1964). Enzyme concentration in the incubation mixture was about 0.2 mg/ml. Arrows indicate the addition of an excess of pyridoxal 5-phosphate before assaying enzyme activity. This was performed spectrophotometrically at 26°C according to Jenkins et al. (1959).

S-(α , β -dicarboxyethyl) group. Work aimed to define the molecular environment of the S-(α , β -dicarboxyethyl)-cysteine residue in the molecule of pig-heart aspartate aminotransferase is in progress in this laboratory.

REFERENCES

Hughes, R. C., Jenkins, W. T., and Fischer, E. H., Proc. Natl. Acad. Sci. (U.S.) 48, 1615 (1962).

Jenkins, W. T., Yphantis, D.A., and Sizer, I. W., J. Biol. Chem. 234, 51 (1959).

Marino, G., Greco, A. M., and Scardi, V., Boll. Soc. It. Biol. Sper. 41, 438 (1965).

Marino, G., Greco, A.M. , Scardi, V., and Zito, R., Biochem. J. 99, 589 (1966 a).

Marino, G., Scardi, V., and Zito, R., Biochem. J., 99, 595 (1966 b).

O'Kane, D. E., and Gunsalus, I.C., J. Biol. Chem., 170, 425 (1947).

Scardi, V., Scotto, P., Iaccarino, M., and Scarano, E., Biochem. J., 88, 172 (1963).

Turano, C., Giartosio, A., Riva, F., and Fasella, P., Biochem. Biophys. Res. Comm., 16, 221 (1964).