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SKELETAL ORGANIC MATRIX: A TARGET FOR TBT TOXICITY

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Trisubstituted organotin compound as tri-*n*-butyltin (TBT) which are widely present as antifouling paints are responsible for many deleterious effects to aquatic organisms. Although several countries have restricted its use, the current contamination of sea water is still substantial in a range of 0.3 to 2 nmol l^{-1} TBT in coastal and harbour of the Mediterranean area. TBT was demonstrated to induce oyster shell abnormalities as early as 1974 (1-3), however, the mechanisms of its action on calcification remain unknown (4). A possible target of these compounds was thought to be the skeletal organic matrix. Indeed, the presence of organic matrix appears to be a prerequisite step for the formation and growth of biominerals (5). Krampitz *et al.* (6) demonstrated that the gelatinous substance contained in the chambers of TBT-induced malformed oysters differed from the organic matrix of healthy organisms by having a lower proportion of aspartic acid. To understand the mechanism of action of TBT on calcification, we investigated its effect using microcolonies of scleractinian corals as a model of biomineralization. Such a model is extensively used in our laboratories for both fundamental (7-10) and applied (11-12) purposes.

Since acidic amino acids are the most abundant amino acids in the organic matrix of scleractinian corals (13), we used labelled aspartic acid as a tracer for organic matrix. Several parameters were followed simultaneously: aspartic acid (^{14}C-asp) and calcium (^{45}Ca) uptake by coral tissues, rate of protein synthesis, rate of organic matrix incorporation into coral skeleton and rate of calcification (as measured by ^{45}Ca incorporation). Figure 1 shows the kinetics of action of 10 µmol l^{-1} TBT on these parameters compared to controls. While TBT only weakly affected asp and calcium uptake (results not shown), this compound dramatically reduced instantaneously the synthesis of proteins and their subsequent incorporation into skeleton, and the calcification process as measured by ^{45}Ca incorporation into skeleton.

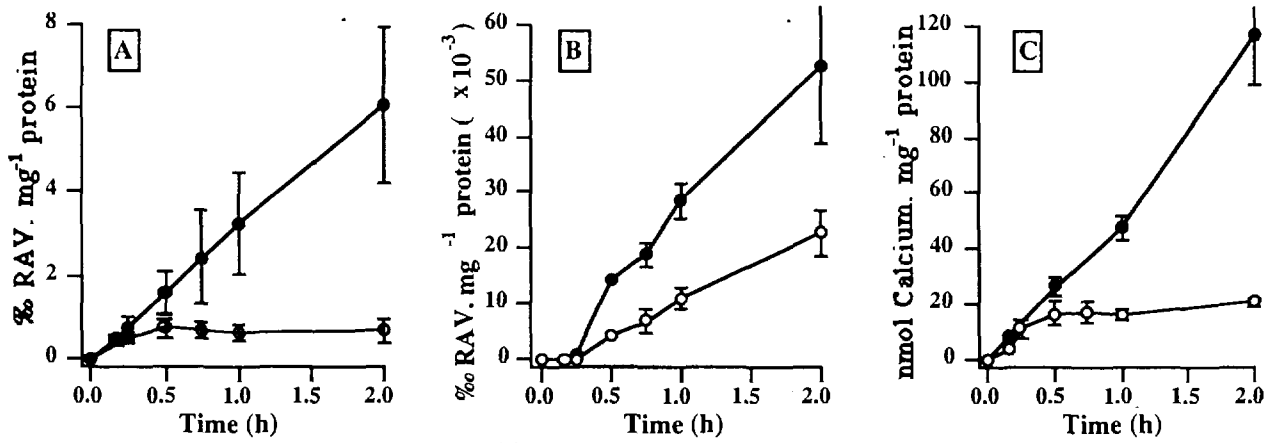

FIG. 1. Effect of tributyltin (TBT) on ^{14}C-asp incorporation into tissue (A) and skeletal (B) proteins and ^{45}Ca incorporation into coral skeleton (C). ●: control, ○: 10 µmol l^{-1} TBT. TBT was added at time 0. Results are expressed as fraction of total radioactivity initially present in the external medium (expressed as ‰) related to the protein content of the sample. Values are means ± S. D., n = 3.

A dose-response of TBT effect showed that the most sensitive process to TBT was protein synthesis (IC_{50} = 0.2 µmol l^{-1}), followed by skeletogenesis (IC_{50} = 3 µmol l^{-1}) and calcium uptake by coral tissue (IC_{50} = 20 µmol l^{-1}). These results suggest that the primary target of TBT could be

protein synthesis. To test this hypothesis, the action of TBT was compared with that of the protein synthesis inhibitor, cycloheximide. We tested the effect of TBT and cycloheximide, either alone or in combination, on protein synthesis and incorporation into skeleton and calcification. TBT was used at 1 µmol l^{-1}, a concentration which allows an incomplete inhibition of protein synthesis. Results shows that their effect was in no case additive, suggesting a common target.

These results suggest that the primary target of TBT in calcifying animals could be protein synthesis whose inhibition led to inhibition of organic matrix formation and consequently reduced skeletogenesis.

REFERENCES

(1) ALZIEU, C. L., THIBAUD, Y., HÉRAL, M., BOUTIER, B., Rev. Trav. Inst. Pêches marit. 44 (1980) 301-348.

(2) ALZIEU, C., HÉRAL, M., THIBAU, D. Y., DARDIGNAC, M. J., FEUILLET, M., Rev. Trav. Inst. Pêches marit. 45 (1982) 101-116.

(3) RODRIGUEZ, C. R., LOPEZ, F. C., Rev. Trav. Inst Pêches marit. 47 (1983) 89-98.

(4) ALZIEU, C., Mar. Environ. Res. 32 (1991) 7-17.

(5) WEINER, S., ADDADI, L., TIBS 16 (1991) 252-256.

(6) KRAMPITZ, G., DROLSHAGEN, H., DELTREIL, J. P., Experientia. 39 (1983) 1105-1106.

(7) GOIRAN C., AL-MOGHRABI S., ALLEMAND D., JAUBERT J., J. Exp. Mar. Biol. Ecol., 199 (1996) 207-225.

(8) AL-MOGHRABI S., GOIRAN C., ALLEMAND D., JAUBERT J., J. Exp. Mar. Biol. Ecol. 199 (1996), 227-248

(9) TAMBUTTÉ, É., ALLEMAND, D., BOURGE, I., GATTUSO, J.-P., JAUBERT, J., Mar. Biol. 122 (1995) 453-459.

(10) TAMBUTTÉ, É., ALLEMAND, D., MUELLER, E., JAUBERT, J., J. Exp. Biol. 199 (1996) 1029-1041.

(11) ROMAINE S., TAMBUTTÉ É., ALLEMAND D., GATTUSO J.-P., Mar. Biol. 129 (1997), 175-182.

(12) ACOSTA, A., FOWLER, S., TEYSSIÉ, J.-L., JAUBERT, J., 8th Int. Coral reef Symp. , Panama June 24-29, (1996) 2.