Aspartate Transcarbamylase from Human Tumoral Cell Lines: Accurate Determination of Michaelis Constant for Carbamylphosphate by Intercept Replots

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ATCase catalyzes the second reaction of the de novo pyrimidine pathway that is the carbamylation of the amino group of aspartate by carbamylphosphate. The kinetic parameters of ATCase were investigated previously in the dialyzed cell-free extracts of 10 different human normal and tumoral cell lines (1). PALA, a transition state analogue of ATCase substrates (6), has been extensively used as an antitumor agent in clinical investigations (3, 7, 8, 17, 18). Previous studies showed that different normal and tumoral cell lines exhibit large differences in sensitivity to PALA which cannot be accounted for by their differences in ATCase specific activities (1, 10, 11, 13). In addition, it has been shown that these differences in sensitivity to PALA cannot be attributed to an intrinsic molecular property of ATCase (1). In particular, in contrast to what had been reported previously using human and rodent cells (2, 4, 9, 12, 14–16), no significant difference could be detected in the affinity for carbamylphosphate of the ATCase present in the extracts of 10 different human normal and tumoral cell lines (1). However, the value obtained in the case of a rectal adenocarcinoma cell line (HRT18) was beyond the S.D. of the results obtained with the 10 cell lines (10 µM compared to 5.9 ± 2.5 µM), and it was interesting to determine whether this difference was significant. The importance of that point derives from the fact that PALA competes with the substrates carbamylphosphate for binding to the catalytic site of ATCase (1, 6, 9). Consequently, the affinity for carbamylphosphate of HRT18 ATCase was accurately reinvestigated in comparison with that of a melanoma cell line (Bell) by the intercept replot method (5).

Saturation curves for carbamylphosphate were determined in the presence of varying concentrations of L-aspartate, and the Kₘ values obtained from the corresponding Lineweaver and Burk double-reciprocal plots were replotted against the inverse of aspartate concentration and extrapolated to the infinite concentration of this substrate (Chart 1). The variation of the apparent Kₘ for carbamylphosphate as a function of aspartate concentration is consistent with the previously reported indication of an ordered mechanism for human ATCase. The values of the ordinate intercepts were calculated using a computerized linear regression program. These values, 6.98 and 5.12 µM, obtained for the ATCases present in the dialyzed cell-free extracts of HRT18 and Bell cells, respectively, are not significantly different and fall into the S.D. calculated previously (1).

In conclusion, it appears that, among all the human normal and tumoral cell lines tested (3 fibroblasts, 4 melanoma, and 3 colorectal carcinomas), the differences in sensitivity of the ATCase activity to PALA cannot result from a difference in affinity of this enzyme for carbamylphosphate. Since these differences cannot be attributed to any of the other tested enzymatic properties of ATCase (1), they might result from variations in the intracellular pools of carbamylphosphate. This hypothesis is currently under investigation.

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