

bited by B₂ at concentrations as low as 5 µg/ml, as shown in Table I. At 25 µg/ml, B₂ became cytotoxic. Viable cells were reduced to 3% of the starting cells scored 72 h later. This cell line is obviously very sensitive to B₂. NBT assay indicated that B₂ is not an inducer of terminal differentiation toward granulocyte lineage (12).

Like other active anticancer components of Antineoplaston formulations, B₂ also showed a profound effect on the cancer isozyme MAT^{LT} purified from HL-60 cells. The inhibitory effect is shown in Fig. 2. The inhibitory effect of B₂ is analogous to acidic peptides of Antineoplaston formulations (1), in that it selectively converted MAT^{LT} into MAT^L while affecting very little the activity of MAT^L, as shown in Table II. Therefore, B₂ can be considered as a selective inhibitor of cancer methylation complex isozymes. This study establishes that B₂ can exert a direct anticancer effect totally unrelated to its major role as coenzymes. The anticancer effect is probably attributable to its selective inhibition of cancer methylation complex isozymes.

Discussion

B₂ is not stable in alkaline solution (14). Urine stored for a prolonged period is usually alkaline. This is why recovery of B₂ and its derivative in Antineoplaston formulations is variable. Although B₂ and its derivative are just very minor active components of Antineoplaston formulations, the anticancer effect of B₂ represents an interesting biological effect apparently unrelated to its major role as coenzymes.

The anticancer effect of B₂ is attributable to a direct inhibitory effect on MAT^{LT}, as demonstrated in this study. B₂ acts as a selective inhibitory effector to convert MAT^{LT} into MAT^L while affecting very slightly the activity of MAT^L. This is the kind of inhibitor the authors have been searching for as selective cancer chemotherapeutic agents. It is

Table II Effect of B₂ on the kinetic parameters of methionine adenosyltransferases.^a

Addition (mg/ml)	MAT ^{LT}		MAT ^L	
	K _m	V _{max} (%)	K _m	V _{max} (%)
None	18.5	11.1 (100)	3.1	0.89 (100)
B ₂ (0.1)	3.8	6.0 (54)	3.1	0.85 (96)
B ₂ (0.25)	3.3	5.6 (51)	3.1	0.82 (92)

^a MAT^{LT} was purified from promyelocytic leukaemia HL-60 cells and MAT^L was purified from mouse spleen by DEAE-cellulose chromatography, as described in a separate paper (9). Aliquots of purified enzymes were allowed to mix with the indicated amounts of B₂ in an ice-bath for 10 min. Aliquots (20 µl) of these enzyme-B₂ mixtures were then added to 80 µl of incubation mixture containing various amounts of [³H]methionine for the determination of its effect on kinetic parameters. Concentrations of [³H]methionine employed for the assay of MAT^{LT} were between 5.6 and 28 µM and the specific activity of [³H]methionine was 1.38 Ci/mmol. Concentrations of [³H]methionine employed for the assay of MAT^L were between 1 and 5 µM, and the specific activity of [³H]methionine was 3.89 Ci/mmol. The amount of enzyme in each tube represented a preparation from HL-60 cells containing 1.2 µg DNA (MAT^L). The incubation and assay were conducted as previously described (13). The unit of K_m is µM methionine, and that of V_{max} is pmol AdoMet formed per min per µg DNA.

apparent that the growth promotion as cofactors of flavoprotein enzymes and the inhibition of neoplastic growth as an inhibitory effector of cancer methylation complex isozymes are unrelated biological effects of B₂. In B₂ deprived hosts, the growth promotion may prevail, whereas in B₂ supplemented hosts the inhibition of neoplastic growth may become apparent. If malignant phenotypes can be modified by B₂ to become non-malignant, it has achieved its therapeutic purpose. Growth promotion by B₂, if proven to be non-malignant type of growth, should not pose a problem for cancer patients. The authors were unable to demonstrate the induction of terminal differentiation in the lineage of granulocyte by B₂. However, B₂ was shown to induce morphological changes examined under the electron microscope