E. coli Dam activity in Hepes buffer asks for a new unit definition

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The *E. coli* DNA-adenine-methyltransferase (Dam) is an enzyme that exerts multiple functions in the cell (1). Formerly Tris/Cl- or Phosphate-buffer have been used for purification and assays of the protein. We found that Hepes/KOH-buffer renders Dam about 3 times more active, than the conventional buffer, which has been used for the unit definition (2; Fig. 1).

Hepes buffer does not only confer higher activity to the enzyme, moreover Dam activity decays considerably slower, than in Tris-buffer. The specificity of Dam is not changed at all, as proved by the incapability of the enzyme to methylate DNA isolated from a *dam+* strain (data not shown). Therefore we suggest new conditions for the unit assessment of the *E. coli* Dam methyltransferase:
50 mM Hepes/KOH pH 7.8, 1 mM EDTA, 1 mM DTT, 200 mM KGlu, 5% glycerol, 0.25 mg/ml calf thymus DNA and 6 μM S-adenosylmethionine. 1 unit Dam methyltransferase catalyses the transfer of 1 pmole 3-H methylgroups to DNA in a form that can be acid precipitated in Whatmann GF/C filters in 10 minutes.

REFERENCES

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ABBREVIATIONS

Hepes	2-[4-(2-Hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid
KGlu	Potassium glutamate
Tris	Trishydroxymethylaminomethane

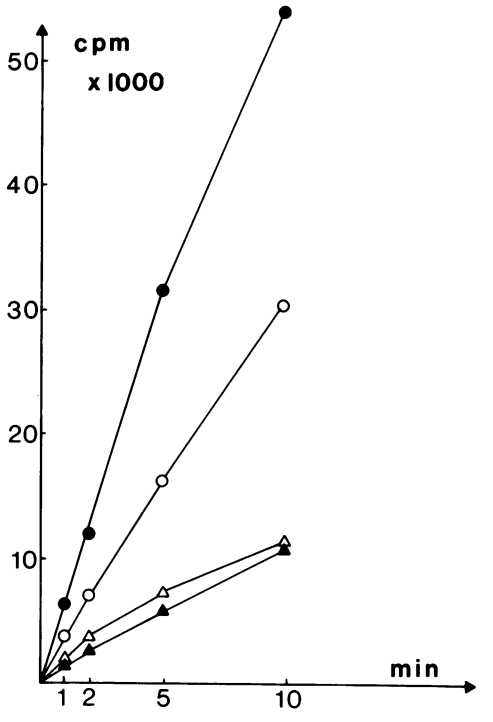

Figure 1. Incorporation of methylgroups into calf thymus DNA catalyzed by the Dam enzyme in Hepes- and Tris/Cl-buffer at steady state conditions. Buffer conditions were: 50 mM Hepes with (●) or without KGlu (○), or 50 mM Tris/HCl with (▲), or without KGlu (△), 1 mM EDTA, 1 mM DTT, 5% glycerol, 0.25 mg/ml calf thymus DNA, 6 μM S-adenosylmethionine. Results comparable to those with Tris- were obtained with phosphate-buffer.