Isolation and stability of ternary complexes of elongation factor Tu, GTP and aminoacyl-tRNA

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Intact, native EF-Tu, isolated using previously described methods and fully active in binding GTP, was never found to be fully active in binding aminoacyl-tRNA as judged by high performance liquid chromatography (HPLC) gel filtration and zone-interference gel-electrophoresis. In the presence of kirromycin, however, all these EF-Tu.GTP molecules bind aminoacyl-tRNA, although with a drastically reduced affinity. For the first time, the purification of milligram quantities of ternary complexes of EF-Tu.GTP and aminoacyl-tRNA, free of deacylated tRNA and inactive EF-Tu, has become possible using HPLC gel filtration. We also describe an alternative new method for the isolation of the ternary complexes by means of fractional extraction in the presence of polyethylene glycol. In the latter procedure, the solubility characteristics of the ternary complexes are highly reminiscent to those of free tRNA. Concentrated samples of EF-Tu.GMPPNP.aminoacyl-tRNA complexes show a high stability.
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