Fluorescent labeling of the nucleotide site in cytosolic rat liver phosphoenolpyruvate carboxykinase

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Reaction of rat liver phosphoenolpyruvate carboxykinase (GTP: oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32) with the alkylating fluorescent probe N-(iodoacetylaminoethyl)-5-naphthylamine-1-sulfonic acid (1,5-I-AEDANS), results in complete loss of enzymatic activity. One mole of the fluorescent reagent is incorporated per mole of the inactivated enzyme. When the modification is carried out in the presence of GDPMn, the enzyme retains 97% of its activity with almost no incorporation of label. The specificity of the reaction is further supported by the detection of a unique fluorescent peptide from the trypsin-treated modified enzyme. Fluorescence emission of enzyme-bound AEDANS shows a broad band centered at 470 nm and presents a monoexponential decay with a lifetime of 19 ns. These data indicate that the probe-binding site is considerably less polar than water and similar in polarity to ethanol. Anisotropy determinations give evidence for restricted rotational freedom