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Facilitated Purification of Hypoxanthine Phosphoribosyltransferase

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Summary: Three major approaches to the complete purification of hypoxanthine phosphoribosyltransferase from human erythrocytes and rat brain are described. Preparative isolelectric focusing which has been used for the isolation of the human enzyme was not fully successful in the case of rat brain. Preparative polyacrylamide-gel electrophoresis in gel blocks yields enzyme samples of high purity as judged by analytical gel electrophoresis, but with a comparatively low specific enzyme activity. The most rapid and convenient method, a modification of the affinity chromatography on GMP agarose first described by Hughes[5] gives hypoxanthine phosphoribosyltransferase which is superior to the other preparations in its homogeneity and its specific activity. All three methods produce an identical enzyme protein detected by polyacrylamide electrophoresis on nondenaturing and sodium dodecylsulfate gels. Molecular data of hypoxanthine phosphoribosyltransferase derived from these studies are: Isoelectric points of 5.60; 5.85 and 5.90 for three isozyme peaks of the rat brain enzyme; and a molecular weight of 72000 for the native rat brain enzyme and of 25000 - 27000 for the subunit of human and rat enzyme. Guanylate kinase does not interfere with the purification of hypoxanthine phosphoribosyltransferase on GMP agarose and moreover is itself partially purified by this chromatography.

Eine verbesserte Methode zur Reinigung der Hypoxanthin-Phosphoribosyltransferase

Zusammenfassung: Drei Methoden zur vollständigen Reinigung der Hypoxanthin-Phosphoribosyltransferase aus menschlichen Erythrozyten und Rattenhirn werden beschrieben. Die zur Isolierung des menschlichen Enzyms bereits beschriebene präparative isoelektrische Fokussierung ist im Falle von Rattenhirn weniger erfolgreich. Präparative Polyacrylamid-Elektrophorese im Gelblock ergibt Enzymproben, die in der analytischen Gelelektrophorese als sehr homogen erscheinen, aber eine verhältnismäßig niedrige spezifische Enzymaktivität aufweisen. Die schnellste und bequemste Methode, eine Modifikation der Affinitätschromatographie an GMP-Agarose nach Hughes[5], erbringt Hypoxanthin-Phosphoribosyltransferase, welche den anderen Präparationen an Reinheit

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Enzymes:
Guanylate kinase, ATP:(d)GMP phosphotransferase (EC 2.7.4.8)
Hypoxanthine phosphoribosyltransferase, IMP:pyrophosphate phosphoribosyltransferase (EC 2.4.2.8)

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