Fri-H-20CD

PROPERTIES OF IMMOBILIZED PYRUVATE KINASE FROM RABBIT SKELETAL MUSCLE.

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Pyruvate kinase (EC 2.7.1.40) was immobilized to CNBr-activated Sepharose 4B, and some properties of the matrix-bound enzyme were examined. It was found that the enzyme could be immobilized successfully with considerable retention of enzyme activity. A comparison of standard enzymological properties e.g. pH optimum revealed considerable similarities between the immobilized enzyme and the native soluble enzyme. The results indicate that the basic enzymological characteristics of the enzyme have not been grossly altered by immobilization, and that the immobilized enzyme may provide a useful model for studying subunit interactions in the enzyme.

Fri-H-22CD

KINETIC CHARACTERISTICS OF HUMAN ERYTHROCYTE MALATE-DEHYDROGENASE
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Human erythrocytes contain a large quantity of malate-dehydrogenase (E.C. 1.1.1.37), but its role in the metabolism of mature red cells is not clear. The pH optima for the forward (6.9) and the backward reaction (8.9) were determined. The apparent Michaelis constants were 42 µmol/l for oxaloacetate and 13 µmol/l for NAD at pH 7.5 and 355 µmol/l for malate at pH 9. Two Km values were obtained for NAD (pH 9): 36 and 113 µmol/l.

Under the steady-state conditions in vivo, MDH is fully saturated with both NAD and NADH, but the malate concentration is by one order of magnitude lower than the corresponding Km value.

Fri-H-24CD

ROBUST ESTIMATION OF ENZYME KINETIC PARAMETERS
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A computer program has been developed for fitting enzyme kinetic equations to experimental data. It can be applied to most of the common equations of enzyme kinetic parameters, such as those for inhibited reactions and reactions with two substrates. It is much less dependent than previously existing programs on assumptions about the experimental error: it is resistant to the harmful effects of observations with very large errors and does not require the user to guess the dependence of the experimental variance on the true rate. It can, however, be used as a conventional least-squares program if desired. It is written in Fortran and can be obtained from A.C.-B. on request.

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Fri-H-21CD

PROTEASE ACTIVITY AND THE GROWTH OF CULTURED HUMAN FIBROBLASTS

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A neutral serine protease has been purified from the plasma membrane fractions of human lymphocytes and granulocytes. A rabbit antiserum to the enzyme inhibits its proteolytic activity. The antiserum and γ-globulin purified from it can retard or completely inhibit the growth of normal human skin fibroblasts in culture. Non-immune rabbit sera or γ-globulin had no effect on control fibroblast cultures.

Fri-H-23CD

PURIFICATION AND SOME PROPERTIES OF RAT UTERINE PEROXIDASE.

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Using a combination of gel filtration, affinity chromatography on immobilized concanavalin A and hydrophobic adsorption chromatography, a peroxidase has been isolated from the uteri of oestrone-primed rats. The enzyme was purified some 300-fold with respect to the uterine extract and to >95% homogeneity as judged by photodensitometric scanning of SDS-polyacrylamide gels. The final product had an apparent molecular weight of 48 000, an absorption maximum at 412 nm (ε12/ε400=0.47) and a specific activity very similar to those of several other pure haemoprotein peroxidases. Some additional characteristics of this enzyme are also reported.

Fri-H-25CD

IMPROVED RESOLUTION IN THE SEPARATION OF THE PROTEINS IN DRIED PAPAYA LATEX EXTRACT, AND DEFINITION OF STANDARD CONDITIONS FOR ION EXCHANGE CHROMATOGRAPHIC IDENTIFICATION OF THE COMPONENTS.


Ion exchange chromatography on Q-Sepharose CL-6B of a lyophilized preparation of the water-soluble part of dried papaya latex using a linear NaCl-gradient revealed 8 well-separated components absorbing at 280 nm against maximally 6 components obtained by the previously established chromatography on CM- or SP-Sephadex using a gradient in acetate buffer. At least 5 of the components showed enzymatic activity towards benzoylarginine ethyl ester. The improved resolution provided a standard chromatographic procedure for tests of homogeneity and identity of commercial and laboratory-prepared papaya enzymes.