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A spectrophotometric method for the determination of 5-phosphoribosyl-1-pyrophosphate

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Summary. A new, sensitive, specific and simple spectrophotometric method for the determination of 5-phosphoribosyl-1-pyrophosphate (PRPP) is presented. PRPP is reacted with excess hypoxanthine in the presence of hypoxanthine-guanine phosphoribosyltransferase. At the end of the reaction, PRPP concentration is measured from the extent of conversion of hypoxanthine to inositate. The concentration of the purine base is determined spectrophotometrically in the presence of xanthine oxidase.

5-phosphoribosyl-1-pyrophosphate (PRPP) is an essential substrate for several different pathways involved in the synthesis of purine, pyridine, and pyrimidine ribonucleotides[1].

Most of the currently used assays for PRPP are radiochemical and utilize the reaction of this compound with a labelled purine base in the presence of the appropriate, purified phosphoribosyltransferase. The corresponding isotopically labelled nucleotide (end product of the reaction) is separated by means of techniques such as paper[2] or thin-layer[3] chromatography or high-voltage electrophoresis[4].

The reaction of PRPP and hypoxanthine in the presence of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) can be followed spectrophotometrically at 245 nm[5]. However, a direct spectrophotometric assay of PRPP at this wavelength is hardly useful for measuring the intracellular concentration of this compound owing to the high absorption of biological samples at 245 nm.

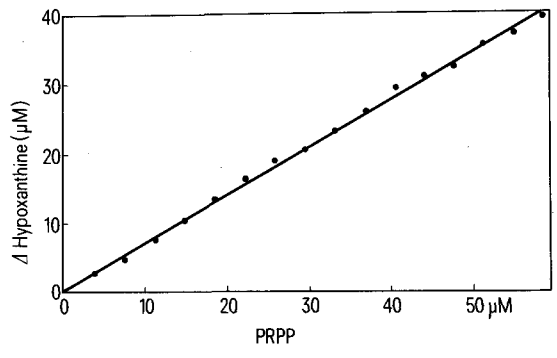
The assay described by Kornberg et al.[6] based on the release of $^{14}CO_2$ from orotic acid in the presence of a mixture of orotate phosphoribosyltransferase (OPRT) and orotidine-5'-monophosphate decarboxylase (ODC) is also employed. The OPRT-ODC reaction has been used[6,7] to measure PRPP concentration with a spectrophotometric technique but this method has not found much application. One reason for this might be that some investigators[8] found a linear response only in the range from 0.01 to 0.1 µmoles of PRPP per ml of reaction mixture. In the present paper, a new, sensitive, specific and simple spectrophotometric assay for PRPP is described.

Materials. HGPRT was purified from human erythrocytes to apparent electrophoretic homogeneity as previously described[5]. PRPP, tetrasodium salt, was purchased from Sigma Inc. 1 mg of the commercial sample contained 1.7 µmoles of PRPP as determined using the enzymatic assay of Kornberg et al.[6]. Paper chromatography according to Wood[9] showed no significant impurity in the commercial sample of PRPP after staining with ammonium molybdate[9]. Xanthine oxidase (XOD) and hypoxanthine were obtained from Boehringer AG. All other reagents were high purity commercial samples from Merck AG.

Methods. PRPP was assayed by the following procedure. PRPP was reacted with excess hypoxanthine in the presence of HGPRT to form the corresponding nucleotide. At the end of the reaction, PRPP concentration was measured from the extent of conversion of hypoxanthine to IMP. Hypoxanthine was determined by the enzymatic, spectrophotometric assay of Kalckar[10].

Step 1: HGPRT catalyzed reaction. The reaction mixture, designed after published procedures[5], contained 0.1 M Tris-HCl, pH 7.4, 0.01 M $MgCl_2$, 8×10^{-5} M hypoxanthine, 0.05 ml of a standard preparation of HGPRT[5], and PRPP ranging from 0 to 5.8×10^{-5} M in a final volume of 1 ml. PRPP was added last to initiate the reaction. All incubations were conducted at 37 °C. The sample containing no PRPP was used as the blank.

Under the experimental conditions employed the reaction went to completion after 15 min when followed as previously described[5]. At the end of the reaction, 0.2 ml of 0.25 M ethylendiamine tetraacetate, sodium salt, pH 7.4, were added to the incubation mixture. This was necessary in order to prevent the HGPRT catalyzed IMP pyrophosphorolysis during step 2[5].



Calibration curve for the PRPP assay. The experimental conditions are described in the text.

Step 2: hypoxanthine determination. A first spectrophotometric reading of the solutions obtained as described above was made at 293 nm to determine the initial absorbance. A 5-µl volume of 4 IU/ml XOD was then added and the conversion of hypoxanthine to uric acid was followed to completion at 293 nm. The reaction went to completion after 30 min at 25 °C. Hypoxanthine concentration was calculated from the optical density variation at 293 nm assuming a molar extinction coefficient of 12,000 for uric acid[10].

PRPP concentration was calculated as follows:

$$[PRPP] = 1.2 \times ([Hypoxanthine]_0 - [Hypoxanthine]_{PRPP}),$$

where $[Hypoxanthine]_0$ was the concentration of the purine base in the blank and $[Hypoxanthine]_{PRPP}$ was the concentration of the purine base in the samples at the end of the HGPRT catalyzed reaction.

Results and discussion. The calibration curve for the PRPP assay obtained by applying the procedure described under "Methods" on PRPP solutions of known concentration is reported in the figure. The relationship between PRPP concentration and the amount of hypoxanthine consumed is linear in the range studied. From the same series of experiments, a standard deviation of $\pm 8 \times 10^{-7}$ M was obtained for the PRPP assay.

Control experiments showed that PRPP at the concentrations employed, IMP and pyrophosphate (products of the HGPRT catalyzed reaction) added in equimolar amounts to PRPP (from 3.5×10^{-6} M to 5.8×10^{-5} M) do not influence the hypoxanthine assay.

The method for the determination of PRPP concentration described above is less time-consuming than the radiochemical procedures involving chromatographic or electrophoretic separation of nucleotides from the corresponding purine bases. The assay is sensitive and specific and responds linearly over the concentration range tested from 3.5×10^{-6} M to 5.8×10^{-5} M. Johnson et al.[8] have stated that no such linearity was found at PRPP concentration lower than 1×10^{-5} M using the alternate spectrophotometric method of Kornberg et al.[6,7].

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Genetic characterization of the new morphological and UV-sensitive mutants in *Coprinus cinereus*. I. A UV-sensitive mutation *rad* 1 associated with elevated frequencies of mitotic and meiotic recombination[1]

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Summary. Studies on the effect of an UV-sensitive mutation, *rad* 1, in meiotic and mitotic recombination in *Coprinus* indicated that, in homozygous condition, *rad* 1 increased the spontaneous meiotic recombination by 50% and UV-induced mitotic intergenic recombination by about 5-fold. The homozygous *rad* 1 diploid was shwon to be much more sensitive to the recombinogenic effects of polyfunctional than of mono- or non-functional alkylating agents.

The existence of relationships between radiation sensitivity, genetic recombination and repair was first suggested when UV-sensitive mutants of *Escherichia coli* were shown to be defective in recombination[3-5]. During the last decade, it has further been demonstrated that at least some of the enzymes responsible for the repair of induced DNA lesions are also involved in genetic recombination of eukaryotes such as *Ustilago*[6], *Neurospora*[7], *Aspergillus*[8], *Saccharomyces*[9-11] and *Drosophila*[12-14]. In *Coprinus*, 4 UV-sensitive mutants have previously been described[15] and tested for their effects on meiotic recombination. 2 were shown to be allelic (*uvs* 3-1 and *uvs* 3-2) and reduce the frequency of meoitic intergenic recombination, whereas the other 2 were mutations of other genes and did not affect meiotic recombination. None were tested for effects on mitotic recombination.

A UV-sensitive mutant (*rad* 1) in *Coprinus*, isolated from UV-irradiated oidial suspension produced from the morphological mutant *den*[2] (AJ1/65), was shown to increase by 50% the normal meiotic recombination frequency between the 2 morphological mutants *den*[2] and *zig* in linkage group III, either in repulsion or coupling (table).

The synthesis of diploid strains of *Coprinus* from 2 recessive morphological mutants in repulsion[16], carrying *rad* 1 mutation either in homozygous or heterozygous condition facilitated greatly the study of the effect of *rad* 1 on mitotic recombination. Thus a comparative study was made of the effect of UV-irradiation on oidial suspension from 3 diploid strains, all of which were heterozygous for *den*[2] and *zig* in repulsion; the strain AJZ4 was homozygous for *rad* 1, the strain AJZ1 heterozygous for *rad* 1 and the strain AJZ homozygous wild type for *rad* 1. The results of these studies were evaluated from the data of the dose-response curves together with the frequency of mitotic segregants expressed as a percentage of colonies on untreated controls. The same data were used to plot the frequency of UV-induced mitotic segregants against the surviving fraction (figure). As is apparent from these