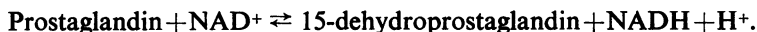


Enzymatic assay of the prostaglandins

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We recently described the purification and properties of a 15-hydroxy prostaglandin dehydrogenase from swine lung (Änggård & Samuelsson, 1966). This enzyme catalyses the oxidation of the secondary alcohol group at C-15 to a ketone in all known prostaglandins with the exception of those containing the dienone structure absorbing at 278 nm (PGB-compounds and their 19-hydroxylated analogues). The enzyme, which is present in the soluble fraction of a homogenate, uses NAD⁺ but not NADP⁺ as cofactor. The reaction can thus be written as follows:



Because the equilibrium favours the oxidation of prostaglandin, it is evident that the enzyme can be used in an enzymatic assay method for the prostaglandins based on the measurement of either of the products formed. Such a method would combine the selectivity of the enzyme with the high sensitivity attainable with fluorimetric measurements of pyridine nucleotides.

In the present communication the general principles for the enzymatic analysis of prostaglandins are discussed. Using direct fluorimetric measurement of the generated NADH, 10^{-10} – 10^{-8} moles of prostaglandins have been assayed. By coupling the prostaglandin dehydrogenase catalysed reaction to an amplifying system (Lowry, Passoneau, Schultz & Rock, 1961), the sensitivity of the assay is further increased to a lower limit of about 10^{-12} moles.

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N¹-Methylnicotinamide uptake by rat kidney slices: reversible and irreversible inhibition

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The identification and isolation of protein components of complex systems has become theoretically feasible through use of site-directed irreversible inhibitors, but has been demonstrated in only a few cases. We are applying this technique to the isolation of proteins involved in the transport of N¹-methylnicotinamide (NMN) by kidneys.

Slices of cortex from kidneys of rats were incubated with ¹⁴C-N¹-methylnicotinamide (NMN) and ³H-p-aminohippurate (PAH) in Robinson's kidney medium for 30 min. The slices were analysed for radioactivity, the uptakes of NMN and PAH computed and corrected for 25 % extra-cellular space. Each result was the average of two determinations. Doses of different inhibitors were added to the system and the change in uptake of NMN and PAH (expressed as % inhibition) was compared with controls.

With a set of homologues of NMN, N¹-alkylnicotinamides (alkyl groups of 2 to 11 carbons) the inhibition of NMN uptake increased with length of alkyl chain from 2 to 6 carbons, did not change from 6 to 9 carbons, and dropped nearly to zero with the 11-

carbon analogue. A set of four 2-halogenoethylamines was similarly tested; inhibition increased with hydrophobic character of the side chain. The inhibition was irreversible for the 2-halogenoethylamines in contrast to the reversible inhibition by the N¹-alkyl-nicotinamides. For both sets of inhibitors, concentrations were found that inhibited uptake of NMN with little or no inhibition of PAH uptake, demonstrating a specificity for the NMN system.

Inhibitors of protein synthesis, puromycin and cycloheximide, were used to find whether the turnover of one or more components of the NMN transport system was sufficiently rapid to be detected. Incubation of slices with either inhibitor resulted in time-dependent inhibition of uptake of NMN. PAH uptake was not inhibited by these agents.

Presence and properties of dihydrofolate reductases within the genus *Trypanosoma*

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Dihydrofolate reductase activity was detected in extracts of seven species representing all four major subgroups of the protozoan genus *Trypanosoma*. The enzyme assay was based on the decrease in absorbency at 340 m μ in the presence of extract, dihydrofolic acid and NADPH (Jaffe & McCormack, 1967). The species studied were rat-adapted bloodstream forms of *T. brucei*, *T. rhodesiense*, and *T. equiperdum* (*brucei* subgroup); *T. congolense* (*congolense* subgroup); *T. vivax* (*vivax* subgroup); *T. lewisi* and *T. cruzi* (*lewisi* subgroup). Dihydrofolate reductase activity was also detected in culture forms of *T. rhodesiense* and *T. cruzi*.

The amount of dihydrofolate reductase per mg of protein appeared to be greater in extracts of trypanosomal populations in the log phase of growth and less in extracts of relatively stationary populations.

When an equimolar amount of folic acid was substituted for dihydrofolic acid in the standard assay system, or when NADH replaced NADPH, very little decrease in absorbency at 340 m μ was observed, indicating the superiority of dihydrofolic acid as substrate and NADPH as hydrogen donor in the reaction mediated by trypanosomal dihydrofolate reductase. Such properties are characteristic of this enzyme from whatever source. The Michaelis constants (*K*_M) for dihydrofolic acid ranged from 3.3×10^{-6} M (*T. brucei*) to 2.3×10^{-5} M (*T. cruzi*); the Michaelis constants for NADPH with respect to those trypanosomal reductases so studied were quite similar, around 1×10^{-5} M.

The trypanosomal reductases, like those from other genera, were strongly inhibited by 4-amino analogues of folic acid, such as aminopterin and methotrexate. For example, the concentration of methotrexate required for 50% inhibition (ID₅₀) ranged from 2×10^{-10} M (*T. equiperdum*) to 1×10^{-5} M (*T. cruzi*).

Burchall & Hitchings (1965) found that dihydrofolate reductases from mammalian sources on the one hand and from bacterial sources on the other exhibited two markedly dissimilar patterns of sensitivity to the inhibitory action of various 2,4-diaminopyrimidines and related heterocyclic compounds. We found that the analogous reductases from the bloodstream form of trypanosomes were also inhibited by this class of compounds and