ENZYMIC SYNTHESIS OF INOSINE-6-O^{18} FOR INFRARED VIBRATIONAL ASSIGNMENT

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Introduction

A specific adenosine deaminase from intestine, which catalyzes reaction (I) was described by Kalckar (1), who employed ultraviolet spectroscopy to follow the reaction (2).

$$\text{Adenosine} + H_2O \xrightarrow{\text{enz.}} \text{inosine} + NH_3 \qquad \text{(I)}$$

We have carried out reaction (I) in D_2O and have made what appears to be the first use of infrared spectroscopy to follow enzyme kinetics.

The primary purpose of our experiments was to synthesize inosine specifically labeled with O^{18} as an aid in infrared band assignment. Advantages of infrared spectroscopy in studies of tautomeric forms of nucleic acid components have been discussed previously (3), and O^{18} substitution as an aid in interpreting vibrational spectra has been employed by a number of investigators (4).

Experimental

Adenosine deaminase (1 µl of a suspension of the enzyme obtained from Sigma Chemical Co.) was added to 100 µl of a 0.0424 M adenosine solution in D_2O^{18} (90.7 atom % O^{18}), buffered with 0.2 M sodium cacodylate, pD 6.9. Infrared spectra were measured in cells of 55µ path length at

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intervals of a few minutes until no further change with time was observed. A Beckman IR-7 spectrophotometer was employed. The temperature was uncontrolled and varied from 32 to 35°. Similar reactions were carried out in D_2O^{16} and D_2O^{18} containing 32.9 atom % O^{18}.

Results and Discussion

The enzymic deamination of adenosine to form inosine-6-O^{18} has been observed directly in the infrared cell (Fig. 1). While the method is less

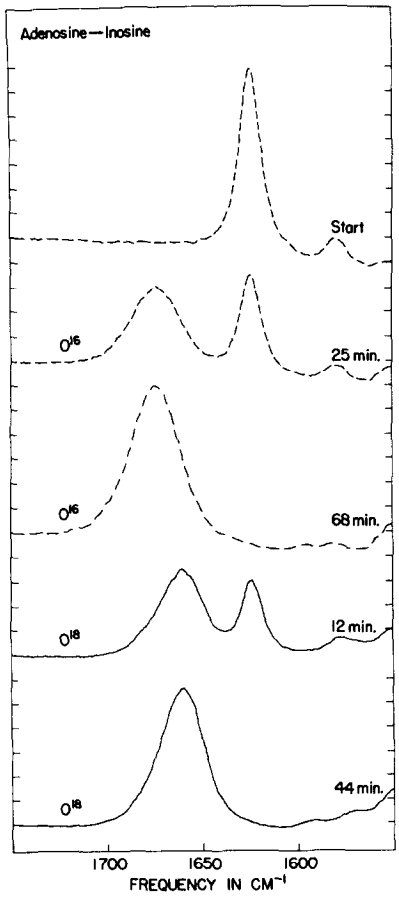

Fig. 1. Enzymic conversion of adenosine to inosine in D_2O^{16} (broken lines) and D_2O^{18} (90.7%, solid lines). The carbonyl absorption band of inosine-6-O^{18} has shifted to a lower frequency by 13 cm^{-1} (1673-1660 cm^{-1}). The faster rate of inosine-6-O^{18} formation was due to a larger amount of adenosine deaminase being present in the reaction mixture. No attempt was made in these experiments to determine an intrinsic effect of isotope on the reaction rate.

convenient than ultraviolet spectroscopy as a method of enzyme assay, it is clearly more convenient and economical of isotope than total chemical synthesis followed by isolation of the compound and measurement of the infrared spectrum. Satisfactory kinetic data can be obtained (Fig. 2). Isotopic exchange of the 6-oxygen of inosine did not occur in two months in neutral solution.

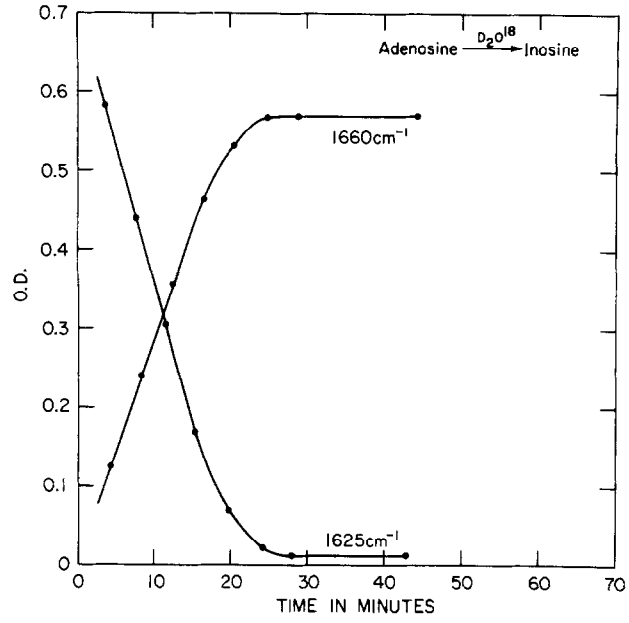

Fig. 2. Kinetics of adenosine deamination (1625 cm^{-1}) and inosine-6-O^{18} formation (1660 cm^{-1}).

The carbonyl absorption frequency shifted 4 cm^{-1} to lower frequency in 32.9% D_2O^{18} and 13 cm^{-1} in 90.7% D_2O^{18} (Fig. 1). Digitized spectra of inosine formed in D_2O^{18} were normalized to an extinction coefficient basis by a computer, and the spectrum of ordinary inosine-6-O^{16} (weighted by the fraction of O^{16} in the water) was subtracted from the spectrum of enzymically formed inosine-6-O^{18}. The difference curves were then renormalized to 100% D_2O^{18}. The same normalized frequency shift ($\Delta\nu$ = 14 cm^{-1}) was found for both the 33% and 91% D_2O^{18} solutions, though,

as would be expected, the quality of the normalized spectrum obtained from the 91% D_2O^{18} solution is much higher. The fact that the same normalized frequency shift is obtained from solutions of such different isotopic content suggests that there is no significant kinetic isotope effect in the reaction. Pure inosine-6-O^{18}, therefore, would have a 14 cm^{-1} shift and ϵ_{max} of 1010 at 1569 cm^{-1}. These data allow us to assign the 1673 cm^{-1} absorption band of inosine-6-O^{16} to a (strongly coupled) carbonyl vibration at position 6, in agreement with previous assignments based on chemical plausibility (3,5).

The application of the techniques described above to the preparation and study of the infrared spectra of inosine-5'-phosphate-6-O^{18} (from adenosine-5'-phosphate) and guanosine-6-O^{18} (from 2,6-diamino-9-β-D-ribofuranosylpurine) will be described at a later date.

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