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단 신

환원형 Flavin의 산소배제 반응장치

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An Apparatus for Monitoring Anaerobic Reactions of Reduced Flavins

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In a study[1] undertaken by the author on adduct formation between certain epoxides and reduced flavins (FlH_2) such as $FMNH_2$, it was necessary to run and monitor the reactions under anaerobic conditions. Since catalytic hydrogenation was chosen for several reasons as the method of reducing oxidized flavin (Fl_{ox}), the conventional spectrophotometric measurements using Thunberg type cuvette could not be utilized for this purpose. Thus, monitoring the reaction by HPLC assay of anaerobically withdrawn reaction mixture was attempted to this end. In doing this, due to instability of FlH_2 (rapid conversion of FlH_2 to Flox by air oxidation)[2], anaerobicity should have been maintained not only during the reaction but also during the withdrawal of portions for reaction mixture. In addition, it was desirable to be able to achieve the reduction (catalytic hydrogenation) of Fl_{ox} to FlH_2 prior to the reaction in the same reaction vessel. Since known reaction vessels[3~5] do not meet all of these requirements, a special reaction vessel shown in *Fig.* 1 was invented. As the apparatus allowed relatively simple and accurate kinetic measurements without destroying the anaerobicity of reaction mixture, it seemed worthwhile to report the results of the experiment so that this could be used for the reactions which require similar conditions.

Following is the procedure taken in the experiment. Epoxide stock solution is taken in the side bulb of the lower tube (B), and a flavin solution and Pt catalyst are placed in the upper tube (A).

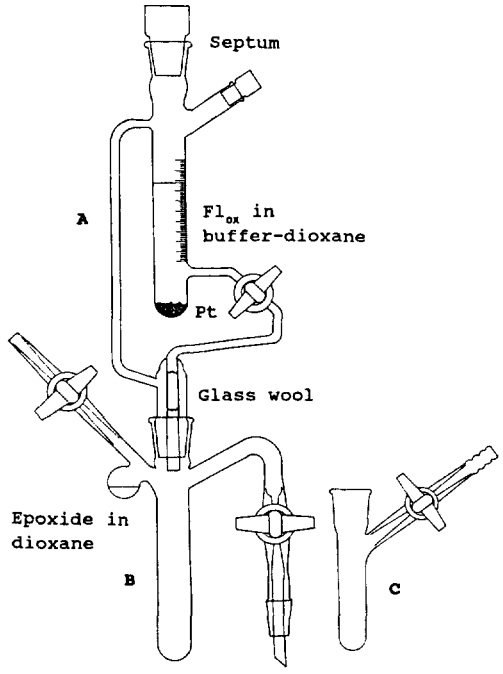

Fig. 1. Reaction vessel used for generation of FlH_2 from Fl_{ox} by catalytic hydrogenation and time course monitoring of reactions of the epoxides with FlH_2. A: part for catalytic hydrogenation of Fl_{ox}, B: part for reaction of the epoxides with FlH_2, C: part for anaerobic withdrawal of portions of reaction mixture.

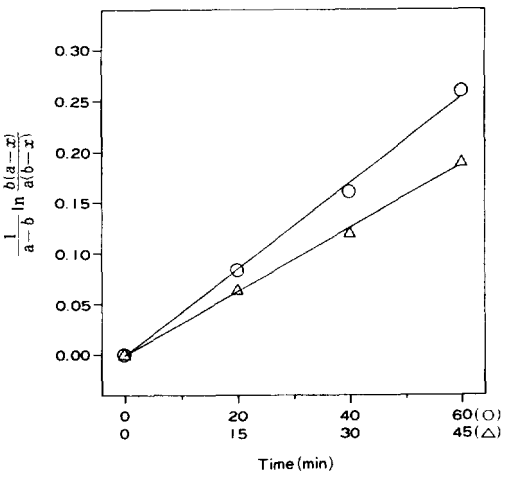

Fig. 2. Plots of $(1/a\text{-}b)\ \ln[b(a\text{-}x)/a(b\text{-}x)]$ *vs.* time for bimolecular adduct formation between 1 mM epoxide, (±)-1a,2,3,7b-tetrahydro-(1aα,2α,3β,7bα)-naphth[1,2-*b*]oxirene-2,3-diol and 3.6 (○) and 7.2 (△) mM $FMNH_2$ in pH 5.85 cacodylate buffer-dioxane at 25℃, ionic strength=0.1 with $NaClO_4$ (a=initial concentration of $FMNH_2$, b=initial concentration of the epoxide, x=concentration of the epoxide reacted). The reaction was carried out using the apparatus described in *Fig.* 1 and aliquots of reaction mixture at the designated time were withdrawn to elute on a C-18 HPLC column. The concentrations of the reactants were determined from the epoxide peak area relative to that of a standard (*cis*-1,2-dihydroxyindane).

After closing the reaction vessel, the flavin solution is deoxygenated by bubbling the solution with an inert gas introduced through a needle via a septum, with the side arm of tube B opened, and the epoxide solution deoxygenated by alternately evacuating and flushing with nitrogen. Then, hydrogen gas is passed through the flavin solution by the same procedure as for the inert gas until fluorescence is no longer seen (blue fluorescence of Fl_{ox} disappears upon its transformation into FlH_2). After a short rest time to allow the catalyst to settle, the FlH_2 solution is drained to the lower tube through a glass wool plug and is preequilibrated at appropriate temperature. Reaction is started by addition of the epoxide solution to FlH_2 solution by tipping. Withdrawal of portions of reaction mixture for HPLC analysis is done by attaching tube C to the side arm of B, transferring a portion of the reaction mixture onto the upper side of the side arm of B by tilting the apparatus, and then transferring the necessary amount of reaction mixture into tube C by opening the stopcock. An example of kinetic data obtained using this apparatus combined with HPLC assay of reaction mixture is shown in *Fig.* 2. The withdrawn reaction mixture could also be kept anaerobic if tube C was deoxygenated before the transfer. By attaching tube A to a cuvette through an adaptor, it was also possible to monitor the reaction by spectrophotometry.

The results of the experiment suggested that the present methodology have the following merits that the conventional spectrophotometric ones do not.

1. Have no difficulty in interpreting the results of measurements (spectrophotometric method frequently suffers from ambiguous spectral changes resulting from multiphasic kinetics or slowness of reaction).

2. Can follow the pathway of individual reactants.

3. No limit in the usable concentration of reactants.

In conclusion, the apparatus described in this report has been demonstrated to be a convenient device for monitoring anaerobic reactions of reduced flavins.

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