Short Communication

Transfer of Hydrogen from NADPH to NAD^+ in Frozen Aqueous SolutionYoichi ASO, Nobuyuki YAMASAKI,*
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Chemical reactions in frozen solutions have attracted a great deal of interest because of their unique characteristics.^{1–4} In the course of the investigation of the ferredoxin: NADP^+

reductase (FNR, EC 1.18.1.2)-catalyzed transfer of hydrogen between NAD(H) and NADP(H) ,⁵ we found that the transhydrogenation proceeded in frozen aqueous solution without FNR. This is the first report describing the nonenzymatic transfer of hydrogen from NADPH to NAD^+ in frozen aqueous solution.

All chemicals used in this study were reagent grade. The concentrations of NAD(H) and NADP(H) were measured spectrophotometrically using their absorption coefficients at 260 nm.⁶ For kinetic studies on the transhydrogenation between the oxidized and reduced nicotinamide adenine dinucleotides, 40- μl samples of the mixture of 1.9 mM NAD^+ and 4.8 mM NADPH in 10 mM Tris-HCl buffer (pH 7.5) was placed in a test tube, frozen at -60°C in an instant, and then kept at -20°C .

Fig. 1. Elution Profiles of the Mixture of the Pyridine Nucleotides.

The samples were analyzed by reverse-phase HPLC on a column of Cosmosil 5C_{18} (4.6×50 mm, Nacalai Tesque) before (A) and after freezing for 6 hr (B). The column was developed with 0.15 M phosphate/citrate buffer (pH 6.8) containing 1 mM EDTA at 40°C at a flow rate of 1 ml/min, as described previously.⁵ The effluents were monitored at 260 nm using a Hitachi 655A Variable Wavelength UV Monitor, and the amounts of NAD(H) and NADP(H) were calculated from the peak areas with the calibration curves prepared for the respective nucleotides. The inset shows the time-dependent formation of NADH at -20°C in the absence (○) and presence (●) of 0.3 M KCl, and at 4°C in the absence of KCl (◐).

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At times, the frozen samples were thawed by immersing the test tubes in a bath kept at 40°C, and 20 μ l of the resulting solution was immediately analyzed by HPLC. HPLC was done on a Hitachi 655A-11 Liquid Chromatograph under the conditions described in Fig. 1.

Figure 1 shows the elution profiles of nicotinamide adenine dinucleotides before and after incubation of the mixture of NADPH and NAD⁺ at -20°C. The sample before freezing gave two major peaks corresponding to NADPH and NAD⁺. In addition, a small peak eluted at 1.5 min was identified as NADP⁺ that was present in the NADPH preparation. When the sample was kept at -20°C, NADP⁺ increased markedly, and at the same time, a new peak appeared at 11.5 min. This was identified as NADH by comparison of the retention time with the authentic one. With incubation time, the peak area of NADPH and NAD⁺ decreased concomitantly with the increase in those of NADP⁺ and NADH. After 6 hr of incubation, 73 nmol of NADH and NADP⁺ were formed concomitantly with the decrease in the same amounts of NADPH and NAD⁺. The hydrogen transfer from NADPH to NAD⁺ was also observed at 4°C but its reaction rate was less than 10% that given for the reaction at -20°C (inset in Fig. 1). These findings indicate that the transfer of hydrogen from NADPH to NAD⁺ was accelerated in frozen aqueous solution.

One of the mechanisms so far proposed for the reactions in frozen aqueous solution is the concentration of reactants in liquid regions

surrounded by ice crystals.²⁻⁴⁾ As demonstrated here, the transhydrogenation between the nucleotides in the frozen state was suppressed in the presence of KCl (inset in Fig. 1). In the presence of KCl the concentration effect of the nucleotides may be weakened by the partial destruction of the ice structure in favor of the liquid solution. From these results, we postulate that acceleration of the transhydrogenation in the frozen state is principally due to the concentration of the nucleotides in the liquid regions in ice. There is evidence indicating that some buffer solutions have a pH change upon freezing.⁷⁾ Although no direct evidence to prove such a pH change was obtained in this case, there is a possibility that the pH change in the liquid regions in ice may also be involved in the acceleration of the transhydrogenation between the nucleotides in the frozen state. This is under investigation to elucidate the mechanism of this reaction.

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