# SOLUBILIZATION OF ENZYME FORMING ADPR POLYMER FROM NAD

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An aggregated enzyme preparation obtained from disrupted nuclei of rat liver, could catalyze the polymerization of ADPR of NAD (Chambon et al., 1966; Fujimura et al., 1967a, b; Nishizuka et al., 1967; Reeder et al., 1967; Sugimura et al., 1967). The enzymatic polymerization was inhibited by nicotinamide but not by isonicotinic acid hydrazide. The possible involvement of a certain type of NADase in this polymerization reaction was suggested (Fujimura et al., 1967b; Nishizuka et al., 1967; Sugimura et al., 1967).

This aggregated enzyme preparation had also the activities of NAD pyrophosphatase, NADase and phosphodiesterase. The phosphodiesterase in the aggregated enzyme preparation was responsible for the hydrolysis of ADPR polymer (Futai et al., 1967). In this paper, the solubilization

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Abbreviation used: NAD, Nicotinamide adenine dinucleotide; ADPR, Adenosine diphosphate ribose, NADase, NAD nucleosidase, DNase, pancreatic deoxyribonuclease.

TABLE I. Solubilization of ADPR Polymerase

| Enzyme | ADPR polymerase[1] | | NADase[1] | Hydrolysis[2] of polymer |
|---|---|---|---|---|
| | Total activity | Specific activity | Total activity | Total activity |
| Aggregated preparation | 340 | 7.26 | 1990 | 7580 |
| Solubilized preparation | 113 | 5.94 | 105 | 248 |

Protein in aggregated enzyme preparation was 47mg. 1/ Activity was expressed as mμmoles of incorporation of NAD into acid-insoluble material or of hydrolysis of NAD. 2/ Activity was experssed as cpm of the conversion of labeled ADPR polymer into acid-soluble form. The specific radioactivity of ADPR polymer was $3.24 \times 10^5$cpm/μmole of adenine. All incubations were carried out under standardized condition for 10 min at 37°C.

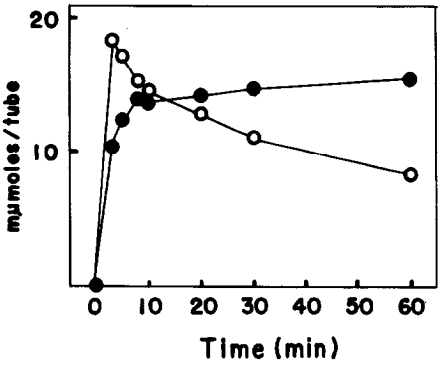


Figure 1. Time course of formation of ADPR polymer

Aggregated enzyme preparation (2.0 mg protein) or solubilized preparation (2.0 mg protein) was incubated with 1.5 μmole of NAD ($3.61 \times 10^4$cpm) and 5.0 μmoles of Tris-HCl buffer (pH 8.0) in 0.5 ml. Each point represents the radioactivity in acid-insoluble from each tube. o—o Aggregated preparation, ●—● Solublized preparation.

of the enzyme responsible for the formation of ADPR polymer from NAD, referred as ADPR polymerase and the partial removal of NADase and phosphodiesterase will be described.

Materials and methods : Aggregated enzyme preparation was made from rat liver as described in details in a previous paper (Fujimura et al., 1967b). The activity of ADPR polymerase was assayed as described previously by using NAD labeled with $^{32}P$, which has been prepared from Escherichia coli grown in the medium containing radioactive inorganic phosphate (Fujimura et al., 1967b). NADase was measured by the method using cyanide (Colowick et al., 1951). The hydrolysis of ADPR polymer was determined by the decrease in acid-insoluble radioactivity using purified labeled ADPR polymer (Futai et al., 1967). Pancreatic deoxyribonuclease was a crystalline preparation from Worthington Biochemicals.

Results and discussion : Aggregated enzyme preparation was suspended in 50mM Tris-buffer (pH 7.8) and 5mM $MgCl_2$ and incubated with a rather high concentration of DNase (DNase 400 $\mu$g/ml, DNase 20 $\mu$g/mg protein) for 10 min at 37°C. Incubation medium was centrifuged for 120 min. at 108,000 g at 2°C and the supernatant was assayed for the activity of ADPR polymerase, NADase and the hydrolysis of ADPR polymer. As shown in TABLE I, about one third of the ADPR polymerase activity was recovered in the soluble form, while only one twentieth of NADase activity was recovered in the soluble fraction. The activity of hydrolysis of ADPR polymer was also lowered appreciably in the soluble fraction in comparison with that in the aggregated preparation. The time course of the formation of acid-insoluble material from labeled NAD with the solubilized preparation also reflected the removal of the activity of hydrolysis of ADPR polymer. As shown in Figure 1, while with aggregated enzyme preparation, the acid-insoluble radioactivity, which reached the maximum within 10 min, decreased rapidly with time possibly due to the hydrolysis

of ADPR polymer, with solublized enzyme preparation the acid-insoluble radioactivity remained constant at its maximum.

The solubilized ADPR polymerase activity was inhibited by nicotinamide but not by isonicotinic acid hydrazide, as was the case of the aggregated enzyme. This may be interpreted to indicate that ADPR polymerase is a certain type of NADase which catalyzes the exchange of nicotinamide of a molecule of NAD with ribose of AMP moiety of another molecule of NAD. However, this type of NADase should be different from the bulk of NADase which was removed from ADPR polymerase by the solubilization of the enzyme.

The solubilized ADPR polymerase was stable at least for five days at 2°C and the activity was efficiently adsorbed and eluted on CM-cellulose column. The purification of ADPR polymerase is now in progress.

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