

NICOTINAMIDE MONONUCLEOTIDE ACTIVATION OF A NEW DNA-DEPENDENT
POLYADENYLIC ACID SYNTHESIZING **NUCLEAR** ENZYME

P. Chambon, J.D. Weill and **P. Mandel**

Institut de Chimie Biologique, Faculté de Médecine, Strasbourg
France

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Previous work in our laboratory showed that addition of NMN to whole kidney nuclei incubated in vitro stimulated C14-adénine incorporation into an ~~RNA~~ fraction (Revel and Mandel, in press). Since we were studying RNA-polymerase from hen liver nuclei (Weill, Busch, Chambon and Mandel, 1963) we decided to check whether NMN affected this enzyme. We discovered that even in the absence of other nucleoside triphosphates, NMN tremendously enhances the activity of a ~~DNA~~ dependent enzyme which incorporates ATP into a product which is presumably polyA ; we wish to report some properties of this enzyme which does not seem to have been described as yet.

Material and method. Particulate fractions of nuclear extracts from hen liver are prepared according to the method previously described (Busch, Chambon, Mandel and Weill, 1962). The incubation medium is indicated in the legend of the table. After 20 minutes at 37°C the incubations are stopped with trichloroacetic acid and the amount of radioactivity incorporated from ATP into acid-insoluble material is measured as related earlier

*Abbreviations : NMN nicotinamide mononucleotide - NAD, nicotinamide adenine dinucleotide - NADP, nicotinamide adenine dinucleotide phosphate - RNA, DNA. ribo- and deoxyribonucleic acids - AMP, ADP, ATP, adenosine mono-, di- and triphosphates - UTP, GTP, CTP, uridine, guanosine and cytidine triphosphates - polyU, polyA, polyuridylic and polyadenylic acids.

Busch, Chambon, Mandel and Weill, 1962). One unit of enzyme corresponds to an incorporation of one millimicromole of radioactive AMP per milligram of proteins in twenty minutes.

Properties of the enzyme. Table I shows that, in the presence of NMN, the incorporation varies from about 5 to about 15 units, while the omission of this compound reduces the activity at least 1000 times. Other properties of the enzyme can be seen in this table, in particular the absence of effect of added nucleoside triphosphates upon incorporation of ATP. The activity is strongly decreased by preincubation with DNAase and can be restored by addition of homologous or heterologous DNA but not by polyA or polyU nor by extensively hydrolysed DNA. If ATP is replaced by ADP (table 1) there is still a certain amount of label incorporated due to a phosphorylation of ADP since the residual activity is almost completely suppressed by addition of glucose and hexokinase; moreover pyrophosphate is inhibitory (table 1) while phosphate is not.

Optimum conditions are : pH 7.5, Mg^{++} 3.10^{-2} M, NMN 2.10^{-3} M. The time curve shows an increase in activity up to at least one hour. The activity is proportional to the amount of proteins up to 3 mg of proteins per incubation. The activity is roughly proportional to the logarithm of NMN concentration up to 2.10^{-3} M and when NMN concentration is 2.10^{-5} M the enzymatic activity still represents 14 percent of the activity measured at 2.10^{-3} M NMN concentration.

Ammonium sulphate (10 percent saturated), p-ohloromercuribenzoate (10^{-3} M), versene (10^{-2} M) are inhibitory.

Nature of the reaction product The product of the reaction has been identified as polyA on the basis of the following Properties : it is acid-insoluble, and insensitive to DNAase and to pancreatic and takadiastase T1 On the other hand,

TABLE 1

N°	Conditions	Incorporation of AMP (units)
1	Normal	6.20
	+UTP, GTP and CTP	5.50
	-NMN	< 0.01
2	Normal	7.20
	+ammonium sulphate (10 percent sat.)	2.88
	preincubation 1h. 37°C	7.05
	pretreated with DNAase 100 µg/ml, 1h., 37°C	1.00
	same DNAase pretreatment, then addition of DNA into incubation	9.00
3	Normal	4.41
	+ versene (0.01M)	0.10
	+ p-chloromercuribenzoate (0.001M)	0.11
4	Normal	14.20
	pretreatment with RNAase, 200 µg/ml, 1h., 37°C	12.40
5	Normal	5.80
	-ATP +ADP + glucose (0.1 M) + hexokinase (200 µg/ml)	3.30
	-ATP +ADP + glucose (0.1 M) + hexokinase (2000 µg/ml)	1.50
		0.80
	+pyrophosphate 0.01M	1.40

Incubation medium : each incubation contains in a final volume of 0.25 ml (in poles) : C^{14} -ATP or ATP- α - P^{32} 0.25 ; tris-phosphate buffer pH 7.5 25.0 ; Mg^{++} 7.5 ; FNa 5 ; KCl 15 ; 2-mercaptoethylamine 0.25 ; NMN 0.5 ; enzymatic extract (1-2 mg proteins).

when AMP 32 is incorporated, alkaline hydrolysis yields at least 97 percent of 2'(3')-AMP while after incorporation of C^{14} -AMP we find 1 percent of C^{14} -adenosine among alkaline hydrolysis products. This would indicate a polyA polymer of a chain length of about a hundred nucleotides.

Discussion. Several bacterial and animal polyA-polymerases have been described (Chung et al., 1960; Edmonds and Abrams, 1960 and 1962; August et al., 1962; Chamberlin and Berg, 1962; Gottesman et al. 1962 ; Smellie, 1962 ; Venkataraman, 1962) ;

none exhibits simultaneously all the properties which distinguish our enzyme : activation by NMN, DNA-dependency, absence of stimulation by polyA, absence of inhibition by the three other nucleoside triphosphates and by various RNAases, nature of the product formed. The same enzyme is present in calf thymus nuclei, in hog and rat liver nuclei, but in crude E. Coli extracts the DNA-dependent synthesis of **polyA** described by Chamberlin and Berg (1962) is not stimulated by NMN. The role of NMN is unknown ; it cannot be replaced by NAD nor by NADP. The DNA-dependency might suggest that this ATP polymerisation is a special feature of RNA-polymerase. However in several respects our enzyme behaves differently from RNA-polymerase : the former is much more stable at 37°C ; it is inhibited by ammonium sulphate (table 1) which under the same conditions stimulates RNA-polymerase ; polyU which acts as primer for RNA-polymerase (Krakow, reported by Vogel et al., 1962) is inactive in our system ; RNA-polymerase needs native DNA as **primer**, while partially degraded DNA is a more efficient primer for our polymerase ; finally actinomycin D (at a concentration of 140 µg per ml of medium containing 120 µg of DNA) fails to inhibit our enzyme while this compound completely stops the activity of RNA-polymerase. Under this respect our enzyme behaves in the same way than bacterial DNA dependent polyriboadenylate polymerases (Goldberg et al., 1962 ; Hurwitz et al., 1962). Purification now in progress is necessary to throw light on the roles of DNA and NMN.

Our enzyme might play a physiological role since it was shown (Kaplan et al., 1956) ; Revel and **Mandel**, 1962) that nicotinamide administration modifies in animals the metabolism of nicotinamide adenine dinucleotide end nuclear RNA's. PolyA could intervene as an ATP precursor storage form and polyA formation

could be a regulation process in these metabolisms. Our enzyme might also catalyze in the nucleus the synthesis of the polyA which acts as primer of the "ATP polymerase" described in thymus nuclei by Edmonds and Abrams (1962). Possible implication of polyA in the control of protein synthesis has been recently discussed by August et al. (1962).

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