NAD^+ kinase: molecular weight determination by low-angle laser-light scattering

Mina K. Gabriel and Eugene T. McGuinness*

Department of Chemistry, Seton Hall University, South Orange, NJ 07079, USA

Received 30 July 1984

Low-angle laser-light scattering (LALLS) was employed to measure the absolute molecular weight of chicken liver NAD^+ kinase (NADK). The weight-average molecular weight ($\bar{M}_w$) was found to be 275000 ± 15000. The corresponding value for the second virial coefficient was -1.65×10^{-3} ml·mol·g^2. The value for M_w is in close accord with estimates reported for pigeon liver (270000) and *C. utilis* (260000) NADK. If the active enzyme is a dimer, the weight difference between pigeon/chicken liver and rabbit liver (136000) NADK would indicate that the latter enzyme is an active monomer unit.

NAD^+ kinase *Absolute molecular weight* *Low-angle laser-light scattering*

1. INTRODUCTION

The enzyme NAD^+ kinase (ATP: NAD^+ 2′- phosphotransferase, EC 2.7.1.23) catalyzes the only known biochemical reaction leading to the synthesis of $NADP^+$. The reaction proceeds by way of the ATP-linked phosphorylation of NAD^+:

$$Mg\text{-}ATP^{2-} + NAD^+ \rightleftharpoons Mg\text{-}ADP^- + NADP^+ \quad (1)$$

NAD^+ kinase (NADK) is a large multimeric complex. Reports of molecular weights range from ~40000 to 650000 for the active enzyme. While a consensus is evident that a subunit of molecular weight of ~30000–35000 is a component of mammalian and yeast enzymes, little or no agreement can be discerned as to the best size estimate of the dominant active specie(s).

Molecular weights of 250000 and 270000, respectively, have been reported for rat [1] and pigeon [2] liver NADK, with an octameric structure inferred for the avian enzyme based on a 34000 Da subunit. A rabbit liver preparation [3,4] is reported to consist of (four) multiple forms spanning the weight range 150000–290000. Denaturant electrophoresis of this NADK revealed the presence of α- (35000) and β- (62000) typer (size) subunits, both of which can produce oligomers differing in molecular weight and catalytic activities. If the active enzyme is a dimer [3], the 136000 molecular weight (rabbit liver) preparation of Chung [5] may represent an active monomer unit.

Structure studies on rabbit skeletal [6] and pigeon heart [7,8] muscle NADK revealed the presence of multiple active forms of the enzyme with weight ranges of 31000–370000 (rabbit) and 90000–270000 (pigeon). Purification of the pigeon heart enzyme to homogeneity results in the removal of a factor, the absence of which prevents the enzyme from acting as a dissociating system of oligomers. Native and (subunit) molecular weights of 124000 (31000) and 260000 (32000) are cited for *S. cerevisiae* [9] and *C. utilis* [10], respectively. In contrast, *N. crassa* NADK is reported to be multimeric [11] with different forms and specific activities evident at different stages of growth and development. Size diversity is also evident in preparations from sea urchin eggs (310000) [12, 13], squash (50000) [14], and *A. vinelandii* (130000) [5].

In view of our lack of knowledge concerning the

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

size of the native enzyme under catalytic conditions, we have used low-angle laser-light scattering to determine the absolute molecular weight of chicken liver NADK in a buffer environment consistent with catalytic activity measurements. The results from these studies, presented here, provide a point of reference for size-estimate by other methods of the enzyme which occupies a center point in pyridine nucleotide metabolism [15].

2. MATERIALS AND METHODS

These experiments were carried out at 25°C on the enzyme (Sigma, lot No. 79C-7120, containing 94% protein) using a Chromatix Model KMX-6 low-angle laser-light scattering photometer [16]. At the small forward scattering angles employed in the KMX-6 and low solute concentration, the relation between the Rayleigh factor and the weight average molecular weight ($\bar{M}_w$) is given by the expression:

$$(K\,c)/\bar{R}_\theta = 1/\bar{M}_w + 2A_2\,c \qquad (2)$$

where c is the solute concentration in g/ml and A_2 is the second virial coefficient. The excess Rayleigh factor ($\bar{R}_\theta$) is the difference in the Rayleigh factor of the solution and that of the pure solvent

$$\bar{R}_\theta = \bar{R}_{\theta\ \text{solution}} - R_{\theta\ \text{solvent}} \qquad (3)$$

and K is the polymer (protein) optical constant defined as:

$$K = (2\pi^2 n^2/\lambda^4\,N)\,(\mathrm{d}n/\mathrm{d}c)^2\,(1 + \cos^2\theta) \qquad (4)$$

where n is the refractive index of the solution at the incident wavelength (λ, in vacuo), N is Avogadro's Number, and θ is the angle of scattered light collection. The specific refractive index increment, $\mathrm{d}n/\mathrm{d}c$ (the change in the solution refractive index as a function of solute concentration), was determined in a separate series of measurements using a Chromatix KMX-16 differential refractometer. $\bar{M}_w$ was obtained from a plot of $(K\,c/\bar{R}_\theta)$ vs c (eq. 2). All measurements of scattered light and the refractive index increment were carried out in

Table 1
Summary of molecular weights for NAD^+ kinase

Source	Molecular weight	Method[a]	Ref.
Liver			
Pigeon	270000	GF	[2]
Rat	250000	UC	[1]
Rabbit	180000–650000	PAGE	[3,4]
	136000	SDGC	[5]
Chicken	275000	LALLS	This report
Muscle			
Rabbit skeletal	40000–300000	TLGF	[6]
Pigeon heart	90000–270000	GF, PAGE	[8]
Plant			
Squash	50000	GF	[14]
Yeast			
S. cerevisiae	124000	SEUC	[9]
N. crassa	203000–338000	PAGE	[11]
C. utilis	260000	GF, PAGE	[10]
Bacteria			
A. vinelandii	130000	SDGC	[5]
Marine			
Sea urchin eggs	310000	GDGC	[12,13]

[a] GF, gel filtration; UC, ultracentrifugation; PAGE, polyacrylamide gel electrophoresis; SDGC, sucrose density gradient centrifugation; LALLS, low-angle laser-light scattering); TLGF, thin-layer gel filtration; SEUC, sedimentation equilibrium ultracentrifugation; GDGC, glycerol density gradient centrifugation.

ultramembrane filtered 0.20 M phosphate buffer, pH 7.4.

3. RESULTS AND DISCUSSION

The $\bar{M}_w$, calculated by linear regression for six concentrations of protein ($3.71–37.1 \times 10^{-5}$ g/ml), for NADK was found to be 275 000 (275 444). The second virial coefficient (A_2 in eq. 2, a potential source of useful information on solute interactions) for this preparation was -1.65×10^{-3} ml $\cdot$ mol $\cdot$ g^{-2}. This molecular weight is in good agreement with the value (270 000) cited by Apps [2] for pigeon liver NADK and Butler and McGuinness [10] for *C. utilis* NADK (260 000). It does not agree with the values reported by Tseng et al [9], Blomquist [12] or Chung [5] for kinase preparations from *S. cerevisiae* (124 000), sea urchin eggs (310 000) or *A. vinelandii* (136 000) and rabbit liver (136 000), respectively. A summary of the range and diversity found among these values and the methods employed for weight determination of NAD^+ kinase is shown in table 1.

ACKNOWLEDGEMENTS

This work was supported, in part, by Allied Corporation, Morristown, NJ 07960. We are grateful to A. Tonelli and L. Stillwagon of Bell Laboratories, Murray Hill, NH 07974, for advice, assistance and use of their facilities in carrying out this investigation.

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