Biochimica et Biophysica Acta, 483 (1977) 248—257

BBA 68194

THE MECHANISM OF ADP-RIBOSYLATION OF ELONGATION FACTOR 2 CATALYZED BY FRAGMENT A FROM DIPHTHERIA TOXIN

DOMINIC W. CHUNG * and R. JOHN COLLIER **

Department of Bacteriology, University of California, Los Angeles, Calif. 90024 (U.S.A.)

(Received January 17th, 1977)

Summary

Measurements of the initial rate of ADP-ribosylation of elongation factor 2 (EF-2) catalyzed by Fragment A from diphtheria toxin support a sequential mechanism and suggest that the reaction proceeds through a central ternary complex involving Fragment A and the substrates, EF-2 and NAD. The Michaelis constants for EF-2 and NAD are 0.15 and 1.4 μM, respectively. As determined by equilibrium gel permeation, EF-2 does not bind Fragment A significantly, alone or in the presence of adenine, ADPribose, nicotinamide or NADH. Based on these and earlier results, we propose an ordered sequential mechanism for the reaction; the sequence of binding of substrates is NAD, followed by EF-2.

Introduction

The inhibitory effect of diphtheria toxin on protein synthesis has been shown to result from the toxin's capacity to promote the ADP-ribosylation of elongation factor (EF-2) [1—3]:

$$\text{EF-2} + \text{NAD} \rightleftharpoons \text{ADPRib-EF-2} + \text{nicotinamide} + \text{H}^+$$

The equilibrium of the reaction is far to the right (Equilibrium constant $6.3 \cdot 10^4$ M at pH 8.0 [2]). The ADP-ribosyl derivative of EF-2 is inactive in promoting the translocation event on ribosomes, and thus peptide chain elongation is blocked after modification of a high percentage of EF-2 in cells.

Studies in recent years [4—7] have shown that the toxin is synthesized and excreted as a proenzyme, composed of a single polypeptide chain, about 60000 in molecular weight. For its enzymic activity to be expressed, the toxin must apparently undergo two covalent alterations of structure. Exposure of the

* Present address: Department of Biochemistry, University of Washington, Seattle, Wash. 98195, U.S.A.

** To whom reprint requests should be addressed.

intact toxin to mild proteolysis with trypsin, for example, produces "nicked toxin", which is also enzymically inactive and which consists of two major fragments, A and B, linked by a disulfide bridge. Reduction of the nicked toxin with thiols then releases the N-terminal A fragment (M_r = 21145 [8]), which can be identified as the enzymically active entity. The C-terminal B fragment (M_r = 39000), has no apparent enzymic activity, but is required for toxicity. There is evidence which suggests that Fragment B is responsible for recognizing and binding the toxin to cell surface receptors [9—11].

The interaction of Fragment A with one of its substrates, NAD, has been studied in detail [12,13]. Fragment A has a single binding site for NAD with a dissociation constant of 8 μM. The binding of NAD is reversible and involves interactions of both the adenine and nicotinamide moieties of NAD with Fragment A. Bound NAD is labilized and hydrolyses slowly into ADPribose and nicotinamide.

We report in this communication, studies of the kinetics of the ADP-ribosylation reaction and the interaction of Fragment A with EF-2. On the basis of results presented here and earlier, we propose that the ADP-ribosylation of EF-2 proceeds by an ordered, sequential mechanism in which Fragment A binds NAD before EF-2.

Materials and Methods

Diphtheria toxin. Partially purified toxin was obtained from Connaught Laboratories, Toronto, Canada. It was purified by column chromatography successively with DEAE-cellulose and Sephadex G-100 as described previously [4,5].

Fragment A. Purified toxin was nicked by limited proteolysis with trypsin. Toxin, at a concentration of 10 mg/ml in 50 mM Tris · HCl, and 1 mM EDTA, was incubated with 1 μg/ml trypsin (Worthington) at 25° C for 45 min. Proteolysis was stopped by adding soybean trypsin inhibitor to a final concentration of 1.5 μg/ml [5]. Under these conditions, close to 95% of the toxin molecules were converted to the nicked form with Fragments A and B as the predominant products. The nicked toxin was concentrated to 40 mg/ml by ultrafiltration in an Amicon concentrator with a PM-10 membrane. Nicked toxin was unfolded in 8 M urea and reduced with 0.1 M dithiothreitol and Fragments A and B were separated by gel filtration in a Sephadex G-100 column (5 × 80 cm) equilibrated with 50 mM Tris · HCl, pH 8.2, 1 mM EDTA, 4 M urea and 50 mM mercaptoethylamine. Protein concentration was monitored by relative fluorescence (excitation 290 nm, emission 340 nm), and enzymic activity was monitored by ADP-ribosylation as descrbed below. Fractions containing pure Fragment A were pooled, and the single free sulfhydryl group on Fragment A was reversibly blocked by dialysis against 0.1 M 2,2′-dihydroxyethyl disulfide. Fragment A monomers were purified on a Sephadex G-100 column (2.5 × 80 cm) equilibrated with 50 mM Tris · HCl, pH 8.2, 1 mM EDTA.

Carboxymethylation of Fragment A. Purified Fragment A (1 mM) was reduced under nitrogen for 2 h at room temperature with 2 mM dithiothreitol in 0.1 M Tris · HCl, pH 8.6, 10 mM EDTA and 4 M urea. Iodo[^{3}H]acetate (Amersham, specific activity, 38 Ci/mol) was added to a final concentration of

5 mM and allowed to react in the dark for 15 min at room temperature. Further reaction was stopped by adding mercaptoethanol to 30 mM. Carboxy-[^{3}H]methyl Fragment A, recovered after desalting on a Sephadex G-25 column, had a measured specific activity of 30 Ci/mol.

Preparation of Fragment A-Sepharose. Sepharose 2B was suspended in an equal volume of water and was activated with CNBr at pH 11 according to Parikh et al. [14]. The activated gel was rapidly washed with citrate buffer (0.2 M sodium citrate, pH 6.3, containing 0.5 M NaCl) and incubated with Fragment A (5 mg/ml in 0.2 M sodium citrate, pH 6.3). The coupled Sepharose was washed batchwise in Tris/EDTA with 4 M urea. A portion of the gel was packed into a column (0.6 × 5 cm) and washed with initial buffer containing 50 mM Tris · HCl, pH 8.0, 1 mM EDTA, 0.25 mg/ml bovine serum albumin and 5 mM dithiothreitol, until the effluent contained no free Fragment A detectable by ADP-ribosylation. The extent of coupling was determined by assaying a portion of the washed gel for enzymic activity.

Purification of EF-2. EF-2 was purified from rabbit reticulocytes. Circulating reticulocytes were collected, washed and lysed as previously described [15,16]. The ribosome-free lysate was adjusted to pH 6.0 with acetic acid and fractionated with ammonium sulfate. Material which precipitated from 40 to 70% $(NH_4)_2SO_4$ saturation at 4°C contained approx. 70% of the total EF-2 content. This fraction was redissolved and dialysed against buffer A (50 mM Tris · HCl, pH 7.2, 1 mM EDTA, 10 mM mercaptoethanol, 5% glycerol and 0.5 μM phenylmethylsulfonyl fluoride [PMSF]). The crude EF-2 was applied to a column of DEAE-cellulose (Bio-Rad, Cellex-D, 2.5 × 30 cm) equilibrated at 4°C with buffer A, and EF-2 was eluted by a linear gradient of NaCl, 0—0.6 M in buffer A. EF-2 was assayed by ADP-ribosylation as described below. Fractions containing EF-2 were concentrated by ultrafiltration over an Amicon XM-50 membrane and dialyzed against buffer B (10 mM sodium phosphate, pH 7.0, 1 mM EDTA, 10 mM mercaptoethanol, and 0.5 μM PMSF). The partially purified EF-2 was subsequently chromatographed on a phosphocellulose column (Whatman, P-11, 1.1 × 20 cm) equilibrated with buffer B. EF-2 was eluted with a linear sodium phosphate gradient, 10 mM to 0.5 M in buffer B. Pooled EF-2 was re-chromatographed on a similar phosphocellulose column. The EF-2 obtained was pure as judged by the appearance of a single protein band in sodium dodecyl sulfate (SDS)-polyacrylamide gels.

Preparation of radioactive NAD. [^{14}C]NAD labelled uniformly in the adenine moiety was synthesized from [^{14}C]ATP and nicotinamide mononucleotide (NMN) and purified on a DEAE-cellulose column as previously described [17]. The specific activity of the final product was 407 Ci/mol. The preparation was isotopically pure as analyzed by thin-layer chromatography on polyethyleneimine cellulose (PEI-cellulose) developed ascendingly with 0.3 M LiCl [18]. Exposure to X-ray film (Cronex 5, Dupont) revealed a single spot corresponding to NAD.

ADP-ribosylation assays. (a) ADP-ribosylation was used to monitor the purification of EF-2 and for total EF-2 determinations. The reaction was performed at 25°C for 15 min in a total volume of 0.1 ml, containing 50 mM Tris · HCl, pH 8.2, 1 mM EDTA and 1 mg/ml bovine serum albumin (Pentex). Fragment A (5 μg/ml) and [^{14}C]NAD (5 μM) were added and incubated with

unknown quantities of EF-2. The reaction was stopped by adding an equal volume of cold 10% trichloroacetic acid. The precipitate was collected on glass fiber filters (Whatman, GF/C) and the amount of radioactivity determined in a gas flow counter. (b) For initial rate measurements, limiting concentrations of Fragment A were used (5 ng/ml). The concentrations of EF-2 and NAD were varied as required in the respective experiments. Under these conditions, the amount of Fragment A was limiting and less than 7% of the substrates were reacted to ensure accurate estimation of initial rates.

Results

Kinetics of the ADP-ribosylation of EF-2

Goor and Maxwell [19] have reported studies on the kinetics of the ADP-ribosylation reaction, but these were performed before discovery of the covalent modifications in structure required for expression of enzymic activity by diphtheria toxin. Also the EF-2 used in these studies was relatively impure. We therefore undertook to define the kinetics more precisely using a system containing only purified components; that is, Fragment A, NAD, and pure EF-2 from rabbit reticulocytes.

We first measured the effects on the initial rate of ADP-ribosylation of varying the concentrations of the two substrates independently. Double reciprocal plots, as shown in Figs. 1A and 1B, gave families of convergent lines intersecting above the abscissa. The vertical coordinates of the points of intersection in Figs. 1A and 1B are identical within experimental error. Secondary plots of the data from Fig. 1 gave Michaelis constants of 1.4 and 0.15 μM for NAD and EF-2, respectively, and a turnover number of about 200 mol of ADPRib-EF-2 per mol of enzyme per min.

The fact that the families of lines in Figs. 1A and 1B are convergent rather than parallel, implies that the ADP-ribosylation reaction involves a sequential mechanism. This conclusion is also supported by the apparent absence of a

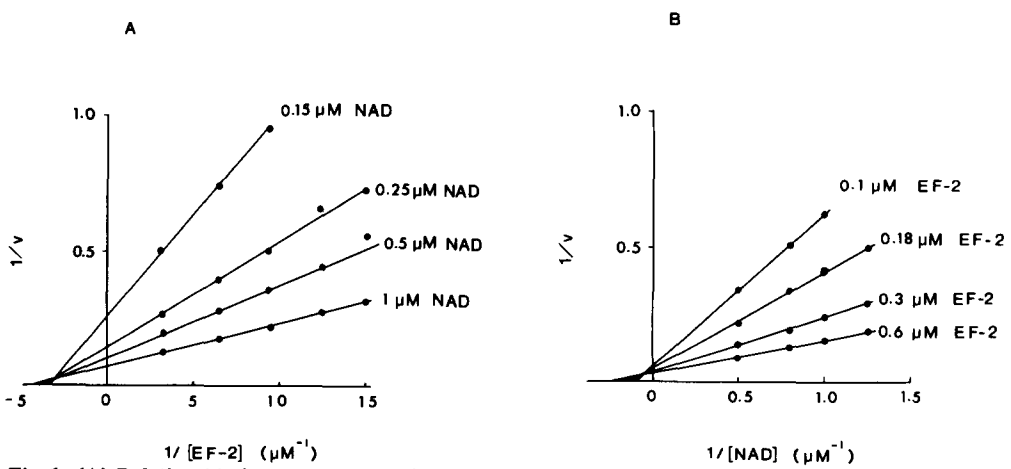

Fig. 1. (A) Relationship between initial velocity and EF-2 concentration, at selected constant NAD concentrations. The initial velocity is in pmol of ADPRib-EF-2 formed per 15 min at 25°C. (B) Relationship between initial velocity and NAD concentration, at selected constant EF-2 concentrations.

stable, covalent ADPRib-Fragment A intermediate, which has been sought by various methods; and by the fact that the NAD-glycohydrolase reaction catalyzed by Fragment A is much slower than the ADP-ribosylation reaction [12]. The latter result implies that the NAD-glycohydrolase reaction could not provide a required intermediate. Furthermore, it has been reported that significant rates of exchange between nicotinamide and NAD occur only in the presence of EF-2 [19,20]. It therefore seems certain that the ADP-ribosylation of EF-2 proceeds via a ternary, non-covalent intermediate containing Fragment A, NAD, and EF-2.

This ternary intermediate might be formed by either of two pathways:

$$\text{NAD} + \text{Fragment A} \rightleftharpoons [\text{Fragment A-NAD}]$$

$$[\text{Fragment A-NAD}] + \text{EF-2} \rightleftharpoons [\text{EF-2-Fragment A-NAD}] \quad \text{(I)}$$

or

$$\text{EF-2} + \text{Fragment A} \rightleftharpoons [\text{Fragment A-EF-2}]$$

$$[\text{Fragment A-EF-2}] + \text{NAD} \rightleftharpoons [\text{EF-2-Fragment A-NAD}] \quad \text{(II)}$$

Since it has been shown unequivocally that NAD can bind to Fragment A, it is reasonable to assume that the first pathway (I) is at least possible. Evidence was lacking, however, for the second pathway (II). Kinetic evidence to distinguish between the two pathways is theoretically obtainable, but in practice, there are formidable obstacles. For example, preliminary experiments (results not shown) indicate that ADPRib-EF-2 has a high inhibition constant, thus ruling out product inhibition measurements because of the prohibitively large amounts of ADPRib-EF-2 required. Isotope exchange experiments are also unsuitable for the same reason and because of the highly unfavorable equilibrium. Thus we undertook experiments to determine directly if Fragment A was capable of binding EF-2 in the absence of NAD.

Chromatography of EF-2 on Fragment A-Sepharose affinity column

Fragment A was coupled to CNBr-activated Sepharose 2B, and after extensive washing, the concentration of covalently bound Fragment A was determined by assay of ADP-ribosylation activity. A column (0.6 × 5 cm) was packed with the Fragment A-Sepharose containing about 0.7 nmol active Fragment A per ml packed gel, and equilibrated with buffer as described under Materials and Methods. A sample, 0.5 ml, of partially purified EF-2 (about 1 μM), was applied to the column, followed by two bed volumes of initial buffer. Essentially none of the EF-2 was retained by the column, and virturally all was recovered in active form at the void volume. Subsequent stepwise elution with Fragment A at 1 and 10 mM, or NaCl at 0.5 M, failed to elute significant amounts of bound EF-2. Similar results were obtained when adenine (0.5 mM) was included in all buffers. Inasmuch as the chromatography was performed under conditions close to those optimal for the ADP-ribosylation reaction, and the Fragment A covalently bound to the Sepharose was known to be active, this experiment suggested that Fragment A does not bind EF-2 strongly in the absence of NAD.

Equilibrium gel permeation chromatography of EF-2 with Fragment A

A more critical test of the interaction of EF-2 with Fragment A was conducted by means of equilibrium gel permeation chromatography, a technique originally described to study the binding of low molecular weight ligands to proteins [21]. To facilitate sensitive detection and accurate quantification of Fragment A, the latter was radioactively labelled by carboxymethylation of its single sulfhydryl group with iodo[^{3}H]acetate, a reaction which did not affect the enzymic activity [4]. A Sephadex G-100 superfine column (0.8 × 33 cm) was equilibrated with buffer containing 0.2 μM carboxy[^{3}H]methyl Fragment A. Pure EF-2 (0.1 μM) was incubated at 25°C for 15 min with excess carboxy-[^{3}H]methyl Fragment A, and the mixture was chromatographed on the pre-equilibrated column. Effluent fractions were assayed for Fragment A and EF-2, the former by scintillation counting of the tritium label, and the latter by adding radioactive NAD and allowing ADP-ribosylation to proceed to completion.

Due to the difference in molecular weights, EF-2 (M_r = about 100000) migrated faster than Fragment A, and since the column was pre-equilibrated with a constant level of free Fragment A, EF-2 migrated through the column in equilibrium with that concentration of free Fragment A. association of Fragment A with EF-2 would have been detected by the presence of a peak of Fragment A above the background level in the equilibrating buffer. However, as shown in Fig. 2, the elution profile showed no detectable binding.

Several variations of this experiment were performed, by including partial structural analogues of NAD, (adenine, ADPribose, NMN, or nicotinamide) in the equilibrating buffer, each at a concentration 10-fold its respective K_i (see Table I). Also the concentration of free Fragment A was varied from 0.2 to

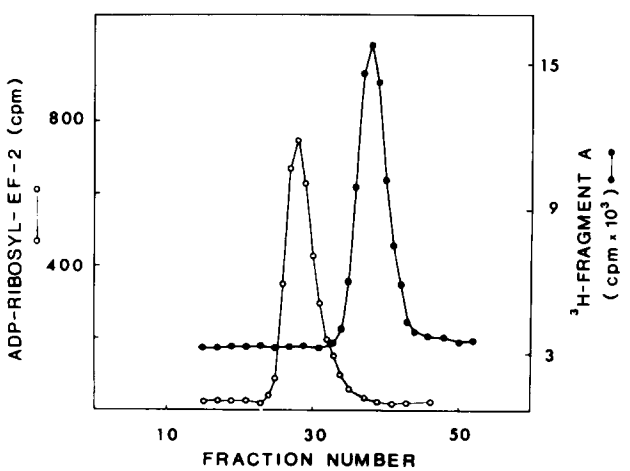

Fig. 2. Equilibrium gel permeation of EF-2 in Sephadex G-100 equilibrated with Fragment A. A Sephadex G-100 superfine column (0.8 × 33 cm) was equilibrated with 50 mM Tris · HCl, pH 8.2, 1 mM EDTA, 5 mM marcaptoethanol, 1 mg/ml bovine serum albumin and 0.2 μM carboxy[^{3}H]methyl Fragment A. EF-2 (0.1 μM) was incubated with excess labelled Fragment A (1 μM) at 25°C for 15 min, and a 0.1 ml sample was chromatographed on the column. Fractios of 0.12 ml were collected, 50-μl samples of which were counted by liquid scintillation for Fragment A quantification. EF-2 was measured by adding radioactive NAD to 50-μl samples of each fraction and allowing ADP-ribosylation to proceed to completion.

TABLE I

COMPARISON OF INHIBITION AND DISSOCIATION CONSTANTS OF ADENINE- AND NICOTINAMIDE-CONTAINING COMPOUNDS

Compound	K_I by inhibition of initial rate of ADP-ribosylation (μM)	K_I by inhibition of initial rate of NAD-glycohydrolase * (μM)	K_D dissociation constant for Fragment A * (μM)
Adenine	30	30	30
Adenosine	300	370	270
ADP ribose	3500	6000	4800
Nicotanamide	200	250	220
NADH	40	—	140

* Data from ref. 12.

1 μM. Under all these conditions, EF-2 was quantitatively recovered from the column, but no detectable binding was observed. Although the concentrations of Fragment A used in these experiments could only detect binding constants of the order of 0.1—1.0 μM, we felt that this was the reasonable range, because, as calculated from the initial rate data, the K_m for EF-2 was about 0.15 μM.

From these experiments, we conclude that if Fragment A is capable of binding EF-2 in the absence of NAD, the binding constant must be outside the range of these experiments, that is, greater than about 10^{-6} M. Such constants would probably not be of significance in the catalytic mechanism. It is therefore probable the formation of the Fragment A, NAD, EF-2 ternary complex does not proceed by pathway (II) at a significant rate. Thus the complex is presumably formed preferentially by Fragment A first binding NAD, followed by EF-2.

Competitive inhibition of ADP-ribosylation

Initial rates of ADP-ribosylation were measured in the presence of fixed levels of a number of partial structural analogues of NAD, and the data were analyzed by double reciprocal plots. For example, as shown in Figs. 3A and 3B, adenine was competitive with respect to NAD and non-competitive with EF-2. Secondary intercept and slope replots show that the inhibition is linear, with a K_i of 30 μM. Similar analyses were performed with adenosine, ADPribose, NADH and nicotinamide which is one of the products of the reaction. Results showed that they were all competitive with NAD and non-competitive with EF-2. Except for NADH, inhibition is linear, which is consistent with the interpretation that competitive binding of these analogues to the single NAD site on Fragment A is the cause of the competitive inhibition. The effectiveness of these analogues in inhibiting the reaction, as shown by their K_i values, should depend, therefore, on their respective binding constants, K_D values, to Fragment A. As shown in Table I, the correlation is apparent. The non-linearity of inhibition by NADH could be due to the presence of trace amounts of NAD which have not been vigorously excluded prior to the experiment. Similar anomalous biphasic inhibition curves with NADH have been previously reported [12].

The fact that adenine is a more potent inhibitor than adenosine, which is in

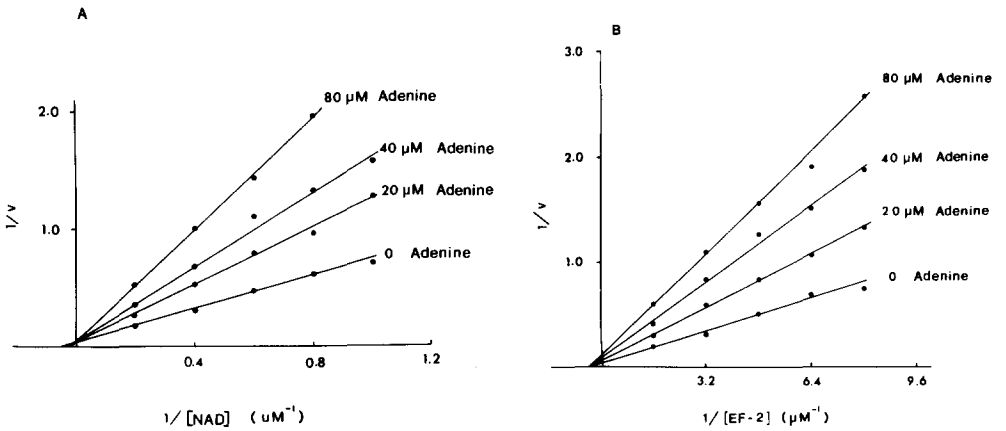

Fig. 3. (A) Effect of adenine on initial velocity at varying NAD concentrations, with EF-2 concentration held constant at 0.15 μM. (B) Effect of adenine on initial velocity at varying EF-2 concentrations, with NAD concentration held constant at 0.2 μM. The initial velocity is in units of pmol of ADPRib-EF-2 per 15 min at 25°C.

turn more potent than ADPribose, has also been reported by others [19]. Studies of binding of NAD to Fragment A indicate that interactions of the adenine and nicotinamide moieties are responsible for most or all of the binding energy. Presumably entropy factors are responsible for the decreased affinity of adenosine or ADPribose, compared with adenine.

Discussion

Our studies, performed with pure Fragment A and EF-2, present a consistent picture of the formal mechanism involved in the ADP-ribosylation of EF-2. Initial rate measurements and other evidence provide convincing evidence that the reaction proceeds through a ternary complex. Such a complex might be formed either by ordered or random binding of the two substrates to Fragment A. Results from direct binding measurements by equilibrium gel permeation on Sephadex strongly suggest that there is a preferential order, and that addition of NAD occurs before EF-2. This is consistent with the evidence available showing that Fragment A binds one molecule of NAD non-covalently in the absence of EF-2. Further evidence to support the proposed order of substrate addition is theoretically obtainable by product inhibition and isotope exchange measurements, but the highly unfavorable equilibrium and large amount of pure EF-2 required, present practical difficulties.

The proposed ordered, sequential mechanism is also supported by calculations from the data in Fig. 1. In such a mechanism, the vertical coordinate of the point of convergence in a Lineweaver-Burk plot is equal to $1/V\ (1 - K_a/K_{ia})$, where K_a and K_{ia} are the Michaelis and dissociation constants of the leading substrate, respectively. In both Figs. 1A and 1B, the points of convergence have vertical coordinate values of 0.018 (pmol ADPRib-EF-2 per 15 min)$^{-1}$. From this value, the V (40 pmol of ADPRib-EF-2 per 15 min), and the Michaelis constant of NAD, one can calculate a dissociation constant of 5 μM for NAD. The fact that this value is close to the value of about 8 μM measured

directly by dynamic dialysis, fluorescence quenching [12], and equilibrium dialysis [22], is consistent with our proposal of NAD being the leading substrate in an ordered sequential mechanism. Similar calculations for EF-2, from its Michaelis constant of 0.15 μM, yield a value of about 0.5 μM for the putative dissociation constant of EF-2 with Fragment A. It is very likely that our experiments on direct binding would have detected association if the actual dissociation constant were in that range.

The apparent lack of binding of EF-2 to Fragment A probably reflects a specific requirement for prior NAD binding to produce a high affinity site on Fragment A. Such a requirement may be analogous to the effect of the obligatory initial binding of NAD to dehydrogenases. In the case of several dehydrogenases [23—25], there is clear evidence for the occurrence of induced conformational changes accompanying the initial binding of NAD. These conformational alterations are necessary for catalysis upon binding of the second substrate. Although upon binding NAD, Fragment A shows no gross conformational changes detectable by velocity sedimentation or rotational relaxation measurements, it has been observed that Fragment A becomes resistant to trypsin and chymotrypsin in the presence of saturating levels of NAD [12]. This increase in resistance may reflect structural shifts necessary for EF-2 binding.

The proposed ordered sequential mechanism also provides a plausible explanation for the NAD-glycohydrolase activity observed with Fragment A. Since Fragment A has a single site for NAD, the active site involved in the highly efficient ADPribose transfer to EF-2 is also responsible for the inefficient transfer to water, leading to NAD hydrolysis. The slow rate of the latter reaction may be due to unproductive substitution of EF-2 by water in forming the ternary complex. Manifestations of substrate specificity in reaction rates have been observed [26,27].

Acknowledgments

We thank Samuel Raeburn for helpful discussions, and Patricia Bernard for excellent technical assistance. This work was supported in part by U.S. Public Health Service research grant AI-07877 from the National Institute of Health.

References

1 Honjo, T., Nishizuka, Y., Hayaishi, O. and Kato, I. (1968) J. Biol. Chem. 243, 3553—3555
2 Honjo, T., Nishizuka, Y., Kato, I. and Hayaishi, O. (1971) J. Biol. Chem. 246, 4251—4260
3 Gill, D.M., Pappenheimer, Jr., A.M., Brown, R. and Kurnick, J.J. (1969) J. Exp. Med. 129, 1—21
4 Collier, R.J. and Kandel, J. (1971) J. Biol. Chem. 246, 1496—1503
5 Drazin, R., Kandel, J. and Collier, R.J. (1971) J. Biol. Chem. 246, 1504—1510
6 Gill, D.M. and Dinius, L.L. (1971) J. Biol. Chem. 246, 1485—1491
7 Gill, D.M. and Pappenheimer, Jr., A.M. (1971) J. Biol. Chem. 246, 1492—1495
8 DeLange, R.J., Drazin, R.E. and Collier, R.J. (1976) Proc. Natl. Acad. Sci. U.S. 73, 69—72
9 Ittelson, T.R. and Gill, D.M. (1973) Nature 242, 330—332
10 Uchida, T., Pappenheimer, Jr., A.M. and Harper, A.A. (1972) Science 175, 901—903
11 Uchida, T., Pappenheimer, Jr., A.M. and Harper, A.A. (1973) J. Biol. Chem. 248, 3851—3854
12 Kandel, J., Collier, R.J. and Chung, D.W. (1974) J. Biol. Chem. 2088—2097
13 Michel, A. and Dirkx, J. (1974) Biochim. Biophys. Acta 365, 15—27
14 Parikh, I., March, S. and Cuatrecases, P. (1974) in Methods in Enzymology (Jakoby, W.B. and Wilchek, M., eds.), Vol. XXXIV, pp. 77—101, Academic Press, New York
15 Allen, E.S. and Schweet, R.S. (1962) J. Biol. Chem. 237, 760

16 Collier, R.J. (1967) J. Mol. Biol. 25, 83—98
17 Kornberg, A. (1950) J. Biol. Chem. 182, 779
18 Randerath, K. and Randerath, E. (1967) In Methods in Enzymology (Grossman, L. and Moldave, K., eds.), Vol. XII, part A, pp. 323—347, Academic Press, New York
19 Goor, R.S. and Maxwell, E.S. (1970) J. Biol. Chem. 245, 616—623
20 Honjo, T., Nishizuka, Y., Kato, I. and Hayaishi, O. (1971) J. Biol. Chem. 246, 4251—4260
21 Hummel, J.P. and Dreyer, W.J. (1962) Biochim. Biophys. Acta 63, 530
22 Montanaro, L. and Sperti, S. (1968) Biochem. J. 105, 635—640
23 Adams, M.J., Buehner, M., Chandrasekhar, K., Ford, G.C., Hackert, M.L., Liljar, A., Rossmann, M.G., Smiley, I.E., Allison, W.S., Everse, J., Kaplan, N.O. and Taylor, S.S. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1968—1972
24 Shore, J.D. and Brooks, R.L. (1971) Arch. Biochem. Biophys. 147, 825—827
25 Tsernoglou, D., Hill, E. and Banaszak, L.J. (1971) Cold Spring Harbor Symp. Quant. Biol. 36, 171—177
26 Trayser, K.A. and Colowick, S.P. (1961) Arch. Biochem. Biophys. 94, 161
27 Inouye, K. and Fruton, J.S. (1967) Biochemistry 6, 1765