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SUBUNIT INTERACTIONS IN RABBIT-MUSCLE GLYCERALDEHYDE-PHOSPHATE DEHYDROGENASE, AS MEASURED BY NAD^+ AND NADH BINDING

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Summary

1. The binding parameters for NADH and NAD^+ to rabbit-muscle glyceraldehyde-phosphate dehydrogenase (D-glyceraldehyde-3-phosphate:NAD^+ oxidoreductase (phosphorylating), EC 1.2.1.12) have been measured by quenching of the fluorescence of the protein and the NADH.

2. The fact that the degree of protein fluorescence quenching by bound NAD^+ or NADH, excited at 285 nm and measured at 340 nm ('blue' tryptophans), is not linearly related to the saturation functions of these nucleotides, leads to a slight overestimation of the interaction energy and an underestimation of the concentration of sites, if linearity is assumed.

3. This is also the case for NADH, but not for NAD^+, when the protein fluorescence is excited at 305 nm and measured at 390 nm ('red' tryptophans).

4. The binding of NAD^+ can be described by a model in which the binding of NAD^+, via negative interactions within the dimer, induces weaker binding sites, with the result that the microscopic dissociation constant is 0.08 μM at low saturation and 0.18 μM for the holoenzyme.

5. The binding of NADH can be described on the basis of the same model, the dissociation constant at low saturation being 0.5 μM and of the holoenzyme 1.0 μM.

6. The fluorescence of bound NADH is not sensitive to the conformational changes that cause the decrease in affinity of bound NAD^+ or NADH.

7. The binding of NAD^+ to the 3-phosphoglyceroyl enzyme can be described by a dissociation constant that is at least two orders of magnitude greater than the dissociation constants of the unacylated enzyme. The affinity of NAD^+ to

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Abbreviation: Mops, 3-(*N*-morpholino)propanesulphonate.

this form of the enzyme is in agreement with the K_i calculated from product inhibition by NAD^+ of the reductive dephosphorylation of 1,3-diphosphoglycerate.

Introduction

In a previous paper the NAD^+-binding properties of a chromatographically purified preparation of rabbit-muscle glyceraldehyde-phosphate dehydrogenase (D-glyceraldehyde-3-phosphate:NAD^+ oxidoreductase (phosphorylating), EC 1.2.1.12) were described [1]. The main finding was that the negative interactions calculated from the NAD binding to this preparation were much smaller than those reported by various laboratories using the classical preparation from rabbit muscle [2—7]. This result suggested that the asymmetrical behaviour of this classical preparation is at least partly due to a modification during isolation of some or all enzyme molecules. However, since also in the new preparation tight and weak sites were detectable, it remains an open question whether the remaining asymmetry is an intrinsic property of the enzyme, or is due to residual affinity heterogeneity [8] or is perhaps only apparent. Since Schlessinger and Levitzki [9] have shown that different types of tryptophan can be detected fluorimetrically in the enzyme (those emitting at higher wavelengths being designated 'red' and those emitting at lower wavelengths 'blue' tryptophans) and that the quenching of the 'blue' tryptophans is not linearly related to the saturation function of NAD^+, it is necessary to examine to what extent the assumption of linearity influences the interpretation of our binding studies with NAD^+, especially with respect to the magnitude of the interaction energy. Furthermore, it was interesting to investigate the possibility that the quenching coefficients of the bound nucleotides are sensitive to the same conformational changes that cause the decrease in affinity of NAD^+ and NADH.

The binding of NADH to the classical preparation can be described with a similar set of four dissociation constants as needed for NAD^+ binding. The recent study of Bell and Dalziel [7] indicated a slightly tighter binding of NADH to the tight sites, when compared with NAD^+, and equally weak binding to the remaining sites. When an enzyme solution containing a small amount of NADH was titrated with NAD^+ an increased fluorescence of the NADH was observed at half-saturation with NAD^+ [10]. Bell and Dalziel [10] concluded that this increased fluorescence reflects a change in conformation of the enzyme from the apo form to the holo form after two molecules of nucleotide have been bound, resulting in a much weaker binding of the last two molecules. Boers and Verhoeven [11] reached a similar conclusion using the charge-transfer band with *N*-methylnicotinamide as probe.

In the light of the much smaller interactions present in our preparation, it was of interest to carry out with this preparation the experiment described by Bell and Dalziel [10].

Materials and Methods

The enzyme was isolated from rabbit skeletal muscle as described before [1]. The preparation used in this study had a specific activity of 185 μmol/min per

mg protein, determined by the method of Ferdinand [12], not correcting for incomplete saturation with P_i.

The tetra-(3-phosphoglyceroyl) enzyme was prepared by addition of a 8—12 fold molar excess of 1,3-diphosphoglycerate (prepared and purified as in Ref. 13) to the holoenzyme. Acylation, followed spectrophotometrically at 360 nm, was found to be complete. No deacylation was detected during the period necessary to perform a complete binding experiment.

All experiments were carried out in a buffer (pH 7.6) containing 5 mM Mops, 1 mM EDTA, 1 mM dithiothreitol and 0.1 M $(NH_4)_2SO_4$,unless indicated otherwise.

Binding of NAD^+ to the acylated enzyme was measured at 18°C using the equilibrium gel filtration technique, as described before [1]. The Sephadex G-50 columns were equilibrated with varying concentrations of NAD^+ (10—100 μM) and 80 μM 1,3-diphosphoglycerate in the Mops/EDTA/dithiothreitol/$(NH_4)_2SO_4$ buffer (pH 7.0) to keep the enzyme in the acylated form during the filtration.

Spectrophotometry was carried out with an Aminco DW-2 spectrophotometer, using thermostatically controlled cells with a light path of 1 cm. All fluorescence measurements were carried out with a Perkin Elmer spectrofluorimeter model MPF 2a, using thermostated cells with a light path of 1 cm.

Results

Fig. 1 illustrates the different responses of the 'red' (observed at 390 nm) and 'blue' (observed at 340 nm) tryptophans [9] to the binding of NAD^+. Linear extrapolation of the first part of trace A ('red' tryptophans), where binding is known to be stoicheiometric at this enzyme concentration, leads to a stoicheiometry of 4.0 sites/tetramer while from trace B ('blue' tryptophans) an apparently lower stoicheiometry is obtained. It may be concluded that the degree of quenching by bound NAD^+ of the 'blue' tryptophans is not linearly

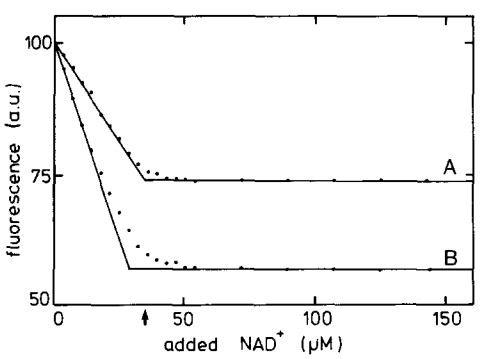

Fig. 1. Quenching of protein fluorescence by added NAD^+. 34.4 μM sites (indicated by arrow) in 5 mM Mops/1 mM EDTA/1 mM dithiothreitol/0.1 M $(NH_4)_2SO_4$ (pH 7.6, 16°C). (A) 'Red' tryptophans, excited at 305 nm (slitwidth 7 nm), emission observed at 390 nm (slitwidth 20 nm). (B) 'Blue' tryptophans, excited at 290 nm (slitwidth 2 nm), emission observed at 340 nm (slitwidth 40 nm). The points shown were corrected for inner-filter quenching by NAD^+ as described before [1]. The continuous lines are drawn to estimate the concentration of binding sites assuming a linear relationship between this signal and the saturation function.

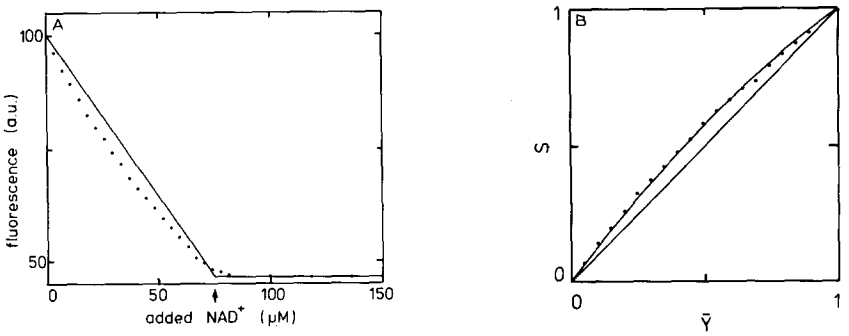

Fig. 2. (A) Quenching of protein fluorescence by added NAD^+. 75.0 μM sites, indicated by arrow. Other conditions as in Fig. 1. Excitation at 285 nm (slitwidth 5 nm), emission at 330 nm (slitwidth 8 nm). The continuous line is the shape of the titration curve expected for a signal with the same amplitude, but linearly related to the saturation function, assuming stoicheiometric binding to 75.0 μM sites. (B) Data of (A) normalized (S) and replotted against the saturation function $\overline{Y}$.

related to the saturation function but decreases as saturation increases. In our previous paper [1] this low stoicheiometry was ascribed to a small errror in the protein determination and/or the presence of impurities.

The relation between the degree of quenching of the 'blue' tryptophans and the saturation function is shown in more detail in Fig. 2A. Since, at the concentration of sites used (75.0 μM), binding is stoicheiometric over at least 80% of the saturation range, a plot of the quenching versus saturation can be constructed directly, after correction for the inner-filter quenching by NAD^+ as previously described [1]. This is shown in Fig. 2B. The deviation from linearity seen in this plot can be described on the assumption that the tightly bound NAD^+ causes 33% of the total quenching (=100%) per site occupied, and that at full saturation ($\overline{Y} = 1$) all molecules of NAD^+ are bound relatively weakly and contribute equally to the signal (see Appendix). We shall use this formalism to describe our titration curves; however, an alternative description of the non-linearity is possible (see Discussion).

In Fig. 3 (upper curve) is shown a titration with NAD^+ carried out at a low

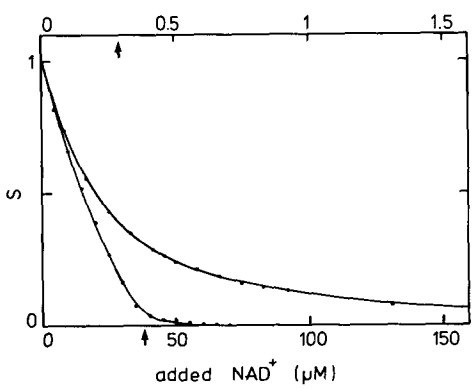

Fig. 3. Quenching of protein fluorescence by added NAD^+. 0.30 μM sites (upper curve, upper abscissa) and 38.2 μM sites (lower curve, lower abscissa), indicated by arrows. Other conditions as in Fig. 1. Excitation at 290 nm (slitwidth 7 nm), emission at 340 nm (slitwidth 5 nm (38.2 μM sites) or 40 nm (0.30 μM sites)). The signal was corrected for inner-filter quenching and normalized. Parameters used in the simulation (continuous line): see text.

enzyme concentration (0.30 μM sites). The quenching coefficient of the tightly bound NAD^+ was determined in a titration at high enzyme concentration (38.2 μM sites) using the same excitation and emission wavelengths and this value (33% of the total signal per tight site occupied) was used in the simulation of the two titrations shown in Fig. 3. Good fits are obtained using a microscopic dissociation constant of 0.08 μM at low saturation and 0.18 μM for the holoenzyme on the basis of a model, in which the binding of NAD^+, via negative interactions within the dimer, induces weaker binding sites. The interaction energy (2 kJ/mol sites) is a little lower than the value (2.6 kJ/mol sites) found assuming a linear relationship between the signal and the saturation function [1].

Binding of NADH

The binding of NADH was studied in the same manner as that of NAD^+. Fig. 4 shows a titration at high and low enzyme concentration, both simulated with the same set of parameters. As in the case with NAD^+ the tightly bound NADH contributes more to the quenching of the fluorescence of the 'blue' tryptophans (33% of the total signal per tightly bound NADH) than does the weakly bound NADH. The binding can be adequately described with a dissociation constant of 0.5 μM for the tight sites and negative interactions across one of the three different areas of contact between the subunits (interaction energy 1.7 kJ/mol), producing weaker binding sites, having a dissociation constant of 1.0 μM.

The response of the 'red' tryptophan fluorescence to NADH binding is shown in Fig. 5. It is clear from this figure that the degree of quenching of these tryptophans, which is exactly linearly related to NAD^+ saturation (Fig. 1), is not linearly related to the saturation of the sites with NADH. The contribution of the tightly bound NADH to the signal is 36% of the total per tight site occupied.

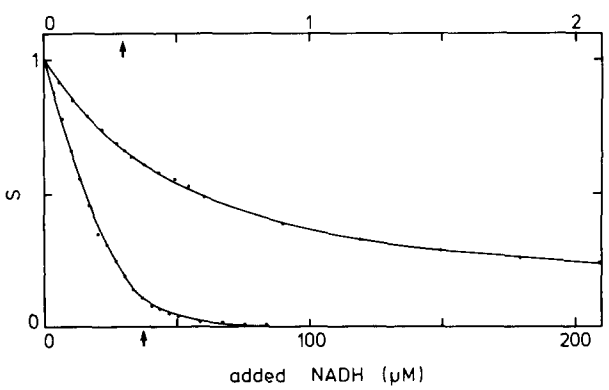

Fig. 4. Quenching of protein fluorescence by added NADH. 0.30 μM sites (upper curve, upper abscissa) and 37.8 μM sites (lower curve, lower abscissa), indicated by arrows, Other conditions as in Fig. 1. Excitation at 290 nm (slitwidth 2 nm (37.8 μM sites) or 7 nm (0.30 μM sites)), emission at 340 nm (slitwidth 40 nm). The signal was corrected for considerable inner-filter quenching and normalized (end level was 52% of starting fluorescence). Other parameters used for the simulation (continuous line): see text.

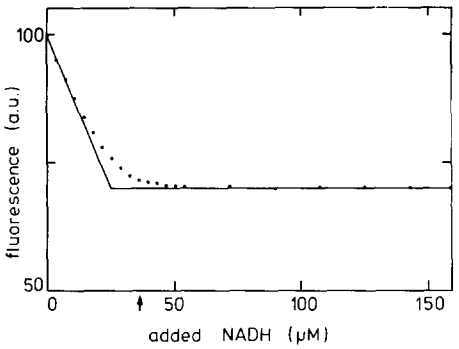

Fig. 5. Quenching of protein fluorescence by added NADH. 36.6 μM sites, indicated by arrow. Other conditions as in Fig. 1. Excitation at 305 nm (slitwidth 7 nm), emission at 390 nm (slitwidth 20 nm) ('red' tryptophans). The continuous line is drawn to show the underestimation of the concentration of sites by linear extrapolation of the signal.

Quenching of NADH fluorescence

The experiment reported in Fig. 6 shows that the fluorescence yield of bound NADH is constant at all levels of saturation within experimental error, as found previously by Kelemen et al. [14] for the sturgeon enzyme. This is clear from the fact that until about 70% saturation, where binding is still stoicheiometric with the enzyme concentration used, the curve can be simulated with a fluorescence yield of bound NADH equal to 54% of the yield of free NADH, and the dissociation constants found by the protein fluores-

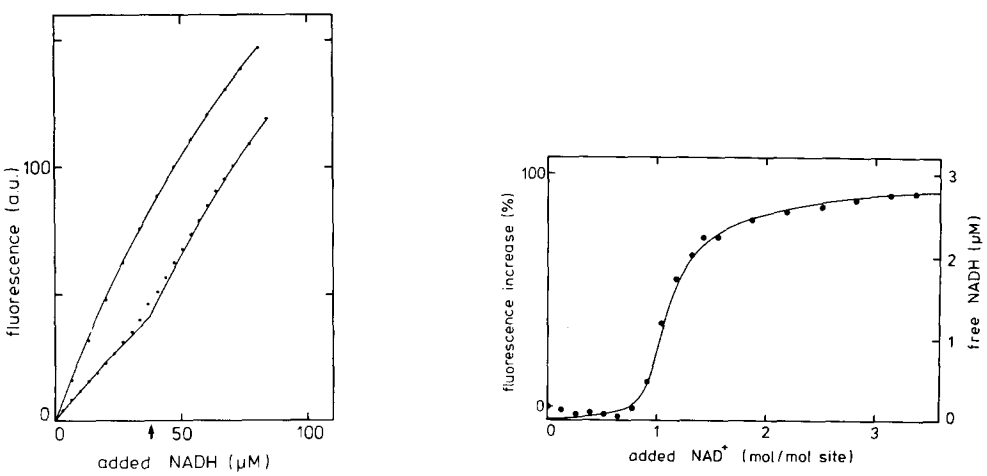

Fig. 6. NADH fluorescence in the absence (uppper line) and presence (lower line) of 37.8 μM sites, indicated by arrow. Other conditions as in Fig. 1. Excitation at 340 nm (slitwidth 8 nm), emission at 450 nm (slitwidth 8 nm). The continuous lines are drawn assuming stoicheiometric binding to 37.8 μM sites and a constant value of the fluorescence yield of bound NADH (54% of the yield of free NADH). The non-linearity is due to the high absorbance of the solution at the excitation wavelength.

Fig. 7. Fluorescence of 3 μM NADH monitored during a titration of 77.3 μM sites with NAD^+. Other conditions as in Fig. 1. Excitation at 340 nm (slitwidth 8 nm), emission at 450 nm (slitwidth 8 nm). The points represent the measured fluorescence, the continuous line the concentration of free NADH (see text for parameters used in this calculation).

cence quenching. The non-linearity of the calibration curve is caused by inner-filter quenching. The fluorescence rises more steeply after 70% saturation due to the higher fluorescence yield of free NADH.

Fig. 7 shows the effect of NAD^+ on the fluorescence of a small amount of NADH bound to the enzyme (3 μM NADH, 77.3 μM sites). In contrast to the finding of Bell and Dalziel [10], no significant change in the NADH fluorescence was observed until about 80% saturation with NAD^+. Upon further addition of NAD^+ a rather sharp rise was seen, due to the higher fluorescence yield of the free NADH displaced from the enzyme by NAD^+. With a large excess of NAD^+ the fluorescence observed was almost as high as that of the same amount of free NADH. Also shown is the concentration of free NADH, calculated using the dissociation constants found by the protein fluorescence quenching method, assuming the same interactions occurring between bound NAD^+ and NADH as those between different NADH molecules.

Binding of NAD to the acylated enzyme

Since our binding studies reveal no weak sites ($K_D > 1$ μM) for either NAD^+ or NADH we studied the product inhibition by both NAD^+ and NADH in the backward and forward reaction, respectively, of the enzyme with the natural substrates. The data of Fig. 8 show that the K_i for the inhibition by NAD^+, measured at 25° C, is of the same order of magnitude (about 150 μM) as found before with the classical preparation of the enzyme [13]. For NADH the K_i was somewhat lower (50 μM). Since there results cannot be incorporated in a sequential mechanism, where the K_i values should be the same as the K_D values of the weak sites, we investigated the binding of NAD^+ to the acylated form of the enzyme. The K_D values for NAD^+, measured at 18° C, varied between 30 and 80 μM (mean 65 μM). Since it has been shown for the sturgeon enzyme that NAD^+ binding to the acylated enzyme is weaker at higher temperature ($K_{25°C}/K_{10°C} = 2.6$) [15] this value is close enough to the value for K_i in the backward reaction of the enzyme to be compatible with a mechanism in which the competition between NAD^+ and NADH takes place for enzyme subunits in their acylated form.

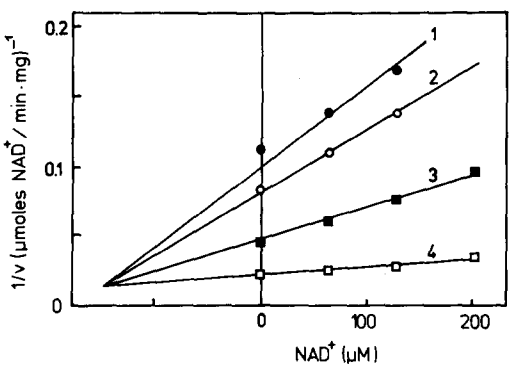

Fig. 8. Product inhibition by NAD^+ of the reductive dephosphorylation of 1,3-diphosphoglycerate measured fluorimetrically. The medium (pH 7.0, 25° C) contained 5 mM Mops, 1 mM EDTA, 1 mM dithiothreitol, 0.1 M $(NH_4)_2SO_4$ and 83 μM 1,3-diphosphoglycerate in 1.5 ml. The reaction was started with the holoenzyme. The concentrations of NADH were 1.3, 2.1, 4.2 and 14 μM in lines 1—4, respectively.

Discussion

First it is important to note that the preparation of rabbit-muscle glyceraldehyde-phosphate dehydrogenase used shows a more homogeneous binding behaviour with respect to both the reduced and oxidized NAD than the classical preparation used in various laboratories [2—7]. This most probably results from the modified isolation procedure as was discussed before [1]. The large decrease in affinity on going from the first to the fourth molecule of NAD^+ or NADH, which has made rabbit-muscle glyceraldehyde-phosphate dehydrogenase the first example of 'negative cooperativity', has almost disappeared in the present preparation.

Even if we may ascribe the presence of tight and weak sites in our preparation to negative interactions across one of the three crystallographic axes in the tetramer (however, see below), these interactions cause a difference in binding free energy of only about 5% between the tight and weak sites (39.3 kJ/mol NAD^+ tightly bound and 37.3 kJ/mol NAD^+ weakly bound; 34.8 kJ/mol NADH tightly bound and 33.2 kJ/mol NADH weakly bound).

This small interaction energy necessarily means that there will be a significant overlap between the occupation of 'tight' and 'less tight' sites during a titration with NAD^+ or NADH, so that no sharp change in a parameter sensitive to this interaction is to be expected at half-saturation. This is illustrated in Fig. 9 where the weakly bound fraction of the bound ligand is plotted against the saturation function for different values of the interaction energy, assuming a dimeric pattern of interactions (see Appendix). A plot of any parameter reflecting these interactions against the saturation function must show a transition around half-saturation. A sharp transition at half-saturation only occurs if the interaction energy is so large that hardly any overlap occurs between the occupation of the tight and weak sites (trace 3 in Fig. 9). Such a sharp transition at half-saturation was seen in a study of Bell and Dalziel [10], who monitored the fluorescence of a small amount of bound NADH during a titration with NAD^+. On the basis of the binding data obtained with their preparation [7], which suggest very large negative interactions, their conclusion seemed justified that the fluorescence yield of bound NADH indeed is a param-

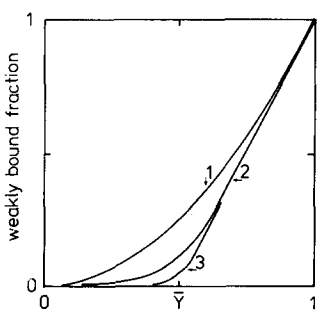

Fig. 9. Weakly bound fraction of bound ligand as a function of the saturation. A dimeric pattern of negative interactions (positive interaction free energy) of varying magnitude is assumed. 1. Vanishingly small interaction free energy. 2. Interaction free energy equal to 5.5 kJ/mol (K_{loose}/K_{tight} = 10). 3. Interaction free energy equal to 11.1 kJ/mol (K_{loose}/K_{tight} = 100).

eter that reflects these interactions, or, in their own terms, that reflects a conformational transition from a relaxed to a tense quaternary structure at half-saturation. In our preparation, however, the NADH probe shows no rise in fluorescence near half-saturation with NAD^+. No rise in fluorescence is seen before the stage is reached, where, according to the calculations, the probe is expected to be displaced from the enzyme by the NAD^+ (Fig. 7). The sharpness of the transition is caused by the significantly weaker binding of NADH compared with that of NAD^+, resulting in an effective displacement of the NADH probe by NAD^+.

Furthermore, if the fluorescence of NADH is used to monitor its binding to the apoenzyme the titration curve can be simulated accurately assuming a constant, low fluorescence of bound NADH at all levels of saturation, indicating that also in this case the fluorescence yield of bound NADH does not reflect the negative interactions resulting in a decrease of the NADH affinity during the titration. The same was observed by Seydoux and colleagues with the enzyme from sturgeon muscle [14].

Our data are, therefore, in agreement with the suggestion made by Schlessinger and Levitzki [9] that signals associated with the nicotinamide part of the coenzyme are linearly related to the saturation function. This is not the case for the degree of protein fluorescence quenching by bound NAD^+ or NADH. It was shown that not all molecules of bound NADH quench the tryptophan fluorescence equally, while the same holds for NAD^+ with respect to the 'blue' tryptophan fluorescence quenching, in qualitative agreement with Schlessinger and Levitzki [9].

There are several ways to explain this non-linear relationship between a signal used to monitor binding and the saturation function. The formalism used to describe our titration curves is that the tightly bound NAD^+ (or NADH) has a higher quenching coefficient than the weakly bound nucleotides. This means that these quenching coefficients reflect inter-subunit interactions across the same crystallographic axis as those causing the decrease in binding affinity. As illustrated in Fig. 9 a plot of such a signal against the saturation function is dependent on the magnitude of the interaction energy. However, if the differences in the quenching coefficients are caused by interactions across a crystallographic axis other than those affecting the affinity of the nucleotides the plot is independent of the interaction energy, and a smooth curve, with no break at half-saturation, will be obtained, as is also the case described in Fig. 9, when the interaction energy is vanishingly small. No clear break in the curve was observed in our fluorescence quenching measurements at high enzyme concentrations (Fig. 2), but, in view of the small value of the calculated interaction energy, it is not possible to decide between these two possibilities.

A third possibility is that the change during binding in quenching coefficients and dissociation constants reflects residual affinity heterogeneity in our enzyme preparation. Gennis [8] has shown that this was indeed the cause of the observed, apparent negative cooperativity in the binding of NAD^+ to the yeast enzyme.

In previous papers from this laboratory [16—18] it was suggested that only the fourth and possibly the third subunit of glyceraldehyde-phosphate dehydrogenase can play a catalytic role. The main argument for this hypothesis

was the fact that the K_i values for product inhibition by NADH and NAD^+ of the oxidative phosphorylation and reductive dephosphorylation reactions, respectively, are the same as the dissociation constants measured for the binding of these nucleotides to the fourth subunit only. The sequential mechanism accepted by most workers at that time [13,19—22] indeed predicts much stronger product inhibition if also the tight binding sites would play a catalytic role. Therefore, it was interesting to see if the K_i values measured with the present preparation (which does not contain the weak binding sites present in the classical preparation) are different from those measured with the classical preparation. The fact that this is not the case adds further evidence in support of a mechanism, in which the competition between NAD^+ and NADH is not for the non-acylated but for the acylated enzyme. Direct binding studies with the fully acylated enzyme indeed show that the binding of NAD^+ to this form of the enzyme can be described with a dissociation constant that is close to the K_i value for product inhibition by NAD^+ of the reductive dephosphorylation of 1,3-diphosphoglycerate, measured under the same conditions, as is predicted by the now generally accepted ping-pong mechanism evolved from the studies of Harrigan and Trentham [23,24], Duggleby and Dennis [25], Seydoux et al. [15] and Meunier and Dalziel [26].

Appendix

The binding data presented in this paper were analyzed in terms of a Koshland type of interaction model [27], with interactions restricted to only one of the three different types of boundaries (designated P, Q and R in the crystallographic description [28]), so that the binding to the tetramer can be treated mathematically as binding to a dimer (of dimers).

The saturation function $\bar{Y}$ is related to the concentration of free ligand as follows:

$$\bar{Y} = (\alpha + p\alpha^2)/(1 + 2\alpha + p\alpha^2) \quad (1)$$

in which

$$\alpha = [\text{ligand}]_{free}/K_{tight}$$

and

$$p = K_{tight}/K_{loose}$$

where K_{tight} and K_{loose} are the microscopic dissociation constants. The interaction free energy is equal to $-RT\ln p$.

In the fluorimetric titrations the observed signal, after correction for inner-filter quenching and normalization, is related to the concentration of free ligand as follows:

$$S = (2\epsilon\alpha + p\alpha^2)/(1 + 2\alpha + p\alpha^2) \quad (2)$$

in which ϵ is the parameter describing the relative contribution of the tightly bound nucleotides to the signal. This parameter can be estimated from the initial part of a titration at a high concentration of binding sites (relative to the dissociation constant of the ligand). With this value of ϵ, an assumed value

for p (usually 1) and the signal S, the quadratic equation (Eqn. 2) can be solved to calculate α and (via Eqn. 1) the value of $\overline{Y}$ corresponding to this signal. The other parameters (K_{tight} and p) are then derived from a Hill plot, and one iteration of the process, using the new value for p, is usually sufficient to obtain a consistent set of parameters, with which the titrations performed at different protein concentrations can be simulated.

The weakly bound fraction (ϕ) is given by

$$\phi = p\alpha^2/(1 + 2\alpha + p\alpha^2)$$

which was calculated for three different values of p, together with the corresponding saturation functions, to construct Fig. 9 of this paper.

Acknowledgements

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References

1 Scheek, R.M. and Slater, E.C. (1978) Biochim. Biophys. Acta 526, 13—24
2 De Vijlder, J.J.M. and Slater, E.C. (1968) Biochim. Biophys. Acta 167, 23—34
3 Conway, A. and Koshland, D.E., Jr. (1968) Biochemistry 7, 4011—4023
4 Velick, S.F., Baggott, J.P. and Sturtevant, J.M. (1971) Biochemistry 10, 779—786
5 Price, N.C. and Radda, G.K. (1971) Biochim. Biophys. Acta 235, 27—31
6 Allen, G. and Harris, J.I. (1975) Biochem. J. 151, 747—749
7 Bell, J.E. and Dalziel, K. (1975) Biochim. Biophys. Acta 391, 249—258
8 Gennis, L.S. (1976) Proc. Natl. Acad. Sci. U.S. 73, 3928—3932
9 Schlessinger, J. and Levitzki, A. (1974) J. Mol. Biol. 82, 547—561
10 Bell, J.E. and Dalziel, K. (1975) Biochim. Biophys. Acta 410, 243—251
11 Boers, W. and Verhoeven, J.W. (1973) Biochim. Biophys. Acta 328, 1—9
12 Ferdinand, W. (1964) Biochem. J. 92, 578—585
13 Furfine, C.S. and Velick, S.F. (1965) J. Biol. Chem. 240, 844—855
14 Kelemen, N., Kellershohn, N. and Seydoux, F. (1975) Eur. J. Biochem. 57, 69—78
15 Seydoux, F.J., Kelemen, N., Kellershohn, N. and Roucous, C. (1976) Eur. J. Biochem. 64, 481—489
16 De Vijlder, J.J.M., Hilvers, A.G., van Lis, J.M.J. and Slater E.C. (1969) Biochim. Biophys. Acta 191, 221—228
17 Boers, W., Oosthuizen, C. and Slater, E.C. (1971) Biochim. Biophys. Acta 250, 35—46
18 Boers, W. and Slater, E.C. (1973) Biochim. Biophys. Acta 315, 272—284
19 Keleti, T. and Batke, J. (1965) Acta Physiol. Hung. 48, 195—201
20 Fahien, L.A. (1966) J. Biol. Chem. 241, 4115—4123
21 Orsi, B.A. and Cleland, W.W. (1972) Biochemistry 11, 102—109
22 Smith, C.M. and Velick, S.F. (1972) J. Biol. Chem. 247, 273—284
23 Harrigan, P.J. and Trentham, D.R. (1973) Biochem.J. 135, 695—703
24 Harrigan, P.J. and Trentham, D.R. (1974) Biochem. J. 143, 353—363
25 Duggleby, R.G. and Dennis, D.T. (1974) J. Biol. Chem. 249, 167—174
26 Meunier, J.C. and Dalziel, K. (1978) Eur. J. Biochem. 82, 483—492
27 Cornish-Bowden, A.J.-C. and Koshland, D.E., Jr. (1970) J. Biol. Chem. 245, 6241—6250
28 Buehner, M., Ford, G.C., Moras, D., Olsen, K.W. and Rossmann, M.G. (1974) J. Mol. Biol. 90, 25—49