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X-RAY SMALL-ANGLE SCATTERING OF YEAST GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AS A FUNCTION OF SATURATION WITH NICOTINAMIDE-ADENINE-DINUCLEOTIDE

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The binding of NAD to the tetrameric yeast glyceraldehyde-3-phosphate dehydrogenase at pH 8.5 and 40°C is positively cooperative. Temperature-jump studies [1] have led to the conclusion that the concerted (or "allosteric") mechanism [2] is the simplest one explaining the kinetic and equilibrium data. This letter reports investigations on the X-ray small-angle scattering of the enzyme as a function of NAD binding under the same conditions as used in the kinetic studies (i.e., at pH 8.5 and 40°C). The results show that the enzyme molecule contracts upon binding NAD. Moreover, the degree of contraction precedes the degree of saturation. This finding unequivocally excludes the sequential mechanism [3] and agrees well with the predictions of the concerted mechanism [2].

Measurements were done at the following degrees of saturation with NAD: 0.00 (=apo-enzyme), 0.23, 0.46, 0.72 and 0.99 (=holo-enzyme). The intermediate values were adjusted by gel filtration of concentrated enzyme solutions over Sephadex at 40°C [4]. The buffer used for equilibrating the column and for eluting the enzyme contained NAD corresponding to the free ligand concentration at the desired degree of saturation. The latter correlation was accurately established by spectrophotometric titration and equilibrium dialysis [10]. The procedure fulfilled the criterium characterizing true equilibrium in ligand binding studies by gel filtration (i.e., a well-defined plateau between the high and low molecular weight peaks). After determining the protein concentration the equilibrium mixture was diluted with the corresponding elution solvent. In this way the degree of saturation remains unchanged. Holo-enzyme was obtained by prolonged dialysis at 4°C versus 10^{-2} M NAD.

The molecular weight of the apo-enzyme, the shape, the volume and the internal solvation were determined using the experimental techniques and the theory published previously [5].

The instability of the enzyme under prolonged exposure to elevated temperatures necessitated the use of a continuous flow device. The cold (4°C) enzyme solution was slowly pumped without recycling through the observation capillary thermostated at 40°C. The enzyme was therefore exposed only briefly to the deleterious conditions. As a control, enzyme activity was always determined before and after exposure. The loss in specific activity rarely exceeded 10%.

The scattering curves were measured for various concentrations of enzyme between 0.25–2.00%. After extrapolating the curves to zero concentration the following quantities were determined:

(1) The molecular weight of the enzyme was obtained from the absolute scattering intensity at zero angle. The value found for the apo-enzyme

Table 1
Im^2-values (counts/sec cm^2) at the following degrees of saturation with NAD.

m (cm)	0.00 (=apo-enzyme)	0.23	0.46	0.72	0.99 (=holo-enzyme)
0.00	0.00	0.00	0.00	0.00	0.00
0.02	0.13	0.13	0.13	0.13	0.13
0.04	0.40	0.40	0.40	0.40	0.40
0.06	0.85	0.85	0.86	0.86	0.86
0.08	1.46	1.46	1.47	1.47	1.47
0.10	2.19	2.19	2.20	2.20	2.20
0.12	2.98	2.99	2.99	3.00	3.00
0.14	3.79	3.80	3.81	3.82	3.83
0.16	4.59	4.61	4.63	4.64	4.65
0.18	5.32	5.35	5.37	5.39	5.41
0.20	5.98	6.01	6.04	6.06	6.08
0.22	6.48	6.53	6.57	6.60	6.63
0.24	6.88	6.93	6.98	7.02	7.04
0.26	7.11	7.19	7.25	7.27	7.31
0.28	7.21	7.30	7.37	7.42	7.45
0.30	7.12	7.23	7.32	7.37	7.42
0.32	6.84	6.97	7.09	7.17	7.23
0.34	6.35	6.49	6.62	6.72	6.78
0.36	5.74	5.89	6.03	6.15	6.22
0.38	5.00	5.20	5.37	5.48	5.56
0.40	4.27	4.49	4.67	4.78	4.84
0.42	3.55	3.75	3.95	4.05	4.12
0.44	2.99	3.15	3.28	3.38	3.45
0.46	2.47	2.60	2.71	2.80	2.85
0.48	2.02	2.14	2.20	2.27	2.32
0.50	1.66	1.74	1.79	1.85	1.89
0.52	1.36	1.42	1.47	1.49	1.52
0.54	1.15	1.18	1.22	1.24	1.26
0.56	0.98	1.01	1.03	1.05	1.06
0.58	0.90	0.91	0.91	0.92	0.92
0.60	0.85	0.85	0.85	0.85	0.85
0.65	0.93	0.93	0.94	0.94	0.94
0.70	1.18	1.18	1.18	1.17	1.16
0.75	1.30	1.31	1.31	1.32	1.33
0.80	1.18	1.20	1.22	1.24	1.25
0.85	0.93	0.96	0.98	1.00	1.03
0.90	0.68	0.70	0.72	0.73	0.75
0.95	0.52	0.53	0.54	0.56	0.58
1.00	0.40	0.41	0.42	0.42	0.43
1.05	0.29	0.30	0.31	0.32	0.33
1.10	0.20	0.21	0.22	0.23	0.23
1.15	0.14	0.14	0.14	0.15	0.15
1.20	0.10	0.10	0.10	0.11	0.11
1.25	0.06	0.06	0.06	0.06	0.06
1.30	0.03	0.03	0.03	0.03	0.03
1.35	0.01	0.01	0.01	0.01	0.01
1.40	0.00	0.00	0.00	0.00	0.00

Table 1 (continued)

m (cm)	0.00 (=apo-enzyme)	0.23	0.46	0.72	0.99 (=holo-enzyme)
Q	2.5820	2.6308	2.6729	2.7041	2.7281
$I_0 f$	249.50	248.45	247.41	246.25	245.05
V (A^3)	2.642×10^5	2.582×10^5	2.530×10^5	2.489×10^5	2.455×10^5
$\bar{R}$	0.00	0.32	0.60	0.82	1.00

(1.41×10^5 daltons) agrees well with the results of other authors (cf. ref. [6]). Occasionally, values as low as 1.0×10^5 daltons were found. These significant deviations are almost certainly due to the presence of large aggregates of protein which do not contribute to the scattering in the accessible range. The decrease in intensity is not accompanied by a change in the shape of the scattering curves. They are superimposable after multiplication by a scale factor. One can therefore conclude that smaller aggregates or dissociation products, which would necessarily interfere with the scattering of the intact enzyme, are absent.

(2) The radius of gyration R was obtained from the tangent to zero angle in the Guinier plot.

(3) The volume V was determined from the zero angle intensity I_0 and the invariant Q according to Porod [7] using the following formulas:

$$V = \frac{\lambda^3 a^3}{4\pi} \times \frac{I_0 f}{Q},$$

$$Q = \int_0^\infty Im^2 \mathrm{d}m,$$

$$m = 2a \sin\Theta,$$

where λ = wavelength CuK_α (= 1.54 Å), a = distance sample-registering plane (= 21.1 cm), I_0 = intensity at zero angle (counts/sec), f = factor allowing for the change of the molecular weight at different degrees of saturation with NAD, I = intensity at m (counts/sec), m = distance in the registering plane from the position at zero angle (cm) and 2Θ = scattering angle (radians).

(4) The shape of the molecule was obtained from a comparison between experimental and theoretical scattering curves.

(5) Finally, the internal solvation was calculated from the volume in solution, and from the volume obtained from the known molecular weight and the partial specific volume.

All scattering curves possess a well-defined isometric sub-maximum with an intensity of about 0.96% of the main maximum. This submaximum is important for the comparison of curves. The middle portions of the scattering curves obtained at various concentrations of NAD differ in a way characteristic of an increase of anisotropy of the enzyme molecule with increasing saturation. In all cases it was possible to find models equivalent in scattering within the experimental error. These can be either quadratic prisms, cylinders or rotation ellipsoids with approximately the same axial ratios.

The comparison of the curves obtained for the apo-enzyme with the theoretical curves based on the assumption of the cylindrial form lead to an axial ratio of the height h to the diameter d of h: d = 0.6:1. With a measured volume of $V = 2.642 \times 10^5$ Å^3 (table 1) the dimensions are h = 49.5 Å and d = 82.4 Å. The radius of gyration R = 32.4 Å calculated from these values agrees sufficiently well with the value determined experimentally (R_{exp} = 32.1 Å). The corresponding quantities of the holo-enzyme as obtained by exactly the same evaluation procedure are $V = 2.455 \times 10^5$ Å^3 and R_{exp} = 31.7 Å.

It should be noted that the 7.08% decrease in volume observed for the transition of apo- to holo-enzyme is accompanied by a corresponding decrease of the radius of gyration of only 1.25%. This fact can only be explained if the axial ratio of the holo-enzyme

is *more* anisotropic (i.e., $h : d = 0.56 : 1.00$) than that of the apo-enzyme. This agrees not only with the qualitative indication mentioned above but also with the shape of the scattering curve. The corresponding dimensions of the holo-enzyme based on this assumption are $h = 46.1$ Å and $d = 82.4$ Å.

Although the above dimensions can vary within certain limits, depending on the particular choice of the scattering model, the relative values can be considered to reflect physical reality because of the identical evaluation procedure used.

The changes in volume and radius of gyration are accompanied by a change in the internal solvation of approximately 26%. Apo-enzyme contains about 0.35 and holo-enzyme 0.26 g solvent per g of protein respectively.

The above findings are in qualitative agreement with other investigations on changes in the intrinsic viscosity and susceptibility towards trypsin digestion [8] and on the variation of gross structural and conformational parameters [9]. The latter results indicate that yeast glyceraldehyde-3-phosphate dehydrogenase becomes more compact upon binding NAD. Moreover, the kind of structural change occurring in the allosteric transition can now be described more precisely in terms of an increase in the inherent anisotropy of the tetrameric apo-enzyme.

The quantitative evaluation of the observed volume changes as a function of saturation with NAD provides a specific test of the mechanism proposed for the cooperative nature of the process [1]. The simple sequential model [3] postulates a direct coupling between binding and isomerization steps. In contrast, the concerted model [2] allows for a discrepancy between the degree of binding and the degree of structural change. Fig. 1 depicts according to table 1 the volume decrease (normalized by the total change between apo- and holo-enzyme) as a function of the degree of saturation with NAD. It is clear that the measurements rule out the sequential scheme. Moreover they agree well with the theoretical curve calculated on the basis of the concerted mechanism with the equilibrium constants obtained from more recent kinetic studies with the temperature-jump method [10]. Similar quantitative results have been obtained from independent measurements of sedimentation velocity and optical rotatory dispersion [9].

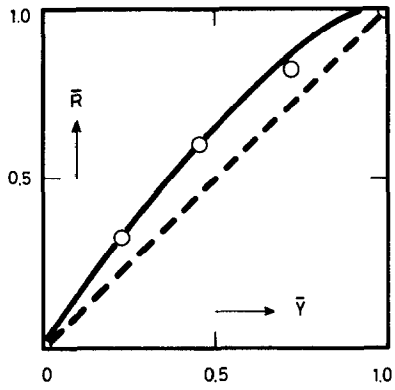

Fig. 1. The degree of volume contraction of yeast apo-glyceraldehyde-3-phosphate dehydrogenase as a function of saturation with NAD. $\bar{R}$ = degree of structural change, $\bar{Y}$ = degree of saturation with NAD. The diagonal represents the prediction for the sequential mechanism of cooperative binding. The curve was calculated on the basis of the concerted mechanism for $n = 4$, $K_R = 0.1$ mM, $K_T = 2.5$ mM, $L_0 = 60$ [10]. o = experimentally observed degree of volume contraction.

The results reported in this letter represent the first independent demonstration of *concerted* structural changes of an allosteric enzyme with the X-ray small-angle scattering technique.

A detailed publication is in preparation and will follow shortly.

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