STEREOSELECTIVE REACTIVITY OF THE SH GROUPS OF YEAST GLYCERALDEHYDEPHOSPHATE DEHYDROGENASE IN THE ALLOSTERIC T AND R STATES

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Yeast glyceraldehyde-3-phosphate dehydrogenase as a typical SH enzyme is inactivated by the antipodes of α-iodopropionic acid and its amide at different rates. The apoenzyme reacts faster with the D(+) antipode of the free α-iodopropionic acid (k_D/k_L = 6.8) and the L(−) antipode of the amide (k_L/k_D = 3). On addition of NAD^+ the stereoselectivity of the SH group towards α-iodopropionic acid is inverted, that towards the amide is enlarged, the rate relationships depending on the NAD^+ concentration.

The results were interpreted by the assumption, that the allosteric T state of the enzyme reacts most rapidly with the D(+) antipodes, whereas the R state favours the L(−) antipodes of the alkylation reagents. The dependence of the reaction rates on the NAD^+ concentration could be fitted to the allosteric function of state $\bar{R}$.

Typical SH-enzymes have SH-functions as an integral part of their active sites. Such enzymes as e.g. papain or yeast alcohol dehydrogenase (Y-ADH) show different reactivities with the antipodes of α-iodopropionic acid and its amide [1-3]. From the evaluation of the kinetic parameters for the different antipodes, conclusions could be drawn concerning the steric surroundings of the reacting SH groups. Most of the NAD^+ dependent dehydrogenases are SH-enzymes, and usually the active site SH groups seem to be involved in coenzyme binding. NAD^+ dependent dehydrogenases are known to have another stereoselective reactivity. They are able to distinguish between the two "enantiotopic" [4] hydrogen atoms in the 4 position of the nicotinamide moiety (A and B specific enzymes) [5]. If the SH group participates in coenzyme binding, the steric behaviour of the essential SH groups should be governed by the spatial arrangement of the groups which form the chiral active center of the enzyme and decide the A/B specificity.

This hypothesis was tested with some NAD^+ dependent dehydrogenases. The reaction rates with the antipodes of α-iodopropionic acid and its amide were determined by logarithmic plots of the residual activity against the reaction time. It was found that the A specific enzymes reacted faster with the D(+) antipodes and the B specific enzymes faster with L(−) antipodes of the reagents [2,3]. The B specific Y-GAPDH showed the opposite behaviour in the reaction with α-iodopropionic acid: the inactivation reaction of the apoenzyme was 6.8 times faster with the D(+) antipode. Only the reaction with the amide showed the expected preference for the L(−) antipode: k_L/k_D = 3.

If NAD^+ was added to the reaction mixture, Y-GAPDH exhibited the expected L stereoselectivity with the acid and the amide. For both reagents the stereoselective preference for the L antipode is enhanced with increasing NAD^+ concentration. In the highest concentration used (2×10^{-2} M) the quotient k_L/k_D attained 18.7 for the acid and 10 for the amide.

Assuming, that any stereoselective reactivity of single chemical functions in proteins must have its origin in specific chiral structures at multipoint binding sites for the reagents, one can see in this effect of NAD^+ on the stereoselectivity a new and strong support for the idea, that the binding of coenzyme induces the formation of the chiral structure of the enzymatically active site of the dehydrogenase. This may be characterized by the stereoselective preference for the L(−) antipodes of the reagents and – possi-

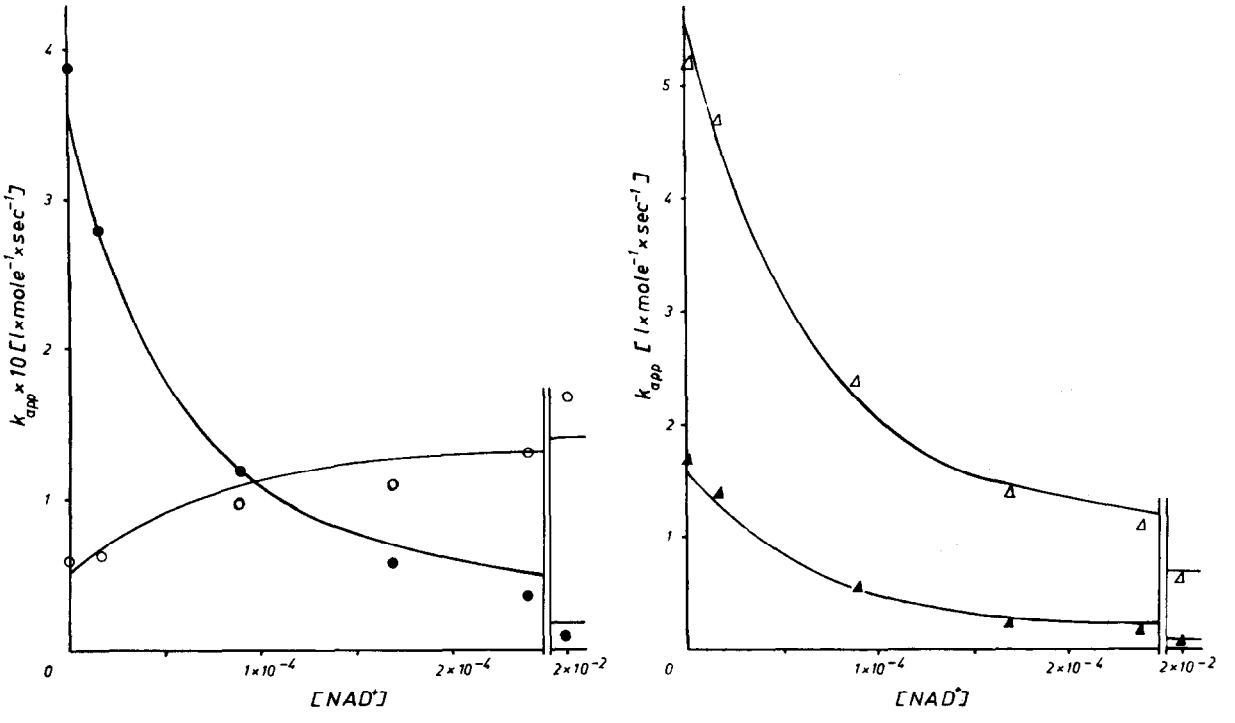

Fig.1. Dependence of the rate constants of the alkylation of Y-GAPDH with the antipodes of α-iodopropionic acid (A) and its amide (B) on the NAD^+ concentration. Black symbols: D(+) antipodes, open symbols: L(−) antipodes
○: α-iodopropionic acid 10^{-2} M,
△: α-iodopropionamide 5×10^{-4} M,
Y-GAPDH 1.5×10^{-6} M. Tris/HCl 0.05 M, NaCl 0.1 M, EDTA 0.01 M, Cysteine 0.001 M. pH 8.5, 25°C. The drawn lines are calculated from eqs. IIa and IIb with the constants given in table 1, the points are experimental.

bly – the B enantiotop of the coenzyme.

As it was shown by Kirschner et al. [6,7], Y-GAPDH is an allosteric enzyme, which according to Monod et al. [8] may exist in two tetrameric conformations T_4 and R_4. Only the R state is enzymatically active [7]. The equilibrium

$$T_0 \overset{L_0}{\rightleftharpoons} R_0$$

is shifted to the right by the binding of NAD^+. To see wether the steric behaviour of the Y-GAPDH SH groups might be explained by the allosteric transition from T_4 to R_4 the reaction rates were plotted against the NAD^+ concentrations (fig. 1).

With the exception of the L antipode of α-iodopropionic acid the reaction rates of all reagents are lowered on the addition of NAD^+ from which in the case of the D(+) antipodes of the reagents reasonable dissociation constants of NAD^+ may be calculated [2]. It was shown however by Kirschner and Johannin [7,9], that the reactivity of the SH groups of Y-GAPDH towards 5,5 dithiobis-benzoic acid (Ellman's reagent) is nearly independent of NAD binding, but strongly depends on the conformational state of the enzyme, the T state reacting about 100 times faster than the R state. If a similar condition is valid in our case, the reaction rates of fig. 1 should be governed by the relative amounts of the enzyme in T and R states. These may be calculated from the function of state $\bar{R}$ as defined by Monod, Wyman and Changeux [7]:

Table 1

Intrinsic rate constants and allosteric constants for the alkylation of Y-GAPDH in presence of NAD^+ with the antipodes of α-iodopropionic acid and its amide. Reaction as in fig. 1.

α-iodopropionic acid	α-iodopropionamide
$k_{T_D} = 0.4$ ($l \times mole^{-1} \times sec^{-1}$)	$k_{T_D} = 1.8$ ($l \times mole^{-1} \times sec^{-1}$)
$k_{T_L} = 0.035$ ($l \times mole^{-1} \times sec^{-1}$)	$k_{T_L} = 6.5$ ($l \times mole^{-1} \times sec^{-1}$)
$k_{R_D} < 0.002$ ($l \times mole^{-1} \times sec^{-1}$)	$k_{R_D} < 0.01$ ($l \times mole^{-1} \times sec^{-1}$)
$k_{R_L} = 0.145$ ($l \times mole^{-1} \times sec^{-1}$)	$k_{R_L} = 0.38$ ($l \times mole^{-1} \times sec^{-1}$)
$K_R = 4 \times 10^{-5}$	
$L_o = 6.0$	
$c = 0.3$	

$$\bar{R} = \frac{(1+\alpha)^n}{L_o(1+c\alpha)^n + (1+\alpha)^n} \quad (1)$$

with $\alpha = (NAD^+)/K_R$, $c = K_T/K_R$ and n = number of subunits. If the allosteric transition is fast as compared to the alkylation reaction, the apparent rate constants may be defined as:

$$K_{app_L} = k_{T_L}(1-\bar{R}) + k_{R_L}\bar{R}, \quad (2a)$$

$$K_{app_D} = k_{T_D}(1-\bar{R}) + k_{R_D}\bar{R}, \quad (2b)$$

The intrinsic rate constants can be extrapolated from the experimental values. K_R at pH 8.5 and 26°C was taken from the literature [10]. With the L_o and c values given in table 1 acceptable agreement with the experimental data was obtained.

The L_o and c values are in the range expected from fast reaction kinetics under similar conditions [9]. The intrinsic rate constants of the D antipodes of both reagents in the alkylation of the T state are about two orders of magnitude higher than in the reaction with the R state. In the reaction with α-iodopropionamide k_{T_L} is only 3.6 times greater than k_{R_L} whereas in the reaction of the acid k_{R_L} is 4.1 times higher than k_{T_L}. The over all reactivity of the apoenzyme is 15 to 35 times higher than that of a cysteine SH group under similar conditions.

The finding, that the T state favours the reaction of the D antipodes and the R state that of the L antipodes of the reagents may be explained by the following assumption: the chirality of the groups, which are responsible for the high reactivity of the SH groups of the T form of Y-GAPDH, is practically inversed during the allosteric transition. As the α-iodopropionate ion is much more affected by this transition, than the neutral α-iodopropionamide, probably charged groups are involved in the specific enhancement of the reactivity of the SH group. The question if this change of chiralty is equivalent to a transition from "A" specific to "B" specific NAD^+ binding cannot be answered by enzymatic experiments because the T state is enzymatically inactive. It may eventually be decided from measurements of optical rotatory dispersion.

References

[1] K.Wallenfels and B.Eisele, European J. Biochem. 3 (1968) 267.
[2] B.Eisele, Dissertation Freiburg/Br. (1967).
[3] B.Eisele and K.Wallenfels, European J. Biochem., in press.
[4] K.Mislow and M.Rabin, in: Topics in Stereochemistry, Vol. 1, eds. N.L.Allinger and E.L.Eliel (Interscience, New York, London, Sidney, 1967) p. 1.
[5] B.Vennesland and F.H.Westheimer, in: "The Mechanism of Enzyme Action", eds. W.D.McElroy and B.Class (John Hopkins Press, Baltimore, 1954) p. 357.
[6] K.Kirschner, M.Eigen, R.Bittmann and B.Voigt, Proc. Nat. Acad. Sci. U.S. 56 (1966) 1661.

[7] K.Kirschner and G.Johannin, Hoppe-Seyl. Z. Physiol. Chem. 384 (1967) 1235.
[8] J.Monod, J.Whyman and J.P.Changeux, J. Mol. Biol. 12 (1965) 88.
[9] K.Kirschner, personal communication.
[10] A.Stockell, J. Biol. Chem. 234 (1959) 1286.