Biochem. J. (1976) **153**, 503–504
Printed in Great Britain

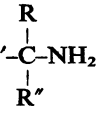
Some Observations on the NADP$^+$-Linked Oxidation of Methylglyoxal Catalysed by 2-Oxoaldehyde Dehydrogenase

By JOHN DUNKERTON* and SYBIL P. JAMES
Department of Biochemistry, University of Birmingham, P.O. Box 363, *Birmingham B*15 2*TT, U.K.*

(*Received* 21 *November* 1975)

In the oxidation of methylglyoxal by 2-oxoaldehyde dehydrogenase, the apparent K_m value for NADP$^+$ was about 2.5 times lower than the corresponding K_m for NAD$^+$; the apparent K_m values for methylglyoxal and for the amine activator L-2-aminopropan-1-ol, with NADP$^+$ as cofactor, were also different from those obtained with NAD$^+$. In the presence of NADP$^+$, the enzyme was not activated by P_i, in contrast with the activation of the enzyme when NAD$^+$ was used. The significance of the results is discussed.

2-Oxoaldehyde dehydrogenase (EC 1.2.1.23) catalyses the oxidation of methylglyoxal to pyruvate (Monder, 1967). The reaction is of interest, since inhibitors of methylglyoxal metabolism may possess cytotoxic properties (Vince *et al.*, 1971; Vince & Daluge, 1971). 2-Oxoaldehyde dehydrogenase was found to be completely dependent on the large concentrations of unusual amines, all of general structure:



where not more than one R group is hydrogen, and it was suggested that such a dependence *in vivo* could provide a useful basis on which to design analogues of the amines that might inhibit the reaction and therefore possess cytotoxic properties (Dunkerton & James, 1975; Dunkerton, 1974). In the presence of L-2-aminopropan-1-ol as activator, 2-oxoaldehyde dehydrogenase was allosterically activated by P_i, with a lowering of the K_m values for methylglyoxal, L-2-aminopropan-1-ol and NAD$^+$, but the kinetic data obtained did not show whether the true substrate of the enzyme was free methylglyoxal or an adduct formed from methylglyoxal and L-2-aminopropan-1-ol (Dunkerton & James, 1975).

In an attempt to elucidate the mechanism of the reaction, some observations were made on the properties of 2-oxoaldehyde dehydrogenase with NADP$^+$ instead of NAD$^+$ as cofactor.

Experimental

2-Oxoaldehyde dehydrogenase was purified from fresh sheep liver by using $(NH_4)_2SO_4$ fractionation and DEAE-cellulose, phosphocellulose, hydroxyapatite and Sephadex G-200 chromatography according to the method of Dunkerton & James (1975). Methylglyoxal was prepared as described by Monder (1967) from the aq. 40% solution obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. L-2-Aminopropan-1-ol was obtained from Ralph N. Emanuel Ltd., Wembley, Middx., U.K., and NAD$^+$ and NADP$^+$ were from Sigma (London) Chemical Co. The specific activity of the purified 2-oxoaldehyde dehydrogenase was 0.24 μmol/min per mg of protein when the assay mixture consisted of methylglyoxal (5mM), L-2-aminopropan-1-ol (40mM), Ches [2-(cyclohexylamino)ethanesulphonic acid] buffer (50mM), NADP$^+$ (0.1mM), potassium phosphate (10mM) and 100 μg of protein at 25°C and pH9.3 in a final volume of 3ml. Solutions of buffer, amine and P_i were adjusted to pH9.3 before use. All enzyme assays were carried out in duplicate and duplicates always agreed to within ±5%. No enzyme activity was detected when L-2-aminopropan-1-ol was omitted from the assay mixture.

Results

Apparent K_m values of NAD$^+$ and NADP$^+$

The apparent K_m values for NAD$^+$ and NADP$^+$ were 0.11mM and 0.0045mM respectively when 100 μg of the enzyme protein was assayed under the conditions described above.

Apparent K_m values of methylglyoxal and L-2-aminopropan-1-ol

The apparent K_m and $V_{max.}$ values found for methylglyoxal and L-2-aminopropan-1-ol in the presence of either NAD$^+$ (0.5mM) or NADP$^+$ (0.1mM) are given in Table 1. It is noteworthy that, although the apparent K_m for methylglyoxal is lower in the presence of NADP$^+$ than in the presence of NAD$^+$, that for L-2-aminopropan-1-ol is much higher.

* Present address: Emscote Lawn School, Emscote Road, Warwick CV34 5QE, U.K.

Table 1. *Apparent K_m and $V_{max.}$ values for methylglyoxal and L-2-aminopropan-1-ol in the presence of either NAD$^+$ or NADP$^+$*

The reaction mixture for the determination of apparent $V_{max.}$ and K_m for methylglyoxal contained methylglyoxal (0.3–5mM), L-2-aminopropan-1-ol (40mM), Ches buffer (50mM), K_2HPO_4 (10mM), NAD$^+$ (0.5mM) or NADP$^+$ (0.1mM) and 100μg of enzyme in a final volume of 3ml at pH9.3 and 25°C. The reaction mixture for the determination of apparent K_m and $V_{max.}$ for L-2-aminopropan-1-ol contained methylglyoxal (5mM), L-2-aminopropan-1-ol (10–160mM), Ches buffer (50mM), potassium phosphate (10mM), NAD$^+$ (0.5mM) or NADP$^+$ (0.1mM) and 100μg of enzyme protein at 25°C and pH9.3 in a final volume of 3ml. Analysis of the results by the double-reciprocal method gave linear plots.

	Methylglyoxal		L-2-Aminopropan-1-ol	
Cofactor	K_m (mM)	$V_{max.}$ (μmol/min per mg)	K_m (mM)	$V_{max.}$ (μmol/min per mg)
NAD$^+$	2.08	0.27	5.0	0.28
NADP$^+$	0.83	0.185	83.0	0.45

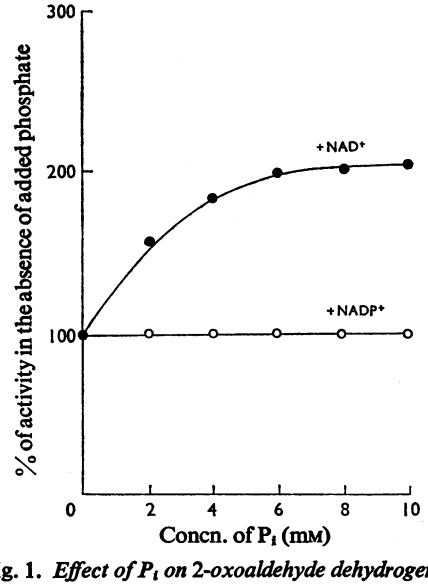


Fig. 1. *Effect of P_i on 2-oxoaldehyde dehydrogenase*

The reaction mixture for the enzyme assay consisted of methylglyoxal (5mM), L-2-aminopropan-1-ol (40mM), Ches buffer (50mM), K_2HPO_4, NAD$^+$ (0.5mM; ●) or NADP$^+$ (0.1mM; ○) and 100μg of enzyme protein at 25°C and pH9.3 in a final volume of 3ml.

Effect of P_i

Fig. 1 illustrates the effect of K_2HPO_4 on 2-oxoaldehyde dehydrogenase in the presence of either NAD$^+$ or NADP$^+$. P_i activates the enzyme when NAD$^+$ is the cofactor, but has no effect when the cofactor is NADP$^+$. A similar result was obtained when $Na_2P_4O_7$ was used instead of K_2HPO_4.

Discussion

It was previously shown (Dunkerton & James, 1975) that, with NAD$^+$ as cofactor and in the presence of L-2-aminopropan-1-ol, 2-oxoaldehyde dehydrogenase was activated by P_i. The activation could possibly be allosteric, since on storage the enzyme lost its response to P_i without losing its catalytic activity. Under the same conditions, but with NADP$^+$ instead of NAD$^+$, P_i has no effect on the activity of the enzyme. Since the K_m value for NADP$^+$ was much lower than that for NAD$^+$, it seems likely that NADP$^+$ may be the true physiological cofactor for the enzyme, and it is possible that an (allosteric) effector of the enzyme operating *in vivo* may be a compound related to P_i.

In the previous study of 2-oxoaldehyde dehydrogenase, it was difficult to establish whether the true substrate was free methylglyoxal or an adduct formed from methylglyoxal and L-2-aminopropan-1-ol (Dunkerton & James, 1975). However, the present observations on the apparent K_m and $V_{max.}$ values for methylglyoxal and L-2-aminopropan-1-ol suggest that it is most unlikely that the substrate is an adduct, since although the apparent K_m for methylglyoxal is, 2.5 times lower when NADP$^+$ is the cofactor used rather than NAD$^+$, the apparent K_m for L-2-aminopropan-1-ol is about 16 times higher when NADP$^+$ is the cofactor. It therefore appears that the true substrate for the enzyme is free methylglyoxal and that the enzyme is activated by free amines of a particular type. However, although the substrate is not an adduct, adduct formation is likely to occur, because of the reactive nature of the substrates at the high pH used in the assay, and this will undoubtedly complicate any observations on the kinetic properties of the enzyme. If NADP$^+$ is the true cofactor for the enzyme *in vivo*, the significance of the high K_m value of 83mM for L-2-aminopropan-1-ol requires further investigation before the physiological role of the enzyme and the nature of the amine-dependence are fully understood.

We thank Dr. E. A. Wren for her help and advice, and J. D. is grateful to the University of Birmingham for a temporary Research Fellowship.

References

Dunkerton, J. (1974) Ph.D. Thesis, University of Birmingham

Dunkerton, J. & James, S. P. (1975) *Biochem. J.* **149**, 609–617

Monder, C. (1967) *J. Biol. Chem.* **242**, 4603–4609

Vince, R. & Daluge, S. (1971) *J. Med. Chem.* **15**, 35–37

Vince, R., Daluge, S. & Wadd, W. B. (1971) *J. Med. Chem.* **14**, 402–404