THE ROLE OF CYANIDE IN THE NON-SPECIFIC STIMULATION OF GLYCEROL-1-PHOSPHATE DEHYDROGENASE

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Received August 21, 1963

Extramitochondrial DPN-specific glycerol-1-phosphate dehydrogenase (GDH) plays a key role in the mechanisms of "glycerol-1-phosphate pyruvate dismutation" and "glycerol-1-phosphate cycle" (Zebe et al.,1957;cf. Bücher,1963). Interestingly enough, extremely low or completely lacking activities of GDH are found in many malignant tumors (Zebe et al.,1957; Delbrück et al.,1959; Boxer and Shonk,1960; Ciaccio et al.,1960; Sacktor and Dick,1960; Borst,1962). With respect to these findings, results recently published by Emmelot and Bos (1962) have caused considerable confusion. The authors described a "stimulatory effect of potassium cyanide on the activity of GDH" which in their opinion results from a protective action of the cyanide anion against inactivation of the enzyme by heavy metal ions. Emmelot and Bos concluded that the low or absent activity of GDH in malignant tumors as reported by other authors primarily results from insufficient test conditions. Since this "stimulatory effect" of KCN could not be reproduced either in our or in other laboratories (Boxer and Devlin,1962; Borst,1963), we felt obliged to study the problem in detail.

Material and Methods

Crystallized GDH and lactic dehydrogenase (LDH) from rabbit muscle, DPNH, TPNH, methylglyoxal, dihydroxyacetone phosphate (DAP), glyceraldehyde phosphate (GAP) and an enzymatically prepared mixture of the two triosephosphates (TP), theoretically containing DAP and GAP at an equimolar ratio, were commercial products. The buffer used in the activity tests was prepared from triethanolamine hydrochloride (TRA) and contained EDTA. KCN solutions were freshly prepared every two hours and adjusted to the pH of the buffer solution (pH 7.6). Tests were run at 25°C in a recording photometer. pH-controls were performed for each sam

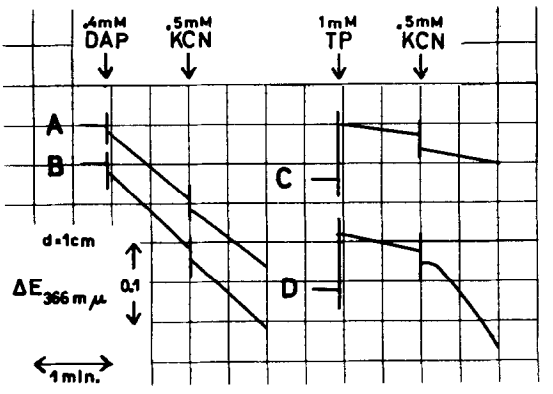

Fig. 1 GDH activity tests in the presence of KCN. Reactions were performed in 1 cm glass cuvettes. Test volume = 1 ml; pH = 7.6. Optical density was recorded at 366 mμ. Reaction mixture : TRA 50 mM, EDTA 5 mM, DPNH 0.2 mM. 0.1 μg of crystallized GDH is present in A and C. 30 μg of protein extracted from white rabbit muscle are present in B and D. Reactions A and B were started by the addition of 0.4 μmoles of DAP. Reactions C and D were started by the addition of 1 μmole of TP (i.e. 0.6 μmoles of DAP + 0.4 μmoles of GAP). 0.5 μmoles of KCN were added at equal intervals.

Results and Discussion

In fig. 1 original graphs are reproduced which represent GDH activity tests performed in a diluted sample of the crystallized enzyme (A and C) and in a sucrose extract from white rabbit muscle (B and D). As can be seen, addition of KCN remains without measurable influence on the reaction rate when DAP is used as substrate (A and B). On the contrary, a considerable effect is noted when, instead of pure DAP, a mixture of the two triosephosphate esters (TP) is used in the activity test. In the case of the pure enzyme (C) only a slight increase (approximately 40 %) in the reaction rate is caused by KCN. However, a KCN-induced acceleration of about 800 % is observed in the reaction catalyzed by the crude extract of rabbit muscle (D). These results are in agreement with the data reported by Emmelot and Bos, who exclusively used TP in their experiments.

As a matter of fact, the marked difference in the "stimulation" observed with the pure enzyme and the crude extract gives reason to suspect that the phenomenon is non-specific and probably results from the interference of another enzyme than GDH. This is emphasized by the finding that only a slight "stimulation" of GDH acti-

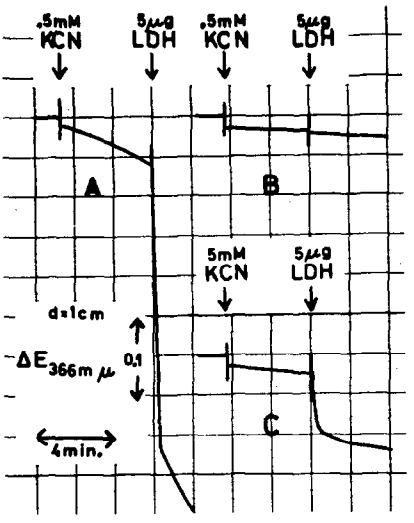

Fig. 2 KCN-induced DPNH oxidation in the presence of different samples of DAP and GAP. Its stimulation by LDH.
Experimental conditions as in fig. 1.
A : TRA 50 mM, EDTA 5 mM, DPNH 0.2 mM, TP 1 mM (i.e. 0.6 mM DAP + 0.4 mM GAP).
B : TRA 50 mM, EDTA 5 mM, DPNH 0.2 mM, DAP 0.6 mM.
C : TRA 50 mM, EDTA 5 mM, DPNH 0.2 mM, GAP 0.4 mM.
Reactions were started by the addition of 0.5 µmoles of KCN.
5 µg of crystallized LDH were added at equal intervals.

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vity by KCN can be demonstrated in crude extracts of flight muscle from locusta migratoria. Since this tissue is known to be almost devoid of LDH (Zebe and McShan, 1957), experiments were performed to study the influence of LDH on the phenomenon described above. They are summarized in fig. 2. Interestingly enough, it was found that the addition of KCN to a reaction mixture containing buffer, EDTA, DPNH and TP causes a measurable oxidation of DPNH in the absence of any enzyme (reaction A, fig.2). The oxidation rate of DPNH is then increased to extreme values after the addition of LDH. In order to clarify this effect, samples of pure DAP (reaction B, fig.2) and GAP (reaction C, fig.2) were incubated separately under identical conditions and at the same concentration as used in reaction A. A slight oxidation of DPNH is also registered after the addition of KCN to the reaction mixture containing DAP (B, fig.2).

Further addition of LDH causes only a small increase in the reaction rate. In contrast, a considerable oxidation of DPNH is set off by KCN in the control cuvette containing GAP instead of DAP (C, fig.2). Similar to reaction A, LDH again produces a marked acceleration in the oxidation rate of DPNH. However, the velocity of the reaction soon diminishes and slowly returns to its initial value.

Obviously, two different effects of KCN have to be distinguished: under its influence an oxidation of DPNH takes place at varying rates in different reaction mixtures containing DAP and GAP at identical concentrations. As could be shown, the same effect occurs if DPNH is replaced by TPNH. A shift in the absorption spectra of the reduced pyridinenucleotides (PN) by formation of a complex with the cyanide, which might explain the decrease in optical density at 366 mu, was ruled out. In a second type of reaction, which could be shown to occur also in the absence of reduced PN, a compound is formed in the presence of KCN; the reduction of this compound is readily catalyzed by LDH. In this reaction, which is specifically catalyzed by LDH, DPNH cannot be replaced by TPNH.
The direct formation of this compound from the two triosephosphates is improbable, since the amount of DPNH oxidized in the three reactions (fig.2) are different, although the concentration of DAP is identical in reactions A and B, and that of GAP in reactions A and C respectively. Therefore, the interference of a substance which is present at different concentrations in the three samples (TP, DAP and GAP) as impurity appears to be more probable.

Since methylglyoxal is a common decomposition product of GAP and DAP, it might be the substrate of the two KCN-dependent reactions. Meyerhof (1925) first described a transformation of methylglyoxal to lactate catalyzed by KCN. As could be shown later on by C.V. Smythe (1932), pyruvate is the primary product of this dismutation reaction. As is evident from fig. 3, an almost identical type of reaction as illustrated in fig.2 is recorded if, instead of TP or GAP, methylglyoxal is used as substrate. The addition of KCN (A, fig.3) leads to a measurable oxidation of DPNH (cf.fig.2) which is markedly accelerated after the addition of LDH. A reverse order in the addition of KCN and LDH (B, fig.3) clearly demonstrates that the formation of pyruvate is catalyzed by KCN. From the initial oxidation of DPNH in the presence of methylglyoxal and KCN

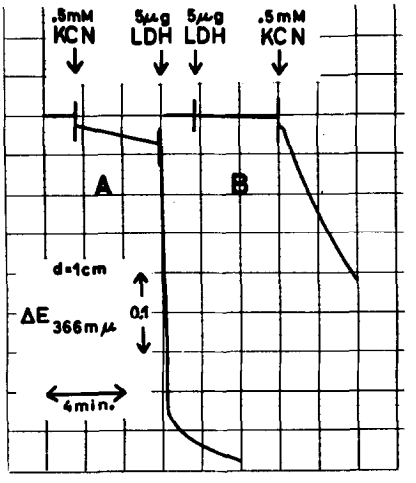

Fig. 3 KCN-induced DPNH oxidation in the presence of methylglyoxal. Experimental conditions as in fig. 1. Reaction mixture : TRA 50 mM, EDTA 5 mM, DPNH 0.2 mM, methylglyoxal approximately 0.1 mM. Reaction A is started by the addition of 0.5 µmoles of KCN, reaction B by the addition of 5 µg of crystalliz LDH.

it may be suspected that a DPNH-dependent transformation of methylglyoxal occurs simultaneously with its dismutation to pyruvate and acetol. As could be shown in a series of orienting experiments, the two reactions occur at different rates at different concentrations of KCN. No further experiments, however, have been performed to identify the products of this interesting reaction.

In summary, it can be stated that the "stimulatory effect of KCN on the activity of GDH" as reported by Emmelot and Bos is a non-specific artifact due to the use of impure substrates in the activity test. Thus, the deficiency in GDH of most malignant tumors remains an undeniable fact which is of greatest interest with respect to a possible deviation from the normal pathway of intracellular hydrogen transfer in malignant cells.

This study was supported by the Deutsche Forschungsgemeinschaft.

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