NAD-GLYCOHYDROLASE[x] (STREPTOLYSIN - O), EC 3.2.2.5 AND ITS ROLE IN CYTOLYSIS

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SUMMARY

Treatment of red blood cells with NAD-glycohydrolase, streptolysin-O, EC 3.2.2.5, resulted in a complete loss of intracellular NAD. During the breakdown of NAD no appreciable hemolysis was observed. Cell lysis was detected first after complete destruction of NAD. It is suggested that enzyme molecules penetrated through the cellular membrane and that the enzymatic breakdown of cellular NAD is the key process in NAD-glycohydrolase induced hemolysis.

INTRODUCTION

The mechanism of bacterial hemolysins has been the subject of numerous investigations (1-5). This is especially true for the oxygen labile cytolysins of gram positive bacteria such as pneumolysin, tetanolysin, cereolysin and streptolysin-O. Within this group of chemically related cytolysins, streptolysin-O is the only substance which was recently isolated in homogenous form (6). At the same time, the identity of NAD-glycohydrolase with streptolysin-O was demonstrated (6,7). Although the possibility of a direct action on the cellular membranes

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x) from group C streptococci, H46A.

has been suggested (5,8), an enzymatic destruction of lecithin, kephalin, sphingomyelin, cholesterol or cholesterolesters could not be confirmed (9). Therefore, the NAD-glycohydrolase specificity was assumed to be directly involved in the hemolytic process.

MATERIAL AND METHODS

Enzyme: NAD-Glycohydrolase, streptolysin-O, EC 3.2.2.5, was purified from crude material, kindly supplied from Behringwerke, Marburg/Lahn, GFR, according to the method reported earlier (6). Cellular NAD was extracted according to Grunicke et al. (10), and measured enzymatically with yeast alcohol dehydrogenase, EC 1.1.1.1., (11). Reactions were stopped by the addition of 4.0 ml $HClO_4$, 0.8 M, and the residual NAD extracted from the cells.

Sheep erythrocytes were washed with isotonic NaCl solution, containing 10 mM of cysteine, and centrifuged at 2000 x g. This solvent was used throughout. The course of hemolysis was measured by following the decrease in extinction at 546 nm in an Eppendorf photometer, coupled to a chart recorder (3 ml-cuvettes, 1 cm light path).

RESULTS AND DISCUSSION

Eight tubes were charged with 1 ml of red cells (1.4×10^{10} cells). Erythrocytes in tubes VII and VIII were treated with purified NAD-glycohydrolase, 170 U/ml, for 5 sec at 30° C, whereas tubes I-VI, without enzyme were run as controls. As can be seen from Table 1, the NAD concentration amounts to an average value of 131 $\pm$ 1.7 nM, assay I-VI, however no NAD could be detected in tubes VII and VIII.

TABLE 1

NAD (nM) extracted from sheep erythrocytes

No. of extract	I	II	III	IV	V	VI	VII	VIII
nM NAD/ ml cells	133	125	129	137	129	134	0	0

Although this short treatment of the cells with NAD-glycohydrolase resulted in a complete loss of cellular NAD no appreciable hemolysis was observed.

Therefore, the rate of hydrolysis of cellular NAD by NAD-glycohydrolase with lower enzyme concentrations was investigated. Seven tubes, containing 1 ml of red cells (1.4×10^{10} cells), and 2.0 ml NAD-glycohydrolase solution, 0.85 U/ml, were incubated at 30° C. The assay was stopped after 1,3,5,8,10 and 19 min, respectively. After 4 min incubation, when all the enzyme was fixed to the cells, 0.1 ml cellsuspension was withdrawn from a control assay which contained the complete charge to study the course of hemolysis. The withdrawn aliquot was diluted 1000 fold with NaCl solution and 3 ml of the dilution were placed in a cuvette.

The rate of NAD breakdown and the course of hemolysis are shown in Figure 1. The data show that 6.5 min after the addition of the enzyme only 50 % NAD, equivalent to 42.5 nM NAD, could be recovered from the cells. However, at this time hemolysis has hardly

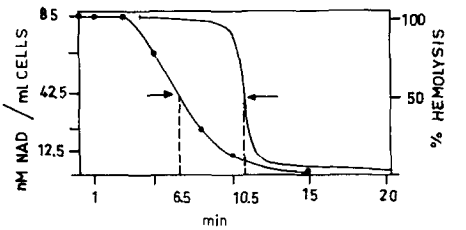

Figure 1. Rate of decrease of intracellular NAD from sheep erythrocytes after treatment with NAD-glycohydrolase (EC 3.2.2.5), streptolysin - O C,H46A (-●-●-). Course of hemolysis of a control assay, using the same amount of enzyme (——); curve starts after 4 min. Black arrows indicate 50 % turnover.

started. Contrary to expectation 50 % of cells were hemolysed first after 10.5 min. Since the loss of 50 % of cellular NAD was observed before hemolysis has started, it is to be supposed that the enzyme generates NAD breakdown in intact cells. The results suggested that NAD-glycohydrolase molecules penetrated through the cellular membrane. Thus, it is to be assussumed that the destruction of NAD by the enzyme is the key-process in the mechanism of NAD-glycohydrolase induced hemolysis and cytolysis.

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REFERENCES

1. Wilbrandt, W., Arch. Ges. Physiol., 245, 22 (1941).
2. Ponder, E., McLachlan, D.G.S., Brit. J. Exp. Path., 8, 267 (1927.
3. Bernheimer, A.W., J. Gen. Physiol., 30, 337 (1947).
4. Bernheimer, A.W., Davidson, M., Science, 148, 1229 (1965).

5. Alouf, J.E., Raynaud, M., in Current Research on Group A Streptococcus, edit. by R. Caravano, p. 192, Excerpta Medica Foundation, New York, Amsterdam, 1970.
6. Fehrenbach, F.J., Eur. J. Biochem., 18, 94 (1971).
7. Fehrenbach, F.J., Z. Naturfschg. 26b, 12, 1336 (1971).
8. Bernheimer, A.W., in Microbial Toxins, edit. by S.J. Ajl, S. Kadis, T.C. Montie, Vol. 1C, p. 183, Academic Press Inc., New York, 1970.
9. Eibl, H.J., Fehrenbach, F.J., in preparation.
10. Grunicke, H., Liersch, M., Hinz, M., Puschendorf, B., Richter, E., Holzer, H., Biochim. Biophys. Acta, 121, 228 (1966).
11. Racker, E., J. Biol. Chem. 184, 313 (1950).