SALT-DEPENDENT CONFORMATIONAL CHANGES OF ALANINE DEHYDROGENASE FROM *HALOBACTERIUM SALINARIUM*

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1. Introduction

The activity of enzymes from halophilic bacteria is largely influenced by the salt concentration [1]. In general a decrease of the salt concentration is followed by a reversible or irreversible loss of activity. In the cases of isocitrate dehydrogenase from *Halobacterium cutirubrum* (EC 1.1.1.42) [2] and glutamate dehydrogenase from a *Halobacterium* sp. from the Dead Sea (EC 1.4.1.2) [3] a correlation between the loss of activity after salt withdrawal and the decrease of α-helix content was established.

In the case of glutamate dehydrogenase the loss of activity after salt removal is irreversible, while isocitrate dehydrogenase can be partially reactivated by controlled readdition of salt. In contrast to these enzymes alanine dehydrogenase from *Halobacterium salinarium* (EC 1.4.1.1) may be subjected to several cycles of inactivation and reactivation by changes of the salt concentration without any loss of activity [4]. This property renders the enzyme especially suitable for studies on the relationship between catalytic activity and conformation.

2. Materials and methods

Pure alanine dehydrogenase from *H. salinarium* was prepared according to [4]. By treatment with different concentrations of NaCl or denaturing agents, 7 different forms of the enzyme were prepared: (a) native enzyme; (b) completely inactivated enzyme after dialysis for 2 h at 4°C against 50 mM potassium phosphate buffer (pH 7.8); (c) completely reactivated enzyme by dialysis of the inactivated enzyme for 14 h at 30°C against 50 mM potassium phosphate buffer (pH 7.8), containing 10 mM 2-mercaptoethanol and 4.3 M NaCl; (d) partially reactivated enzyme; the procedure was the same as under (c), but NaCl was 3.0 M instead of 4.3 M; (e) partially reactivated enzyme; the procedure was the same as under (c), however, 2-mercaptoethanol was omitted from the reactivation buffer; (f) denatured enzyme; the procedure was the same as under (b); in addition the inactivation solution contained 6.0 M urea; (g) partially renatured enzyme; first the enzyme was treated as under (f); subsequently reactivation was performed as under (c).

2-Mercaptoethanol is required for inactivation and complete reactivation, but not for the stability of the native or reactivated enzyme [5]. As this compound interferes with CD spectroscopy, it is removed by extensive dialysis (48 h at 30°C) against the corresponding buffer without 2-mercaptoethanol.

The activity of the preparations after the different treatments was assayed by the standard procedure [4]. Protein determinations were made following [6].

CD spectra were recorded by a Cary 60 spectropolarimeter equipped with an accessory for CD measurements or by a Dichrograph 3. Calibration was performed with a solution of (+)-10-camphorsulfonic acid. The measurements were carried out in 0.5 and 10.0 mm quartz cells at 26°C at 50–60 μg protein/ml.

From the amino acid composition (D.K., unpublished) a mean residue weight of 110 was derived. This value was taken for the calculation of the molecular ellipticities (θ). The content of α-helical, β- and ρ-structure of the different samples was estimated as in [7] on the basis of the standard data [8]. The values of the molecular ellipticities between 225 and 208 nm were evaluated. A fit better than 5% between computed and experimental spectra at all wavelengths

was achieved by means of a least-squares program. The calculations were carried out with an Olivetti-Programma 101.

3. Results and discussion

The CD spectra of the different active forms of alanine dehydrogenase in the ultraviolet range are shown in fig.1. The spectra of the active enzyme (curve a) and of the completely reactivated enzyme (curve c) are identical. They exhibit two characteristic minima of negative molecular ellipticity at 208 and 220 nm. Enzymes which were only partially reactivated either by dialysis against a buffer with NaCl reduced to 3.0 M (curve d), or a buffer without 2-mercaptoethanol (curve e) show markedly lower negative ellipticities. Inactivated enzyme (curve b) has largely, and denatured enzyme (curve f), completely lost the negative molecular ellipticity. Renaturation (curve g) leads to partial reconstitution. The protein contains a relatively small number of aromatic amino acids and only 2 tryptophan residues per mol. wt 58 000 (D.K., unpublished). This may account for the absence of the characteristic CD double spectral bands due to these amino acids in the near ultraviolet range. The results of the estimation of α-helical, β- and ρ-structure are given in table 1.

From these data the following conclusions may be drawn:

(i) For all forms of active enzyme the quotient α-helix content to relative activity is constant.

(ii) Inactivation by salt removal leads to a reduction of the α-helix content to <2% whereas the β-structure content is only reduced by ~50%.

(iii) Partially reactivated forms of the enzyme show a complete reconstitution of the β-structure and α-helix contents which are strictly proportional to the relative activity.

(iv) Treatment with 6 M urea leads to complete

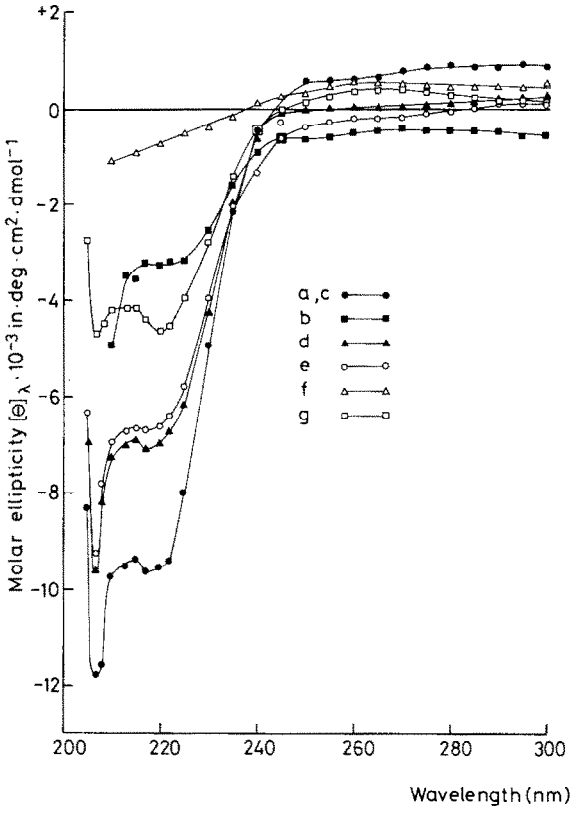

Fig.1. Circular dichroism of halophilic alanine dehydrogenase. Experimental details are given in the text.

Table 1

The content of α-helix, β- and ρ-structure of alanine dehydrogenase, calculated from the CD spectra between 225 and 208 nm

Protein	% α-Helix	% β-structure	% ρ-structure	% relative activity	α-Helix/relative activity
a. Native enzyme	22.6	24.8	52.6	100	0.226
b. Inactive enzyme	1.2	12.2	86.6	0	–
c. Completely reactivated enzyme	22.6	24.8	52.6	100	0.226
d. Partially reactivated enzyme (dialysed against 3.0 M NaCl)	15.8	24.6	59.6	70	0.225
e. Partially reactivated enzyme (without 2-mercaptoethanol)	12.3	24.5	63.4	55	0.224
f. Denatured enzyme	0	0	100	0	–
g. Renatured enzyme	6.1	8.1	85.8	27	0.226

inactivation and to total loss of both, α-helix and β-structure.

(v) Partial renaturation is linked to a corresponding reappearance of α-helix and of β-structure.

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References

[1] Lanyi, J. K. (1974) Bacteriol. Rev. 38, 272–290.
[2] Wulff, K., Hubbard, J. S. and Miller, A. B. (1972) Arch. Biochem. Biophys. 148, 318–319.
[3] Leicht, W., Werber, M. M. and Eisenberg, H. (1978) Biochemistry 17, 4004–4010.
[4] Keradjopoulos, D. and Holldorf, A. W. (1979) Biochim. Biophys. Acta 570, 1–10.
[5] Keradjopoulos, D. and Wulff, K. (1974) Can. J. Biochem. 52, 1033–1037.
[6] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[7] Saxena, V. P. and Wetlaufer, D. B. (1971) Proc. Natl. Acad. Sci. USA 68, 969–972.
[8] Chen, Y. H., Yang, J. T. and Martinez, H. M. (1972) Biochemistry 11, 4120–4131.