Volume 260, number 2, 297-300 FEB 08079 January 1990

Mapping of the immunodominant regions of the NAD-dependent formate dehydrogenase

Anna V. Bogdanova*, Tatyana V. Cherednikova*, Tsezi A. Egorov+, Emil G. Harutyunyan°, Nataleya A. Kurochkina†, Victor S. Lamzin*, Alexsander P. Savitskiy*, Igor A. Shumilin* and Vladimir O. Popov*

*A.N. Bakh Institute of Biochemistry, USSR Acad. Sci., Leninskiy pr. 33, 117071 Moscow, +V.A. Engelgard Institute of Molecular Biology, USSR Acad. Sci., Vavilov str. 32, 117984 Moscow, °A.V. Shubnikov Institute of Crystallography, USSR Acad. Sci. Leninskiy pr. 59, 117333 Moscow and †Institute of Protein Research, USSR Acad. Sci., 142292 Puschino, Moscow region, USSR

Received 4 December 1989

A panel of 4 monoclonal antibodies and 7 polyclonal antisera against NAD-dependent formate dehydrogenase from methylotrophic bacterium *Pseudomonas* sp. 101 has been obtained. The reactivity of the 37 overlapping proteolytic peptides with the monoclonal antibodies and polyclonal antisera has been studied with ELISA test. The data obtained were interpreted residing on the structural model of the formate dehydrogenase at 3 Å resolution. The immunodominant regions in the formate dehydrogenase molecule and the epitopes for the monoclonal antibodies were elucidated.

Epitope mapping; Antigenic determinant; Immunodominant region; Formate dehydrogenase

1. INTRODUCTION

The study of the antigenic structures contributes to the understanding of the protein–protein interactions [1–4], constructing of synthetic vaccines [5,6], detecting gene products and isolating proteins [7]. In spite of recent advances in the elucidation of the factors essential for antigenicity [3,4,8–11], there still remains some controversy concerning the molecular mechanism of antigen-antibody recognition. The database on the complexes between protein antigens and antibodies is restricted now to only a few structures [1,2,12–14] and the need for expanding on it seems rather urgent.

NAD-dependent formate dehydrogenase from methylotrophic bacterium *Pseudomonas* sp. 101 is a dimer (M_r ~86 000) composed of two identical subunits. This protein has been recently sequenced and its tertiary structure at 3 Å resolution resolved [15–17].

Here we report the first experiments on the antigenic properties of formate dehydrogenase. Polyclonal antisera have been used for mapping the immunodominant regions in the protein molecule. A panel of 4 monoclonal antibodies against the native formate dehydrogenase has also been obtained and their corresponding epitopes elucidated. Structural models of the enzyme was used for the spatial presentation of the immunological studies.

Correspondence address: V.O. Popov, A.N. Bakh Institute of Biochemistry, USSR Acad. Sci., Leninskiy pr. 33, 117071 Moscow, USSR

2. MATERIALS AND METHODS

2.1. *Preparation of peptides*

Formate dehydrogenase was purified by a standard procedure [15]. To obtain a set of overlapping peptides, the formate dehydrogenase was digested at lysine, arginine and glutamate residues by specific proteases [15]. In some cases, longer peptides originating from the BrCN cleavage at methionine residues were used. Peptides were purified by a double or triple HPLC using gel-filtration and C_8 columns. Purity of the peptides was verified by amino acid analysis or direct sequencing as described earlier [15].

About 7% of the amino acid sequence of the formate dehydrogenase was not tested for the antigen properties because of the low yields or insufficient length of the corresponding peptides.

2.2. *Monoclonal antibodies and polyclonal antisera*

Polyclonal antibodies were obtained by immunization of BALB/c mice with formate dehydrogenase according to 7 different immunization protocols.

Four hybridoma cell lines were obtained by fusion of the immune mice splenocytes with the Sp2/0 myeloma cell line by a standard procedure [18]. Monoclonal antibodies were purified by affinity chromatography on immobilized formate dehydrogenase.

2.3. *ELISA*

From 10 to 80 (usually 20–30) pmol of the peptide was applied to the nitrocellulose filter strip. The strips were blocked with 5 mg/ml BSA/0.3% Tween-20 for 1 h followed by a thorough rinsing in 0.01 M Na-phosphate buffer, pH 7.4, containing 0.15 M NaCl and 0.3% Tween-20 (PBS). Then the strips were incubated in the antibody solution for 1 h, rinsed and incubated in the solution containing conjugate of peroxidase with rabbit anti-mouse antibodies for another 1 h. After rinsing, the nitrocellulose strips were developed in the substrate mixture of 0.5 mg/ml diaminobenzidine/0.02% $CoCl_2$/30% H_2O_2 (15 μl/100 ml) in PBS.

2.4. *Structural model of the formate dehydrogenase*

Recently the three-dimensional structure of formate dehydrogenase

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

at 3 Å resolution was elucidated and the fold of the main chain was determined [16]. In this work we used nonrefined coordinates of the main chain atoms (unpublished data). A novel method of 'molecular cartography' [19] was used to visualize the results of immunochemical studies.

3. RESULTS AND DISCUSSION

3.1. *Visualisation of the results*

In accordance with [19], the formate dehydrogenase molecule was approximated by an ellipsoid. The semiaxes were fitted out as 33, 50 and 31 Å. The areas including superficial amino acid residues were projected on the surface of the ellipsoid. Only the residues forming part of the 'positive' tested peptides were taken into consideration. Suitable representation of immunodominant regions and epitope areas as an 'unfolded' ellipsoid is shown in fig.1A–E. Angle increment between meridians is 30°. As mentioned above, the formate dehydrogenase molecule is a dimer composed of two-identical subunits [16]. The molecular two-fold symmetry axis coincludes with z-axis of molecular coordinate system xyz (see fig.1E). For more convenient representation only one of the epitopes is shown in fig.1A–D.

3.2. *Immunodominant regions*

The amino acid sequence of the formate dehydrogenase is presented in fig.2. The peptides used for the immunological studies are listed in table 1. The response of these peptides to the ELISA test is also shown in table 1.

Comparing the peptides from table 1 with each other, it is possible in some cases to locate relatively short segments of polypeptide chain that presumably contain antigenic determinants of formate dehydrogenase. Of course, this approach reveals only the so-called sequential determinants [20].

Our assignment of the potential antigenic regions in formate dehydrogenase molecule is based on the assumption that the residues contributing to the antigenic determinants should be as a rule (but not necessarily [4]) accessible from the solvent. Seven sites, residues 107–115, 214–224, 269–275, 318–322, 333–343, 360–363 and 379–393, comprising about 16% of the total length of the formate dehydrogenase subunit emerge as immunodominant (the peptides incorporating them are always positive with all the 7 antisera tested). Three other regions may be located with less confidence. These are residues 27–40, 57–61 and 85–91 (~7%).

The distribution of the immunodominant regions over formate dehydrogenase molecule is presented in fig.1E.

3.3. *Epitopes for monoclonal antibodies*

The majority of the monoclonal antibodies against native proteins are considered to be directed against

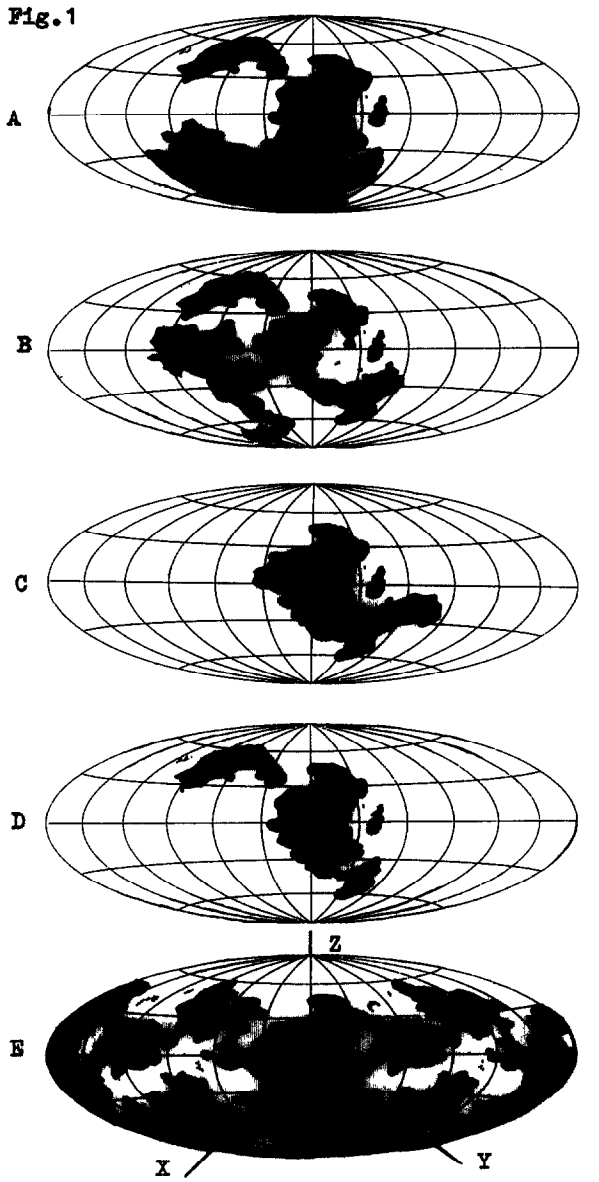

Fig.1. The schematic representation of the epitope areas of the monoclonal antibodies (A) for C_3, (B) for G_{11}, (C) for G_2, and (D) for G_{10}, and the immunodominant regions of the formate dehydrogenase (E). Molecular coordinate axes are shown.

discontinuous conformational epitopes [2]. Assuming that the peptides representing contiguous parts of the conformational antigenic determinants will still bind to the respective monoclonal antibodies (but probably with much less affinity), we decided to try to map the epitopes for monoclonal antibodies on the surface of formate dehydrogenase.

The additivity test showed that all the 4 antibodies compete for one and the same site on the protein surface. The additivity coefficient [21] varied from −7% for the G_{10}/G_2 pair to 21% for G11/C3. Table 1 confirms a low degree of additivity of the monoclonal antibodies under study. All 4 antibodies give strong positive reactions with at least 4 polypeptide fragments: residues 27–40, 56–63, 333–343 and 370–379.

```
                  10                  20                  30                  40
A K V L C V L Y D D P V D G Y P K T Y A R D D L P K I D H Y P G G Q T L P T P K
                  50                  60                  70                  80
A I D F T P G Q L L G S V S G E L G L R K Y L E S N G H T L V V T S D K S G P D
                  90                 100                 110                 120
S V F E R E L V D A D V V I S Q P F W P A Y L T P E R I A K A K N L K L A L T A
                 130                 140                 150                 160
G I G S D H V D L Q S A I D R N V V T A E V T Y V N S I S V A E H V V M M I L S
                 170                 180                 190                 200
L V R N Y L P S H E W A R K G G W N I A D C V S H A Y D L E A M H V G T V A A G
                 210                 220                 230                 240
R I G L A V L R R L A P F D H V L H Y T D R H R L P E S V E K E L N L T W H A T
                 250                 260                 270                 280
R E D M Y P V C D V V T L N C P L H P E T E H M I N D E T L K L F K R G A Y I V
                 290                 300                 310                 320
N T A R G K L C D R D A V A R A L E S G R L A G Y A G D V W F P Q P A P K D H P
                 330                 340                 350                 360
W R T M P Y D G M T P H I S G T T L T A Q A R Y A A G T R E I L E C F F E G R P
                 370                 380                 390
I R D E Y L I V Q G G A L A G T G A H S Y S K G N A T G G S E E A
```

Fig.2. The amino acid sequence of formate dehydrogenase [15].

Table 1

Peptide	Polyclonal antisera							Monoclonals			
	I	II	III	IV	V	VI	VII	G_2	G_{11}	G_{10}	C_3
3-17	+ −	+ −	− −	− −	− −	− −	− −	− − +	− − −	− − −	− − −
17-26	− −	− −	− −	− −	− −	− −	− −	− − +	− + −	− − −	− − −
18-40	− −	− −	− +	− −	+ −	+ +	+ +	− − +	− + +	+ + +	+ − +
27-40	+ −	+ +	+ −	+ −	+ −	+ +	+ +	+ + +	+ + −	+ + −	+ + +
41-56	− −	− −	− −	− −	− −	− −	− −	− − −	− − −	− − −	− − −
41-60	+ +	− +	− +	+ +	+ +	+ +	+ +	+ − +	+ − +	+ − −	+ − +
62-76	+ −	− −	− −	− −	− −	+ −	− −	− − −	− − −	− − −	− − −
65-84	− −	+ +	+ +	− −	− +	− −	− −	− − −	− − +	− − −	− − −
73-85	− −	− −	− −	− −	− −	+ −	− −	− − −	− − −	− − −	− − −
85-106	+ +	+ +	+ +	− −	− −	− −	+ +	− − −	− − −	− − −	+ − −
107-141	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ + −	+ + +	+ + −	+ + +
116-135	− −	− −	− −	− −	− −	− −	− −	+ − −	+ − −	+ − −	+ − −
164-173	− −	− −	− −	− −	− −	− −	− −	+ − −	+ − −	+ − −	+ − −
175-230	*	*	*	*	*	*	*	− − *	+ − *	− − *	− − *
191-230	+ +	+ +	+ +	+ +	+ +	+ +	+ +	− − −	− − −	+ − −	+ − −
202-208	− −	− −	− −	− −	− −	+ +	+ −	− − −	− − −	− − −	+ − −
223-231	+ +	+ +	+ +	+ +	+ +	+ +	+ +	− − −	− − −	+ − −	+ − −
225-231	− −	− −	− −	− −	− −	− −	− −	− − −	− − −	+ − −	+ − −
232-241	− −	− −	− −	− −	− −	+ +	+ −	− − −	− − −	− − −	+ − −
232-271	− −	+ −	+ −	+ −	− −	− −	− −	− + *	+ + *	− − *	− − *
242-271	− −	− −	− −	− −	− −	+ −	− −	− − −	− + −	− − −	− − −
243-260	− −	+ −	− −	− −	− −	− −	− −	− − −	− − −	+ − −	− − −
263-268	*	*	*	*	*	*	*	− − *	− − *	− − *	− − *
269-298	+ +	+ +	+ +	+ +	+ +	+ +	+ +	− − −	− − −	− − −	− − −
275-286	+ +	+ +	− −	+ −	− −	− −	+ −	+ − +	+ − −	+ − −	+ − −
276-284	− −	− −	− −	− −	− −	− −	− −	+ − −	− − −	+ − −	+ − −
287-317	− −	− −	− −	− −	− −	− −	− −	− − −	− − −	− − −	− − −
318-322	+ +	+ +	+ +	+ +	+ +	+ +	+ +	− − −	+ − −	+ + +	+ + +
318-383	*	*	*	*	*	*	*	+ **	+ **	+ **	+ **
323-332	− −	+ +	− +	+ +	− −	− −	+ −	+ + −	+ + +	+ + −	+ + +
333-343	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ − +	+ − +	+ − +	+ − +
350-359	− −	− −	− −	− −	− −	− −	− −	− − −	− − −	+ − −	+ − −
358-364	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ − −	+ − −	+ − −	+ − −
360-379	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ + +	+ + +	+ + −	+ + +
363-379	+ +	− +	− +	+ +	+ +	+ +	− +	+ + +	+ + +	+ + −	+ + +
365-391	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ + −	− + −	+ + −	+ + +
370-379	+ −	+ −	+ −	− −	− −	− −	+ −	+ − *	+ + *	+ + *	+ + *
380-383	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ + +	− − +	+ + +	+ + +

+ = positive; − = negative; * = not determined

Inspection of the spatial model of the formate dehydrogenase shows that these segments of the polypeptide chain cluster within the limited area on the protein surface (fig.1A–D). It may be hypothesized that amino acid residues located in these segments contribute to the antigenic determinant that is shared by all the antibodies obtained.

The epitope areas comprise not simply the spatially adjacent polypeptide fragments from the same polypeptide chain, but are formed by the amino acid residues located in different subunits.

The schematic representation of the epitope areas for monoclonal antibodies, including 'positive' tested peptides, is shown in fig.1A–D.

The presented epitope mapping provides some insight into the inhibitory effect of the antibodies tested. Inhibition studies showed that only one of the antibodies (G_2) partially suppressed the enzyme catalytic activity (80% inhibition).

The G_2 antibody differs from the other 3 in that respect that it reacts with the N-terminal peptides (3–17 and 18–26). These elements of the protein structure are shielding the substrate cleft from the solution. Antibody binding may somehow affect the conformation of this mobile segment of polypeptide chain and prevent proper positioning of the elements contributing to the enzyme active site. Such and indirect mechanism of enzyme inhibition is sustained by its only partial origin.

Thus, the present study shows that if the tertiary structure of the protein is known, the use of a sufficiently large panel of peptides provides a fast and reliable method for its epitope mapping.

Acknowledgements: The authors thank Prof. Yu.N. Chirgadze for helpful discussions. This work was supported in part by grants from the State Committee for Science and Technology.

REFERENCES

[1] Amit, A.G., Mariuzza, R.A., Phillips, S.E.V. and Poljak, R.J. (1986) Science 233, 747–753.
[2] Colman, P.M., Laver, W.G., Varghese, J.N., Baker, A.T., Tulloch, P.A., Air, G.M. and Webster, R.G. (1987) Nature 326, 358–363.
[3] Geysen, H.M., Tainer, J.A., Rodda, S.J., Mason, T.J., Alexander, H., Getsoff, E.D. and Lerner, R.A. (1987) Science 235, 1184–1190.
[4] Getsoff, E.D., Geysen, H.M., Rodda, S.J., Alexander, H., Tainer, J.A. and Lerner, R.A. (1987) Science 235, 1191–1196.
[5] Paoletti, D.E., Lipinskas, B.R., Samsonoff, C., Mercer, S. and Panicali (1984) Proc. Natl. Acad. Sci. USA 81, 193.
[6] Arnon, R. (1986) in: Current Topics in Microbiol. Immunol. vol. 130, pp. 1–12.
[7] Lerner, R.A. (1982) Nature 299, 592–596.
[8] Berzofsky, J.A. (1985) Science 229, 932–940.
[9] Thornton, J.M., Edwards, M.S., Taylor, W.R. and Barlow, D.J. (1986) EMBO J. 5, 409–413.
[10] Tainer, J.A., Getsoff, E.D., Alexander, H., Houghten, R.A., Olson, A.J. and Lerner, R.A. (1984) Nature 312, 127–134.
[11] Westhof, E., Altschuh, D., Moras, D., Bloomer, A.C., Klug, A. and Van Regenmortel, M.H.V. (1984) Nature 311, 123–126.
[12] Davies, D.R., Sheriff, S. and Padlan, E.A. (1988) J. Biol. Chem. 263, 10541–10544.
[13] Bjorkman, P.J., Saper, M.A., Samraoui, B., Bennet, W.S., Strominger, J.L. and Wiley, D.C. (1987) Nature 329, 506–512.
[14] Bentley, G.A., Boulod, G., Navaza, J. and Poljak, R.J. (1989) 7th International Congress of Immunology, W. Berlin, p. 1, Gustav Fisher, Stuttgart.
[15] Popov, V.O., Shumilin, I.A., Ustinnikova, T.B., Lamzin, V.S. and Egorov, Ts.A. (1990) Bioorg. Chem. (USSR) in press.
[16] Lamzin, V.S., Aleshin, A.E., Popov, V.O. and Harutyunyan, E.H. (1990) Bioorg. Chem. (USSR) in press.
[17] Lamzin, V.S., Aleshin, A.E., Shumilin, I.A., Ustinnikova, T.B., Egorov, Ts.A., Harutyunyan, E.A. and Popov, V.O. (1990) Bioorg. Chem. (USSR) in press.
[18] Kohler, G. and Milstein, C. (1975) Nature 256, 495–497.
[19] Chirgadze, Yu.N., Kurochkina, N.A. and Nikonov, S.V. (1989) Protein Engineering in press.
[20] Atassi, M.Z. (1975) Immunochemistry 12, 423–438.
[21] Friquent, B., Ohaniance, L.D. and Ragen, J. (1983) J. Immunol. Methods 60, 351–358.