THE DPNH-BINDING CAPACITY OF VARIOUS DEHYDROGENASES

Gerhard Pfleiderer and Ferdinando Auricchio[+]

Institut für Biochemie im Institut für Organische Chemie
J.W. Goethe-Universität
Frankfurt am Main (Germany)

Received April 14, 1964

Some time ago we suggested that the DPN-dehydrogenases might be built up from units of approximately the same size (Jaenicke and Pfleiderer, 1962) since the majority of these enzymes could bind one coenzyme molecule for approximately 40,000 g protein. This suggestion has become more plausible since the recent demonstrations from various laboratories that high molecular weight dehydrogenases can be split into sub-units of approximately this size by exposure to strong solutions of urea, guanidine, acids, or alkalis (Elödi et al., 1960; Sund, 1960; Appella and Markert, 1961; Jaenicke, 1963 and 1964). It is possible to measure the coenzyme-binding capacity of an enzyme by means of fluorimetric or equilibrium dialysis techniques or with the help of an ultracentrifuge. Since these methods are in part laborious and time consuming and sometimes give different results, the Sephadex gel filtration technique, used by Hummel and Dreyer (1962) to measure the binding of nucleotides to ribonuclease, and by us to deter-

[+]Postdoctoral Research Fellow, Consiglio Nazionale delle Ricerche, Roma (Italia)

mine the DPNH-binding capacity of lactate dehydrogenase (Pfleiderer, 1963; Warzecha, 1963), has been extended to study other dehydrogenases.

MATERIALS AND METHODS

Except for lactate dehydrogenase which was isolated pure as we have previously described (Pfleiderer and Jeckel, 1957), all dehydrogenases were obtained from C.F. Boehringer & Soehne, Mannheim, as was DPNH. Sephadex G-50 fine (Pharmacia, Uppsala, Sweden) was vigorously stirred in water and after frequent washing in 0,07 M phosphate buffer pH 7,2 was allowed to stand in the same buffer overnight. After removal of air under vacuum, the gel was packed into a column (40 x 1 cm). Enzymes were dialysed for 12 hours at 2° against the above buffer and any insoluble material removed by centrifuging at 12,000 g. The protein contents of the supernatants were determined by the biuret method. The Sephadex column was equilibrated with 0,07 mM DPNH in phosphate buffer. Approximately 10 mg enzyme dissolved in 0,07 mM DPNH-phosphate buffer was added to the column which was then developed with the same DPNH-phosphate buffer. The absorption of the eluate at 253 mμ was continually recorded by a Uvicord Flow Photometer (LKB-Produkter AB, Stockholm, Sweden). Figure I shows a typical elution curve. The peak was the enzyme-DPNH-complex which appeared within the exclusion-volume of the column. The " trough " which followed corresponded to the amount of DPNH bound by the enzyme. After equilibrium between bound and free coenzyme had been established, the registered absorbance returned to the starting value. The eluate was sampled by a fraction

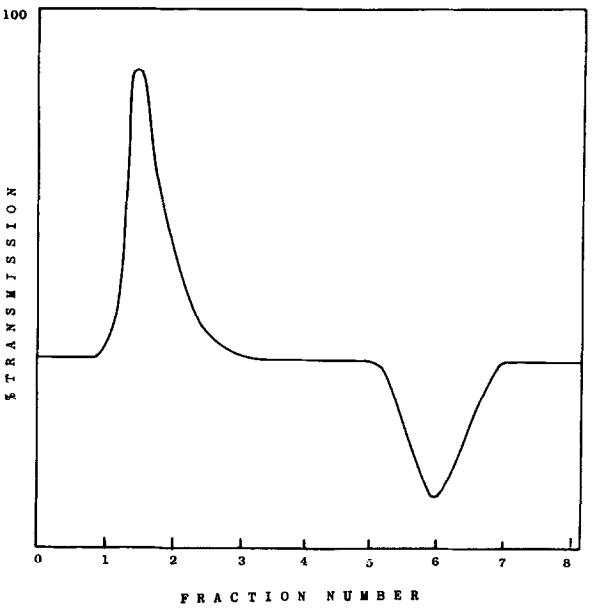

Fig.1. Elution profile (253 mμ) after passage through a Sephadex gel column being equilibrated with 0,07 mM DPNH.

collector and the samples corresponding to the " trough " in the absorption trace were combined. The difference between the DPNH present in this solution and that in an equal volume of unchanged eluate (determined enzymatically using pyruvate and lactate dehydrogenase) was the amount of DPNH bound by the enzyme. Using the molecular weight of the enzyme, the bound DPNH per molecule of enzyme was calculated.

RESULTS AND DISCUSSION

Table I shows the DPNH-binding capacities of various dehydrogenases determined by this method. There is agreement in most cases with the published capacities determined by other methods. Differences, which were reproducible, were noticed in the cases of α-glycerophosphate dehydrogenase and glutamate dehydrogenase. In the case of the former

Table I

Enzyme	Molecular weight	Mole bound DPNH/Mole Enzyme		Molecular weight/ 1 bound DPNH
		untreated	after treatment with charcoal	
α-Glycerophosphate dehydrogenase from rabbit muscle	78.000	2,0		39.000
Malate dehydrogenase from pig heart	85.000[+]	1,80	2,0	42.000
Alcohol dehydrogenase from horse liver	84.000	1,70	2,18	38.000
Glutamate dehydrogenase from beef liver	1.000.000	20,00-20,80		49.000
Lactate dehydrogenase from pig heart	118.000	2,3	3,0-3,1	39.000
Alcohol dehydrogenase from yeast	150.000	4,95-5,15		29.000
Glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle	118.000	2	3	39.000

[+]Auricchio, unpublished.

enzyme Ankel et al. (1959) observed only one DPNH-binding site / molecule whereas van Eys et al. (1959) using the equilibrium dialysis method found 33 bound DPNH molecules, two of which were very strongly bound. The literature values for glutamate dehydrogenase (Kubo et al., 1957; Sund, 1961) were lower than those reported here. We have measured the DPNH-binding capacities at different enzyme concentrations, although not at very high dilutions since then method is too inaccurate. The DPNH-binding capacity remained constant. It is possible that in more dilute solutions the coenzyme-binding capacity might be higher since Sund (1961) has shown that on dilution glutamate dehydrogenase dissociates thereby exposing more DPNH-binding groups. If the dehydrogenase contained secondary products of DPNH or nucleotides, as we and others have previously reported (Ankel et al., 1959; Wieland et al., 1962; Pfleiderer and Stock, 1962; Pfleiderer et al., 1962), these were removed by treatment with charcoal. For example: we found that the DPNH-binding sites of lactate dehydrogenase were raised from 2,3 to 3,1 (Warzecha, 1963) by a charcoal treatment which according to Wieland et al. (1963) should have removed the DPNH-X. The high loss involved in this treatment of the more expensive enzymes prevented its use in every case so that small corrections might have to be made to the values in Table I.

The results in the last column of Table I show that 6 of the DPN-dehydrogenases have one active sub-unit of molecular weight 30 - 50,000 as measured by the gel filtration method. The values for the DPN-binding sites of the seventh enzyme, glyceraldehyde-3-phosphate

dehydrogenase of rabbit muscle, after charcoal treatment, were taken from Velick (1953) and Pfleiderer (1962). We believe, especially after considering the newly discovered enzyme sub-units such as are involved in the lactate dehydrogenase isozymes (Markert and Appella, 1961), that a general building principle can be recognised for the dehydrogenases. Unfortunately it has not yet been possible to isolate a sub-unit of 30 - 40,000 molecular weight which is still enzymatically active. We have already used the Sephadex method with success to correlate the number of coenzyme sites with enzymic activity during the reaction of an enzyme with specific amino acid reagents (Warzecha, 1963).

REFERENCES

Ankel, H., Bücher, Th., and Czok, R.: Biochem. Z. 332, 315 (1959).

Appella, E., and Markert, C.L.: Biochem. Biophys. Res. Comm. 6, 171 (1961).

Elödi, P., Jécsai, G., and Mozolooszky; Acta Physiol. Hung. 17, 165 (1960).

Elödi, P., and Jécsai, G.: Acta Physiol. Hung. 17, 175 (1960).

van Eys, J., Nuenke, B.J., and Patterson jr., M.K.: J. Biol. Chem. 234, 2308 (1959).

Frieden, C.: Biochim. Biophys. Acta 47, 428 (1961).

Hummel, J.P., and Dreyer, W.J.: Biochim. Biophys. Acta 63, 530 (1962).

Jaenicke, R., and Pfleiderer, G.: Biochim. Biophys. Acta 60, 615 (1962).

Jaenicke, R.: Habilitationsarbeit Frankfurt a.M., 1963.

Jaenicke, R.: Biochim. Biophys. Acta, in press.

Kubo, H., Yamano, T., Iwatsubo, M., Watari, H., Soyama, T., Shiraishi, J., Sawada, S., and Kawashima, N.: Proceedings of the International Symposium on Enzyme Chemistry, Tokyo and Kyoto 1957 (K. Ichihara ed.), Academic Press, Inc., New York, p. 345.

Pfleiderer, G., and Jeckel, D.: Biochem. Z. 329, 370 (1957).

Pfleiderer, G., and Stock, A.: Biochem. Z. 336, 56 (1962).

Pfleiderer, G.: 14. Mosbacher Kolloquium 1963, Springer-Verlag, Heidelberg, in press.

Pfleiderer, G., Hohnholz-Merz, E., and Gerlach, D.: Biochem. Z. 336, 371 (1962).
Sund, H.: Acta Chem. Scand. 15, 940 (1961).
Velick, S.F.: J. Biol. Chem. 203, 56 (1953).
Warzecha, K.: Dissertation Frankfurt a.M., 1963.
Weichselbaum, T.E.: Amer.J.Chem.Path.Techn.Soc. 10, 40 (1946).
Wieland, Th., Duesberg, P., Pfleiderer, G., Stock, A., and Sann, E.: Arch. Biochem. Biophys., Suppl. 1, 260 (1962).