Volume 223, number 1, 33–36 FEB 05190 October 1987

Enzyme and organic solvents: horse liver alcohol dehydrogenase in non-ionic microemulsion: Stability and activity

Kang Min Lee and Jean-François Biellmann

Laboratoire de Chimie Organique Biologique, UA no. 31, Institut de Chimie, Université Louis Pasteur, 1 rue Blaise Pascal, 67008 Strasbourg, France

Received 25 August 1987

In a microemulsion made with Triton X-100, the stability of the enzymatic activity was higher than in ionic microemulsions. The stability increased with water content. The kinetic constants (Michaelis constant of NAD^+ and maximum velocity) were close to those found in the previously studied microemulsions. The Michaelis constant of NAD^+ expressed with respect to the buffer volume was higher than in water. The pH dependence of the kinetic constants in this microemulsion was determined. The activity determined by NAD^+ reduction decreased with water content, whereas the redox activity determined via butanol oxidation coupled to retinal reduction was only slightly reduced.

Alcohol dehydrogenase; Enzyme stability; Microemulsion; Organic solvent; Surfactant; Water content

1. INTRODUCTION

The use of enzymes in reverse micelles and microemulsions is a developing area [1–3]. In [4], we described the behavior of horse liver alcohol dehydrogenase in microemulsions made of ionic detergents: SDS and cetyltrimethylammonium bromide. We describe here the reactivity of this enzyme in a microemulsion made with the non-ionic detergent Triton X-100 [5].

2. METHODS AND RESULTS

2.1. *Materials*

Materials were as in [4]. Triton X-100 (Sigma) was used without further purification. Retinal was purchased from Aldrich. The buffer was 50 mM Tes, pH 7.5, unless stated otherwise.

Correspondence address: K.M. Lee, Laboratoire de Chimie Organique Biologique, UA no.31, Institut de Chimie, Université Louis Pasteur, 1 rue Blaise Pascal, 67008 Strasbourg, France

2.2. *Microemulsions*

The compositions of the systems are given in table 1. Microemulsions were prepared at 20°C by adding the various constituents under constant stirring (N, non-ionic surfactant; b, 1-butanol as cosurfactant). The ratio of surfactant to cosurfactant was kept constant.

2.3. *Activity tests*

In the Nb microemulsions, 1-butanol had two functions: cosurfactant and enzyme substrate. The kinetic data were plotted according to Eadie [6].

2.4. *Enzyme stability in microemulsions*

Experiments were performed in microemulsions Nb-1 to Nb-4. For each system, two microemulsions were prepared containing (i) buffer only and (ii) the coenzyme: NAD^+, 1 mM for Nb-1 and

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

Table 1

Composition of the microemulsions

	Cyclo-hexane	Water (buffer)	Surfactant Triton X-100	Cosurfactant 1-butanol
Nb-1	76	≥0	12	12
Nb-2	73	3	12	12
Nb-3	69	7	12	12
Nb-4	66	10	12	12

The ratios are expressed in weight (%) (w/w). Buffer was 50 mM Tes, pH 7.5

3 mM for Nb-2 to Nb-4 (overall concentration). The enzyme (0.15 nmol) was incubated in microemulsion (i) (0.40 ml). At intervals, microemulsion (ii) (0.30 ml) was added to microemulsion (i) and the absorption increase was followed at 340 nm.

The stability of the activity increased with water content. Even in microemulsion Nb-1 containing the least amount of water, the half-life was 12 h and in microemulsion Nb-4, 4 days (fig.1).

2.5. *Michaelis constant of NAD$^+$ and maximum velocity of NAD$^+$ reduction in buffer*

Increasing amounts (5 to 50 μl) of buffer solution containing 0.1 M 1-butanol and 10 mM NAD^+ were added to 0.1 M 1-butanol solution. The reaction was initiated by addition of the enzyme solution (75 pmol). The results are listed in table 2.

2.6. *Michaelis constant of NAD$^+$ and maximum velocity of NAD$^+$ reduction in microemulsions and their pH dependence in microemulsion Nb-4*

The Michaelis constant (K_m) and maximum velocity (V_{max}) of NAD^+ were determined in microemulsions Nb-2 to Nb-4, in which the water content was varied from 3 to 10% (w/w). For each determination two microemulsions were prepared containing (i) only buffer and (ii) coenzyme NAD^+ (3 mM). Varying amounts of microemulsion (ii) (5 to 50 μl) were added to microemulsion (i) to give a final volume of 0.7 ml. The reaction was initiated by addition of the enzyme solution (0.15 nmol) (table 2).

The pH dependence of the kinetic constants was

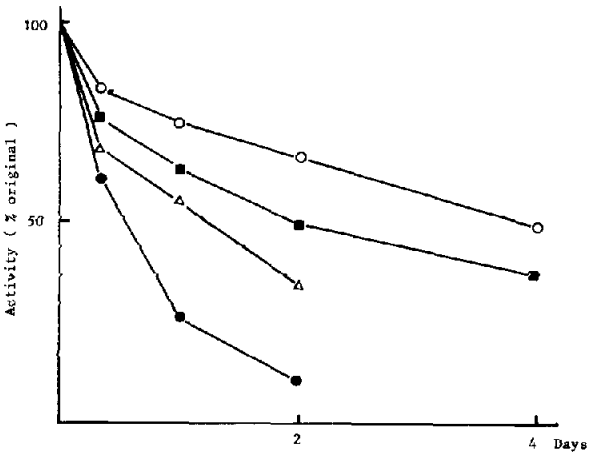

Fig.1. Alcohol dehydrogenase activity in microemulsions Nb-1 to Nb-4 as functions of time. Activity expressed in % to the initial activity in microemulsion: Nb-1 (●); Nb-2 (△); Nb-3 (■); Nb-4 (○).

determined in microemulsion Nb-4 prepared with 50 mM buffer, pH 6.8, 7.5 and 8.5. The results are summarised in fig.2.

The K_{mwp} (K_m expressed with respect to the buffer volume) of NAD^+ was almost constant over 3–10% water content of the microemulsions and had a larger value than in buffer. The maximum velocities increased with water content and in microemulsion Nb-4 amounted to 33% of that in buffer. The constants varied only slightly with pH.

Table 2

Michaelis constant of NAD^+ and maximum velocity of NAD^+ reduction with alcohol dehydrogenase in buffer (1-butanol) and microemulsions Nb-2 to Nb-4

Micro-emulsions	K_{mov} (μM)	K_{mwp}	V_{max} (μmol·min^{-1}·mg enzyme^{-1})
Nb-2	35	1400	80
Nb-3	70	1150	170
Nb-4	130	980	420
Buffer 100 mM 1-butanol		91	1400

The Michaelis constant was expressed with respect to the total volume (K_{mov}) and with respect to the buffer volume (K_{mwp}). Buffer was 50 mM Tes, pH 7.5

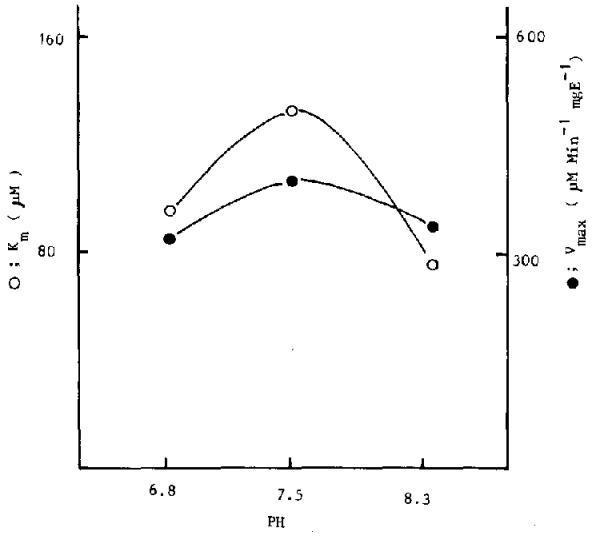

Fig.2. Michaelis constant (○) and maximum velocity (●) of NAD^+ in microemulsion Nb-4 as a function of pH.

2.7. *Redox activity*

Since binding and dissociation of the coenzyme and thus the activity may be perturbed in microemulsions with low water content, we determined the redox activity in a reaction which does not involve the coenzyme binding dissociation. The ultraviolet absorption at 280 nm of the surfactant complicates the utilization of cinnamaldehyde (λ_{max} = 320 nm), which has been used previously for this purpose [4]. We therefore carried out the redox activity determination with retinal as oxidant and the cosurfactant butanol as reductant. Retinal is a substrate for this enzyme [7,8], and absorbs at 370 nm (ϵ = 1690) [9], well away from the surfactant absorption (fig.3).

Microemulsions Nb-1 to Nb-3 containing NAD^+ (0.14 mM) were prepared, enzyme (50 μg for 1 ml) added and the activity determined at 340 nm. For measurement of the redox activity, the microemulsions contained NAD^+ and retinal. Enzyme was added and the absorption decrease was determined

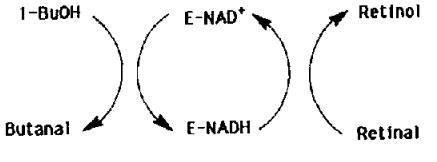

Fig.3. Redox reaction catalyzed by HLADH.

Table 3

NAD^+ reduction rate and 1-butanol/retinal exchange rate as a function of w_0 (water content) in non-ionic microemulsion Nb-1 to Nb-3 containing 0.14 mM NAD^+ and 50 μg/ml enzyme

Micro-emulsion	Water content (%)	NAD^+ reduction rate ($mmol \cdot min^{-1} \cdot mg\ enzyme^{-1}$)	1-BuOH/retinal (10 μM) exchange rate ($mg\ enzyme^{-1}$)
Nb-1	>0	0.038	0.13
Nb-2	3	0.079	0.14
Nb-3	7	0.12	0.17

The reduction and exchange rate were determined at 340 and 370 nm, respectively

at 370 nm. The results are presented in table 3. The redox activity decreased much less at lower water content than did the overall activity.

3. DISCUSSION

Since the critical micellar concentration of Triton X-100, a non-ionic surfactant, is substantially lower than that of most ionic surfactants [10], the enzyme should be more stable in microemulsions made with Triton X-100 than in those made with ionic detergent. Indeed, we found that horse liver alcohol dehydrogenase showed a higher activity stability in Triton X-100 than in ionic microemulsions. The activity determined by NAD^+ reduction was significant in a microemulsion of low water content (labelled 0) (table 3). Microemulsion Nb-1 contained the water of the buffer with the enzyme and the water present in the detergent. In contrast, the activity determined with the redox test was quite high in microemulsion Nb-1. It is likely the binding dissociation process of the coenzyme to enzyme which is perturbed. Indeed, the coenzyme as well as its binding site on the enzyme contains a polar group. Hence, as the medium becomes less solvating, binding of the coenzyme to the enzyme becomes stronger [4]. Therefore, the activity determined by NAD^+ reduction involving binding and dissociation of the coenzyme may be reduced whereas the binding dissociation of the substrate and the redox reaction have high rates.

The Michaelis constant of NAD^+ and the maximum velocities were determined. The Michaelis constant of NAD^+ expressed relative to the water content (K_{mwp}) was increased over the value in buffer, as we had found in ionic microemulsion [4]. For alcohol dehydrogenase from *Thermoanaerobium brockii*, the Michaelis constant expressed in the same manner had a value close to that found in buffer [11]. The mechanism of liver alcohol dehydrogenase seems to be modified in the microemulsions. The pH dependence of the Michaelis constant of NAD^+ and of maximum velocity was close to that found in buffer. Microemulsions made with a non-ionic detergent may be very useful media for synthetic transformations using enzymes acting on substrates of low water solubility [12–14].

ACKNOWLEDGEMENT

This work was supported by Elf Aquitaine (contract GRL no.5636).

REFERENCES

[1] Luisi, P.L. (1985) Angew. Chem. Int. Ed. Engl. 24, 439–450.
[2] Martinek, K., Levashov, A.V., Klyachko, N.L., Khmelnitski, Y.L. and Berezin, I.V. (1986) Eur. J. Biochem. 155, 453–468.
[3] Waks, M. (1986) Proteins 1, 4–15.
[4] Samama, J.P., Lee, K.M. and Biellmann, J.F. (1987) Eur. J. Biochem. 163, 609–617.
[5] Kumar, C. and Balasubramanian, D. (1980) J. Colloid Interface Sci. 74, 64–70.
[6] Eadie, G.S. (1942) J. Biol. Chem. 146, 85.
[7] Reynier, M. (1969) Acta Chim. Scand. 23, 1119–1129.
[8] Pocker, Y. and Raymond, K.W. (1980) Adv. Exp. Med. Biol. 132, 137–150; Pocker, Y., Li, H. and Page, J.D. (1987) in: Enz. Mol. Biol. of Carbonyl Comp. (Weiner, H. and Flynn, T.G. eds) pp.217–225, Liss, New York.
[9] Plack, P.A., Kon, S.K. and Thompson, S.Y. (1959) Biochem. J. 71, 467–476.
[10] Makino, S., Reynolds, J.A. and Tanford, C. (1973) J. Biol. Chem. 248, 4926–4932.
[11] Lee, K.M. and Biellmann, J.F. (1987) Nouv. J. Chim., in press.
[12] Bello, M., Pievic, H.A., Thomas, D. and Legoy, M.D. (1986) CR Acad. Sci. Paris 303, 187–192.
[13] Lee, K.M. and Biellmann, J.F. (1986) Bioorg. Chem. 14, 262–273.
[14] Lee, K.M. and Biellmann, J.F. (1986) Nouv. J. Chim. 10, 675.