(15) J. T. Edsall and J. Wyman, "Biophysical Chemistry," vol. 1, Academic, New York, N.Y., 1958, p. 495.
(16) K. Tomino, *Chem. Pharm. Bull.*, **6**, 648(1958).
(17) L. H. Conover, Chem. Soc. Special Publication No. 5, 1956, p. 48.
(18) R. A. Morton and A. L. Stubbs, *J. Chem. Soc.*, **1940**, 1347.
(19) K. Andrae, *Chem. Ber.*, **101**, 1013(1968).
(20) K. Andrae, *Z. Anorg. Allg. Chem.*, **1968**, 254; through *Chem. Abstr.*, **69**, 102663k.
(21) D. J. Clive, *Quart. Rev.*, **22**, 439(1968).
(22) L. A. Mitscher, A. C. Bonacci, B. Slater-Eng, A. K. Hacker, and T. D. Sokoloski, *Antimicrob. Ag. Chemother., 1969*, **1970**, 111.

ACKNOWLEDGMENTS AND ADDRESSES

Received August 1, 1975, from the *Department of Chemistry, University of Iowa, Iowa City, IA 52242*

Accepted for publication February 13, 1976.

The partial support of Pfizer, Inc. is gratefully acknowledged.

* Present address: Department of Chemistry, Augustana College, Rock Island, IL 61201

‡ Present address: 3M Center, 3M Co., St. Paul, MN 55075

§ Present address: Department of Chemistry, College of Charleston, Charleston, SC 29401

ˣ To whom inquiries should be directed.

Lipid–Protein Interactions: Enhancement of Enzyme Activity of L-Glutamic Acid Dehydrogenase by Nonionic Detergents

DAVID H. KEMPNER and BRIAN J. JOHNSON ˣ

Abstract □ Five nonionic detergents enhanced the activity of L-glutamic acid dehydrogenase [L-glutamate:nicotinamide adenine dinucleotide phosphate oxidoreductase (deaminating) (EC 1.4.1.3)]. These detergents activated the enzyme toward α-ketoglutaric acid reduction, causing a decrease in the sensitivity of the enzyme to allosteric regulation by guanosine 5-triphosphate. There was also a diminution of the enhancing effect of the modifier adenosine 5-diphosphate on the enzyme's L-glutamic acid dehydrogenase activity. These detergents may cause a conformational change in the enzyme, and this change could lead to an increase in the binding of the substrates for the α-ketoglutaric acid reduction. Accompanied with this conformational change would be a decrease in the binding of the modifier guanosine 5′-triphosphate, with no concomitant change in the binding of the adenosine 5′-diphosphate modifier.

Keyphrases □ Lipid–protein interactions—L-glutamic acid dehydrogenase activity, effect of various nonionic detergents □ L-Glutamic acid dehydrogenase—effect of various nonionic detergents on activity □ Detergents, nonionic—effect on activity of L-glutamic acid dehydrogenase □ Enzyme activity—L-glutamic acid dehydrogenase, effect of various nonionic detergents

Nonionic detergents have been used for the isolation of membrane proteins (1, 2). However, the use of detergents can sometimes produce materials that possess altered activity. Thus, the cell surface antigen Thy-1.2 found on mouse thymocytes loses all activity when isolated with a polyoxyethylene ether nonionic detergent (3) whereas its activity is retained when it is solubilized by papain digestion (4, 5).

These changes in the biological activity of a protein in the presence of nonionic detergents prompted the investigation of the effects of such materials on a defined protein possessing an easily assayable biological activity. The enzyme L-glutamic acid dehydrogenase [L-glutamate:nicotinamide adenine dinucleotide phosphate oxidoreductase (deaminating) (EC 1.4.1.3)] was chosen as a model because it shows activity toward various substrates (6) and its activity is altered by allosteric modifiers (7).

EXPERIMENTAL

The concentration of L-glutamic acid dehydrogenase solutions was determined using an $E^{1\%}_{280}$ of 10.0 (8). Quantitation of the enzyme in detergent solutions and of ovalbumin was determined by fluorescamine (9).

L-Glutamic Acid Dehydrogenase Activity—This reaction was assayed in the direction of α-ketoglutaric acid reduction. A 6-μl aliquot of the enzyme, 0.4 mg/ml, was added to 3 ml of phosphate buffer (10^{-4} *M* ethylenediaminetetraacetic acid and 0.04 *M* sodium phosphate, pH 7.6) containing 3×10^{-4} mole of nicotinamide adenine dinucleotide phosphate reduced form, 1.5×10^{-3} mmole of α-ketoglutaric acid, and 3×10^{-1} mmole of ammonium chloride. The assays were monitored by recording the disappearance of the chromophore at 340 nm. An ϵ_{340} of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$ was used. All initial rates were calculated between 15 and 30 sec after the start of the assay, during which time the reaction rate was linear.

L-Glutamic Acid Dehydrogenase Activity in Presence of Detergent[1]—This reaction was also assayed in the direction of α-ketoglutaric acid reduction. The L-glutamic acid dehydrogenase solutions in 25% (v/v) detergent were prepared by adding 0.5 ml of detergent to a 1.5-ml aliquot of a 0.53-mg/ml solution of the enzyme in phosphate buffer (1×10^{-4} *M* ethylenediaminetetraacetic acid and 0.04 *M* sodium phosphate, pH 7.4). A control was prepared by adding 0.5 ml of deionized water to a 1.5-ml aliquot of the same enzyme solution.

All samples were incubated at 37° for 15 min and then at 4° for 24 hr. Then 6-μl aliquots of this enzyme preparation were added to 3-ml aliquots of the phosphate buffer such that the detergent concentration in the assay mixture was 0.05%, and the assays were monitored as already described. The assays were performed immediately upon addition of the detergent solution of enzyme and at various time intervals over 42 hr.

A further control consisting of L-glutamic acid dehydrogenase solutions in 0.05% (v/v) detergent was prepared by adding 0.75 μl of detergent to a 1.5-ml aliquot of a 0.53-mg/ml solution of the enzyme in phosphate buffer (1×10^{-4} *M* ethylenediaminetetraacetic acid and 0.04 *M* sodium phosphate, pH 7.4). Then 6-μl aliquots of this enzyme mixture were added to 3-ml aliquots of the phosphate buffer con-

[1] The following nonionic detergents were purchased from Sigma Chemical Co.: Triton X-100, Triton X-102, Triton X-35, Triton X-45, Triton X-65, Triton CF-54, Triton CF-32, Triton N-101, Triton DF-12, Triton QS-15, Triton B-1956, Tween 20, Tween 40, Tween 60, Tween 80, Brij 96, and NP-40.

Table I—Chemical Composition of the Nonionic Detergents[a]

Detergent	Hydrophile Polyoxyethylene n[b]	Other	Lipophile
1[c]	20	Sorbitan	Monolaurate
2[c]	20	Sorbitan	Monopalmitate
3[c]	20	Sorbitan	Monostearate
4[c]	20	Sorbitan	Monooleate
5[c]	10	—	Oleyl alcohol
6[d]	3	Sorbitan	Octylphenyl
7[d]	5	Sorbitan	Octylphenyl
8[d]	—	—	Alkyl polyether alcohol
9[d]	9.5	—	Octylphenyl
10[d]	12.5	—	Octylphenyl
11[d]	9.5	—	Nonylphenyl
12[d]	—	—	Arylaryl polyether
13[d]	—	—	Modified polyether adduct
14[d]	—	—	Modified polyethoxylated alcohol
15[d]	—	—	Amine polyglycol condensate
16[d]	—	—	Sodium salt of amphoteric surfactant
17[d]	—	—	—
18[e]	9	—	Octylphenyl

[a] The nonionic detergents had the general formula of hydrophile–lipophile. The hydrophilic portion was a polyoxyethylene moiety and/or sorbitan. The lipophilic portion consisted of monolaurate, *etc.* The difficulty encountered in arriving at a comprehensible chemical name for each detergent led to the assigning of numbers as given in the left-hand column. Their respective trade names are Tween 20, Tween 40, Tween 60, Tween 80, Brij 96, Triton X-35, Triton X-45, Triton X-65, Triton X-100, Triton X-102, Triton N-101, Triton CF-10, Triton CF-54, Triton DF-12, Triton CF-32, Triton QS-15, Triton B-1956, and NP-40. [b] Number of ethylene oxide units. [c] From the General Characteristics of Atlas Surfactants, Atlas Chemical Industries, Wilmington, Del. [d] From Rohm and Haas Surfactants Technical Bulletin, Rohm and Haas Co., Philadelphia, Pa. [e] From Shell Oil Co. Data Sheet 2A003.

taining 0.05% (v/v) detergent. The assays were monitored as already described.

Determination of V_m and K_m of L-Glutamic Acid Dehydrogenase—Aliquots of 6 μl of the enzyme in a 25% (v/v) detergent solution were added to 3-ml aliquots of the following assay mixtures. Incremental amounts of a solution of nicotinamide adenine dinucleotide phosphate reduced form at a concentration of 2×10^{-4} *M* in a buffer solution (1×10^{-4} *M* ethylenediaminetetraacetic acid and 0.04 *M* sodium phosphate, pH 7.6) were added to a similar buffer solution containing 5×10^{-4} *M* α-ketoglutaric acid and 1×10^{-1} *M* NH_4Cl such that the concentration of the nicotinamide adenine dinucleotide phosphate reduced form was varied from 1×10^{-4} to 5×10^{-5} *M*. The assays were monitored as already described.

Inhibition of L-Glutamic Acid Dehydrogenase Activity Using Guanosine 5′-Triphosphate in Presence of Detergent—Incremental amounts of a guanosine 5′-triphosphate solution at a concentration of 4×10^{-3} *M* were added to a phosphate buffer solution (1×10^{-4} *M* ethylenediaminetetraacetic acid and 0.04 *M* sodium phosphate, pH 7.6) such that the concentrations of nicotinamide adenine dinucleotide phosphate reduced form, α-ketoglutaric acid, and ammonium chloride were 1×10^{-4}, 5×10^{-4}, and 1×10^{-1} *M*, respectively.

The final concentrations of guanosine 5′-triphosphate in the assay mixture were 0, 1, 2, 5, and 8×10^{-6} *M*. Three-milliliter aliquots of this solution were taken, and 6 μl of a 25% (v/v) detergent solution of the enzyme was added. The assays were monitored as already described.

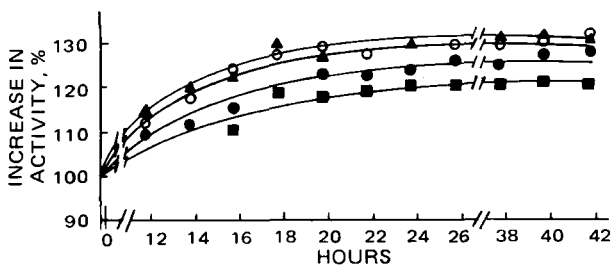

Figure 1—*Activity of L-glutamic acid dehydrogenase in detergent solutions with time. The assay conditions are given in the text. Key:* ▲, *enzyme in Detergent 10;* ●, *enzyme in Detergent 13;* ○, *enzyme in Detergent 18; and* ■, *enzyme in Detergent 2.*

Table II—Enzyme Activities of L-Glutamic Acid Dehydrogenase in Detergents[a]

Detergent	Activity[b], %	K_{GTP}, $M \times 10^{-6}$	Activity[c], %
—	100	1.55 ± 10%	100
12	0	—	—
14	0	—	—
16	8	—	—
1	120	4.3 ± 4.5%	120
2	118	3.4 ± 9.9%	97
3	10	—	—
18	130	2.65 ± 12%	43
9	94	—	—
10	130	9.25 ± 3%	103
15	74	—	—
13	125	4.9 ± 5%	110

[a] Assay conditions as stated in the text. [b] Measured in the direction of α-ketoglutaric acid reduction. [c] Measured as a monocarboxylic acid dehydrogenase.

Activation of L-Glutamic Acid Dehydrogenase Activity Using Adenosine 5′-Diphosphate in Presence of Detergent—Incremental amounts of an adenosine 5′-diphosphate solution at a concentration of 2×10^{-1} *M* were added to a phosphate buffer solution (1×10^{-4} *M* ethylenediaminetetraacetic acid and 0.04 *M* sodium phosphate, pH 7.4) such that the concentrations of nicotinamide adenine dinucleotide phosphate reduced form, α-ketoglutaric acid, and ammonium chloride were 1×10^{-4}, 5×10^{-4}, and 1×10^{-1} *M*, respectively.

The final concentrations of adenosine 5′-diphosphate in the assay mixture were 0, 0.5, 1.0, 3, and 6×10^{-4} *M*. Three-milliliter aliquots of this solution were taken, and 6 μl of a 25% (v/v) detergent solution of the enzyme was added. The assays were monitored as already described.

Monocarboxylic Acid Dehydrogenase of L-Glutamic Acid Dehydrogenase Activity in Presence of Detergent—This reaction was assayed in the direction of pyruvate reduction. Aliquots of 250 μl of a 25% (v/v) detergent solution of the enzyme were added to 2.75 ml of phosphate buffer (10^{-4} *M* ethylenediaminetetraacetic acid and 0.04 *M* sodium phosphate, pH 9.0) containing 1.28×10^{-4} mmole of nicotinamide adenine dinucleotide phosphate reduced form, 3×10^{-1} mmole of ammonium chloride, and 1.2×10^{-1} mmole of sodium pyruvate. The assays were monitored as already described.

RESULTS AND DISCUSSION

In preliminary screening experiments, 18 detergents (Table I) were investigated for their effects on L-glutamic acid dehydrogenase. Of those in which the enzyme was soluble, many destroyed much or all enzyme activity. These detergents were 3, 12, 14, and 16 (Table I). This loss could have been due to denaturation or disruption of the enzyme into its nonactive subunits, possessing molecular weights of 55,000 (10). Detergents 4, 5, 6, 7, 8, 11, and 17 did not solubilize the enzyme under the described conditions. Detergents 9 and 15 solubilized the enzyme, but these solutions showed some loss in enzyme activity.

Five detergents, 1, 2, 10, 13, and 18, gave an enhanced activity of the enzyme when α-ketoglutaric acid was used as the substrate. This change in enzyme activity was observed when the enzyme was initially incubated in a 25% (v/v) detergent solution and then diluted to the assay concentration of 0.05% detergent (Table II). When the assays were repeated such that the enzyme and substrates were only in an environment of a 0.05% detergent solution, there were no changes in enzyme activity. Thus, it appears that when the enzyme is incubated in 25% detergent solution, some change is produced that enhances the enzyme's activity toward the α-ketoglutaric acid reduction.

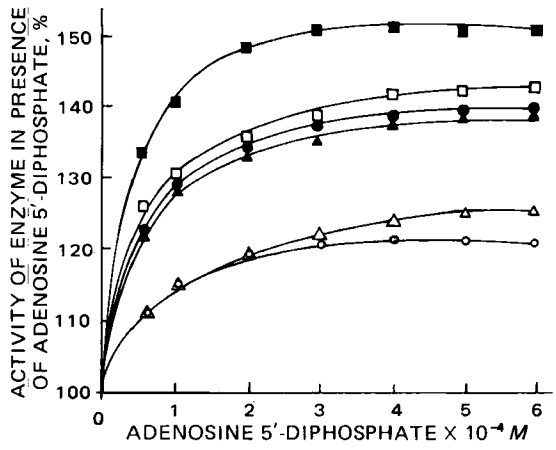

Figure 2—*Effect of detergent on the activation of* L-*glutamic acid dehydrogenase by adenosine 5′-diphosphate. The assay conditions are given in the text. Key:* ■, *enzyme in buffer;* □, *enzyme in Detergent 18;* ●, *enzyme in Detergent 13;* △, *enzyme in Detergent 10;* ▲, *enzyme in Detergent 1; and* ○, *enzyme in Detergent 2.*

The stability of the enhanced activity in detergent solution over time was investigated (Fig. 1). A similar enhancement was obtained with the nonionic detergent, 1, which showed a maximum activity of 120%. Possibly, the effects shown by the detergents on the catalytic activity of the enzyme were caused by a change in the Michaelis–Menton constant, K_m, or in the maximum number of moles of substrate that 1 mole of enzyme can convert per minute, the V_m of the enzyme. An investigation of these parameters was undertaken. The initial enzyme velocities obtained from the various enzyme assays were obtained graphically (11). The K_m and V_m of the enzyme control were 6.8×10^{-5} *M* and 17,000, respectively. Detergents 2, 10, 1, and 13 had K_m values of 8.0×10^{-5}, 8.3×10^{-5}, 4.8×10^{-5}, and 7.6×10^{-5} *M* and V_m values of 19,825, 21,525, 14,000, and 21,000, respectively. All of these detergents showed parallel changes in their V_m and K_m values.

The influence of the detergents on the regulatory properties of L-glutamic acid dehydrogenase was investigated. In general, any modifier of the enzyme that produced an increase in its activity toward the α-ketoglutaric acid conversion caused a decrease in the monocarboxylic acid dehydrogenase activity of the enzyme and vice versa. It is known that adenosine 5′-diphosphate causes an increase in the activity of the enzyme toward the α-ketoglutaric acid conversion and a decrease in its monocarboxylic acid dehydrogenase activity. The allosteric modifier guanosine 5′-triphosphate stimulates the monocarboxylic acid dehydrogenase activity of the enzyme and inhibits its activity toward the α-ketoglutaric acid conversion. Both of these materials probably mediate the equilibrium between the two modifications of the enzyme (7, 12).

In these studies with detergent solutions of the enzyme and the modifier adenosine 5′-diphosphate and guanosine 5′-triphosphate, all detergents lowered the activating effect of adenosine 5′-diphosphate (Fig. 2). The guanosine 5′-triphosphate inhibition of the α-ketoglutaric acid conversion activity of detergent solutions of the enzyme was quantitated by calculation of the reciprocal of the affinity of guanosine 5′-triphosphate (GTP) for the enzyme, the K_{GTP} in each detergent (13). The presence of detergent decreased the sensitivity of the enzyme to inhibition of the α-ketoglutaric acid conversion activity by guanosine 5′-triphosphate. In the absence of guanosine 5′-triphosphate, the monocarboxylic dehydrogenase activity of the enzyme (6, 14) was not altered greatly by detergents, with the exception of Detergent 18 which produced a decrease in activity (Table II). This decrease could have been due to this detergent influencing the equilibrium between the α-ketoglutaric acid conversion and the monocarboxylic acid dehydrogenase modifications.

A possible explanation for this enhanced enzyme activity in detergent solutions, when α-ketoglutaric acid was used as the substrate, could be related to the relative rates of the α-ketoglutaric acid reduction and the glutamate oxidation. It was shown previously that the former reaction is an order of magnitude faster than the latter reaction (15). This information indicates that the substrates for the glutamate oxidation are not bound as well as the substrates for the α-ketoglutaric acid reduction. The binding of detergents may cause a slight conformational change in the enzyme, thereby causing an increase in the binding of the substrates for the α-ketoglutaric acid reduction and accounting for the enhanced activity of the enzyme. It could be construed that this change in conformation is sufficient to lower the binding of guanosine 5′-triphosphate and, thereby, reduce this modifier's inhibitory ability of the α-ketoglutaric acid conversion activity of the enzyme. This conformational change in the enzyme could reduce the ability of the adenosine 5′-diphosphate to enhance the α-ketoglutaric acid conversion activity but not reduce the binding of this modifier with the enzyme.

REFERENCES

(1) A. Helenius and K. Simons, *Biochim. Biophys. Acta*, **415**, 29(1975).

(2) J. N. Umbreit and J. L. Strominger, *Proc. Nat. Acad. Sci. USA*, **70**, 2997(1973).

(3) E. S. Vitetta, E. A. Boyse, and J. W. Uhr, *Eur. J. Immunol.*, **3**, 446(1973).

(4) B. J. Johnson, U. N. Kucich, and J. C. Bennett, *Fed. Proc.*, **34**, 553(1975).

(5) U. N. Kucich, J. C. Bennett, and B. J. Johnson, *J. Immunol.*, **115**, 626(1975).

(6) J. Struck and I. W. Sizer, *Arch. Biochem. Biophys.*, **86**, 260(1960).

(7) G. M. Tomkins, K. L. Yielding, J. Curran, M. R. Summers, and M. W. Bitensky, *J. Biol. Chem.*, **240**, 3793(1965).

(8) M. W. Bitensky, K. L. Yielding, and G. M. Tomkins, *ibid.*, **240**, 668(1965).

(9) S. Udenfriend, S. Stein, P. Bohlen, W. Dairman, W. Leimgruber, and W. Weigele, *Science*, **178**, 871(1972).

(10) N. Talal, G. M. Tomkins, J. F. Mushinski, and K. L. Yielding, *J. Mol. Biol.*, **8**, 46(1964).

(11) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658(1934).

(12) J. Monod, J. Wyman, and C. J. Changeux, *J. Mol. Biol.*, **12**, 88(1965).

(13) M. Dixon, *Biochem. J.*, **55**, 70(1953).

(14) G. M. Tomkins, K. L. Yielding, and J. Curran, *Proc. Nat. Acad. Sci. USA*, **47**, 270(1961).

(15) D. A. Olson and C. B. Anfinsen, *J. Biol. Chem.*, **197**, 67(1952).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 24, 1975, from the *Department of Microbiology, University of Alabama Medical School, Birmingham, AL 35294*

Accepted for publication February 19, 1976.

Supported in part by Grant AI 11970 from the National Institutes of Health.

The encouragement of Dr. K. Lemone Yielding is gratefully acknowledged.

[x] To whom inquiries should be directed.