Reversible Dissociation of L-Asparaginase of *Escherichia coli* B

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Abstract □ A large conformational change can be induced in L-asparaginase of *Escherichia coli* B, purified by heat treatment, by the addition of 0.0026 *M* sodium dodecyl sulfate to a 3.8–7.4 × 10^{-6} *M* enzyme solution. Optical rotatory dispersion and circular dichroism measurements indicated that conformational change occurs simultaneously with dissociation of the tetrameric enzymes to the size of a dimer ($s^0_{20,w}$ = 4.05), as shown by ultracentrifugal measurements, and with a decrease of activity *in vitro*. This change can be reversed by the addition of sodium sulfate, sodium citrate, or dipotassium hydrogen phosphate. Simultaneously, the enzyme regains its original molecular weight of 130,000 daltons ($s^0_{20,w}$ = 8.75) and its full activity *in vitro*.

Keyphrases □ L-Asparaginase of *Escherichia coli* B—reversible dissociation, conformational changes, activity □ *Escherichia coli* B—reversible dissociation, conformational changes, and activity of L-asparaginase □ Conformational changes—L-asparaginase of *Escherichia coli* B, reversible dissociation, activity

Some conflicting data appear in the published reports concerning the physical properties of L-asparaginase (L-asparaginase amidohydrolase, (E.C. 3.5.1.1) from *Escherichia coli* B. For example, Kirschbaum *et al.* (1), using heat-treated enzyme prepared from *E. coli* B essentially by the method of Whelan and Wriston (2), reported an $s^0_{20,w}$ value of 8.7 S, molecular weights of 255,000 and 130,000 daltons, and a subunit molecular weight of 22,000 daltons. Arens *et al.* (3) and Wagner *et al.* (4) purified L-asparaginase from *E. coli* ATCC 9637 and 11303, using heat, acetone, and 2-methylpentane-2,4-diol, and they reported an $s^0_{20,w}$ value of 7.5 S with molecular weights of 240,000 and 120,000 and, for the subunit, 21,000 daltons. However, amino acid end-group analysis indicated a molecular weight of 35,000 for the subunits. Frank *et al.* (5), using crystalline L-asparaginase purified from *E. coli* by the aqueous ethanol method of Ho *et al.* (6), reported an $s^0_{20,w}$ value of 7.6 S, a molecular weight of 133,000 daltons (with higher molecular weight aggregates present), and a subunit molecular weight of 33,000 by ultracentrifugation and amino acid analyses.

Since then the present authors were able to find an $s^0_{20,w}$ value of 7.6–7.75 S for L-asparaginase purified by alcohol; when the enzyme from the same strain of organism was purified with the aid of heat, the $s^0_{20,w}$ value was 8.6 S. A sample of L-asparaginase purified by Ho *et al.* (6) was found in this laboratory to dissociate with acid, sodium dodecyl sulfate (I), or sodium chloride to give a molecular weight with $s^0_{20,w}$ value of 3.6 S. The heat-purified enzyme gave an $s^0_{20,w}$ of 4.0 S under the same conditions of measurement.

These findings prompted the authors to investigate the molecular weight again, especially in reference to the association–dissociation behavior of the subunits of the heat-treated enzyme in the presence of different salts and I. The effects of the different agents were followed by activity measurements *in vitro*, molecular weight determinations, optical rotatory dispersion (ORD), and circular dichroism (CD). A 60,000-molecular weight dimer with an $s^0_{20,w}$ value of 4.0 S was found when L-asparaginase was treated by 0.0035 *M* I.

MATERIALS AND METHODS

Experiments were performed with a heat-treated enzyme preparation that had been shown to be homogeneous by Mashburn and Landin (7), using an isoelectrofocusing procedure, unlike preparations from other laboratories.

Activity measurements were made by a modified method of Campbell *et al.* (8). Reagents used in the various experiments were dissolved in 1.7 ml. of 0.05 *M* tromethamine buffer (pH 8.4) prior to the addition of 0.2 ml. substrate and 0.1 ml. enzyme solution. Blank assays were made by adding the enzyme after the reaction had been quenched with trichloroacetic acid. To assure that the different reagents did not interfere with the Nesslerization reaction, blanks were also run by adding these reagents after quenching a normal test. No interference was assumed if Nesslerization showed full activity. Accuracy of the activity measurement is ±10%.

Gel filtration was carried out by the method of Andrews (9). Sephadex-150, in a 2.5 × 50-cm. tube, was equilibrated with 0.05 *M* tromethamine buffer (pH 8.4) or with the same buffer containing either 0.0035 *M* I or 0.077 *M* L-cysteine, 6 days prior to preparation of the column. Effluent volume was measured to the nearest 2 ml. Markers dissolved in 0.3 ml. eluting solvent were: blue dextran 2000[1], bovine serum albumin[2], ovalbumin[3], papain[4], γ-chymotrypsin[5], and α-globulin[2].

Molecular weight determinations, with the aid of an analytical ultracentrifuge (Spinco), were performed as described previously by Kirschbaum *et al.* (1).

ORD and CD spectra were recorded at 27 ± 1°, employing a spectropolarimeter (Cary 60) equipped with a CD attachment (Cary 6001). Calibrations were performed by the method of Cassim and Yang (10), using *d*-10-camphorsulfonic acid as a standard.

RESULTS

The effect of different salts, urea, L-cysteine, and I on the activity *in vitro* of L-asparaginase is summarized in Table I. All compounds,

[1] Pharmacia.
[2] American National Red Cross.
[3] Mann Research.
[4] Hog stomach, Mann Research.
[5] Worthington.

Table I—Effect of Different Reagents on Activity *In Vitro* of L-Asparaginase[a]

Treatment	Concentration, *M*	Activity, %
Dipotassium hydrogen phosphate	8	18
	3	70
	2.5	100
Sodium citrate	3.6	32
	1.6	87
	0.5	95
Sodium sulfate	4.8	100
	1.3	100
Sodium chloride	5	37
	2	90
	1	100
Urea	8	0
	5	27
	2	78
	1	85
L-Cysteine	0.077	37
	0.03	80
Sodium dodecyl sulfate (I)	0.07	20
	0.034	32
	0.014	40
	0.007	50
	0.0035	68
	0.0014	94

[a] Standard test conditions = no preincubation. 100% activity = activity of the enzyme at standard test conditions without additives.

except sodium sulfate, reduced the activity to some extent. The activity *in vitro* of L-asparaginase was diminished to 32% of the control value by the addition of 0.034 *M* I and to other values by the addition of different amounts of I (Table I). These suppressions of activity could not be observed if 0.05 *M* sodium citrate, 1.3 *M* sodium sulfate, or 2.5 *M* dipotassium hydrogen phosphate was simultaneously added. However, simultaneous addition of sodium chloride reduced the activity *in vitro* in an additive way.

Compounds from the metabolic pathway, in which L-asparaginase is involved, were tested for an effect on the activity of L-asparaginase *in vitro*. Although L-aspartic acid, L-methionine, and L-threonine had no apparent effect, 0.077 *M* L-cysteine reduced the activity about 60%. This reduction of activity by L-cysteine could not be compensated by the addition of sodium sulfate, sodium citrate, or dipotassium hydrogen phosphate, as was the case with I. Mercaptoethanol (0.01 *M*), a disulfide-reducing agent, had no effect on the activity *in vitro* of L-asparaginase. This was shown to be the case also by Jayaram *et al.* (11).

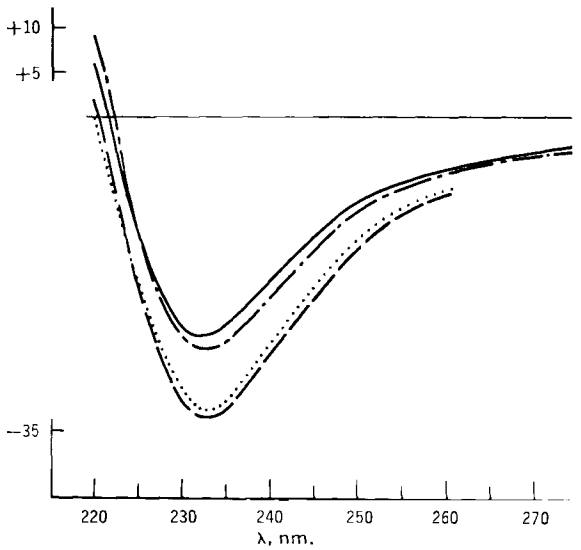

Figure 1—*ORD spectrum of* L-*asparaginase in:* (——) *0.0762* M *tromethamine,* (– –) *0.0762* M *tromethamine plus 0.0052* M *sodium dodecyl sulfate,* (. . .) *0.0381* M *tromethamine plus 0.5* M *sodium sulfate plus 0.0026* M *sodium dodecyl sulfate, and* (– – –) *0.0381* M *tromethamine plus 2.0* M *sodium sulfate plus 0.0026* M *sodium dodecyl sulfate.*

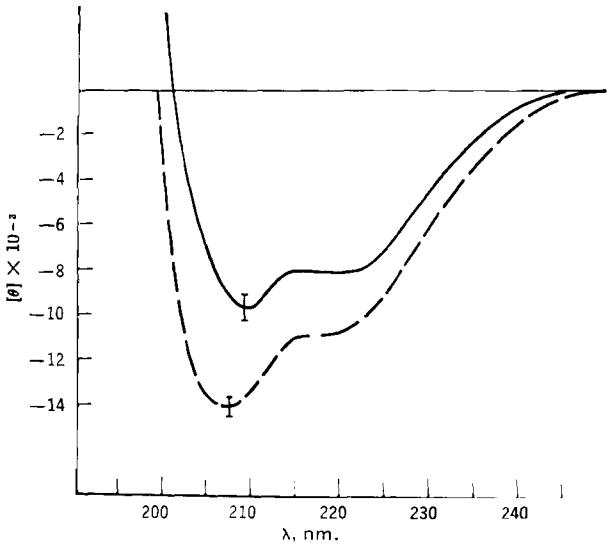

Figure 2—*CD spectrum of* L-*asparaginase in:* (——) *0.0762* M *tromethamine, and* (– –) *0.0762* M *tromethamine plus 0.0026* M *sodium dodecyl sulfate.*

The molecular weights of the active control, the I-treated forms, and the L-cysteine-treated forms were determined by gel filtration on Sephadex-150 columns. The molecular weight of untreated L-asparaginase was 136,000. Enzyme activity appeared in the same fraction when concentrations of 0.5 mg. enzyme/ml. (3.8×10^{-6} *M*), 0.04 mg./ml. (3×10^{-7} *M*), and 0.003 mg./ml. (2.2×10^{-8} *M*) were applied to the column. The concentration of L-asparaginase used in the determinations of activity *in vitro* was 0.002–0.001 mg./ml., indicating that the form of L-asparaginase active *in vitro* has a molecular weight of 136,000 with an $s^0_{20,w}$ value of 8.7 S. This molecular weight was also found for L-asparaginase deactivated by L-cysteine in the corresponding L-cysteine-containing column. A third, similar Sephadex-150 column was prepared, using 0.05 *M* tromethamine buffer (pH 8.4) containing 0.0035 *M* I. From our calibration curve, the molecular weight of L-asparaginase, independent of concentration, appeared to be 60,000.

The 0.0035 *M* I-treated form of L-asparaginase, with reduced activity, a molecular weight of 60,000 daltons, and an $s^0_{20,w}$ value of 4.0 S, as determined by gel filtration and ultracentrifugation, had the original activity in the presence of 1.3 *M* sodium sulfate, or 0.5 *M* sodium citrate, or 2.5 *M* dipotassium hydrogen phosphate. The molecular weight of this fully active enzyme, in the presence of I and subsequent addition of one of these inorganic salts, was 136,000 daltons with an $s^0_{20,w}$ value of 8.7 S, as determined by ultracentrifugation.

The ORD spectrum of 7.4×10^{-6} *M* L-asparaginase in 0.0762 *M* tromethamine buffer (pH 8.5) exhibits a trough at 232 nm. ([R′]232 = 2600), a crossover at 222 nm., and a peak at 200 nm. ([R′]2200 = 14000). Incremental addition of I up to 0.0052 *M* increased by about 40% the magnitude of the trough at 232–233 nm. (0.001 *M* I, [R′]232 = 3500). Adding sodium sulfate up to the concentration of 2 *M* and performing the identical dilution resulted in a dramatic decrease ([R′]233 = 2650) (Fig. 1).

Changes in the CD spectra of 2.3×10^{-5} *M* L-asparaginase in 0.0762 *M* tromethamine buffer (pH 8.5) (Fig. 2) demonstrated that the reversible changes in the ORD trough were not due solely to solvent-induced shifts of the UV absorption that give rise to overlapping dispersion bands.

DISCUSSION

The dissociation of L-asparaginase (molecular weight = 136,000 daltons) into a dimer, with a molecular weight of approximately 60,000, can be induced by I or 5 *M* sodium chloride, as shown by gel filtration and ultracentrifugation. During this dissociation into its dimer, L-asparaginase undergoes conformational changes and its catalytic activity is reduced. Conformational changes induced by I

can be reversed, however, by the addition of sodium sulfate, sodium citrate, or dipotassium hydrogen phosphate, as shown by ORD measurements. It was also shown by ultracentrifugation that the addition of these salts counteracts the dispersing forces of I and the subunits are reassociated to the 136,000-molecular weight form. The ionic environment significantly influences the dissociation of the heat-treated enzyme into its subunits, concomitantly with changes in activity *in vitro*.

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Calculation of Partial Molal Volume in Micellar Systems

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Abstract □ The partial molal volume of a large number of surfactants in the micellar state was calculated by the addition of partial atomic values and the inclusion of a term to account for the hydrocarbon liquid-like nature of the micelle interior. With micellar systems, such numbers often are difficult to obtain experimentally and this approach provides a means of determining relatively accurate values by simple calculation.

Keyphrases □ Partial molal volume—calculation, surfactants in the micellar state □ Surfactants in the micellar state—calculation of partial molal volume □ Micellar systems—calculation of partial molal volume of surfactants

The molecular volume of a surfactant in a micelle is a useful parameter for the study of properties of micelles. For example, it can be used, along with hydrodynamic and light-scattering data, to determine the amount of solvent associated with the micelle (1, 2), to determine the surface charge density and the surface potential of a micelle from its size (3), or, conversely, to determine its size from potentiometric titration data (4). The key to the usefulness of the molecular volume is that it does not change significantly with changes in the solution, such as pH, temperature, and ionic strength (5). Therefore, it can be used to relate the micelle radius and aggregation number, both of which are influenced by the stated conditions.

Table I—Partial Molal Volumes of Some Common Atoms and Groups

Atom	Partial Molal Volume, cm.^{3}/mole[a]	Group	Partial Molal Volume, cm.^{3}/mole[a]
C	9.9	CH_3	19.3
H	3.1	CH_2	16.2
H^+	−4.5	NH_2	7.7
N	1.5	$N(CH_3)_3^+$	66.3
N^+	8.4	COOH	19.0
O (=O or —O—)	5.5	COO^-	11.5
O (—OH)	2.3	C_2H_5	35.3
O (diol)	0.4	C_3H_7	51.7
S	15.5	C_4H_9	67.9
P	17.0	C_6H_{13}	100.3
P^+	28.5	C_8H_{15}	132.7
Li^+	−5.2	$C_{10}H_{21}$	165.1
Na^+	−5.7	$C_{12}H_{25}$	197.5
K^+	4.5	$C_{14}H_{29}$	229.9
Cl^-	22.3	OCH_2CH_2	37.9
Br^-	29.2	One ring	−8.1
I^-	40.8	Two fused rings	−26.4

[a] To convert to Å^3/molecule, divide by 0.6023.

DISCUSSION

The molecular or molar volume of a solute cannot be measured, but the experimentally measured partial molal volume (p.m.v.) is a