Biologic and Physical Properties of Succinylated and Glycosylated Acinetobacter Glutaminase-Asparaginase*

(Received for publication, October 17, 1974)

JOHN S. HOLCENBERG,† GOTTfried SCHNER, AND DAVID C. TELLER

From the Departments of Medicine, Pharmacology, Laboratory Medicine and Biochemistry, University of Washington School of Medicine, Seattle, Washington 98195

JOSEPH ROBERTS

From the Sloan-Kettering Institute for Cancer Research, Rye, New York 10580

SUMMARY

Acinetobacter glutaminase-asparaginase was chemically modified by succinylation and glycosylation with glycopeptides from human fibrin and γ-globulin. These modifications markedly prolonged the half-lives of the enzyme in mice, rats, and rabbits. The plasma half-life in mice increased with decreasing isoelectric point. Glycosylation caused greater prolongation in rodents than succinylation.

The kinetic properties of the modified enzymes were unchanged. Succinylation protected the enzyme from trypsin digestion. Glycosylated preparations had less heat inactivation than native and succinylated enzyme.

Sedimentation equilibrium studies on a succinylated preparation showed reversible dissociation to a dimer (71,400 g/mol) with an association constant of 1.3 × 10^6 liters/mol. This dissociation was identical with native enzyme, except for a 3% increase in molecular weight due to succinate groups. Sedimentation equilibrium studies on glycosylated preparations showed mixtures of molecular weight from 60,000 to over 180,000. Gel filtration and active enzyme sedimentation showed active polymers, but no active species smaller than tetramer.

The biochemical and physical properties of Acinetobacter glutaminase-asparaginase have been extensively studied (1, 2). The enzyme has a ratio of V_{max} for L-glutamine and L-asparagine of 1.2 and K_{m} for both substrates of less than 10^{-5} M. It is a tetramer of 138,000 g/mol, which undergoes reversible dissociation to an inactive dimer during gel filtration and ultracentrifugation. Treatment of experimental animals with the enzyme causes marked regression of certain transplantable tumors in mice and human leukemic cells in vitro.

* This investigation was supported by United States Public Health Service Grants CA 11881, CA 15674, GM 13401, CA 08748, and CA 15860.

† Career development awardee, National Cancer Institute, CA 25976.

The plasma half-life of the enzyme is only 1 to 2 hours in normal mice, but is increased to 12 to 18 hours in mice infected with lactate dehydrogenase-elevating virus (3). Preliminary studies also indicated a short half-life in the rat, rabbit, and monkey.

The plasma half-life of both Escherichia coli and Erwinia asparaginase preparations have been increased by deamination, acylation, and carbodiimide reactions with free amino groups (6, 7). In most cases plasma half-life increased with decreasing isoelectric point. Glycosylation has not been investigated as a means to increase the half-life of these enzymes. In order to improve the plasma half-life and hopefully the distribution and biologic effect of Acinetobacter glutaminase-asparaginase, we investigated various chemical modifications. This paper reports the effects of succinylation and glycosylation of the enzyme on its plasma half-life and biologic and physical properties.

EXPERIMENTAL PROCEDURE

Materials and Methods—Crystalline bovine albumin and 65% clottable fibrinogen were purchased from Miles Laboratories; grade C pronase, glutaraldehyde, soybean trypsin inhibitor from Sigma; catalase from Boehringer Mannheim; thrombin from Upjohn; and trypsin from Worthington. Glutaraldehyde was stored over barium carbonate, as Diao 1-oxonorvaline was a gift from Dr. R. E. Handschumacher, Yale University School of Medicine.

Imidazole-NaCl buffer contained 0.15 M NaCl and 0.02 M imidazole-HCl, pH 7.35. Plasminogen was prepared from human plasma by affinity chromatography as described by Deutsch and Mertz (8) and was stored at -90° as 50 CTA units/ml of imidazole-NaCl buffer. Plasminogen was activated to plasmin by streptokinase (9). Escherichia coli asparaginase was a gift from Merck Sharp and Dohme. Lot ABI-2, containing 200 i.u./mg of powder. Erwinia carotovora asparaginase was a gift from National Cancer Institute, Lot MRE 10/9/70.

Glutaminase-asparaginase was isolated from Acinetobacter glutaminisiceps (ATCC 27197), cultured, and supplied as a frozen paste by Grain Processing Co. according to our method (1). Enzyme was purified to homogeneity by the following modifications of the method of Roberts et al. (1). The pellet from the initial sonicate was extracted with 30% ammonium sulfate to increase the enzyme yield, and the DEAE-Sephadex chromatography step was performed in 0.005 to 0.01 M potassium phosphate buffer, pH 8. This buffer provided more stability than the sodium salt during

4165
the subsequent lyophilization. All enzyme preparations had a specific activity greater than 120 i.u./mg of protein. Enzyme activity was assayed by ammonium formation from asparagine or glutamine. K_m determinations were performed with C14-labeled substrate. Radioisotopic substrate and product were separated on Dowex 1 (Cl) columns (0.4 x 4 cm) (1). Activity with 5-diao-4-oxonorvaline was monitored spectrophotometrically (10).

Protein concentration was determined by absorbance at 280 and 260 nm or by alkaline hydrolysis and ninhydrin reaction (11). Neutral sugar was assayed by ammonia formation from asparagine or glutamine. Z-spectrophotometric determinations were performed with C14-labeled substrate. Radioisotopic substrate and product were separated on Dowex 1 (Cl) columns (0.4 x 4 cm) (1). Activity with 5-diao-4-oxonorvaline was monitored spectrophotometrically (10).

Protein concentration was determined by absorbance at 280 and 260 nm or by alkaline hydrolysis and ninhydrin reaction (11). Neutral sugar was assayed by ammonia formation from asparagine or glutamine. Z-spectrophotometric determinations were performed with C14-labeled substrate. Radioisotopic substrate and product were separated on Dowex 1 (Cl) columns (0.4 x 4 cm) (1). Activity with 5-diao-4-oxonorvaline was monitored spectrophotometrically (10).

Gel disc electrophoresis was performed by modification of the method of Littgitti and Drysdale (14). The gels contained 4.5 g of acrylamide, 0.2 g of bisacrylamide, 2.0 g of Pharmacia ampholytes, pH 3.0 to 10, 0.08 mg of riboflavin, and 5 ml of glycerol/100 ml. Gels were washed twice with 10% trichloroacetic acid and then suspended in water prior to addition of the phenol and sulfuric acid reagents. Fibrinogen concentration was determined by the method of Links (12).

Enzyme activity and pH were determined on each eluate. Fibrinogen concentration was determined by the method of Habeeb (18). Fibrinoglycopeptide Preparation—Ten grams of bovine fibrinogen (65% clottable) was dissolved in 500 ml of imidazole-NaCl buffer and precipitated at room temperature by addition of solid ammonium sulfate to 25% saturation. After a 30-min incubation at room temperature, the solution was centrifuged at 10,000 x g for 15 min and dissolved in 500 ml of imidazole-NaCl buffer. The resulting fibrinogen was 95% clottable. Two thousand National Institutes of Health units of bovine thrombin were added to the stirred solution of fibrinogen. After incubation for about 1 min at room temperature, the fibrin clot was removed with a wooden spatula. Excess fluid was removed by squeezing the clot in filter paper or gauze. The clot was suspended in 150 to 200 ml of imidazole-NaCl buffer and digested in a shaker for 24 hours at 37°C with 1,000 CA units of plasmin. Further digestion was achieved by incubation for 24 hours with the addition of 200 mg of pronase to the stirred solution of fibrinogen. The original sample was adjusted with a 1:25 volume of 0.1% trichloroacetic acid. After a 10-min incubation at room temperature, the precipitate was removed by centrifugation for 5 min at 20,000 x g and 5°C. The supernatant containing the fibrinoglycopeptides was immediately chromatographed on a Sephadex G-25 column (4 x 100 cm) previously equilibrated with 0.1 sodium borate buffer, pH 8.4. The glycopeptides eluted in the exclusion volume without appreciable dilution as a sharp peak. This 200-ml pool was incubated for 60 min at 37°C without stirring with a 100-fold weight excess of glutaraldehyde relative to the neutral sugar content of the glycopeptide. A large excess of glutaraldehyde was used to minimize cross-linking of the glycopeptides.

The glutaraldehyde-activated glycopeptides were separated from free glutaraldehyde on the same G-25 column eluting in the void volume in 200 to 250 ml. These activated glycopeptides were used immediately for reaction with enzyme or stored frozen at -20°C.

For glycopeptide coupling, the activated glycopeptides were stirred with enzyme for 18 hours at room temperature at weight ratios of equality to 2-fold excess of glycopeptide. The original weight ratio of enzyme to glycopeptide was determined, since the glutaraldehyde-activated glycopeptide gave a 50% increase in the color formation with this reagent. The glycopeptide coupled to enzyme was used immediately for reaction with enzyme or stored frozen at -20°C.

Purification of the γ-globulin glycopeptides, activation with glutaraldehyde, and coupling to enzyme were performed as with the fibrinoglycopeptides.

RESULTS

Biologic Properties—The plasma half-life in CD female mice and in ileostomy point were determined with 18 preparations of succinylated and 15 preparations of glycosylated enzyme. Duplicate determinations were usually performed. The average pi and plasma t½ in each mouse for these modified enzymes is plotted.
FIG. 1. Half-life and isoelectric point of modified enzymes after intraperitoneal injection. ○, values and standard deviation for unmodified enzyme; ●, determinations in each mouse for succinylated enzyme preparations; ---, linear regression through these points described by $t_{1/2} = -1.9 (\pm 0.2) pI + 16.7 (\pm 1.3)$. $t_{1/2}$ in each mouse for enzyme glycosylated with fibrin and γ-globulin glycopeptides are shown by △ and ○, respectively. ---, linear regression through these points: $t_{1/2} = -3.0 (\pm 0.5) pI + 28.2 (\pm 3.0)$.

In Fig. 1. Untreated enzyme (○) had a mean $t_{1/2}$ of 1.1 ± 0.3 hours ($N = 19$) and $pI$ of 8.2. The $t_{1/2}$ increased with decreasing $pI$ with both types of modification. Succinylated preparations (open symbols) have longer $t_{1/2}$ at any isoelectric point than succinylated preparations (●). Maximal increases in $t_{1/2}$ were about 15- and 9-fold with the two modifications. The enzyme preparations coupled to γ-globulin glycopeptides (△) have slightly longer $t_{1/2}$ than those coupled to fibrin glycopeptides (○).

Injection of uncoupled glycopeptide with Acinetobacter glutaminase-asparaginase did not prolong the $t_{1/2}$ of the enzyme. Infection of these mice with lactate dehydrogenase-elevating virus increased the $t_{1/2}$ of untreated enzyme to 16 hours, succinylated preparations to 20 hours, and glycosylated preparations to 17 hours.

Erwinia and Escherichia coli asparaginase preparations had $pI$ of 8.0 and 5.0 by this isoelectric focus method and plasma $t_{1/2}$ of 2.9 and 4 hours, respectively. These results are similar to other reports (20, 22).

In all these experiments, 20- to 30-g mice were bled about 0.2 ml for each time point. This volume represented a significant portion (about 10%) of the blood volume and therefore the enzyme within the animal. This systematic error should decrease the calculated $t_{1/2}$. Therefore, Fig. 1 presents minimal $t_{1/2}$ values.

The plasma enzyme activity can be corrected by the packed cell volume (PCV) at each bleeding, if one assumes that the enzyme is distributed only within the plasma space and that a constant blood volume is rapidly reconstituted. The corrected plasma enzyme activity is given by:

$$C_t = C_0 \cdot \frac{PCV_t}{PCV_0} \cdot \frac{1-PCV_t}{1-PCV_0} \cdot e^{-0.693/t_{1/2}(T_2-T_1)}$$

In this equation, $C_0$ and $C_t$, $PCV_0$, and $PCV_t$, and $T_1$ and $T_2$ are the enzyme activity per ml of plasma, the packed cell volume, and time of bleeding, respectively. Correction of these experiments by this equation increased the calculated $t_{1/2}$ values 1- to 2-fold. The chemical modifications still produced marked prolongation of the plasma $t_{1/2}$.

Several preparations were tested intravenously in rats and rabbits to overcome the problems of significant loss of enzyme during bleeding and possible differences in absorption of the modified enzymes from the peritoneal cavity. Duplicate determinations in Sprague-Dawley male rats showed that untreated Acinetobacter glutaminase-asparaginase had a $t_{1/2}$ of approximately 1 hour, while a succinylated preparation ($pI = 4.7$) was 5.1 hours, and a glycosylated preparation ($pI = 4.7$) was 8.2 hours. E. coli asparaginase had a $t_{1/2}$ of 3.4 hours. The log of enzyme activity in plasma decreased linearly with time in both mice and rats injected with these preparations.

The enzyme preparations showed a two phase decay of activity in the plasma samples from New Zealand male rabbits (Fig. 2). The succinylated and glycosylated preparations persisted much longer than the native enzyme. The slow component of this decay had a $t_{1/2}$ of 4.3 hours with untreated Acinetobacter glutaminase-asparaginase, 19 hours with the glycosylated enzyme, and 33 hours with the succinylated enzyme. Similar disappearance curves were seen with preparations of fibrinogen and γ-globulin glycopeptide coupled to glutaminase-asparaginase.

**Biochemical Properties**—Preparations of modified enzymes with isoelectric points of 4.7 to 5.1 were compared with native glutaminase-asparaginase. There were no appreciable differences in the ratio of activity with glutamine, asparagine, and diazooxonvaline, pH for optimal activity, and $K_m$ for glutamine. The degree of heat denaturation varied with the specific activity of...
the preparations. At equal protein concentrations, the glycosylated preparations seemed more stable than the native or succinylated enzyme to heating at 45°C for 10 min. The neutral sugar content of the fibrin and γ-globulin glycopeptide-coupled preparations used for physical properties were 7.1 and 141%, respectively. Free amino residues were decreased 16 to 22% with succinylation and with glycosylation.

The results of trypsin inactivation of modified and native enzyme are shown in Fig. 3. The succinylated preparation was degraded much slower than the untreated enzyme. The enzyme glycosylated with γ-globulin glycopeptides lost activity slightly faster than Acinetobacter glutaminase-asparaginase. In other experiments, fibrin glycopeptide-coupled enzyme was denatured at the same rate as native enzyme.

The succinylated enzyme preparation was also stable in mouse plasma in vitro. After 18 hours of incubation at 37°C in 50% mouse plasma under sterile conditions, the enzyme had 94% of its initial activity and had the same isoelectric point. The enzyme lost over half its activity on incubation without serum.

Physical Properties—The molecular weight of these modified preparations was studied by gel filtration, equilibrium sedimentation, and active enzyme sedimentation.

The molecular weight profiles of the succinylated and glycosylated enzyme preparations are shown in Fig. 4. The molecular weight for each channel is plotted at various fringe displacements. Open symbols show results with the succinylated preparation after centrifugation for 25 hours at 16,000 rpm and 20°C in 0.01 M potassium phosphate buffer, pH 7.4, 0.2 M NaCl, and 0.02% NaN3. The initial concentrations of enzyme were 0.93, 0.62, and 0.31 mg of protein/ml. The profiles of both $M_n$ and $M_w$ show lower molecular weight averages at lower equilibrium concentrations. The profiles from the three sectors and from pictures taken after 49 hours of centrifugation are superimposable. Therefore, the succinylated enzyme appears to undergo reversible dissociation.

These profiles were analyzed by a computer program that calculated the root mean square error of fitting the data to a dimer-tetramer dissociation for various molecular weights of dimer (23).

This calculation gave an average dimer molecular weight of 71,400 ± 300 (root mean square error) and an association constant of $1.27 \pm 0.17 \times 10^6$ liters/mol. The solid line in Fig. 4 shows the calculated molecular weight average distributions for the succinylated enzyme from this dimer weight and association constant.

Our previous equilibrium studies on Acinetobacter glutaminase-asparaginase showed similar molecular weight average distributions for sectors with different initial concentrations, but they did not superimpose. Furthermore, the molecular weight averages decreased with time (2). This pattern is consistent with both reversible and irreversible dissociation. These studies were repeated with current enzyme preparations that were lyophilized in potassium phosphate buffers. The enzyme was centrifuged at 16,000 rpm and 20°C. The profiles from the three sectors and from pictures taken after 49 hours of centrifugation are superimposable. Therefore, the succinylated enzyme appears to undergo reversible dissociation.

These profiles were analyzed by a computer program that calculated the root mean square error of fitting the data to a dimer-tetramer dissociation for various molecular weights of dimer (23).

**Fig. 3.** Effect of trypsin on enzyme preparations. Solutions containing 2 i.u. of enzyme and 0.02 mg of trypsin per ml were incubated at 37°C. At various times aliquots were treated with 0.02 mg/ml of soybean trypsin inhibitor and assayed for residual enzyme activity. Solid figures represent one experiment, open figures another. Squares are values for unmodified enzyme, circles for succinylated enzyme, triangles for enzyme glycosylated with γ-globulin glycopeptide.

**Fig. 4.** Molecular weight distributions for modified Acinetobacter glutaminase-asparaginase preparations. A, weight average molecular weight ($M_w$); B, number average molecular weight ($M_n$). Open symbols are data from a succinylated preparation taken from a photograph at 25 hours at 16,000 rpm and 20°C. Δ, ○, and □ represent the channel pairs of the Yphantis centerpiece with 0.31, 0.62, and 0.93 mg/ml of initial protein concentrations, respectively; ---, calculated molecular weight distributions for a protein with a dimer molecular weight of 71,400 g/mol and association constant of $1.27 \times 10^6$ liters/mol. The closed symbols are data from a preparation glycosylated with γ-globulin glycopeptide containing 14% sugar by weight. The photograph was taken at 22 hours at 16,000 rpm and 20°C. Δ, ○, and □ represent the channel pairs with 0.25, 0.50, and 0.75 mg/ml of initial protein concentration, respectively. Solvent was 0.01 M potassium phosphate buffer, pH 7.4, 0.2 M NaCl, and 0.02% NaN3.
are not in chemical equilibrium. The molecular weight averages monomer. We have been seeking enzymatically active species as small as dimers and larger than tetramers, but these species were seen in sectors with the lowest initial concentration. This pattern indicates that the glycosylated enzyme consists of species as small as dimers and larger than tetramers, but these species are not in chemical equilibrium. The molecular weight averages showed identical profiles after 48 hours of centrifugation. The pattern with enzyme coupled to fibrin glycopeptide enzyme was very similar, but had slightly lower molecular weight distributions.

The molecular weight averages below two fringes suggested that a large proportion of the glycosylated enzyme was dimer or monomer. We have been seeking enzymatically active species with a molecular weight less than 80,000 for antitumor testing (16). Therefore, this preparation was tested by active enzyme sedimentation.

In this technique 5 to 10 μl of enzyme are layered simultaneously into two sectors of a sedimentation velocity cell. One sector contains 0.06 to 0.1 mM diazooxonorvaline as substrate in 0.01 M potassium phosphate buffer, pH 7.4, 0.2 M NaCl, and 3% sucrose. The other sector contains only solvent. The sedimentation coefficient of the enzyme form that degrades this substrate is determined from time difference spectra (2). Concentration of enzyme and substrate are selected so that less than 50% of the diazooxonorvaline is degraded as the zone of enzyme sediments through the sector.

Such studies on the γ-globulin glycopeptide-coupled enzyme showed very complex profiles due to active polymers, which rapidly sedimented throughout the diazooxonorvaline solution. Ultraviolet scans of the sectors showed a gradual decrease in the plateau concentration of substrate and broad time difference spectrum. Equivalent zone calculations of this spectrum (16) yielded $s_{n,0}$ of over 9 S.

Consequently, this glycosylated preparation was chromatographed on P200 to isolate the smaller molecular weight species. Fig. 5 shows the activity and protein absorbance of the eluates from this calibrated column. The letters V, a, b, and c indicate elution position of the peak absorbance of blue dextran, catalase, untreated E. coli, and bovine serum albumen, respectively. The glycosylized enzyme has two major peaks of activity. The first smaller peak elutes at a position corresponding to a molecular weight of 180,000. No peak was present at the position of native enzyme. Tubes 42 to 47 of this and a similar gel filtration of the same glycosylated enzyme were pooled. The protein was precipitated with ammonium sulfate, dialyzed in potassium phosphate buffer, pH 7, and concentrated under vacuum in a collodion bag. The chromatography of this concentrate on the P200 column revealed a symmetrical peak that eluted at a position corresponding to a molecular weight of 165,000.

Active enzyme sedimentation of this preparation produced a broad time difference spectrum. The sedimentation coefficient of the whole active zone was 10 S. The trailing edge sedimented at a velocity of 7.2 S. Thus the glycosylated enzyme contains active polymers even in the leading edge of the smallest P200 peak. Furthermore, there was no evidence of any active species smaller than tetramer.

An enzyme preparation glycosylated with fibrin glycopeptides (pI = 5.9) also showed a double elution pattern on P200 almost identical with Fig. 5.

**DISCUSSION**

Several workers have reported an increase in plasma half-life after chemical modification of the isoelectric point of asparaginase enzymes. Native E. coli asparaginase has an average pI of 4.9 to 5.1. Longer half-lives have been reported for deaminitated preparations or natural isomers with lower pI (6, 24). In contrast, Ilare and Handschumacher (20) noted a prolongation of $t_{1/2}$ only with modifications that increased the pI. Shifrin and Grochowski (17) showed that succinylation of more than 40% of the lysine residues of E. coli asparaginase caused dissociation of the tetramer and loss of enzyme activity.

Erwinia asparaginase has a pI of over 8. Chemical modification of this enzyme by acylation, deamination, and carbodiimide reactions has been reported to increase the $t_{1/2}$ in rabbits to a maximum of 10 hours at a pI of 6. Unmodified enzyme had a $t_{1/2}$ of 10 hours. Preparations with pI greater than 8.6 or lower...
than 5 had much shorter half-lives (7). Unlike E. coli asparaginase and other oligomeric enzymes, this enzyme could be extensively succinylated without dissociation or loss of enzyme activity (26).

*Acinetobacter* glutaminase-asparaginase also has a high native pI and can be succinylated without loss of enzyme activity or change in its physical properties. Unlike the *Erwinia* enzyme, the t½ increased with lowering the pI below 6. The stability of succinylated *Acinetobacter* glutaminase-asparaginase to trypsin digestion and the constant pI of 4.5 to 4.9 on successive reactions with succinic anhydride suggest that all of the surface free amino groups have been modified by our procedure. Shifrin has suggested that these more basic enzymes may better neutralize the negative charges introduced by succinylation (26).

Most circulating proteins in mammals are glycoproteins and many have very long plasma half-lives (27). In addition, the sugar sequence may direct the protein to specific tissues (28, 29). In preliminary experiments we synthesized polymers of L-glucose, L-mannose, and L-rhamnose and coupled them to *Acinetobacter* asparaginase with cyanogen bromide. The t½ of these glycopeptides was longer than native enzyme. Similar coupling techniques with *Acinetobacter* glutaminase-asparaginase caused extensive loss of enzyme activity. Gentler methods produced variable results. Nevertheless, intraperitoneal injection of some of these glycosylated preparations produced more prolonged depletion of plasma glutamine and asparagine than native enzyme.

Preparations of these glycopeptides coupled to *Acinetobacter* glutaminase-asparaginase showed 4- to 15-fold increases in the t½ in mice, rats, and rabbits. In the rodents, the glycosylated preparations had a longer t½ than succinylated preparations with comparable isoelectric points. The two glycopeptides produced similar biologic effects. Physical studies reveal that these preparations are polydisperse with active polymers and inactive disassociated forms. The glycopeptides used for coupling are also mixtures. The fibrin preparation is probably more uniform, since specific hydrolysis by plasmin preceded the pronase digestion. The rapid hydrolysis of these preparations by trypsin may be due to long peptide arms remaining after pronase digestion or partial denaturation of these preparations. Further studies are underway with more uniform glycopeptide fractions.

Little is known about the mode of clearance of bacterial asparaginase-glutaminase enzymes in animals. The t½ of these enzymes is prolonged by lactate dehydrogenase-elevating virus infection in mice (33). Blockage of the reticuloendothelial system by zymosan does not affect the t½ in dogs and guinea pigs (34). Our results indicate that both glycosylation and lowering the pI markedly increase the t½. One effect may be due to inhibition of trypsin-like digestion by modification of surface lysine residues. The additional effect by glycosylation may be due to recognition of the sugar residues by specific receptors. We are currently studying the effect of species differences in the glycopeptide and modification of the sugar sequence on the t½.

Effective enzyme therapy of susceptible tumors requires prolonged circulating levels of glutaminase-asparaginase enzymes. Chemical modification of these enzymes can markedly prolong the plasma half-life. Hopefully, other modifications can alter the immune response to these proteins and direct them to specific tissue sites.

Acknowledgments—We wish to thank Philip Lu, Beth Laine, Esther Tang, and Jerry Krys for excellent technical help.

REFERENCES

21. Deleted in proof
25. Deleted in proof
Biologic and physical properties of succinylated and glycosylated Acinetobacter glutaminase-asparaginase.
J S Holcenberg, G Schmer and D C Teller


Access the most updated version of this article at http://www.jbc.org/content/250/11/4165

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/11/4165.full.html#ref-list-1