The pore-forming protein of the outer membrane of *Escherichia coli*, porin, was chemically modified with acetic anhydride, succinic anhydride, and glycinamide. Extensive modification of amino groups of the functional porin trimers caused reduced diffusion rates of the negatively charged solutes such as p-nitrophenyl phosphate and AMP, but did not reduce significantly the diffusion of positively charged molecules carboxyl groups of trimers caused decreased diffusion rates of the positively charged solutes more significantly than the diffusion rates of negatively charged solutes. The results suggest that the ionic interactions play an important role for the diffusion of charged solutes through the porin pore. The diffusion of p-nitrophenyl α-D-glucoside, an uncharged solute, was not influenced significantly by modification of either amino or carboxyl groups. This observation suggests that modifications only occurred in areas outside of the narrowest portion of the pore or, alternatively, that amino and carboxyl groups are exclusively located at noncylindrical area of the pore. The structural integrity of the acetylated and the succinylated trimers seemed well preserved. On the other hand, modification of carboxyl groups decreased the thermal stability of trimers and extensive modifications caused the dissociation of trimers into monomers at 37 °C.

The outer membrane of Gram-negative bacteria, such as *Escherichia coli* and *Salmonella*, contain several diffusion mechanisms for solutes (1). These diffusion systems may be classified into three categories; (i) the specific diffusion systems facilitating the diffusion of Fe<sup>2+</sup>-chelator complexes or vitamin B<sub>12</sub>; (ii) the diffusion pores having relatively low solute specificity such as r-receptor and T<sub>4</sub>-receptor proteins for vitamins B<sub>1</sub> and B<sub>12</sub>; (iii) the nonspecific diffusion pores for diffusion of maltodextrins and nucleosides, respectively (2-4); and (iii) the nonspecific diffusion pores for diffusion of small hydrophilic molecules of less than 600 daltons (5, 6).

**MATERIALS AND METHODS**

**Bacterial Strain and Culture Conditions**—The bacterial strain used throughout this study was *E. coli* B, producing a single species of porin (6, 10). Cells were grown in a medium containing 1% Bacto-tryptone, 0.5% yeast-extract, and 0.5% NaCl in either Erlenmeyer flask or in a 100-liter jar fermentor under vigorous aeration.

**Preparation of Porin**—Porin trimers were prepared according to the procedures described earlier (7) except that 10 mM phosphate buffer, pH 7.0, was used throughout instead of 10 mM Tris-HCl buffer, pH 7.5.

**Acetylation of Porin Trimer**—The purified trimers (50.5 nmol) in 1 ml solution containing 45 μM sodium phosphate buffer, pH 6.0 or 7.0, 0.1% SDS, and 3 mM NaN<sub>3</sub> were mixed with a total of 90.5 μmol of [C<sup>14</sup>]acetic anhydride (specific activity 0.169 mCi/μmol) by adding one-sixth volume of the reagent at every 10 min up to 60 min, and were kept at 25 °C for 60 min. The pH of the mixture was maintained to either 6.0 or 7.0. The protein in the mixture was separated from unreacted free reagents by gel filtration on a Sephadex G-25 column (0.9 × 56 cm, Pharmacia, Fine Chemicals), equilibrated with a solution containing 0.1% SDS and 10 mM sodium phosphate buffer, pH 6.0 or 7.0, and the column was eluted with the same solution. The number of amino groups modified was calculated from the radioactivity of the column eluates.

**Succinylation of Porin Trimer**—To purified porin trimers (2 mg) in 1.1 ml of solution containing 45 mM sodium phosphate buffer, pH 8.0, and 0.1% SDS, we added 6.5-μl portions of a solution of [C<sup>14</sup>]succinic anhydride (specific activity 10 μCi/66.8 pmol/0 μl) of dioxane-acetone mixture, prepared freshly from [C<sup>14</sup>]succinic anhydride in dioxane and nonradioactive succinic anhydride in acetone, every 10 min up to 60 min at 35 °C. The pH of the mixture was maintained at 7.5 to 8.0 throughout. The mixture was passed through a Sephadex G-75 column (0.9 × 60 cm) equilibrated with a solution containing 10 mM sodium phosphate buffer, pH 7.0, and 0.1% SDS. Ammonium formate and sodium formate were washed five times with 0.1% Triton X-100 by centrifugation

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1. The abbreviations used are: SDS, sodium dodecyl sulfate; EDAC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; Cbz-Gly-Pro-Arg-nitrilamide, carboxybenzyl-glycyl-prolyl-arginine-p-nitrilamide; tosyl- Pro-Arg-Arg-nitrilamide, tolylsulfonyl-glycyl-prolyl-arginine-p-nitrilamide.
at 80,000 × g for 30 min in order to replace SDS with Triton X-100. This material (6.4 mg of protein) was mixed with 2 μmol glycineamide (pH was maintained at about 5 with NaOH) at a final molar ratio of protein to glycineamide of 1 to 28,600. The mixture was divided into six equal portions and the pH of each portion was adjusted to 4.75, 5.0, 5.3, 5.6, 6.0, or 6.8, with either 1 N HCl or NaOH. The amidation of appropriate pH. The mixture was dialyzed, 10 min later, against a reaction was started by adding dropwise, 100 μl of freshly prepared EDAC (4.9 × 10⁻⁴ mol) under controlled pH. The reaction was stopped after 2 h at 25 °C by adding 1.5 volumes of 1 M acetic buffer of appropriate pH. The mixture was dialyzed, 10 min later, against a large excess of distilled water at 4 °C for 36 h and the contents of the dialysis bag were collected by centrifugation at 80,000 × g for 60 min, suspended in the solution containing 1% SDS, 0.4 mM EDTA, 0.0510 mercaptoethanol, 10 mM sodium phosphate buffer, pH 7.5, and 3 mM NaCl. The centrifuged supernatant (80,000 × g for 60 min) contained the modified porin trimers (7). The number of carboxyl groups modified was calculated from the increment of glycine residues as determined by amino acid analysis after hydrolysis.

**Thermal Stability of Porin Trimer**—Porin trimers (50 μg) in 50 μl of 0.8 mM sodium phosphate buffer, pH 8.0, containing 2% SDS were placed in an Eppendorf Microtube (capacity 1.5 ml) (Eppendorf Geratebau), heated in a water bath at desired temperature for 5 min, and chilled immediately in crushed ice. A portion of the sample (4 μg of protein) was subjected to acrylamide gel electrophoresis in SDS and the stained protein bands were traced by Shimadzu TLC-Scanner LS-900 (Shimadzu Seisakusho Ltd.).

**Preparation of Anti-porin Antibody and Immunodiffusion Test**—Liposomes containing purified porin trimers were made as reported earlier (13), from 30 μmol of phospholipids extracted from E. coli B and 3 mg of purified porin trimers, except that lipopolysaccharide was omitted and 0.1 M NaCl was used as suspending medium. Rabbits (Albino J. W. female, about 2.5 kg) were injected subcutaneously with liposomes containing 500 μg of porin, and 10 days later, they were boosted with liposomes containing 1 mg of porin. Twenty days after the first immunization, the rabbits were bled and serum was obtained. Serum was fractionated with 45% saturation of (NH₄)₂SO₄, and the precipitates were dissolved in a small amount of 0.1 M NaCl containing 10 mM sodium phosphate buffer, pH 8.0. The precipitates were dialyzed against a large excess of the same buffer. A portion of the dialyzed material (410 mg of protein) was subjected to gel filtration by a Sepharose 4B column (2.5 × 90 cm) equilibrated with the above buffer and the column was eluted with the same buffer. The eluates corresponding to IgG fraction were pooled, concentrated by dialysis against dry Ficoll 400, and dialyzed against a large excess of 50 mM sodium phosphate buffer, pH 8.0, containing 0.15 M NaCl at 4 °C overnight.

**Double-diffusion immunoprecipitation test** was carried out in an agar plate (1.0 mm thickness) containing 0.15 M NaCl, 0.5% SDS, and 3 mM NaCl at room temperature for 24 h.

**Other Methods**—Circular dichroism spectra were recorded with a JASCO J-20 automatic recording spectropolarimeter as reported (7). The mean residue molecular weight of porin was calculated from the amino acid composition (9). Amino acids were analyzed as reported earlier (9) except that 6 N HCl instead of 4 N methanesulfonic acid was used for hydrolysis. Number of O-acetyltyrosine residue of acetylated porin was quantified according to the method described by Simpson et al. (14). Phospholipids were extracted from E. coli B by the method of Bligh and Dyer (15), and were quantified by the method of Marinetti (16). Protein was quantified either by the method of Lowry et al. (17), or by absorption at 278 nm. Absorption coefficient (A₂₇₈)₅₀ of native, acetylated, succinylated, and amidated porin trimers were 1.69, 1.69, 1.67, and 1.84, respectively. Procedure for acrylamide gel electrophoresis in SDS was described earlier (18).

**Chemicals**—Reagents and enzymes were obtained from the following sources. [1-¹⁴C]Acetic anhydride (Amersham), [1,4-¹⁴C]Sucinic anhydride (NEN), acrylamide and SDS (B.D.H), egg yolk phosphatidylycerol and Cbz-Gly-Pro-Arg-nitranilide (Sigma), EDAC (Nakarai) bacterial alkaline phosphatase, and trypsin (Worthington) α-glucosidase and tosyl-Gly-Pro-Arg-nitranilide (Boehringer), dioxane (water content 0.01%, Merck). Other chemicals used were of the highest grade commercially available.

**RESULTS**

**Modification of Amino Group of Porin Trimer**—We have used acetic anhydride and succinic anhydride for modification of amino groups because of the following reasons: (i) since the reagents are relatively small, we hoped that they can modify the amino groups located deep within the pores; (ii) acetic anhydride converts positively charged amino groups into electrically neutral groups and should confer hydrophobicity to the pore wall. (iii) Succinic anhydride replaces positively charged amino groups with negatively charged carboxyl groups. When porin trimers were treated with acetic anhydride at pH 6.0 or 7.0, 15 or 27 amino groups/trimer, respectively, were acetylated. Similarly 40 residues were modified by succinic anhydride at pH 8.0. It is unlikely that these reagents modified the amino groups embedded in a lipophilic environment, since less than one amino group of the trimer (total 57 residues) incorporated [¹⁴C]phenylisothiocyanate, a lipophilic reagent that only attacks unprotonated amino groups, as examined using isolated outer membrane at pH 7.0, at 37 °C for 2 h.² Porin trimers treated with acetic anhydride or succinic anhydride at appropriate conditions appeared as single protein bands migrating slightly above (relative mobil-

² M. Tokunaga and T. Nakae, unpublished result.

![Figure 1. Electrophoretic mobilities of the modified porin trimers.](image-url)
Diffusion of negatively charged and neutral solutes through the vesicle membranes reconstituted from modified porin trimers

Membrane vesicles were reconstituted from 6 μmol of egg yolk phosphatidylcholine and 5 nmol of porins as reported earlier (12). Membrane vesicles for assaying the diffusion rate of p-nitrophenol phosphate, AMP, and NADPH were prepared in 200 μl of the buffer solution containing 9.8 units of alkaline phosphatase, 0.1 M NaCl, 10 mM Tris-HCl buffer, pH 8.0. Those for the assay of p-nitrophenyl α-glucosidase diffusion was made in 200 μl of the solution containing 10 units of α-glucosidase, 0.1 M NaCl, 5 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid/NaOH buffer, pH 7.0. Subsequent treatment of membrane vesicles, substrate concentrations used, and the assay procedure were similar to that described in earlier publication (see legend to Table II of Ref. 12). The diffusion rate of p-nitrophenol α-glucosidase was monitored at 405 nm at the substrate concentration of 5 mM at pH 7.0 and at 30°C. The permeability efficiency was computed from the enzyme activity (nanomoles substrate hydrolysis per min per μmol of phosphatidylcholine) of the vesicle and of the detergent-solubilized vesicles as described (12).

| Porins                  | No. residues modified | p-Nitrophenyl phosphate | AMP | NADPH | p-Nitrophenyl α-α-glucopyranoside |
|-------------------------|-----------------------|-------------------------|-----|-------|----------------------------------|
| Native porin trimer     |                       | 0.42 ± 0.014 (100)       | 0.54 ± 0.023 (100) | 0.05 ± 0.001 (100) | 0.69 ± 0.040 (100) |
| Acetylated porin trimer |                       | 0.42 ± 0.017            | 0.63 ± 0.018       | 0.06 ± 0.001       | 0.64 ± 0.013 (93)  |
| Succinylated porin trimer |                     | 0.26 ± 0.030 (62)       | 0.21 ± 0.007 (39)  | 0.01 ± 0.003 (20)  | 0.59 ± 0.028 (86)  |
| Amidated porin trimer   |                       | 0.10 ± 0.000 (24)       | 0.09 ± 0.002 (17)  | 0.02 ± 0.004 (40)  | 0.60 ± 0.010 (87)  |

* The total number of amino and carboxyl groups per trimer was 57 and 147, respectively.
* The permeability efficiency (PA) values presented in the table were the average ±S.D. of at least three independent assays.
* The fluctuation of relative diffusion rate (%) of NADPH was probably due to lower permeability efficiency values for this solute.

### Table II

Diffusion of positively charged solutes through the vesicle membranes reconstituted from modified porin trimers

Membrane vesicles were prepared from 6 μmol of egg yolk phosphatidylcholine and 5 nmol of porins. Vesicles were suspended in 200 μl of a solution containing 50 μM Tris-HCl, pH 8.0, 50 mM NaCl, and 2 mg of trypsin and were passed through a Sepharose CL-6B column as reported earlier (12). Solute diffusion was monitored at 405 nm at the substrate concentration of 5 × 10⁻⁴ M at pH 8.0 at 25°C.

| Porins                  | No. residues modified | Permeability efficiency (PA) |
|-------------------------|-----------------------|------------------------------|
| Chz-Gly-Pro-Ar-alienide | 0.39 ± 0.021 (130)    | 10⁻³ × PA (cm²/min) (% native trimer) |
| Tosyl-Gly-Pro-Ar-nitranilide | 0.53 ± 0.012 (100)     |
| Acetylated porin trimer at pH 7.0 | 0.46 ± 0.007 (117) | 0.32 ± 0.008 (60) |
| Succinylated porin trimer | 0.70 ± 0.031 (179)   | 0.72 ± 0.005 (135) |
| Amidated porin trimer at pH 5.6 | 0.72 ± 0.045 (69) | 0.25 ± 0.011 (47) |

* The trypsin resistant property of trimers was unchanged even after modifications as determined by gel electrophoresis in SDS after trypsin treatment (not shown).

### Table III

Diffusion rates of positively charged solutes through the vesicle membranes reconstituted from modified porin trimers

The fluctuation of relative diffusion rate (%) of NADPH was probably due to lower permeability efficiency values for this solute.

The modified trimers have altered permeability properties for certain solutes. The diffusion rate of p-nitrophenyl phosphate and AMP through the vesicle membranes reconstituted from acetylated porin trimers (15 modified residues), fell to about 62%, and 39%, respectively, of the diffusion rates of these compounds through the membrane containing the native trimers. As the extent of acetylation reached to 27 residues out of 57 available amino groups, the diffusion rates of p-nitrophenyl phosphate and AMP through the membranes containing these porins dropped to 50% and 22%, respectively, of the control (Table I). The succinylation reaction yielded the trimers, of which 41 amino groups were modified, that allowed the diffusion of p-nitrophenyl phosphate and AMP at only 24% and 17%, respectively, of the rate obtained with native trimers. Conversely, the diffusion rates of positively charged compounds such as Chz-Gly-Pro-Ar-alienide and Tosyl-Gly-Pro-Ar-nitranilide through the membrane containing acetylated or succinylated trimers appeared to be 17% and 179% or about 60 and 135%, respectively, of the rates through the native trimers (Table II).
the modified trimers in SDS. Carboxyl groups were modified as described in the text and a portion of salt-liberated porins were subjected to acrylamide gel electrophoresis in SDS at room temperature without heat treatment. The Coomassie brilliant blue-stained gels were traced with Shimadzu TLC scanner. Number of carboxyl groups modified was determined by amino acid analysis after hydrolysis.

Modification of Carboxyl Group of Porin Trimer—The method employed first activates carboxyl groups with EDAC and glycinamide in the presence of 0.1% SDS or 0.1% Triton X-100 at pH 4.75 resulted in an extensive dissociation of trimers into monomers and since the same treatment in 0.1% Triton X-100 at pH 5.6 produced aggregates of the modified trimers, we used the peptidoglycan-associated porin trimers, rather than free trimers, for modification in the presence of 0.1% Triton X-100. (The trimers are stable under these conditions down to pH 2 at 25 °C). When the reaction was carried out at pH 4.75, 5.0, 5.3, 5.6, 6.0, or 6.8, 78, 75, 69, 60, 54, or 27 carboxyl groups, respectively, per trimer were found to be modified. Extensive modification of carboxyl group resulted in the dissociation of a significant fraction of trimers into monomers (Fig. 2), as determined by slab gel electrophoresis in SDS. Porin trimers, modified in up to 60 carboxyl groups, appeared as a sharp single protein band moving slightly more slowly (relative mobility to dye front was 0.18) than native trimers (Fig. 1). The modification reaction carried out without EDAC yielded no detectable reaction product, and the reaction without glycinamide resulted in extensive cross-linking of the trimers and the formation of amorphous aggregates.

Vesicle membranes containing the modified trimers (27 carboxyl groups were modified per trimer) allowed the diffusion of $p$-nitrophenyl phosphate, AMP, and $p$-nitrophenyl $\alpha$-glucoside as rapidly as native trimers did (Table I). The vesicles containing the trimers, in each of which 60 carboxyl groups on average were modified, showed only 31, 28, and 13% decrease in their permeability to $p$-nitrophenyl phosphate, AMP, and $p$-nitrophenyl $\alpha$-glucoside, respectively, in comparison with those containing the native trimers. On the other hand, the diffusion rate of NADPH, a slowly diffusing substrate, through the membrane containing the modified porins increased about 2- to 2.5-fold. The diffusion rates of Cbz- and tosyl-Gly-Pro-Arg-nitranilide through the membrane containing amidated trimers appeared to be 69 and 47% of those in vesicles containing native trimers (Table II). These results suggested several possible interpretations (see "Discussion"). Porin trimers modified at pH 4.75 conferred little permeability to the vesicles. This result is explained by the fact that trimers are dissociated almost completely into monomers (Table I and Fig. 2).

Structural Integrity of the Chemically Modified Porin Trimer—Fig. 3 depicts the CD spectra of acetylated, succinylated, and amidated porin trimers as well as that of native trimers. The CD spectra of the modified porins showed profiles fairly close to those of native trimers, suggesting $\beta$-rich conformations (Fig. 3). The molar ellipticity at 217 nm of the acetylated, succinylated, amidated, and the native trimers were calculated to be $-7,800$, $-7,600$, $-8,350$, and $-8,180 \deg cm^2 dmol^{-1}$, respectively. Since we often observed that the extensive modification of carboxyl groups tend to disaggregate trimers into monomers in a solution containing 1% SDS, 0.4 M NaCl, 10 mM sodium phosphate buffer (pH 7.5), and $3 \times$ concentrated NaCl.

Fig. 2. Modification of carboxyl groups and the stability of the modified trimers in SDS. Carboxyl groups were modified as described in the text and a portion of salt-liberated porins were subjected to acrylamide gel electrophoresis in SDS at room temperature without heat treatment. The Coomassie brilliant blue-stained gels were traced with Shimadzu TLC scanner. Number of carboxyl groups modified was determined by amino acid analysis after hydrolysis.

Fig. 3. Circular dichroic spectra of the modified trimers. The spectra were recorded using a cell of 0.02-cm light path at scanning speed 2 nm/min at room temperature and the results were expressed in terms of molar ellipticity. Porins were dissolved in 50 mM phosphate buffer, pH 8.0, containing 0.1% SDS, except that amidated porins were dissolved in a solution containing 1% SDS, 0.4 M NaCl, 5 mM EDTA, 0.08% mercaptoethanol, 10 mM sodium phosphate buffer, pH 7.5, and 3 mM NaCl.

Fig. 4. Thermal stability of modified porin trimers. Native and chemically modified trimers (same samples used for the experiment in Fig. 1) were treated as described in the text. $O$—$O$, the native trimers; $\triangle$—$\triangle$, the acetylated trimers; $\bullet$—$\bullet$, amidated trimers.
phosphate buffer, pH 7.0, we wondered if the structural integrity of the modified trimers was preserved, although CD spectra showed little difference. When the amidated trimers (60 carboxyl groups per trimer were modified) were heated at various temperatures in the presence of SDS, they began to dissociate at 40 °C, and a temperature causing 50% dissociation under our conditions appeared to be about 48 °C. In contrast, the native, acetylated, and succinylated trimers began to dissociate at about 62 °C and the complete dissociation was attained at 70 °C. The temperature causing 50% dissociation of the native and amino group-modified porins were indistinguishable from each other, about 67 °C (Fig. 4). These results clearly indicated that the elevated thermal sensitivity of the modified trimers can be attributed to the introduction of glycaminide onto the carboxyl groups of trimer.

Immunodiffusion Test—Antibody raised against native porin trimers was used for agar gel precipitation with modified and native porins. As shown on Fig. 5, anti-trimer antibody formed precipitation lines with the native and the modified trimers but not with the heat-dissociated monomers. Although immunoprecipitation lines of the acetylated and succinylated trimers fused completely, the lines between the native trimers and either the acetylated or succinylated trimers produced a fused line and a spur, suggesting that modification of amino groups blocks at least one immunodeterminant present in the surface of the native trimers (Fig. 5). Similarly, the precipitation lines between native and the amidated trimers produced one fused line and a spur, and that between carboxyl and amino group-modified trimers produced one fused line and one spur over each well (data not shown). The results suggested that the modifications altered a part of trimer surface, but the modified trimer unlike the monomer, still retained a gross conformation close to native trimer.

**DISCUSSION**

The permeability of small hydrophilic molecules through the outer membrane of Gram-negative bacteria is a passive diffusion event through nonspecific diffusion pores made of porins (5, 6, 13). The amino acid analysis of porins from E. coli (10) and Salmonella (9) showed that the proteins are rich in polar amino acids in spite of the fact that they are intrinsic membrane proteins. This apparent inconsistency is probably due to the fact that porins need relatively large numbers of polar amino acid residues in order to construct the water-filled pores (9). Recently, it was shown that the smallest functional unit of porin is a trimeric aggregate of homologous porin subunits (7, 8, 19, 20). It was therefore of interest to modify the amino and carboxyl groups of the functional porin trimers and to see the effect of modifications on permeability properties of the pores.

As regards the effects of chemical modifications on the permeability of solutes, we found essentially that the extensive modification of amino groups caused lowered diffusion rates of the negatively charged compounds, and the modification of carboxyl groups resulted in reduced diffusion of positively charged solutes. The modification of amino or carboxyl group had little influence on the diffusion of uncharged molecule. We favor the following interpretation of these results. Since acetylation and succinylation of the trimer makes the net charge of the pore interior and/or the rim more negative, the decreased diffusion rate of the negatively charged solutes are most likely due to electrostatic repulsion between the solutes and the pore wall or rim. The reduced diffusion rates of positively charged compounds through the pore of amidated trimers offered an additional evidence to support the role of electrostatic forces for diffusion of charged molecules. That the reduced diffusion rates of charged solutes were solely due to the physical blockades formed at the interior or the rim of the pores appear unlikely, since the diffusion rates of p-nitrophenyl α-glucoside, a relatively large and uncharged molecule were less altered. However, the result do not exclude a possibility that the modifications only occurred at the rim but not the interior of the pore wall, or that the narrowest portion of pore wall contained little amino or carboxyl groups. The observation that the amidated trimers allowed the less altered or even faster diffusion of p-nitrophenyl phosphate, AMP, and NADPH and that the acetylated and succinylated trimers allowed accelerated diffusion of positively charged compounds may be explained similarly by the ionic interactions between pore wall or rim and the solutes. The possibility that the decreased diffusion rate of certain solutes through the membranes containing modified porins are simply due to an inefficient incorporation of modified porin trimers into liposome membranes is unlikely, since the diffusion of p-nitrophenyl α-glucoside through these membranes was not reduced.

Modification of carboxyl groups tended to destabilize the integrity of the trimers as shown in Figs. 2 and 4. Several explanations are possible. (i) Negative charges of carboxyl groups form cross-bridges with cationic groups of the neighboring subunit via ionic interactions. (ii) Carboxyl groups are clustered at the periphery of the trimer and they shield the interior from the attack of an ionic surfactants such as SDS. (iii) Neutralization of the negatively charged groups by amidation strengthen electrostatic repulsions between positively charged groups causing distortion of the trimer structure. (iv) Introduction of glycaminide exerts mechanical distortions on the quaternary structure of the trimers. Possibly it appears unlikely, since the trimers are quite stable in a solution containing SDS and moderate concentration of NaCl (7). We are not happy with explanation ii, since the treatment of the trimers with a cationic surfactant, cetyltrimethylammonium bromide, did not cause a significant dissociation of the trimers, and the trimers modified with taurine, 2-aminoethanesulfonic acid, appeared to be unstable in SDS.7 We have no concrete evidence to support or to reject explanations iii and iv. Since the trimers dissociate into monomers along with the increased amidation of carboxyl groups (Fig. 2) but not by the extensive modification of amino groups, it seems reasonable to assume that certain numbers of carboxyl groups play a crucial role in maintaining the quaternary structure of the trimer.
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