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CHARACTERIZATION OF HIGHLY PURIFIED NATIVE STREPTOKINASE AND ALTERED STREPTOKINASE AFTER ALKALINE TREATMENT

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Summary

Physical and chemical data are reported for highly purified native streptokinase (staphylokinase, EC 3.4.99.22) (Kabikinase) and streptokinase treated with an alkaline agent (altered streptokinase). The mol. wts. were similar and were determined to be 50 200 by sedimentation equilibrium methods, polyacrylamide gradient gel electrophoresis and sodium dodecylsulphate-polyacrylamide gel electrophoresis. The sedimentation coefficient $s^0_{20,w}$ of native and altered streptokinase was found to be 3.37 S. The frictional ratio and the absorptivity ($A^{1\%}_{1cm}$) at 280 nm of native streptokinase was found to be 1.29 and 7.5, respectively. Native streptokinase showed essentially a single band in the isoelectrofocusing pattern (p*I* 5.2), while altered streptokinase showed at least two separate bands. Polyacrylamide gel electrophoresis in the presence of Triton X-100 exhibited one band for native streptokinase but altered streptokinase showed two bands. At pH 12 the biological and immunological activity of streptokinase was markedly decreased in a time-dependent reaction. The amino-terminal amino acid of the two streptokinase forms was isoleucine and the carboxyl-terminal amino acid of native streptokinase was tyrosine. Peptide analysis showed that some peptides in altered streptokinase exhibited higher mobility compared to native streptokinase. The data suggest that streptokinase undergoes a conformational change when incubated in alkaline media, but no simultaneous loss of peptides was observed.

Introduction

The bacterial protein streptokinase (EC 3.4.99.22) is not a proteolytic enzyme since it has no titrable active site [1]. The protein is specific for plasminogen activation, however [2,3—8]. Streptokinase has long been known

to cause lysis of human fibrin clots and the objective of thrombolytic therapy is to digest a fibrin clot without inducing a generalized proteolytic state. Streptokinase has been deemed to be capable of achieving this effect with a loading dose followed by maintenance doses of sufficient magnitude.

Commercial samples of streptokinase have been further purified [1,2,4] by various procedures primarily employing ammonium sulphate precipitation, ion-exchange chromatography and gel filtration. Such protein material has been used for chemical and biological characterization. Streptokinase is found to be a single-chain molecule with a molecular weight of about 47 000 [1,2,9]. Half-cysteine residues have not been identified [10]. Lysine [9] and isoleucine [2] are considered to be in the C- and N-terminal positions, respectively. The isoelectric points reported ranged between pH 4.7 and 5.2 [1,2,4,11].

The purpose of the present study was to characterize highly purified streptokinase (Kabikinase) and to determine some physicochemical properties of this substance. We give here a summary of these data and, in addition, we have made a study of the effect of alkaline treatment of streptokinase with respect to some physicochemical properties, electrophoretic properties and biological/immunological activities.

Materials and Methods

Reagents. All chemical reagents used were of reagent grade or better. Sodium dodecylsulphate (SDS) was obtained from BDH Chemicals Ltd., Poole, U.K. and Triton X-100 from Sigma Chemical Co., St. Louis, U.S.A. Sephadex and Sepharose products were bought from Pharmacia Fine Chemicals, Uppsala, Sweden. DEAE-cellulose Whatman DE 32 was purchased from Whatman Biochemicals Ltd., U.K. The agarose used for the electroimmunoassay of streptokinase was bought from Litex, Glostrup, Denmark.

2-Hydroxy-nitro-benzyl bromide was obtained from EGA-Chemie, F.R.G. *N,N,N',N'*-tetramethylethylenediamine, 2-mercaptoethanol, acrylamide, *N,N'*-methylenebisacrylamide and 3-dimethyl-aminopropionitrile were products of Eastman Organic Chemicals. Polyacrylamide gradient gels PAA 4/30 were purchased from Pharmacia Fine Chemicals. Polyacrylamide gels for isoelectric focusing, Ampholine polyacrylamide gels plates, with a pH gradient of 3.5—9.5 were purchased from LKB Aminkemi, Stockholm, Sweden.

Human serum albumin was obtained from AB Kabi. Antisera to streptokinase were raised by hyperimmunization of rabbits with several subcutaneous injections of native streptokinase together with Freund's complete adjuvant.

Purification of streptokinase. Streptokinase (Kabikinase) is isolated from a β-haemolytic streptococcus, Lancefield group C, strain H 64. Partially purified streptokinase produced by the standard procedure for the manufacture of Kabikinase (Swedish patent 210473, U.S. patent 2098015) was taken as a source of protein for the preparation of the highly purified native streptokinase used in the investigation. $(NH_4)_2SO_4$-fractionated material was gel filtered at 4°C on Sephadex G-150 or Sephadex G-100 (K 50/100, flow rate 85 ml/h) with 0.02 M sodium phosphate (pH 7.4). Fractions of about 8 ml were collected and the absorbance at 280 nm was measured. The fractions of the middle protein

peak were pooled and used in the final purification step on DEAE-cellulose (Whatman DE 32) or DEAE-Sepharose CL-6B. This chromatographic step is a minor modification of the final ion-exchange chromatography step used for the preparation of Kabikinase. The specific activity of the highly purified streptokinase was 650—750 I.U./μg of protein nitrogen [12].

Total amino acid analysis. Samples of streptokinase were hydrolysed, using 6 N hydrochloric acid, in vacuo at 110°C for 24 and 72 h. The amino acid compositions were determined on a Durrum D-500 automatic amino acid analyser [13]. The tryptophan content of streptokinase was determined spectrophotometrically using 2-hydroxy-5-nitrobenzyl bromide, following the procedure of Karkhanis et al. [14].

Peptide analysis. Highly purified native and alkali-treated streptokinase was incubated (pH 7.4) with trypsin for 2 h at 40°C. By addition of diluted hydrochloric acid the reaction was stopped. The obtained peptide mixture was investigated [15] by thin-layer chromatography (cellulose), by paper chromatography and by paper electrophoresis. Detection was performed by the use of a ninhydrin reagent.

Thin-layer chromatography was run for 40 min on Merck Fertigplatten (Cellulos F) in two different systems: butanol/ethylacetate/acetic acid/water (1/1/1/1) and butanol/pyridine/water/acetic acid (75/50/60/15). Paper chromatography was run overnight in butanol/acetic acid/water (4/1/5) and paper electrophoresis for 2 h at 1500 V in pyridine/acetic acid/water (1/1/48), pH 4.7.

C-terminal and N-terminal amino acid analysis. The phenylisothiocyanate degradation method of Edman [15] was employed manually for N-terminal determinations. The phenylthiohydantoins were identified by thin-layer chromatography on silica gel containing an ultraviolet fluorescent indicator [16]. For the C-terminal determination, carboxypeptidase A and carboxypeptidase Y were used to digest streptokinase essentially by the method of Hugli [17]. After preset intervals of time up to 20 h, aliquots of the digested sample were run on a Durrum D-500 automatic amino acid analyser.

Protein determinations. The streptokinase concentration was determined by the method of Lowry et al. [18], using human serum albumin as a standard, or spectrophotometrically at 280 nm utilizing the absorptivity value ($A^{1\%}_{1cm}$) of 7.5 determined in this investigation.

The absorption coefficient of highly purified streptokinase was calculated from absorbance measurements at 280 nm (1 cm cell) in 0.02 M phosphate buffer, pH 7.2. The protein content of the solutions was determined by total amino acid analysis, Lowry protein determination, micro-Kjeldahl and elementary analysis.

The biological assay of streptokinase was performed mainly by the clot lysis assay method described in the European Pharmacopoeia [19]. Fibrin clots are rapidly formed in the presence of fixed levels of streptokinase and plasminogen and the time required for clot dissolution is measured automatically by mechanical means.

Electrophoretical techniques. Quantitative immunological streptokinase determinations were performed by electrophoresis on agarose gel containing rabbit antiserum by the method of Laurell [20]. A highly purified lyophilized

streptokinase sample stabilized with human serum albumin was used as a standard. The potency of the internal standard was obtained by comparison with the international streptokinase standard (WHO).

All gel electrophoresis procedures were carried out in the GE-4 gel electrophoresis apparatus manufactured by Pharmacia Fine Chemicals. The runs were done with 0.09 M Tris-HCl/0.08 M boric acid/0.003 M sodium EDTA (pH 8.35) for 4 h. Proteins in the gels were stained with Amido Black 10 B for 30 min in 7% acetic acid, followed by electrophoretic destaining in the same solvent. Densitometry of the stained gels was carried out at 610 nm with an intergrating densitometer (DCD-16, Gelman Instrument Co.).

A mixture of protein markers was electrophoresed in the gels when molecular weights were determined. The markers included ribonuclease A (13 500), light chain immunoglobulin G (26 000), ovalbumin (43 000), heavy chain immunoglobulin G (54 000), and human serum albumin (66 000).

The polyacrylamide gradient gel electrophoresis was performed mainly as described by Andersson et al. [21]. The applied streptokinase sample volume was 15 μl (100 000 I.U./ml).

The sodium dodecylsulphate(SDS)-polyacrylamide gel electrophoretic system of Weber and Osborn [22] and Fairbanks et al. [23] was employed in the main. The runs were carried out on 7.5% polyacrylamide gel plates in the buffer described above with the addition of 0.2% SDS. The applied streptokinase sample volume was 25 μl (60 000 I.U./ml) and was stabilized with sucrose prior to application.

Analytical polyacrylamide gel electrophoresis was carried out in the presence of the non-ionic detergent Triton X-100 according to the method described by Singh and Wassermann [24]. The runs were carried out on 7.5% polyacrylamide gels containing 0.2% Triton X-100 in the buffer system described above. An aliquot of 15 μl of streptokinase (80 000 I.U./ml), stabilized with sucrose, was applied.

For isoelectric focusing the procedure of Vesterberg and Svensson [25] was employed with some modifications. The isoelectric focusing run was carried out at 8°C with a potential difference of 400 V for 3 h on an instrument obtained from AB Analysteknik, Vallentuna, Sweden. The runs were performed on Ampholine polyacrylamide gel plates with a pH range of 3.5–9.5. The applied sample volume was 25 μl (80 000 I.U./ml).

In the carrier-free electrophoresis method [26] convection is minimized by rotation of the horizontal electrophoresis tube about its axis. 50 μl of an alkali-treated (see below) streptokinase sample with a protein concentration of 3.2 mg/ml were introduced with the aid of a microsyringe. The buffer used was 0.1 M phosphate (pH 7.4) at 900 V and 10 mA. The temperature of the cooling water was 16°C. The tube was scanned for absorbing substances at 15-min intervals and the final scan was made after 3 h.

Ultracentrifugal analysis. Ultracentrifugation was carried out at 20°C in a Spinco Model E Analytical Ultracentrifuge equipped with an RTIC unit, electronic speed control, and Schlieren, interference and ultraviolet optics.

Sedimentation runs were performed in 12-mm double-sector cells at 48 000 and 52 000 rev./min. The moving boundary was studied using Schlieren and ultraviolet optics. The protein concentrations were 2.3 and 3.2 mg/ml.

Sedimentation coefficients were calculated by standard procedures; the values were corrected for viscosity and density of the medium [27].

The long column meniscus-depletion method of Chervenka [28] was employed for sedimentation equilibrium runs. The runs were made in 12-mm double-sector synthetic capillary boundary cells. The purified native streptokinase (1.6 mg/ml) was analysed at 22 000 rev./min and streptokinase after alkaline treatment (0.64 mg/ml) at 28 000 rev./min. Equilibrium was attained after 18 h. The interference patterns were analysed in a microcomparator (Nikon Model 6CT2). Molecular weights were calculated from plots of log fringe displacement versus r^2 (r = distance from the centre of rotation) using the least-square method and a test computer. The partial specific volume ($\overline{V}$) was determined to be 0.73 ml/g from the amino acid composition of the purified streptokinase preparation [27].

Alkaline treatment of streptokinase, various incubation times. Two times 5.0 ml homogeneous sample of native streptokinase (1.6 mg/ml) were desalted at 4°C on a column of Sephadex G-25 medium (5 × 26.5 cm) equilibrated in distilled water. To a series of test tubes was added 1.0 ml of the desalted protein solution, which was then brought to pH 12 by the dissolution of trisodium phosphate crystals to a concentration of 0.06 M. The tubes were incubated at different times (0, 2, 5, 10, 15, 30, 60, 120 min) at 4°C. The reaction was stopped by adding about 10 mg of crystalline boric acid to the respective protein solutions and a pH of about 8 was reached.

Alkaline treatment of streptokinase, variation of pH. A series of small columns of Sephadex G-25 medium (PD-10) were equilibrated at 4°C in 0.06 M sodium phosphate buffers with different pH values (8.5, 9.5, 10.0, 10.5, 11.0, and 12.0). To each column was added 1.0 ml of a homogeneous sample of highly purified streptokinase (1.6 mg/ml) in order to attain the desired pH. The protein was eluted in the respective solutions and the eluate was collected according to the manufacturer's instructions. After 1 h incubation at 4°C the reaction was stopped by adding about 10 mg crystalline boric acid/ml eluate. In some experiments the neutralization with boric acid was performed after 5 h of incubation.

Results

Highly purified native streptokinase

Electrophoretic characterization. Various electrophoretic methods were employed to characterize the highly purified streptokinase preparations. Analytical isoelectric focusing showed one band located at a pH of 5.2 (Fig. 3, 1), which is in agreement with the value obtained by Brockway and Castellino [2]. Furthermore, only a single component could be demonstrated by polyacrylamide gradient gel electrophoresis, SDS-polyacrylamide gel electrophoresis and Triton X-100-polyacrylamide gel electrophoresis. All these data indicate that the highly purified streptokinase is quite homogeneous, consisting of only a single protein component.

Amino acid composition. The amino acid composition was determined for a highly purified streptokinase preparation, and the results are summarized in Table I. The values of the 24 and 72 h hydrolysis showed only slight differ-

TABLE I

AMINO ACID COMPOSITION OF HIGHLY PURIFIED STREPTOKINASE

The content in streptokinase is based on a molecular weight of 50 200 for streptokinase.

Amino acid	Content in streptokinase (mol/mol)
Aspartic acid	71
Glutamic acid	48
Serine	26
Threonine	32
Proline	23
Glycine	21
Alanine	23
Half-cystine	0
Valine	23
Methionine	4
Isoleucine	23
Leucine	42
Tyrosine	23
Phenylalanine	15
Histidine	9
Lysine	35
Arginine	21
Tryptophan *	1

* Determined spectrophotometrically.

ences, and the data in Table I are the averages given as molar ratios of the amino acids. All common amino acids with the exception of cysteine and cystine were found to be present. The proportion of glutamic acid and aspartic acid is high. One tryptophan residue was found by a separate spectrophotometric analysis.

N-terminal and C-terminal amino acid determination. The amino-terminal amino acid sequence of native streptokinase was found to be Ile-Ala-Gly-, which agrees with the data presented previously [2,9]. Carboxyl-terminal end-group analysis by carboxypeptidase Y and carboxypeptidase A digestions of native streptokinase resulted in a C-terminal amino acid sequence of -Ser-Ala-Leu-Tyr. The demonstration of tyrosine as the C-terminal amino acid of native streptokinase contradicts the findings of Brockway and Castellino [2] and Morgan and Henschen [9], who found lysine to be the C-terminal amino acid.

Physical properties. The molecular weight of the highly purified native streptokinase was found to be 50 200 by ultracentrifugation using a value of 0.73 ml/g for the partial specific volume. Further, the molecular weight found by SDS-polyacrylamide gel electrophoresis was 50 250 ± 1250 and by gradient polyacrylamide gel electrophoresis about 50 000. The sedimentation coefficient, $s^0_{20,w}$, was found to be 3.37 S. The diffusion constant, $D^0_{20,w}$, was calculated to $6.87 \cdot 10^{-7}$ cm^2/s and the frictional ratio f/f_0 to 1.29. Stokes' radius was determined to 3.11 nm based on a molecular weight of 50 200 for the protein. The hydrodynamic properties indicate that highly purified streptokinase has a fairly symmetrical shape with a low degree of hydration.

The absorptivity ($A^{1\%}_{1cm}$) at 280 nm for our streptokinase preparation was determined to be 7.5. This value is an average of several separate determina-

tions. The protein concentration of the streptokinase solutions was established by various protein determination methods (Materials and Methods) in order to minimize methodological errors.

Alkali-treated highly purified streptokinase

Rate of inactivation by alkaline treatment and effects of different pH values. When highly purified streptokinase was treated with 0.06 M trisodium phosphate (pH 12) at various intervals of time, its biological and immunological activity was markedly reduced. The biological and immunological streptokinase activities decrease approximately in parallel, and already after 2 min of incubation a marked decrease is observed. Both types of streptokinase activity have reached about 80% of the original activity after 15 min of incubation. Longer incubation times resulted in a further loss of activity, although not as rapid as in the beginning. On treating streptokinase with a less alkaline buffer, this dramatic decrease was not observed. On incubation at pH 11.0 for 1 h a slight loss of biological and immunological activity was also found.

When streptokinase was incubated in 0.06 M sodium phosphate buffers of different alkalinity for 1 h the biological activity was markedly affected. At pH about 10 the biological activity appears to reach a maximum, approximately 15% higher compared to the activity of the starting material. In contrast, at pH 12 there was a considerable decrease in the biological activity, approximately 25% compared to the original activity.

Electrophoretic characterization. Polyacrylamide gel electrophoresis in Triton X-100 demonstrates (Fig. 1) that highly purified native streptokinase exhibits a single band while streptokinase treated with alkali at pH 10.5 or higher for 1 h shows two well-separated protein bands. The new component formed at pH 10.5, with higher electrophoretic mobility, was estimated after staining to constitute about 10% of the total amount of the protein. Alkaline treatment of streptokinase at pH 12 for 1 h caused approximately 45% of the original protein to be altered. By isoelectric focusing of streptokinase in the pH

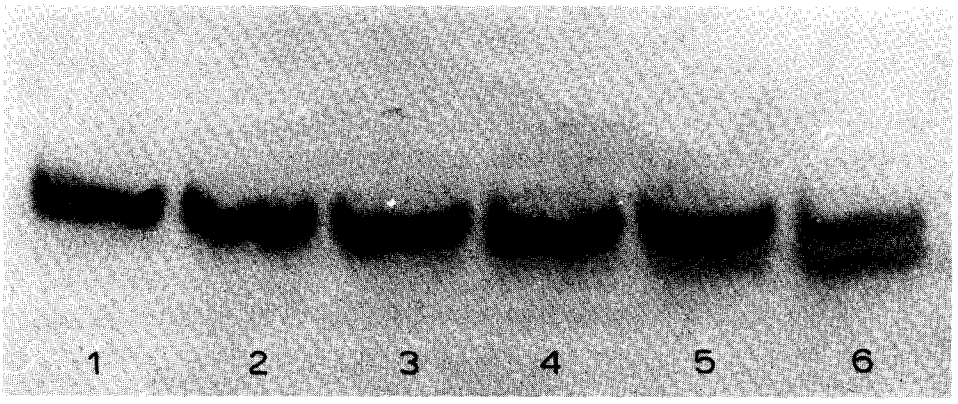

Fig. 1. Triton X-100-polyacrylamide gel electrophoresis of highly purified streptokinase incubated with different alkaline pH values for 60 min at 4°C. The pH values are: 1, pH 8.5; 2, pH 9.5; 3, pH 10.0; 4, pH 10.5; 5, pH 11.0, and 6, pH 12.0. Anode at the bottom.

range 3.5—9.5 after treatment for 1 h with 0.06 M sodium phosphate buffers with different pH values the same type of results were obtained. The native streptokinase shows one well-defined band with p*I* 5.2 (Fig. 3, 1). However, at pH 10.5 it was possible to detect, besides the major protein band (p*I* 5.2), one weak band located more anodally. This new band became stronger the more alkaline the medium was that streptokinase was treated with.

Fig. 2 shows polyacrylamide gel electrophoresis in the presence of Triton X-100 and Fig. 3 the isoelectric focusing pattern of streptokinase before and after treatment with 0.06 M trisodium phosphate at pH 12 for different incubation times. It is evident that the formation of the new band, detected by both techniques, is rapid. After no more than 2 min it is possible to detect this component as a weak band with slightly higher electrophoretic mobility compared to native streptokinase. It is obvious that prolonged incubation at pH 12 resulted in an increase in the anodal component at the expense of the major component with p*I* 5.2. After treatment for 5 h at pH 12 two new distinct bands located more anodally than native streptokinase are found. Consesequently, the alteration of streptokinase is a relatively rapid process and is directly related to the pH of the buffer medium and also to the time used for incubation.

SDS-polyacrylamide gel electrophoresis and gradient polyacrylamide gel electrophoresis revealed only one protein band for all the alkali-treated streptokinase samples. Thus no heterogeneity according to size could be demonstrated by those techniques.

The results from free zone electrophoresis indicated some heterogeneity of the sample but real dissolution into different protein peaks was not noted. This might be due to hydrophobic interaction between the components.

N-terminal determination. By Edman degradation only one N-terminal amino acid sequence, that is, Ile-Ala-, could be demonstrated in the alkali-treated streptokinase sample. Since this is the same as for the native streptokinase the results further support the suggestion that the heterogeneity is not due to protein fragmentation.

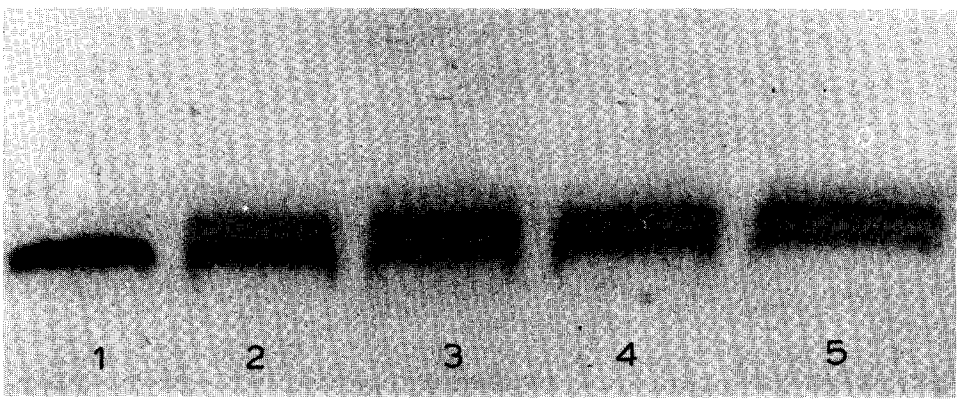

Fig. 2. Triton X-100-polyacrylamide gel electrophoresis of highly purified streptokinase incubated with alkali at pH 12.0 for various intervals of time at 4°C. The incubation times are: 1, 0 min; 2, 15 min; 3, 30 min; 4, 60 min, and 5, 120 min. Anode at the top.

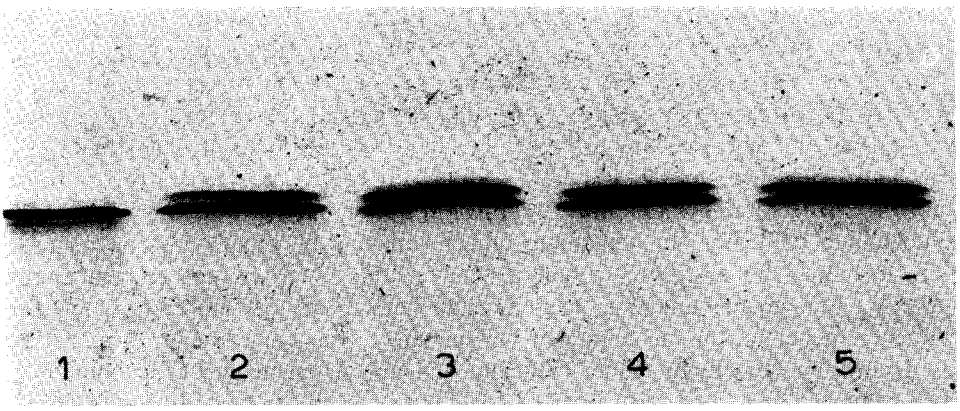

Fig. 3. Isoelectric focusing in the pH range 3.5—9.5 of highly purified streptokinase incubated with alkali at pH 12.0 for various intervals of time at 4°C. The incubation times are: 1, 0 min; 2, 15 min; 3, 30 min; 4, 60 min, and 5, 120 min. Anode at the top.

Peptide analysis

Limited digestions of native and alkali-treated streptokinase with trypsin, followed by thin-layer chromatography and paper chromatography clearly showed that the peptides obtained in the two streptokinase preparations were quite different with separate peptide mobilities. It is evident that some peptides in altered streptokinase exhibit higher mobility compared to native streptokinase. This effect might be due to the loss of amide groups from peptide fragments. By employing paper electrophoresis no acceptable separation was obtained between the trypsin digests of native and alkali-treated streptokinase.

Physical properties. The molecular weight and the sedimentation coefficient determined by ultracentrifugation were found to be about the same for the alkali-treated as for the native streptokinase. Furthermore, no heterogeneity indicating fragmentation could be demonstrated. The protein peak was quite symmetrical throughout the different experiments, although the base of the peak became fairly broad. The molecular weight found by SDS-polyacrylamide gel electrophoresis and gradient polyacrylamide gel electrophoresis was about 50 000.

Discussion

The streptokinase starting material was quite homogeneous according to various electrophoretic and ultracentrifugation techniques and, in addition, it exhibited homogeneity by immunoelectrophoresis and showed high clot lysis activity. The determined physicochemical data of the highly purified protein indicate some differences compared with previously reported data on streptokinase of the same origin. The molecular weight of our highly purified streptokinase (50 200) is somewhat higher than the value (44 000) reported by Brockway and Castellino [2]. To some extent, this discrepancy might be a result of a minor error involved in the calculation of the partial specific volume from the amino acid composition data and uncertainty in the determination of

the sedimentation coefficient obtained by ultracentrifugation. However, we also found a molecular weight of about 50 000 by SDS-polyacrylamide gel electrophoreis and gradient polyacrylamide gel electrophoresis in good agreement with the ultracentrifugation data, suggesting that the difference in molecular weight of the two preparations reflects two separate protein fractions.

The sedimentation coefficient (3.37 S) of our highly purified streptokinase was found to be higher than 3.03 S reported by Brockway and Castellino [2], which further supports the suggestion that our highly purified streptokinase preparation may be a slightly larger protein molecule than that characterized previously. End-group analysis also indicated definite differences, since the carboxyl-terminal amino acid in our preparation was tyrosine, in contrast to lysine found by Brockway and Castellino [2] and Morgan and Henschen [9]. All the preparations, however, possess isoleucine as an amino-terminal residue. The reason for the differences between our preparations characterized earlier might be due to a limited degradation of some of the older streptokinase preparations with a simultaneous loss of a peptide sequence. The amino acid composition data of our streptokinase demonstrated the presence of all common amino acids except cystine or cysteine in conformity with earlier findings [2,9]. We found just one tryptophan residue in streptokinase, which is in accordance with a previous finding [9], but in contrast to another [2]. Amino acid decomposition data on streptokinase from another origin also indicate one tryptophan residue [1].

The absorptivity factor (7.5) or our highly purified streptokinase was identical with the value reported by Loch et al. [29] on their preparation of streptokinase. However, those determined values are significantly lower than previously reported values (10 [1] and 9.49 [4]) for preparations of streptokinase from another origin. It cannot be excluded that this difference could be that various strains of streptococci might produce streptokinase of different chemical compositions, but more likely the higher values are due to determinations on crude preparations and/or uncertainty in the methods used.

The shape of streptokinase is indicated by the hydrodynamic measurements. Within the limits of the experimental error a frictional ratio of 1.29 was determined for this protein. This value is somewhat higher than the values for typical globular proteins, which have frictional ratios of 1.1—1.25 [30]. Clearly, the hydrodynamic behaviour indicates a minor deviation from a globular shape. Assuming zero solvation, an f/f_0 value of 1.29 would correspond to a prolate ellipsoid with an axial ratio of 6.3/1, but it should be stressed that this axial ratio probably does not represent the true dimensions of the molecules since the calculations are based on certain assumptions regarding shape and degree of hydration.

Treatment of the highly purified native streptokinase with an alkaline agent (pH 12) caused a rapid, although time-dependent, and irreversible inactivation of the clot lysis activity of the protein. This effect might be due to an altered geometrical arrangement of some of the amino acids involved in the specific binding of streptokinase to plasminogen. At the same time a drastic decrease in the immunological activity was also observed, indicating perturbation of the antigenic determinants, and this alteration strongly suggests a marked conformational change in the protein structure.

Taking all data into consideration, the most likely interpretation of the alkaline treatment of streptokinase centres on the consequences of deamidation and tyrosyl ionization. Both these effects might contribute to breaking of non-covalent bonds and thus unfolding of the protein structure, even if the unfolding appears to be limited. The electrophoretic data indicate the production of more acidic components in altered streptokinase, which supports the suggestion of deamidation in alkaline media. This also holds true when the results from the various analyses of peptides are considered. Furthermore, the amount of amides in streptokinase is large [2,9,31]. It has also been proposed previously [29] that when incubated in alkaline solutions, streptokinase undergoes an irreversible and structural transition as a result of tyrosyl ionization. Finally, one cannot completely exclude the possibility of other effects being responsible for the described behaviour of native streptokinase in alkaline media.

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