Eur Biophys J (1992) 20:355–361

European Biophysics Journal

Streptokinase is a flexible multi-domain protein

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Received August 19, 1991 / Accepted in revised form November 15, 1991

Abstract. The structure of streptokinase in solution has been studied by dynamic light scattering, small-angle X-ray scattering and circular dichroism spectroscopy. The Stokes' radius and radius of gyration of the protein monomer are 3.58 nm and 4.03 nm, respectively. The maximum intraparticle distance of the molecule is 14 nm. More than half of the amino acids of the molecule are organized in regular secondary structures. The X-ray scattering curve, the results from dynamic light scattering, and the finding that at least 50% of the amino acid residues are organized in regularly folded secondary structures are consistent with the following structural model. Streptokinase consists of four compact, separately folded, domains linked by mobile segments of the protein chain. The molecule exhibits the conformation of a flexible string-of-beads in solution.

Key words: Streptokinase – Protein structure – X-ray scattering – Dynamic light scattering

Introduction

Streptokinase is naturally produced and secreted by various strains of hemolytic streptococci. The clinical importance of streptokinase was first noted by Tillet and Garner (1933), who discovered that this bacterial protein caused the lysis of human blood clots. Streptokinase is a potent activator of plasminogen, the inactive precursor of plasmin (Schick and Castellino 1974; Bajaj and Castellino 1977). Streptokinase is currently used in clinical medicine as a therapeutic agent in the treatment of thromboembolic blockages, including coronary thrombosis.

The mature protein has a molecular mass of 47 000 Daltons. It is a monomeric protein and was found to be composed of 414 amino acid residues (Taylor and Botts 1968; Brockway and Castellino 1974; Jackson and Tang 1982). Its primary structure is known (Jackson and Tang 1982; Malke et al. 1985). Radek and Castellino (1989) have reported on studies of conformational properties of streptokinase by spectroscopic and calorimetric techniques as well as by secondary structure prediction methods. According to their results, streptokinase is a typical $a+\beta$ protein containing about 17% a-helices, 28% β-sheets, 21% β-turns and 34% disordered structure. To gain a more detailed insight into the structural properties of streptokinase, we have investigated this protein by small-angle X-ray scattering (SAXS) and dynamic light scattering (DLS). We were very surprised by the unexpected results. To compare the structure of streptokinase with that of a typical, compact globular protein, we have measured SAXS of cytochrome c (horse heart) using the same diffractometer and identical data treatment. Additionally, we have compared the structure of streptokinase with the known structure of phosphoglycerate kinase from yeast. This protein consists of 415 amino acids and comprises two well-separated domains (Watson et al. 1982). The introduction of the comparison of protein structure by X-ray scattering with the application of scaling laws, viz. streptokinase and cytochrome c, and without scaling laws, viz. streptokinase and phosphoglycerate kinase, is a novel method for investigating protein structures.

Materials and methods

Streptokinase

Streptokinase was isolated from group C streptococci (*Streptococcus equisimilis*) as described by Gerlach and Köhler (1977). Lyophilized streptokinase was further purified by gel filtration on Sephadex-G 100 (2.5 × 100 cm) in 10 mM sodium phosphate, pH = 7.5. Pooled fractions were concentrated in a SARTORIUS collodium bag (SM 13 200). Aliquots of the protein stock solution were diluted to the desired concentrations by addition of appropriate buffer volumes. Concentrations of streptokinase were determined photometrically using $A^{1\%}_{1cm} = 9.8$ at 277 nm. This value was obtained from the nitrogen content of

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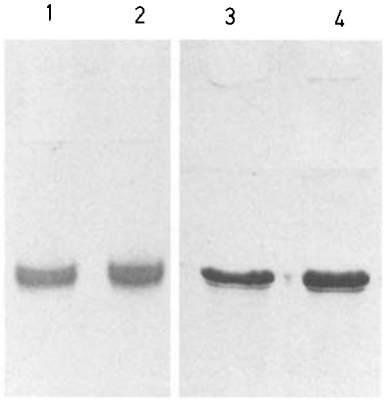

Fig. 1. PAGE of Streptokinase sample ($c = 2.3$ g/l in 10 mM sodium phosphate, pH = 7.5) before and after small-angle X-ray measurement. *Lanes 1 and 2:* non-denaturing conditions; *lanes 3 and 4:* denaturing conditions; *lanes 1 and 3*: before SAXS; *lanes 2 and 4:* after SAXS

streptokinase determined according to Jaenicke (1974). The protein samples were analysed before and after the SAXS and DLS measurements by SDS PAGE according to the method of Laemmli (1970) as well as under non-denaturing conditions in 10% polyacrylamide gels using 90 mM *Tris*, 90 mM boric acid, 2.5 mM EDTA, pH = 8.3 as buffer system. Figure 1 shows that streptokinase does not degrade and does not aggregate during the X-ray experiments.

Cytochrome c

Cytochrome *c* was prepared from horse heart by the method described by Margoliash and Walasek (1967). The X-ray measurements were performed in 20 mM sodium phosphate, pH = 7.0.

Circular dichroism

Measurements of streptokinase were performed on a JASCO-41A spectropolarimeter (Japan) in the far-ultraviolet region (198–240 nm) at a protein concentration of 1.3 g/l and at a path-length of 0.148 mm.

Dynamic light scattering

The DLS spectrometer and particulars of sample preparation have been described previously (Gast et al. 1986). All measurements were performed at wavelength $\lambda = 514.5$ nm, at a scattering angle of 90° and at 20 °C. The translational diffusion coefficients D were derived from the photon-counting autocorrelation functions by performing an inverse Laplace transformation using the constrained regularization method (Provencher 1982 a, b) and the corresponding program CONTIN of Provencher (1984). The diffusion coefficients were corrected to standard conditions (water at 20 °C). Hydrodynamic effective Stokes' radii R_s were determined from D via the Stokes-Einstein relation $R_s = kT\ (6\pi\eta\ D)^{-1}$, where k is Boltzmann's constant, T the temperature in Kelvin and η the solvent viscosity.

Small-angle X-ray scattering

SAXS was measured on a SAXS-WAXS diffractometer (Freiberger Präzisionsmechanik GmbH, FRG) with slit geometry. The CuKα-radiation was collimated by five slits and Soller slits. The methods of data processing and desmearing have been described previously (Müller et al. 1986; Glatter 1982). The absolute intensities needed for the determination of molar masses were obtained using a Lupolen standard (Glatter 1982).

Results

Circular dichroism

The circular dichroism spectrum of streptokinase obtained by us is nearly identical with that published by Radek and Castellino (1989) for streptokinase in 10 mM sodium phosphate, pH = 7.4. Therefore, we omit to show the spectrum in this work. The close agreement of our circular dichroism spectrum with that measured by Radek and Castellino (1989) proves that at least half of all amino acids of the streptokinase in our samples are organized in regularly folded secondary structures, viz. α-helices, β-sheets and β-turns (see: Introduction).

Dynamic light scattering

DLS measurements on streptokinase were performed in the concentration range 0.5–2.5 g/l in 10 mM sodium phosphate, pH = 7.5. The diffusion coefficient depends only weakly on protein concentration. The dependence is given by $D = D^0_{20,w}\ (1 + 0.017\,c)$, where c is the protein concentration in g/l. Extrapolation of the measured diffusion coefficients to zero protein concentration yielded $D^0_{20,w} = (5.97 \pm 0.06)\ 10^{-7}\ cm^2\ s^{-1}$ and the corresponding Stokes' radius $R_s = (3.58 \pm 0.04)$ nm. This value of the radius is rather large for a compact globular protein with a molecular mass of 47 000 Da. To check whether our samples contained protein dimers, we calculated the molar mass $M_{s,D}$ via the Svedberg equation using our value of $D^0_{20,w}$ and the sedimentation constant $s^0_{20,w} = 3.11$ S and the apparent partial specific volume $\bar{v} = 0.719$ ml/g taken from Barlow et al. (1984). We obtained $M_{s,D} = 45\,200$ g/mol. This value is strong evidence for the monomeric state of streptokinase in our samples. Assuming the molecular mass of streptokinase to be 47 000 Da and using $\bar{v} = 0.719$ ml/g, we calculated the radius R_{min} of the streptokinase molecule. R_{min} is the radius of the unsolvated molecule, the shape of which is assumed to be an ideal sphere. We obtained $R_{min} = 2.38$ nm and the frictional ratio $f/f_{min} = R_s/R_{min} = 1.5$.

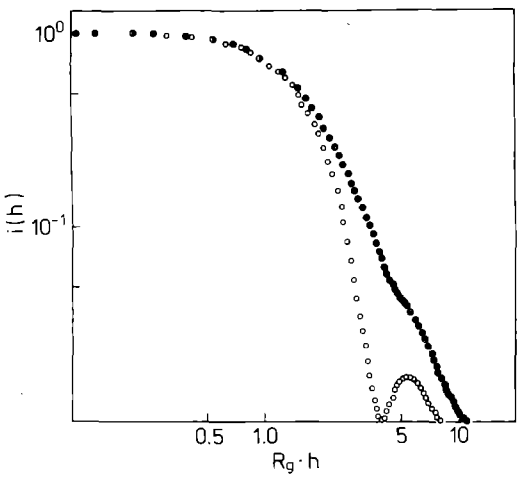

Fig. 2. Small-angle X-ray scattering curves extrapolated to zero protein concentration [$\log I(h)$ versus $\log(R_G h)$] for streptokinase (•), $R_G = 4.03$ nm, and cytochrome c (o), $R_G = 1.48$ nm. $h = 4\pi\lambda^{-1}\sin\theta$ = scattering vector, λ = wavelength of CuKα-radiation = 0.154 nm, 2θ = scattering angle, I = scattered intensity, R_G = radius of gyration

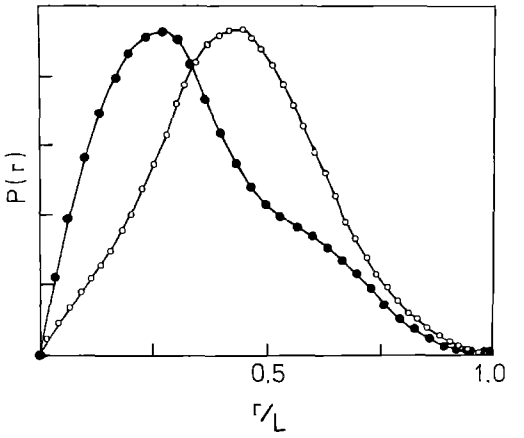

Fig. 3. Distance distribution functions [$p(r)$ versus rL^{-1}] for streptokinase (•) and cytochrome c (o). The maximum diameter amounts to $L = 14$ nm and $L = 4.55$ nm for streptokinase and cytochrome c, respectively. The most frequent electron distances are $R_e = 4.0$ nm and $R_e = 1.85$ nm

Small-angle X-ray scattering

The scattering curves of streptokinase were measured at different concentrations, viz. $c_1 = 1.0$ g/l, $c_2 = 2.3$ g/l, $c_3 = 4.15$ g/l and $c_4 = 5.15$ g/l. Each curve was separately desmeared. From these desmeared curves, a curve extrapolated to zero protein concentration was constructed. This is shown in Fig. 2 together with the scattering curve of cytochrome c obtained in the same manner. The distance distribution functions of both proteins (Fig. 3) were calculated using the integral transformation

$$p(r) = (2\pi^2)^{-1} \int_0^\infty I(h) \cdot (hr) \sin(hr)\, dh \qquad (1)$$

$h = 4\pi\lambda^{-1}\sin\Theta$, λ-wavelength, Θ-Bragg angle, I-scattered intensity.

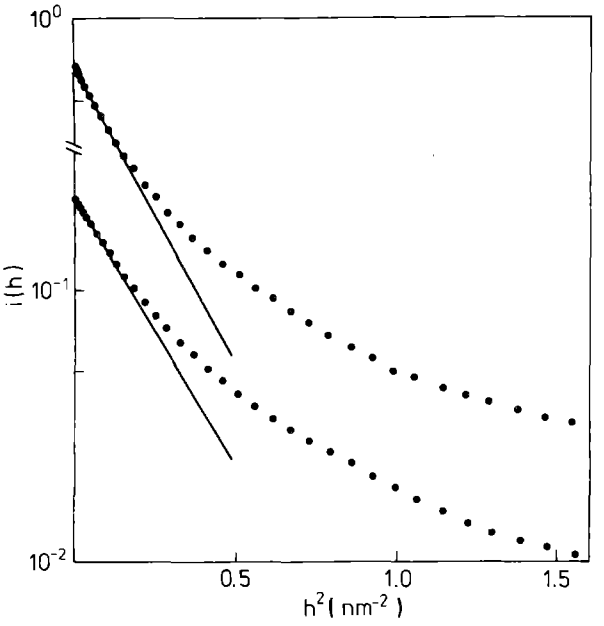

Fig. 4. Guinier plots [$\log I(h)$ versus h^2] of the small-angle scattering from streptokinase at $c = 5.15$ g/l (*top*) and 4.15 g/l (*bottom*). The apparent radii of gyration are $R_G = 4.11$ nm and $R_G = 3.93$ nm, respectively

The maximum intraparticle distance L of the streptokinase molecule was determined from the distance distribution function to be $L > 14$ nm. We obtain for cytochrome c the value $L = 4.55$ nm. We cannot exclude a value somewhat larger than 14 nm for streptokinase, because in this case, in contrast to the curve for cytochrome c, $p(r)$ runs very flatly into the abscissa (Figs. 3, 5). The radii of gyration R_G were determined separately for each protein concentration by the asymptotic Guinier approximation

$$I(h) = I(0) \exp\left[-\frac{h^2 R_G^2}{3}\right] \qquad (2)$$

for the inner part of the scattering curves (Fig. 4). These concentration dependent apparent radii of gyration were extrapolated to zero protein concentration. For $c < 5$ g/l, the apparent radii of gyration depend only weakly on the concentration. Additionally, we have calculated the radius of gyration from the distance distribution function for zero protein concentration (Fig. 3) by

$$R_G^2 = \int_0^L p(r) \cdot r^2\, dr \cdot \left[2 \int_0^L p(r)\, dr\right]^{-1}. \qquad (3)$$

Both methods yield for the radius of gyration of streptokinase the value $R_G = (4.03 \pm 0.1)$ nm. We obtain for cytochrome c $R_G = (1.48 \pm 0.05)$ nm from both methods.

The molar masses, determined from the $I(0)$ values extrapolated to zero concentration, amount to $M = 11\,200$ g/mol and $M = 46\,500$ g/mol for cytochrome c and streptokinase, respectively. Both values agree with the chemical molar masses within the limits of error. Thus, these results demonstrate the monomeric state of the proteins under the conditions of the X-ray investigations. The results for streptokinase obtained from all methods are summarized in Table 1.

Table 1. Physical parameters of streptokinase

Parameter	Value of the parameter
Diffusion coefficent	$D^0_{20,w} = 5.97 \cdot 10^{-7}$ cm^2 s^{-1}
Sedimentation constant	$s^0_{20,w} = 3.11$ S[a]
Molar mass	$M = 46\,500$ g/mol[b]
	$M_{s,D} = 45\,200$ g/mol[c]
	$M = 47\,000$ g/mol[d]
Radius of gyration	$R_G = 4.03$ nm
Stokes' radius	$R_S = 3.58$ nm
Maximum intraparticle distance	$L \geq 14$ nm
Most frequent electron distance	$R_e = 4.0$ nm
Frictional ratio	$f/f_{min} = 1.5$
ϱ-Factor	$\varrho = R_G/R_S = 1.13$

[a] from (Barlow et al. 1984)
[b] SAXS result
[c] DLS result
[d] chemical molar mass

Discussion

The radius of gyration of streptokinase being $R_G = 4.03$ nm is unusually large for a monomeric protein having a molar mass of 47 000 g/mol. We have analysed the relationship between the radius of gyration R_G in water and the number of amino acid residues N for 34 monomeric single-chain, crystalline proteins using the Protein Data Bank (Bernstein et al. 1977). We obtained the scaling law

$$R_G = (0.290 \pm 0.014)\, N^{1/3} . \quad (4)$$

According to this scaling law, one should expect a radius of gyration of $R_G = (2.16 \pm 0.1)$ nm for the streptokinase molecule comprising 414 amino acid residues. However, the experimentally determined radius of gyration $R_G = 4.03$ nm is nearly double this value.

For monomeric proteins, the structure of which have been determined by X-ray crystallography, the maximum intraparticle distance and the number of amino acid residues N are related by the scaling law

$$L = (0.895 \pm 0.069)\, N^{1/3} \quad (5)$$

Assuming the structure of streptokinase to be similar to the structure of compact, globular proteins, one would expect a maximum intraparticle distance $L = (6.7 \pm 0.4)$ nm. The experimentally determined value $L = 14$ nm is more than twice as large. The ratio of the radius of gyration to the Stokes' radius

$$\varrho = R_G \cdot R_s^{-1} \quad (6)$$

is a sensitive indicator of the conformation of polymers. This ratio amounts to $\varrho = 0.775$ for spherical molecules. We have calculated a mean value of $\varrho = 0.8$ for globular proteins with known crystal structure. On the other hand, we obtained the ratio $\varrho = 1.55$ for unfolded proteins (acid denatured) (Damaschun et al. 1991). The experimentally determined value for streptokinase is $\varrho = 1.13 \pm 0.04$ (see Table 1). Comparing the physical parameters, the scaled scattering curve for cytochrome c (Fig. 2) and the scaled distance distribution function for cytochrome c (Fig. 3) with the corresponding functions for streptokinase, one can exclude the possibility that streptokinase is a compact single-domain protein.

Phosphoglycerate kinase from yeast is a bilobal protein consisting of a single polypeptide chain with 415 amino acids. The two widely separated domains are of almost equal size (Watson et al. 1982). The domains are able to fold or unfold independently under certain conditions (Griko et al. 1989; Adams et al. 1985). The radius of gyration R_G and the Stokes' radius R_s of the enzyme are $R_G = 2.33$ nm (Pickover et al. 1979) and $R_s = 2.97$ nm [own unpublished results], respectively. This results in $\varrho = 0.785$. Streptokinase ($N_A = 414$) and phosphoglycerate kinase ($N_A = 415$) consist of nearly the same number of amino acids. Therefore, comparing the radii of gyration, the Stokes radii, and the ϱ-factors as 4.03 nm and 2.33 nm, 3.58 nm and 2.97 nm, and 1.13 and 0.785 for streptokinase and phosphoglycerate kinase, respectively, we can exclude the possibility that streptokinase has a bilobal structure similar to that of phosphoglycerate kinase.

The ultimate proof of this conclusion is the comparison of the distance distribution function for streptokinase, calculated from the X-ray scattering data, with the distance distribution function of phosphoglycerate kinase, calculated from the atomic co-ordinates of this protein (Fig. 5). The shapes of the two curves are totally different. The maximum intraparticle distances $L > 14$ nm (streptokinase) and $L = 8$ nm (phosphoglycerate kinase) show that the latter protein is significantly more compact than streptokinase. The distance distribution function for phosphoglycerate kinase was calculated from the atomic co-ordinates taken from the Brookhaven Protein-Data-Bank (Bernstein et al. 1977) by applying the algorithms described by Müller et al. (1990).

The hydrodynamic parameters of the streptokinase molecule (see Table 1) – the translational diffusion coefficient, the sedimentation constant and the frictional ratio $f/f_{min} = 1.5$ – are indicative of either a pronounced anisotropy of the dimensions of the streptokinase molecule and/or of another kind of expansion or unfolding of the molecule.

The shape of the scattering curve and the distance distribution function (Figs. 2, 3, 4, 5) contain detailed information on the shape of the streptokinase molecule. The Guinier plot of the scattering data is remarkable for the divergence of the scattering curve from linearity already observed at small scattering angles. Scattering curves of globular proteins do not show such behaviour. Figure 2 shows double-logarithmic plots of the scattering curves of both streptokinase and cytochrome c. Log (R_G h) is chosen as the abscissa to correct for the different molar masses of the two proteins. In contrast to the scattering curve of cytochrome c, that of streptokinase does not exhibit a range where $I \propto h^{-4}$. The Guinier range (1) is immediately followed by a region where $I \propto h^{-2}$. Such scattering behaviour is atypical for rigid, globular proteins having a well-defined boundary between the globular protein with hydrophobic core and hydrophilic

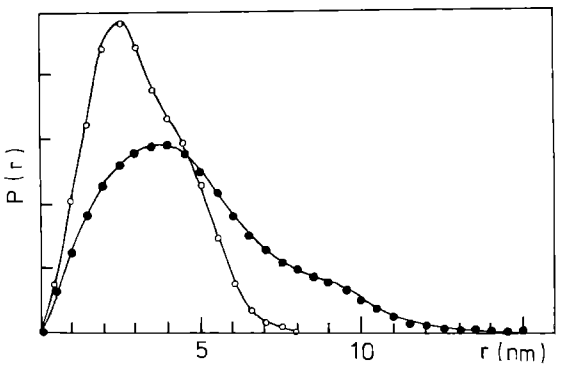

Fig. 5. Comparison of the distance distribution function for streptokinase (•) with that for phosphoglycerate kinase (o). The maximum intraparticle distances amount to $L>14$ nm and $L=8$ nm. The maximum in the distance distribution function of phosphoglycerate kinase at $r=2.5$ nm (most frequent electron distance) is due to the shape of the entire protein molecule; the shoulder at 4 nm is caused by the distance between the two domains of the bilobal protein. The maximum in the distance distribution function for streptokinase at $r=4$ nm and the shoulder at 9 nm are due to frequent distances between the domains of the streptokinase molecules. They can be assigned to the mean distances between a domain and its nearest and second-nearest neighbours, respectively. Model calculations using homogeneous bodies have shown that the maximum at 4 nm cannot be due to the shape of the entire molecule

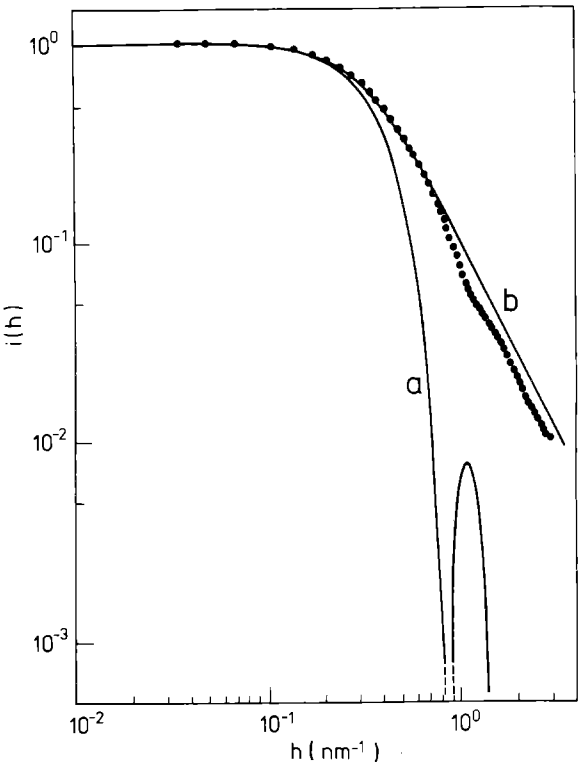

Fig. 6. Comparison of the small-angle scattering curve for streptokinase with calculated curves for a compact sphere with a radius $R=5.20$ nm corresponding to a radius of gyration $R_G=4.03$ nm (*a*) and for a Debey coil with a radius of gyration $R_G=4.03$ nm (*b*)

surface and the solvent. However, such scattering curves are typical for flexible molecules (Glatter 1982).

The circular dichroism spectra and the prediction of the secondary structure (Radek and Castellino 1989) from the amino acid sequence indicate that streptokinase is a folded, compactly organized protein molecule. Therefore, one cannot assume streptokinase to have the conformation of an unfolded protein chain under our experimental conditions. It is known that proteins may adopt the conformation of rigid helices or coiled-coils of helices accessible to the solvent. However, such structures would cause scattering curves with $I\alpha h^{-1}$. About 28% of the amino acid residues of streptokinase are organized in the form of β-sheets. The formation of β-sheets is connected with the formation of a hydrophobic protein core. Out of the 414 amino acid residues of streptokinase, 170, i.e. 41% are hydrophobic. Therefore, we must assume that the streptokinase molecule consists of one or several folded domains each containing a hydrophobic core.

To get more insight into the shape of the streptokinase molecule, we have calculated scattering curves of shape models and have compared these curves with the experimental one. The calculations of scattering curves of models of streptokinase are based on the following plausible assumption. The volume of the model body equals, or is no more than 15% bigger than, the excluded volume of the molecule calculated from the partial specific volume and the molar mass. We found that there is no ellipsoid of rotation, no cylinder, and no rigid arrangement of n spheres ($n\leq 6$) for which experimental and calculated scattering curves would coincide. These negative results agree with the experience of Sjöberg et al. (1991), who also found that scattering curves which are similar to that of a Gaussian coil (see below) cannot be approximated by scattering curves of rigid bodies, e.g. solid three-axial bodies.

In contrast to negative results, we found that the experimental scattering curve is similar to the scattering function (Fig. 6)

$$I(h)=I(0)\cdot 2(R_G^2 h^2)^{-1}\,[\exp(-R_G^2 h^2)-1+R_G^2 h^2] \qquad (7)$$

This scattering function was derived by Debye (1947) and describes the scattering from an angular chain of small spheres, each sphere being free to rotate on the basis circle of a cone independently of the neighbours (Flory 1969). We conclude from the similarity between the scattering curve of streptokinase and the scattering curve of this model that the streptokinase molecule consists of several domains forming a flexible chain. The dimensions, and therefore also the number of domains forming the streptokinase molecule, can be estimated from the shape of the scattering curve in the range $h\cdot R_G>4$. This range is dominated by the mean value of the scattering of the domains (Fig. 6). The interferences of the waves scattered from the individual domains disappear. In this region, we have fitted the scattering curves of a sphere, of slightly eccentric ellipsoids, and the scaled scattering curve of cytochrome *c* (Fig. 7) to the scattering curve of streptokinase, the radius of gyration being the free parameter in each case. The mean radius of gyration of the domains amounts to $R_G=(1.35\pm 0.06)$nm. On the other hand, the mean molecular mass of the domains M_D can be estimated from the radius of gyration by (4). We obtain $M_D=0.24$ M Thus, the mean molecular mass of the domains of strep-

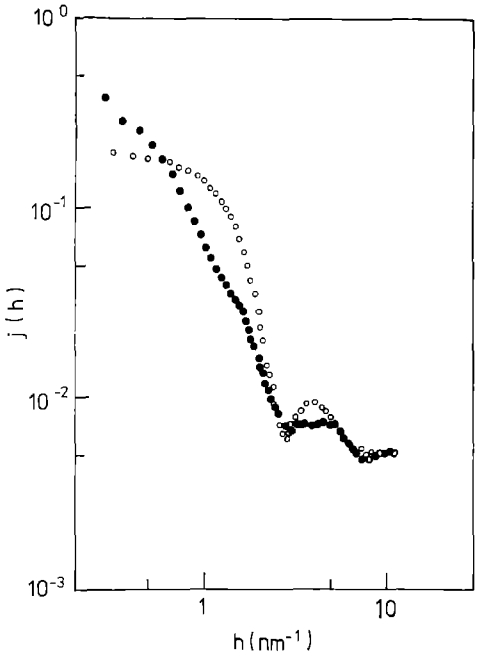

Fig. 7. Double-logarithmic plots of the intermediate-angle X-ray scattering curves for streptokinase (•) and for cytochrome *c* (o). The latter scattering curve is scaled to a radius of gyration of $R_G = 1.35$ nm. The scattering curve for streptokinase exhibits damped oscillations around the curve for the globular protein with a radius of gyration of $R_G = 1.35$ nm in the range $h < 5\ \text{nm}^{-1}$. In the adjacent range $5\ \text{nm}^{-1} < h < 10\ \text{nm}^{-1}$, the scattering curves are identical within the limits of errors. Therefore, the subsidiary maximum of the scattering curve for streptokinase in the region $3\ \text{nm}^{-1} < h < 8\ \text{nm}^{-1}$ is caused by the shape of the subunits and not by the shape of the entire molecule. Thus, the dimensions (R_G) of the subunits can be determined from the course of the scattering curve in this range and from the position of the subsidiary maximum (see text)

tokinase is only insignificantly lower than that of cytochrome *c*. The domains are typical globular proteins as can be concluded from a comparison of their scattering curve with that for cytochrome c in the range $s > 4\ \text{nm}^{-1}$ (Fig. 7). This implies that the streptokinase molecule consists of four compact domains. However, we cannot exclude the existence of five domains for the moment. Four compact, globular domains linked by three flexible segments are consistent with the experimental value of $\varrho = 1.13$ being a value between those for one compact domain ($\varrho = 0.8$) and for an infinite flexible chain of beads ($\varrho = 1.51$).

Conclusions

Deriving a working model of the structure of streptokinase, we must take into consideration the following findings.

(*i*) At least 50% of the amino acids are folded into regular secondary structures.
(*ii*) The overall structure of streptokinase is, at low resolution, very similar to that of a Gaussian coil. However, the radius of gyration of this coil, $R_G = 4.03$ nm, is significantly smaller than the radius of gyration of unfolded streptokinase at the theta-point, $R_G \geq 7.9$ nm.
(*iii*) The shape of streptokinase is unlike the shape of any protein contained in the Protein-Data-Bank (Bernstein et al. 1977) that consists of one or two folded domains.
(*iv*) The scattering curve of streptokinase exhibits damped oscillations around the scattering curve of a folded protein domain having a radius of gyration of $R_G = 1.35$ nm. This corresponds to a protein domain consisting of about 100 amino acids.

These findings indicate that streptokinase is a protein with unusual structure. Its structure is unlike any protein structure described in the Protein-Data-Bank (Bernstein et al. 1977). All present findings are consistent with the following working model. The streptokinase molecule consists of four or possibly five compact domains containing individual hydrophobic cores. The domains are linked by three (or four) flexible segments of the protein chain. The compact domains move relative to each other in solution, so that the molecule as a whole can adopt a great variety of conformations ranging from the compact form to that of an extended chain. The molecule behaves like a statistical coil consisting of four monomer units. Therefore, the structure of the molecule is similar to a flexible string-of-beads.

The small-angle X-ray scattering curve for the streptokinase molecule and the distance distribution function are similar to the small-angle neutron scattering curve and the distance distribution function for the open form of the plasminogen molecule (Mangel et al. 1990), provided that the curves are corrected with respect to the differences in the radii of gyration, viz. $R_G = 4.11$ nm and $R_G = 5.6$ nm for streptokinase and plasminogen, respectively. Plasminogen also exhibits a flexible structure, which can be described approximately by a Debye random coil consisting of five kringle domains and one protease domain. The six compact domains of plasminogen and the flexible links have been identified in the amino acid sequence of the molecule (Sottrup-Jensen et al. 1978). This has not been achieved for streptokinase until now. The identification of the compact and flexible segments of streptokinase by means of biochemical methods is an important future task. It remains an open question whether there exists a compact (closed) form of streptokinase besides the flexible (open) form similar to the corresponding forms of plasminogen. We have not been able to find this hypothetical compact form by increasing the ionic strength up to $I = 0.2$

The flexibility of the open form of plasminogen may be important in its role in fibrinolysis (Mangel et al. 1990). One might suggest that the flexibility of the open form of streptokinase may also be important in its specific role in fibrinolysis. The interaction of two flexible molecules is a novel interesting phenomenon in the field of molecular life processes. Here, the simple idea of the lock and key principle governing the interaction of biological macromolecules must fail.

Acknowledgements. The authors are indebted to Dr. Yu. S. Venyaminov (Pushchino, USSR) for performing CD measurements and to Mrs. B. Lucke and Mrs. B. Poppe for skilful technical assistance. This work was supported by grants from the Bundesministerium für Forschung und Technologie (BMFT) and from the Fonds der chemischen Industrie.

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