Small-angle X-ray Scattering-based Three-dimensional Reconstruction of the Immunogen KLH1 Reveals Different Oxygen-dependent Conformations*

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For decades the respiratory protein keyhole limpet hemocyanin (KLH1) from the marine gastropod Megathura crenulata has been used widely as a potent immunostimulant, useful hapten carrier, and valuable agent in the treatment of bladder carcinoma. Although much information on the immunological properties of KLH1 is available, biochemical and structural data are still incomplete. Small-angle x-ray scattering revealed the existence of two conformations, an oxy state being slightly more compact than the deoxy state. Based on small-angle scattering curves, a newly developed Monte Carlo algorithm delivered a surface representation of proteins. The massive changes of the surfaces of reconstructed didecamer KLH1 molecules are explained as a twist of the two non-covalently associated didecameric half-molecules. Upon oxygenation, the KLH1 molecule becomes longer and skinnier. This study provides the first real evidence how a molluscan hemocyanin changes conformation during an allosteric transition.

Hemocyanins are respiratory proteins found extracellularly in the hemolymph of arthropods and molluscs. Although the structures of arthropod and mollusc hemocyanins are different, the oxygen is reversibly bound at the active site in the same side-on coordination between the two copper atoms (1, 2). Up to 160 active sites are cooperatively coupled when binding oxygen with the highest Hill coefficient observed for natural biomolecules (3–5). The molecular basis especially in molluscan hemocyanins is still not known. One of the most prominent hemocyanins from mollusc, KLH, is that from the marine gastropod keyhole limpet Megathura crenulata. KLH has widely been used as an immunostimulant, hapten carrier, and valuable agent in the treatment of cancer. However, not much is known regarding the immunological (6–10) and biochemical as well as structural properties (11–16).

Two different hemocyanin isoforms, KLH1 and KLH2, have been found in the hemolymph of the keyhole limpet M. crenulata with molecular masses of ~8 MDa. Electron microscopy revealed similar quaternary structures as hollow cylinders with a D5 symmetry. It is composed of two isologously dimerized decamers consisting of 10 large subunits, which fold into ~7–8 copper-containing functional units (FU’s) of ~50 kDa being heterogeneous in their sequences (15, 16). Each FU binds one molecule of oxygen (1, 16, 17). Based on x-ray structures of FU-g from the octopus O. dofleini (1) and FU-e from Rapania thomassiana (18), a FU contains two subdomains with different folding motifs, a N-terminal α-helical part containing the oxygen binding center and a C-terminal β-rich part (1, 17, 18). Depending on the species, covalently bound carbohydrates are located at the interface between the two subdomains (1) or at the N-terminal domain (18). KLH was also found to be glycosylated (19, 20). These carbohydrates are important epitopes to enhance the immune responses to administered antigens and for immunotherapy of bladder and renal carcinoma (6–9). Antigenic cross-reactivity has also been reported between the antigens from KLH and Schistosoma mansoni, indicating a strong carbohydrate similarity (21, 22). However, the exact location of the carbohydrates on the surface of native KLH is not known. Indeed, the arrangement of the subunits and their 160 FUs within the decamers is still under discussion, although a 15-Å resolution three-dimensional reconstruction of KLH1 didecamer has been obtained from low temperature transmission electron microscopy (11).

Materials and Methods

Sample Preparation—KLH1 was kindly provided by Dr. J. Markl (University of Mainz). After purification according to the procedure described by Harris et al. (12), we verified the didecameric state of the molecules by transmission electron microscopy.

The deoxygenation of the KLH1 samples was performed with nitrogen gas in a glove box. Because of the high oxygen affinity of KLH1, the partial oxygen pressure had to be kept below 1 torr to reach complete deoxygenation within 45 min. We judged the deoxygenation to be complete when the oxygen-dependent absorbance at 340 nm disappeared. During this procedure, the protein concentration increased by evaporation of some water. To work with the same concentration for the oxy- and deoxygenated sample, an aliquot of the deoxygenated protein solution was reoxygenated in a small test tube within minutes. The protein concentration was measured by light absorption at 278 nm. Using the extinction coefficient of 0.62 cm⁻¹ mg⁻¹ ml for KLH1, the protein concentration was determined to be 6.3 mg/ml for both the oxygenated and deoxygenated samples.

Scattering Experiments and Data Treatment—The small-angle x-ray scattering (SAXS) data were measured at the beamline B1 of the HASYLAB synchrotron facility at the Deutsches Elektronen Synchrotron (DESY) at distances of 3.6 and 1.8 m. A wavelength λ of 1.38 Å was used, calibrated with the CuKα edge. Scattering intensities I(q) were obtained in the range of 0.008 Å⁻¹ < q < 0.27 Å⁻¹ with q = 4πnsin(θ)/λ. The protein solutions and the buffer were measured in a flow-cuvette at a temperature stabilized to 20 °C. Intensities were recorded on a multi-wire proportional counter with a size of 20 x 20 cm² and with...
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256 × 256 pixels of electronic resolution. The actual resolution function of the detector and the conversion factor from pixel to length units was measured by placing a metallic plate with 30 × 30 pinholes in front of the detector and irradiating it with an isotropic scattering sample. The sensitivity of the detector was determined by fluorescent scattering of a Cu sample. The data were normalized to the intensity of the primary beam and to transmission and corrected for noise, detector response, and the buffer contribution. Details of the experimental technique and the evaluation procedure are described elsewhere (23, 24). Deconvolution of the radially averaged intensities was done by indirect Fourier transformation (24, 25) using the measured geometric resolution function. This simultaneously gives the radial distribution function \( \rho(r) \).

The radii of gyration \( (R_g) \) were calculated from the second moments of the intensity distribution functions (24–26). The integral of the protein and after the experiment was checked by comparing different time frames of the measured intensities and by transmission electron microscopy. Neither aggregation nor dissociation of the KLH1 molecules was found in the samples used for the SAXS measurements.

Monte Carlo annealing algorithm-based Three-dimensional Reconstruction of Small-Angle Scattering Curves (MCSAS)—In this section, we give a short description of the MCSAS algorithm as it was applied to the reconstruction of the KLH1 structure. In more detail, the procedure is described elsewhere (27). Applying the algorithm to simulated scattering curves, we were able to reconstruct excellent low resolution models (27).

At first, a cylinder was created obeying D5 symmetry and with dimensions that certainly include the KLH1 molecule with dimensions as obtained by electron microscopy. An asymmetric unit was filled with an electron density in the form of point charges (scattering points) randomly distributed. The whole cylinder was created by applying D5 symmetry operations. As it was shown previously (28), the small-angle scattering intensity of a molecule can be calculated effectively from a random sample of the excess density. In contrast to a regular grid of densely packed scattering spheres (29, 30), the random arrangement of scattering points allows any symmetry restriction. No systematic holes or overlaps are generated at the interfaces among the asymmetric units. To minimize statistical density fluctuations on a length scale above the resolution limit, the mean distance between neighboring points was adjusted to one-third of the resolution of the data defined as \( d_{\text{min}} \sim 2\pi/q_{\text{max}} \). By chance, a relative scattering amplitude or scattering length \( f = 0 \) or 1 was assigned to each point. Only points with a scattering amplitude \( f = 1 \) contribute to the small-angle scattering and to the shape of the molecule. From the points with \( f = 1 \), the radial distribution function \( \rho(r) \) was calculated and the scattering intensity of the model, \( I_{\text{mod}}(q) \), was calculated with a sine transformation of \( \rho(r) \) as shown in Equation 1.

\[
I_{\text{mod}}(q) = 4\pi \int_0^{\infty} \rho(r) \sin(qr) qr \, dr \quad (\text{Eq. 1})
\]

After scaling the modeled intensities to the experimental intensities \( I_{\text{exp}} \), the discrepancy factor \( \chi^2 \) was computed as shown in Equation 2,

\[
\chi^2 = \frac{1}{n - 1} \sum_{i=1}^{n} \left( \frac{I_{\text{mod}}(q_i) - I_{\text{exp}}(q_i)}{\sigma(q_i)} \right)^2 \quad (\text{Eq. 2})
\]

where \( n \) is the number of experimental intensities with standard deviation \( \sigma \). Currently, in a cyclic Monte Carlo procedure, new configurations were created, gradually improving the correspondence between the calculated and measured intensities. A point was selected by chance, its scattering amplitude was inverted (if \( f = 1 \) then \( f = 0 \) and vice versa), and \( \Delta \chi^2 \), the change of \( \chi^2 \), was calculated. To allow the algorithm to search in the total configuration space, consistent with the experimental data, and not to become stuck in local minima, a criterion similar to the one used by Metropolis et al. (31) was applied to the new configurations. The configuration with a probability \( p = 1 \). For \( \Delta \chi^2 > 0 \), the probability for accepting the configuration is then given by \( P = \exp(-\Delta \chi^2) / a \). The parameter \( a \) formally corresponds to an annealing temperature. It was gradually decreased from 0.3 to 0.03 during one run. After equilibration typically reached with a total of 50,000 iterations, \( \chi^2 \) did not further decrease in the mean but was still fluctuating. Five configurations of the molecules were saved, each after an additional 100,000 iterations. The whole procedure was repeated 20 times/data set with different seeds of the random number generator, giving a total of 100 models for oxygenated and deoxygenated KLH1. The speed of searching in the total configuration space was increased by randomizing the distribution of points and their scattering amplitudes from time to time. Prominent features of the KLH1 molecule were present in all of the individual models, but at a smaller scale artificial density, fluctuations showed up. To reduce the statistical fluctuations, all of the individual models were superimposed and averaged density distributions were calculated for oxygenated and deoxygenated KLH1 molecules. Because small-angle data contain no information regarding the handedness of the molecule, each model was also tested with inverted coordinates and two enantiomorphous classes were created and averaged individually. To compare the different structures for oxygenated and deoxygenated KLH1, correlation coefficients were calculated for the two different combinations of the enantiomorphous class averages. Based on these values, the more similar enantiomers were selected for comparison of oxygenated and deoxygenated KLH1 in Fig. 2. This procedure compares only the relative handedness of the two different enantiomorphous classes. The absolute handedness was selected in such a way that the models, at least the model for deoxygenated KLH1 (compare “Results” and “Discussion”), are more similar to the transmission electron microscopy (TEM) reconstruction (11).

The mean standard deviations \( \sigma \) for the averaged density distributions and the difference density were derived in the following manner. First, for each grid point, a standard deviation \( \sigma_i \) was calculated from the deviations of the individual densities \( \rho_{m,i} \) of the M models from the mean density \( \bar{\rho}_i \) as shown in Equation 3a.

\[
\sigma_i = \frac{1}{M} \sum_{m=1}^{M} (\bar{\rho}_i - \rho_{m,i})^2 \quad (3a)
\]

For the mean standard deviation \( \sigma \), we used a root-mean-square average over all of the \( N \) grid points with density \( \bar{\rho} \) as given in Equation 3b.

\[
\sigma = \left( \frac{1}{N} \sum_{i=1}^{N} \sigma_i^2 \right)^{1/2} \quad (3b)
\]

The mean ± S.D. of the difference density (Fig. 2e) was calculated by error propagation from the \( \sigma \) values for oxygenated and deoxygenated KLH1, \( \sigma_{\text{diff}} = \sqrt{\sigma_{\text{oxy}}^2 + \sigma_{\text{deoxy}}^2} \).

We would like to mention that the above definitions of error levels are conservative choices. Using Equation 3b, all of the grid points contributing to the original model volume and not only grid points with density greater zero would lower the \( \sigma \) value. Furthermore, an average of \( \sigma \) would give a lower value for \( \sigma \) than the root of the mean of variances (Equation 3b). In addition, we would like to point out that a different definition for \( \sigma \) is used in crystallographic papers where the error level is not calculated from different models but is estimated from the variation within one density distribution or difference density distribution, respectively.

RESULTS AND DISCUSSION

We investigated a solution of KLH1 by SAXS in the absence and presence of molecular oxygen. The minima and maxima in the scattering curves are less developed for the oxygenated form, and they are shifted to higher \( q \) values by 1–2% (Fig. 1a). In the case of cylindrically shaped molecules, deeper minima correspond to a hollower cylinder, whereas shallow minima indicate a more filled cylinder. Thus, a comparison of the experimental intensities of deoxy-KLH1 and oxy-KLH1 revealed changes in the quaternary structure upon oxygenation, indicating that oxy-KLH1 is more compact. The difference between the scattering curves of the oxy- and deoxy-KLH1 is shown in Fig. 1b. The differences are well out of the range of experimental error. As a thorough test, the differences in the scattering curves were diminished by least squares fit of one scattering curve (oxy) to the other (deoxy) with a variable contribution of scattering from protein, buffer, empty cuvette, and background as fit parameters. The differences in the scattering curves of the oxygenated and deoxygenated protein decreased only slightly by this fit, thus they can be regarded as highly significant.

The demonstrated existence of different conformational
states of KLH was expected from the fact that KLH with its 160
oxygen binding centers binds dioxygen, cooperatively serving
as an oxygen carrier in vivo (14).

The radii of gyration were determined from the SAXS data to be 163.8 ± 0.3 and 164.7 ± 0.3 Å for oxygenated and deoxy-
genated KLH1, respectively. These values are in good agree-
ment with the values recently determined by small-angle neu-
tron scattering, 163.7 ± 0.5 Å for oxygenated and 165.0 ± 0.6 Å for deoxygenated KLH1 (32). Again, KLH1 seems to be
slightly more compact in the oxygenated than in the deoxygen-
ated state. Indirect Fourier transformation of the intensities
gave distance distribution functions with a maximum size of d_{max} for KLH1 of ~430 ±10 Å for both oxygenation states.

To be able to compare direct experimental results from SAXS with the three-dimensional model obtained by TEM of stained KLH1 samples (11), we used the electron density distribution of the TEM model (kindly provided by Orlova et al. (11)) to calculate the radius of gyration, distance distribution function, and small-angle scattering intensity for the TEM model. Standard procedures were applied for these calculations (24, 26). The scattering curve was calculated with the Debye equation. The distance distribution function from the spherically averaged
auto-correlation function of the density distribution and the
radius of gyration was calculated with Equation 4a (see below)
in which the scattering amplitudes have to be replaced with
density values in this case. A comparison of the simulated scattering curve (data not shown) with the SAXS intensities showed that the minima and maxima of the scattering curve calculated from the TEM model are shifted to lower q values by a factor of 0.88, which is equivalent to an increase in real space dimensions of ~13%. This is reflected by the maximum dis-
tance taken from the distance distribution function. We deter-
mined a maximum distance of 490 Å for the TEM model, ~14% larger than those obtained with SAXS. For the radius of gyra-
tion of the TEM model, we calculated a value of approximately
185 Å, which is 12% larger than the values obtained with
small-angle scattering. This comparison reveals that the KLH1
molecule is smaller in solution than in the stained form when studied by TEM.

Modeling of Oxygenated and Deoxygenated KLH1—Based on
the SAXS data, we constructed models of KLH1 applying a new
developed MCSAS. The well established D5 symmetry of moli-
lluscan hemocyanins (11) was applied to all of the models. Fig.
2 shows three-dimensional surface representations of KLH1 in

\[ \frac{d_{\max}}{q} \]

Fig. 1. SAXS experiments of KLH1 in presence and absence of oxygen. a, experimental (deconvoluted) small-angle scattering of oxygenated KLH1 (filled cir-
cles), deoxygenated KLH1 (open circles), and the Monte Carlo fits (lines). The in-
tensities are on an arbitrary scale and separated from each other for sake of clar-
ity. b, relative difference between the scattered intensities for deoxygenated and oxygenated KLH1 (detector distance 3.6 m). In contrast to part a, the intensi-
ties have not been corrected for instrumental smearing. The propagated exper-
imental means ± S.D. are shown for a few points.
the oxygenated and deoxygenated state. Surprisingly, the shapes of the surface are rather different for the oxy and deoxy forms. In contrast to oxygenated KLH1, the deoxygenated molecule shows a pronounced chirality. Five bulges extending over the whole length of the molecule are twisted around the cylindrical axis. This finding is in agreement with the first electron microscopical pictures of a snail hemocyanin 30 ago (33). No mass was detected at the center of the cylinder (Fig. 2d) as discussed previously (34). In addition, in both oxygenation states masses are concentrated at the two ends of the cylindrical molecules, forming the internal “collars.”

To reveal differences between the modeled structures with respect to the length and the radius of the cylindrical KLH1 molecules, the radius of gyration as shown in Equation 4a,

$$ R_g = \sqrt{\frac{\sum_{i=1}^{N} f_i r_i^2}{\sum_{i=1}^{N} f_i}} $$  \hspace{1cm} (4a) 

was split in a component $R_z$ along the axes of the cylinder as shown in Equation 4b,

$$ R_z = \sqrt{\frac{\sum_{i=1}^{N} f_i z_i^2}{\sum_{i=1}^{N} f_i}} $$  \hspace{1cm} (4b) 

and a radial component $R_{xy}$ as shown in Equation 4c.

$$ R_{xy} = \sqrt{\frac{\sum_{i=1}^{N} f_i (x_i^2 + y_i^2)}{\sum_{i=1}^{N} f_i}} $$  \hspace{1cm} (4c) 

Here $r_i$ denotes the center of mass distances of the N scattering points, $x_i$, $y_i$, and $z_i$ are the components of the distance vectors, and $f_i$ is the scattering amplitude. It should be mentioned that for a model consisting of scattering points, Equation 4a is equivalent to the commonly used calculation of $R_g$ from the second moment of the electron density distribution (24) where the scattering amplitudes in Equation 4a are replaced by electron densities at grid points. By averaging the values of all of the individual models, we calculated the mean values and standard deviations for the model $R_z$ and its components. For the deoxygenated molecule, we determined the values to $R_z = 105.8 \pm 1.5 \text{ Å}$ and $R_{xy} = 126.7 \pm 1.1 \text{ Å}$ and for the oxygenated molecule to $R_z = 108.6 \pm 1.3 \text{ Å}$ and $R_{xy} = 122.9 \pm 1.0 \text{ Å}$. Thus, the mass-weighted length of the modeled oxy-KLH1 molecule is greater than that for the deoxygenated form, whereas the mean diameter is smaller. Qualitatively, this change of the overall structure is also reflected in difference density maps (Fig. 2e). While in the wall region of the cylindrical molecule, the deoxy model dominates the difference density (Fig. 2e, side view) and the density of oxygenated KLH1 is higher at the ends of the cylinder (top view). For the total radius of gyration averaged over the individual MC models, we calculated values of $R_g = 165.1 \pm 0.4 \text{ Å}$ for deoxygenated and $R_g = 164.0 \pm 0.3 \text{ Å}$ for oxygenated KLH1, which are in good agreement with the experimental values.

The reconstructed models of KLH1 (Fig. 2) provide important information regarding the flexible in-solution structure. Although the changes of the overall dimensions of KLH1 are only $\sim 3\%$, a large translocation of masses occurs at the surface of the molecule during the conformational switch. The transition can be explained in the following way. In the deoxygenated molecule, the rod-shaped parts of the subunits forming the wall of the cylinder are inclined relative to the axes of the cylinder, producing the helical surface pattern (Fig. 2a). In oxygenated KLH1, the two decameric non-covalently and isologously associated half-molecules (11) are twisted against each other along the 5-fold symmetry axis, reducing the inclination angle of the subunits. Because of the more parallel arrangement of the subunits with respect to the axes of the cylinder, the length of the cylinder is increased accompanied by a reduction of the diameter. In this way, the helical surface pattern changes to a shape with a striped pattern (Fig. 2b).

Our study provides the first real evidence as to how a molluscan hemocyanin changes during an allosteric transition. Thus, one can really look at allosteric transitions of these large proteins, which were inaccessible or not interpretable by other techniques.

The surface of the 15-Å three-dimensional reconstruction of KLH1 based on TEM (11) shows a similar helical pattern as we
deduced for the deoxy form from the SAXS data. This is surprising because for the TEM measurement KLH1 was stained in presence of atmospheric oxygen. It seems that exposure of specimen to high vacuum in the TEM experiments results in a rapid release of the reversibly bound oxygen within milliseconds as reported for the molluscan hemocyanin HtH1 (35). Therefore, KLH1 molecules contributing to the three-dimensional reconstruction by TEM are most probably in the deoxy state. This seems to be the case for all of the molluscan hemocyanins investigated by electron microscopy (11, 34). Thus, SAXS may be considered as a very useful complementary method to TEM.

As a consequence of these conformational transitions of KLH1 molecules, a strong influence on surface located epitopes is thought to be involved, which may result in differences in the immunological properties of this molecule extensively used in biotechnology and therapeutic medicine. A strong influence of small changes on the surface of proteins such as conformational switches on the effectiveness of epitopes is indeed well documented for other proteins such as myoglobins (36, 37). However, this was never investigated or even considered for KLH. Antigenic properties are known to change drastically on the surface of proteins such as conformational changes on the surface of proteins such as conformational transitions of KLH1 molecules, a strong influence on surface located epitopes may be different in the oxygen and deoxy form of the KLH1 structure and the accessibility of any epitopes on the surface late a similar effect for KLH1. It is easy to imagine that the rapid release of the reversibly bound oxygen within milliseconds as reported for the molluscan hemocyanins investigated by electron microscopy (11, 34).

REFERENCES
1. Cuff, M. E., Miller, K. I., van Holde, K. E., and Hendrickson, W. A. (1998) J. Mol. Biol. 276, 855–870
2. Magnus, K. A., Hazes, B., Ton-That, H., Bonaventura, C., Bonaventura, J., and Hol, W. G. (1994) Proteins 19, 302–309
3. van Holde, K. E., and Miller, K. I. (1995) Adv. Protein. Chem. 47, 1–81
4. Decker, H., and Sterner, R. (1990) J. Mol. Biol. 211, 281–283
5. Ellerton, H. D., Ellerton, N. F., and Robinson, H. A. (1983) Prog. Biophys. Mol. Biol. 41, 143–249
6. Harris, J. R., and Markl, J. (1999) Micron 30, 591–623
7. Jurincic-Winkler, C. D., von der Kammer, H., Beuth, J., Scheit, K. H., and Klippel, K. F. (1996) Anticancer Res. 16, 2105–2110
8. Livingston, P., Zhang, S., Adlari, S., Yao, T. J., Graeber, L., Ragupathi, G., Helling, F., and Fleisher, M. (1997) Cancer Immunol. Immunother. 43, 244–330
9. Lamm, D. L., DeHaven, J. I., Riggs, D. R., and Ebert, R. F. (1995) J. Urol. 149, 648–652
10. Wierczyn, I., Suturkova-Milosevic, L., Briani, C., and Latov, N. (1995) Cancer Immunol. Immunother. 40, 307–310
11. Orlova, E. V., Dube, P., Harris, J. R., Beckman, E., Zemlin, F., Markl, J., and van Heel, M. (1997) J. Mol. Biol. 271, 417–437
12. Harris, J. R., Gebauer, W., Sohngen, S. M., and Markl, J. (1995) Micron 26, 201–212
13. Sohngen, S. M., Stahlmann, A., Harris, J. R., Muller, S. A., Engel, A., and Markl, J. (1997) Eur. J. Biochem. 248, 602–614
14. Swerdlow, R. D., Ebert, R. F., Lee, P., Bonaventura, C., and Miller, K. I. (1996) Comp. Biochem. Physiol. 113, 537–548
15. Senn, M. N., and Briggs, M. (1989) Comp. Biochem. Physiol. 94, 195–200
16. Lieb, B., Altenheim, B., Markl, J., Vincent, A., van Olden, E., van Holde, K. E., and Miller, K. I. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 4546–4551
17. Miller, K. I., Cuff, M. E., Lang, W. F., Varga-Weisz, P., Field, K. G., and van Holde, K. E. (1998) J. Mol. Biol. 278, 827–842
18. Perbandt, M., Guthohrlein, E. W., Rypniewski, W., Idakieva, K., Stoeva, S., Voelter, W., Genov, N., and Betzel, C. (2003) Biochemistry 42, 6341–6346
19. van Kuik, J. A., van Halbeek, H., Kamerling, J. P., and Vliegenthart, J. F. (1985) J. Biol. Chem. 260, 1384–1395
20. Stoeva, S., Schutz, J., Gebauer, W., Hundsdoerfer, T., Manz, C., Markl, J., and Voelter, W. (1999) Biochim. Biophys. Acta. 1435, 94–109
21. Markl, J., Nour el Din, M., Winter-Simanzowski, S., and Simanzowski, U. A. (1991) Naturwissenschaften 78, 30–31
22. Kantelehardt, S. R., Wahrer, M., Dennis, R. D., Doenhoff, M. J., Bickle, Q., and Geyer, R. (2002) Biochem. J. 366, 217–223
23. Haubold, H. G., Gruenhagen, K., Wagener, M., Jungbluth, H., Heer, H., Pfeil, A., Rongen, H., Brandenburg, G., Moeller, R., Matzerarth, J., Hiller, P., and Halling, H. (1989) Rev. Sci. Instrum. 60, 1943–1946
24. Glattier, O., and Kratky, O. (1982) Small Angle X-Ray Scattering, Academic Press, London
25. Svergun, D. I., Koch, M. H., Pedersen, J. S., and Serdyuk, I. N. (1994) J. Mol. Biol. 240, 78–86
26. Feigl, L. A., and Svergun, D. I. (1987) Structure Analysis by Small-angle X-Ray and Neutron Scattering (Taylor, G. W., ed), Plenum Publishing Corp., New York
27. Hartmann, H., and Decker, H. (2004) in Methods Enzymol, in press
28. Henderson, S. J. (1996) Biophys. J. 70, 1618–1627
29. Chacon, P., Moran, F., Diaz, J. F., Pantos, E., and Andreu, J. M. (1998) Biophys. J. 74, 2760–2775
30. Svergun, D. I. (1999) Biophys. J. 76, 2879–2886
31. Metropolis, N., Rosenbluth, A. W., Rosenbluth, N., Teller, A. H., and Teller, E. (1953) J. Chem. Phys. 21, 1087–1092
32. Hartmann, H., Bongers, A., and Decker, H. (2001) Eur. Biophys. J. 30, 471–475
33. van Driel, R., Brunori, M., and Antonini, E. (1974) J. Mol. Biol. 89, 103–112
34. Melelina, J. E., and Klug, A. (1972) Nature 239, 146–150
35. Mesnser, U., Dube, P., Harris, J. R., Stark, H., and Markl, J. (2000) J. Mol. Biol. 298, 21–34
36. Berezovsky, J. A., and Berkower, I. J. (1984) in Fundamental Immunology (Paul, W. E., ed) pp. 595–644, Raven Press, Ltd., New York
37. Crompton, M. J. (1974) in The Antigens (Sela, M., ed) pp. 1–79, Academic Press, Orlando, FL