Localization of Proteins and tRNA Molecules in the 70S Ribosome of the Escherichia coli Bacteria with Polarized Neutron Scattering†

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(Received 23 July 1996; accepted 28 January 1997)

Abstract

Isotopic substitution methods are widely used in neutron scattering for the determination of the in situ structure of macromolecular components in quaternary structures. The contrast created by the substitution of the hydrogen isotope 1H (proton) by 2H (deuterium) is the most prominent example. A further increase of contrast by a factor of three is possible by polarized neutron scattering from polarized nuclear spins. This offers the possibility of measuring small labels, such as proteins, which contribute less than 0.5% to the whole ribosomal mass, or weakly contrasted molecules, such as tRNA ligands, in the 70S ribosomes. In this study, the positions of the proteins S6 and S10 of the Escherichia coli ribosome with respect to the whole 70S ribosome have been determined by nuclear-spin contrast variation. Furthermore, the localization of two weakly contrasted tRNA molecules bound to the pre- and post-translocational 70S ribosome, respectively, is presented. So far, no other technique has allowed the determination of the in situ structures of these molecules.

1. Introduction

Protein synthesis is one of the fundamental processes of life. Therefore, ribosomes, which are the sites for the protein synthesis in each cell, are of great interest. A large number of investigations have provided information about the composition of ribosomes and their compounds (e.g. Wittmann, 1982). Hypotheses have been presented to describe the course of protein synthesis (e.g. Nierhaus, 1990). Nevertheless, it is still not precisely known how the ribosomes actually function. For a better understanding, it is necessary to obtain the structure of the ribosome, which was done with several possible methods [electron microscopy (e.g. Frank, Penczek, Grassucci & Srivastava, 1991; Wagenknecht, Grassucci & Frank, 1988), immuno electron microscopy (IEM) (e.g. Stöffler-Meilicke & Stöffler, 1990), cross-link methods (e.g. Brimacombe et al., 1990), crystallography (e.g. Lindahl et al., 1994; Kim et al., 1974), electron microscopy on two-dimensional crystals (e.g. Yonath & Berkovitch-Yellin, 1993)]. Using small-angle neutron scattering (SANS) combined with isotopic substitution and label triangulation, a map of the positions of the proteins of the 30S subunit has been developed (Capel et al., 1987) and seven proteins of the 50S subunit have been localized (May, Nowotny, Nowotny, Voss & Nierhaus, 1992). To date, it has not been possible to localize proteins in situ in the whole 70S ribosome of the E. coli bacteria using these methods. This is because the contrast is not sufficient to visualize such small components with respect to a very large ribosomal matrix.

Apart from structure determination approaches, biochemical methods can be used to localize molecules which participate in the process of protein synthesis, such as mRNA or tRNA. The aim is to understand how the synthesis is carried out inside the ribosome. However, in the case of structure research, it has not yet been possible to find the in situ positions of the mRNA or tRNA molecules in the elongating ribosome. Conventional SANS methods also fail because mRNA and tRNA molecules are less contrasted than proteins.

Nuclear polarization-dependent small-angle neutron scattering ('spin-dependent' SANS), a method which has been developed over the past few years, could help to overcome this problem. Based on the classical methods of contrast variation and isotopic substitution, the contrast is increased by a factor of three by using polarized neutron scattering from polarized nuclear spins of biological samples. Furthermore, only one sample is needed to locate one label.

The first results of spin-dependent SANS were obtained during the localization of the proteins L3 (22.3 kDa), L4 (22.1 kDa) and L1 (24.6 kDa) in the 50S subunit of the E. coli ribosome (Zhao, 1995; Willumeit, 1996). Since these proteins contribute more than 1% of the ribosomal subunit mass (1.4 MDa), they were used to test the method of spin-dependent SANS. The next step was to localize the proteins that contribute much less than 1% of the total mass of the 70S ribosome (2.4 MDa) and was performed for the proteins S6 (15.7 kDa) and S10

† This paper was presented at the Tenth International Conference on Small-Angle Scattering, Campinas, Brazil, 21–26 July, 1996.
(11.7 kDa) in the 70S ribosome of E. coli and will be presented here.

An even more challenging problem is the elucidation of the sites of the two tRNA molecules during protein synthesis. Using spin-dependent SANS, ribosomes in the pre- and post-translocational state were analysed. The results will also be shown here.

2. Materials and methods

2.1. General principles of spin-dependent SANS

Omitting magnetic scattering, the interaction of neutrons with matter arises from the interaction between the incident neutron and the nucleus. The strength of this interaction is expressed by scattering amplitudes $b_0$ and $B$, which depend on the isotope and its spin state. $b_0$ is the isotopic nuclear scattering length of an ensemble of unpolarized nuclei and $B$ is a measure of the polarization-dependent amplitude (spin-dependent scattering length).

A pronounced change in the scattering lengths is found for the two isotopes of hydrogen. For $^1$H (protons), $b_0 = -0.374 \times 10^{-12}$ cm, while it is $0.667 \times 10^{-12}$ cm for $^2$H (deuterons). A very large $B$ value, $5.824 \times 10^{-12}$ cm, is encountered for protons, while it is $0.57 \times 10^{-12}$ cm for deuterons. With polarized neutron scattering by polarized proton spins, the large $B$ leads to impressive results: if the spins of the neutrons and $^1$H are parallel, the scattering length is equal to $1.08 \times 10^{-12}$ cm; in the antiparallel case it is equal to $-1.83 \times 10^{-12}$ cm. The other nuclei that are present in a biological sample show very little change in the scattering length with spin polarization (see Table 1).

The large $B$ of $^1$H is also the reason for the strong incoherent scattering [scattering cross section 79.9 b (1 barn = $10^{-28}$ m$^2$)]; it is much smaller using $^2$H (2 b). Samples are therefore largely deuterated. For ribosomes, this means that only a small part of the ribosome remains protonated (e.g. the protein S6 in the deuterated 70S ribosome).

For experiments using nuclear spin-contrast variation, an instrument for polarized neutron scattering, which is the small-angle scattering facility SANS1 (Zhao & Stuhrmann, 1993) and a special sample environment are required. A prototype has been installed at the GKSS research reactor FRG1, Geesthacht (see Fig. 1). To polarize nuclear spins, a high magnetic field and very low temperatures are necessary. The main parts of the SANS1 target station at GKSS are thus a 2.5 T electromagnet and a $^3$He/$^4$He dilution refrigerator, originally designed for high-energy physics experiments by CERN, Geneva. Inside the sample cell, temperatures of 130–150 mK can be reached. To obtain high nuclear spin polarization in a short time, the nuclear spins are polarized dynamically [dynamic nuclear polarization, DNP (Abragam & Goldman, 1982)]. This technique requires that the sample is doped with paramagnetic centres [sodium bis(2-ethyl-2-hydroxybutyrate)oxochromate(V) monohydrate, EHBA-Cr(V)]. The spins of the nuclei are aligned in one direction with respect to the external magnetic field by irradiating the frozen sample with 4 mm microwaves corresponding to a frequency of 69 GHz. The fine tuning of the frequency specifies whether the nuclear spins are aligned parallel or antiparallel with respect to the external magnetic field. Although the nuclear spins of all nuclei are affected by DNP, only those of $^1$H and $^2$H are significantly changed. The polarization is determined by measuring the integrated continuous-wave nuclear magnetic resonance (NMR) absorption signal using a series-tuned Q-meter circuit. Since the data treatment is simplified if only one isotope has been polarized, the same NMR system is used to destroy any unwanted polarization from other isotopes. Thus, only $^1$H spins (proton spin target) or only $^2$H spins (deuteron spin target) are kept polarized in the sample during the measurements.

2.2. Sample preparation

The preparation of the ribosomes was carried out by the group of Professor K. H. Nierhaus, MPI of Molecular Genetics, Berlin. Deuterated and protonated ribosomes and tRNA molecules were obtained from two strands of E. coli bacteria grown in deuterated and protonated media. A protonated short mRNA fragment (46 nucleotides) was synthesized by transcription of a defined gene. For preparation of the deuterated 70S with protonated proteins, the proteins of the small subunit (30S) were separated in protonated and deuterated form. In the next step, the set of deuterated proteins with one or two proteins missing were incubated with the protonated version of the missing protein. Self-assembly led to a 30S subunit which was bound to a native 50S subunit to form a 70S ribosome [for a detailed description see the work of Burkhardt (1996)].

The preparations of the pre- and post-translocational ribosomes loaded with tRNA and mRNA molecules were far more complicated; for a description of the techniques the reader should look elsewhere (Wadzack, 1994).

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Spin</th>
<th>$b_0$</th>
<th>$B$</th>
<th>$\uparrow\uparrow$</th>
<th>$\uparrow\downarrow$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>$\uparrow$</td>
<td>-0.374</td>
<td>5.824</td>
<td>1.08</td>
<td>-1.83</td>
</tr>
<tr>
<td>$^2$H</td>
<td>$\uparrow$</td>
<td>0.665</td>
<td>0.57</td>
<td>0.94</td>
<td>0.38</td>
</tr>
<tr>
<td>$^{12}$C</td>
<td>$\uparrow$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$^{14}$N</td>
<td>$\uparrow$</td>
<td>0.937</td>
<td>0.28</td>
<td>1.08</td>
<td>0.80</td>
</tr>
<tr>
<td>$^{16}$O</td>
<td>$\uparrow$</td>
<td>0.580</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$^{31}$P</td>
<td>$\uparrow$</td>
<td>0.51</td>
<td>0.44</td>
<td>0.72</td>
<td>0.40</td>
</tr>
<tr>
<td>$^{32}$S</td>
<td>$\uparrow$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The symbol $\uparrow\uparrow$ indicates that neutron and nuclear spins are parallel to each other ($P_r P_i = 1$); $\uparrow\downarrow$ is the symbol used for an antiparallel spin orientation ($P_r P_i = -1$).
Table 2. Properties of the samples (Pre = pre-translocational ribosome, Post = post-translocational ribosome)

<table>
<thead>
<tr>
<th></th>
<th>D70S (S6)</th>
<th>D70S (S10)</th>
<th>D70S (S6S10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal of the deuterated protein</td>
<td>&gt;95%</td>
<td>&gt;90%</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Substitution by the protonated protein</td>
<td>&gt;95%</td>
<td>90 (5%)</td>
<td>&gt;90-95%</td>
</tr>
<tr>
<td>Biological activity compared to native particles:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tested with rRNA binding</td>
<td>72%</td>
<td>74%</td>
<td>72%</td>
</tr>
<tr>
<td>Tested with peptidytransferase</td>
<td>77%</td>
<td>90%</td>
<td>81%</td>
</tr>
<tr>
<td>Label (deuterated tRNA)</td>
<td>Pre</td>
<td>Post</td>
<td></td>
</tr>
<tr>
<td>Substitution by the protonated tRNA</td>
<td>48%</td>
<td>49%</td>
<td></td>
</tr>
<tr>
<td>Second tRNA bound to the A site</td>
<td>87%</td>
<td>23%</td>
<td></td>
</tr>
<tr>
<td>Second tRNA bound to the P site</td>
<td>13%</td>
<td>77%</td>
<td></td>
</tr>
</tbody>
</table>

All ribosomes were checked for their biological activity and the degree of substitution by the protonated label was determined (see Table 2).

The ribosome solutions were kept frozen until they were used to prepare the target material. During preparation of the functional tRNA complex targets, the ribosomal solutions were kept on ice, since ribosomes with the complex of mRNA and tRNA are particularly unstable.

For the sample solution, about 13 mg of sodium bis(2-ethyl-2-hydroxybutyrato)oxochromate(V) monohydrate \{Na[Cr(C₆H₁₀O₃)₂O]H₂O; EHBA-Crv\} were mixed with 150 mg of D₂O. In another glass tube, 820 mg of deuterated glycerol (deuteration 98.7 \% \( w/v \)) were diluted with 50 μl of 166 mmol MgCl₂ and 1660 mmol KCl in D₂O. The addition of this mixture is necessary because 70S ribosomes are very sensitive to changes in ionic conditions. The deuterated ribosomal solution (70S ribosomes in D₂O, about 500 mg, \( pD \) 7.9) was then added to the glycerol solution. This procedure prevents the 70S ribosome from dissociating into its subunits. During the final step, the concentrated Crv solution was rapidly diluted in the ribosomal solution and the mixture frozen within 10 s using a liquid-nitrogen-cooled brass mould. This procedure resulted in a dark red transparent glassy sample plate with dimensions 17 × 17 × 3 mm.

2.3. Data collection and data treatment

The measurements of the deuterated 70S with one or two protonated proteins were performed over a week. The data collection for the 70S ribosomes with the functional complex lasted 2 weeks. During this time, data sets were accumulated at three different detector-to-sample distances (0.7, 1.8 and 4.5 m). The samples were measured with unpolarized nuclear spins and polarized proton spins (proton spin target). To determine the position of the protein S6 and the tRNA molecules within the 70S ribosome, the deuteron spin target was also measured. During the data accumulation with polarized nuclear spins, the relative orientation of the neutron and hydrogen spins was parallel or antiparallel. The data were accrued with respect to the detector-to-sample distance, the polarization of the sample and the flipper state. From the two-dimensional scattering pattern recorded by a position-sensitive area detector, the one-dimensional intensity curves were calculated. After subtraction of solvent and incoherent scattering effects, the three basic scattering functions were derived. The data sets were corrected for the influence of wavelength distribution and collimation.

The basic scattering functions contribute to the total intensity of spin-dependent neutron scattering in the following way (Abragam & Goldman, 1982):

\[
I(Q) = |U(Q)|^2 + 2P_n \text{Re}[U(Q)V'(Q)] + |V'(Q)|^2, \tag{1}
\]

where \( P_n \) is the neutron polarization, \( Q = |Q| = 4\pi \sin \theta / \lambda \), with \( \theta = \) half the scattering angle, \( U(Q) \) is the scattering amplitude which does not depend on the nuclear spin polarization and \( V(Q) \) is the spin-dependent scattering amplitude. \( P_n \) only influences the cross term \( \text{Re}[U(Q)V'(Q)] \). \( |U(Q)|^2 \) is the scattering intensity obtained from the un polarized target. The spin polarization of the nuclear spins \( P_I \) is included in \( V(Q) \) and influences both the cross term \( \text{Re}[U(Q)V'(Q)] \) and \( |V(Q)|^2 \). \( |V(Q)|^2 \) varies with \( P_I^2 \). The cross term \( \text{Re}[U(Q)V'(Q)] \) is proportional to \( P_I \). The basic scattering functions are calculated (Knop et al., 1991) from the measurements using unpolarized nuclear spins and those using parallel or antiparallel neutron and nuclear spins.

To determine the position of a label with respect to the 70S ribosome, the data analysis starts from a model. The calculated scattering curves obtained from the model are fitted to the measured data. The model is based on all available structural information about the ribosome. This information consists of the shape of the 70S ribosome as determined by electron microscopy (Frank, Penczek, Grassucci & Srivastava, 1991) and the most likely distribution of the protein and the rRNA phase. The label is assumed to be a sphere in the first approximation. For the localization of the tRNA molecules within the ribosome, the shape of the tRNA molecules as determined by crystallography (e.g. Kim et al., 1974) can also be used.

During the calculation of the theoretical scattering curves, it is considered that each scattering amplitude \( U(Q) \) and \( V(Q) \) is the sum of the partial scattering amplitudes of each phase [total ribosomal proteins (TP), tRNA and label]. This means, for example,

\[
U(Q) = [c_{TP} U_{TP}(Q)] + [c_{tRNA} U_{tRNA}(Q)] + [c_{label} U_{label}(Q)], \tag{2}
\]

where \( c_{TP} \), \( c_{tRNA} \) and \( c_{label} \) are the known contrasts in the
unpolarized case. To calculate the partial scattering amplitudes \( U_{TP}(Q) \), \( U_{RNA}(Q) \) and \( U_{label}(Q) \), the multipole expansion is used (see e.g. Stuhrmann, 1993). The scattering amplitude \( V(Q) \) is developed in a similar way.

Each partial amplitude \( U_{TP}(Q) \) and \( U_{RNA}(Q) \) (and respectively for \( V \)) is calculated once from the electron-microscopy model. The label amplitudes \( U_{xabe\[l]t}(Q) \) and \( V_{lab\[l]}(Q) \) are calculated during the fitting procedure. The best least-squares fit should give the position of the label. In this fit, the parameter

\[
R = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(I_{\text{measured},i} - I_{\text{calculated},i})^2}\]

is minimized for 38 intervals of \( Q \) in the range 0.015–0.2 and for all basic scattering functions.

The meaning of the result depends on the validity of the ribosome model with respect to the partial amplitudes \( U \) and \( V \). This raises the question whether the ribosome in frozen solution has the same structure as at room temperature. For this purpose, the scattering curves of the 70S particle at the two temperatures were compared. There is good agreement among the corresponding curves. The radii of gyration, \( R_g \), were determined to be 92 (1) Å for the 70S ribosome and 81 (1) Å for its rRNA (Stuhrmann et al., 1995).

### 3. Results and discussion

#### 3.1. Localization of the proteins S6 and S10 in the 70S E. coli ribosome

The following samples were investigated: (i) deuterated 70S E. coli ribosomes with the protein S6 protonated [D70S(S6)]; (ii) deuterated 70S E. coli ribosomes with the protein S10 protonated [D70S(S10)]; (iii) deuterated 70S E. coli ribosomes with the proteins S6 and S10 protonated [D70S(S6S10); for details of preparation see Burkhardt (1996)].

In principle, it should be possible to determine the position of a protein from only one sample. However, since the shape of the 70S ribosome is almost spherical it is difficult to find a unique solution. Therefore, the possible positions for S6 and S10 were determined independently from D70S(S6) and D70S(S10) and compared with the data obtained from the doubly labelled 70S ribosome D70S(S6S10).

For the protein S6, the best \( R \) value for the proton spin target was 1.829. The distance between this protein and the centre of mass of the 70S ribosome was found to be 104 (2) Å. The radius of gyration of S6 was determined to be 15.6 (5) Å. This is in good agreement with other neutron scattering measurements of S6 in the 30S subunit [14 (1) Å; Capel et al., 1987]. The coordinates of the best positions are given in Table 3. Other possible solutions can be obtained from a ‘minimum map’. This map presents the \( R \) values in a polar coordinate system up to distance \( r = 130 \) Å (in 5 Å steps) and for \( \theta \) from 0 to 180° in 10° steps and for \( \phi \) from 0 to 360° in 20° steps. As an example, see Fig. 2. Beside the position with a minimal \( R \)

![Fig. 1. The polarized target station at the beamline SANS1, GKSS, Geesthacht (Zhao & Stuhrmann, 1993).](image)

![Fig. 2. Minimum map for the protein S6.](image)

<table>
<thead>
<tr>
<th>Protein</th>
<th>( x (\text{Å}) )</th>
<th>( y (\text{Å}) )</th>
<th>( z (\text{Å}) )</th>
<th>( r (\text{Å}) )</th>
<th>( \theta (\circ) )</th>
<th>( \phi (\circ) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>S6 (PT)</td>
<td>-22 (1)</td>
<td>-65 (3)</td>
<td>-78 (2)</td>
<td>104 (2)</td>
<td>2.419</td>
<td>4.392</td>
</tr>
<tr>
<td>S6 (DT)</td>
<td>-24 (1)</td>
<td>-60 (3)</td>
<td>-81 (2)</td>
<td>104 (2)</td>
<td>2.470</td>
<td>4.336</td>
</tr>
<tr>
<td>S10 (PT)</td>
<td>103 (12)</td>
<td>3.5 (1)</td>
<td>-51 (10)</td>
<td>115 (4)</td>
<td>2.0265</td>
<td>0.034</td>
</tr>
<tr>
<td>S10</td>
<td>80 (12)</td>
<td>3.5 (1)</td>
<td>-71 (10)</td>
<td>107 (4)</td>
<td>2.298</td>
<td>0.044</td>
</tr>
<tr>
<td>S10 average</td>
<td>92 (12)</td>
<td>3.5 (1)</td>
<td>-61 (10)</td>
<td>111 (4)</td>
<td>2.163</td>
<td>0.039</td>
</tr>
<tr>
<td>S6S10 (PT)</td>
<td>-13 (3)</td>
<td>-61 (6)</td>
<td>-73 (5)</td>
<td>104 (3)</td>
<td>2.444</td>
<td>4.451</td>
</tr>
<tr>
<td>S6</td>
<td>99 (1)</td>
<td>-6 (1)</td>
<td>-37 (6)</td>
<td>103 (3)</td>
<td>1.8769</td>
<td>-0.065</td>
</tr>
<tr>
<td>tRNA</td>
<td>Pre</td>
<td>-13</td>
<td>-54</td>
<td>-12</td>
<td>57</td>
<td>1.780</td>
</tr>
<tr>
<td>Post</td>
<td>-15</td>
<td>-66</td>
<td>-15</td>
<td>68</td>
<td>1.676</td>
<td>4.486</td>
</tr>
</tbody>
</table>
value, other regions of slightly higher $R$ values are found. These imply that there are three regions where $S_6$ could be located (Willumeit, 1996). From the single proton spin target data of D70S(S6), no unique solution could be found.

A first test to verify the best position of $S_6$ was the inclusion of the deuteron spin target. This analysis of the deuteron spin target gave the same distance to the centre of mass [$104(2)$ Å], the same radius of gyration [$16.0(5)$ Å] and almost an identical position for $S_6$ (see Table 3). The two positions are about 6 Å apart. However, as in the case of the proton spin target, the solution is not unique (Willumeit, 1996). To determine precisely the position of $S_6$, it was necessary to take into account the data of D70S(S6S10).

Before considering the D70S(S6S10) sample, the $S_{10}$ derivative will be discussed. The data analysis of D70S(S10) was based only on the proton spin target. The best $R$ value was 1.635 (for coordinates see Table 3). The distance between the centre of mass of the 70S ribosome and the protein $S_{10}$ was 111 (4) Å and the radius of gyration of $S_{10}$ was 17 (1) Å. Measurements of $S_{10}$ in the 30S subunit gave a value for the radius of gyration of 13 (6) Å (Capel et al., 1987). As in the case of $S_6$, the solution for the position of $S_{10}$ was not unique, as indicated from the minimum map (Willumeit, 1996). A second-best $R$ value of 1.674 gave an alternative position for $S_{10}$, 31 Å from the first position (for coordinates see Table 3). The distance between the most likely position of $S_6$ and the most likely position of $S_{10}$ was calculated to be 131 (10) Å. From measurements of the 30S subunit, the distance between these two proteins was determined to be 133 Å (Capel et al., 1987).

The data analysis of the proton spin target for D70S(S6S10) excluded solutions found in the previous data analysis. The best fit ($R_{\text{min}} = 2.314$) occurred for the most likely positions found by the D70S(S6) and D70S(S10) calculations. The coordinates determined independently for $S_6$ and $S_{10}$ changed slightly (see Table 3) but remained in the same region of the ribosome (see Fig. 3). The distance of the centre of mass of the ribosome from $S_6$ was determined to be 103 (3) Å; the corresponding distance from $S_{10}$ was 104 (3) Å. The distance between the two proteins was calculated to be 130 (8) Å for D70S(S6S10). The radii of gyration for $S_6$ and $S_{10}$ were determined to be 13 (3) and 12 (3) Å, respectively.

As an example, Fig. 4 compares the basic scattering functions $R e(U(Q)V(Q))$ for all three samples. A detailed discussion of the other basic scattering functions is given elsewhere (Willumeit, 1996).


Fig. 4. Comparison of the basic scattering function $R e(U(Q)V(Q))$ for the samples D70S(S6), D70S(S10) and D70S(S6S10). Lines: basic scattering function, least-squares fit. Blue circles: basic scattering function $R e(U(Q)V(Q))$, measurement D70S(S6). Red circles: basic scattering function $R e(U(Q)V(Q))$, measurement D70S(S10). Green squares: basic scattering function $R e(U(Q)V(Q))$, measurement D70S(S6S10). The scattering functions have been multiplied by $-1$. The unit of intensity is $10^{-3}$ n s $^{-1}$ (sample-to-detector distance 0.70 m, collimation 2 m, wavelength 8.5 Å, pixel size of the detector 9 $\times$ 9 mm).

Fig. 5. The positions of tRNA molecules as determined by spin-contrast variation with respect to the 70S ribosome model (Frank, Penczek, Grassucci & Srivastava, 1991).
3.2. Localization of tRNA molecules in the 70S E. coli ribosome

To determine the positions of the two tRNA molecules (50 kDa) in the 70S ribosome, the following samples were prepared: (i) deuterated 70S E. coli ribosome, in a pre-translocational state with a short protonated mRNA and protonated tRNA molecules; (ii) deuterated 70S E. coli ribosome, in a post-translocational state with a short protonated mRNA and protonated tRNA molecules [for details of preparation see the work of Wadzack (1994)].

Unlike the localization of proteins in a matrix where the label is assumed to be a sphere, the data analysis started from a simple model of the shape of the tRNA molecule. In this model, the tRNA is assembled by four spheres representing an ‘L’ according to the overall structure of tRNA as determined by crystallography (e.g. Kim et al., 1974). The long arm is made of three spheres with the anticodon stem at one end. The short arm represents the CCA end and comprises one sphere with another sphere shared with the long arm (see Fig. 5). The allosteric three-site model (Nierhaus, 1990) states that two tRNA molecules are bound to the ribosome during the elongation process. From the functional point of view, the extremes of the two L-shaped tRNA molecules have to be close together (anticodons and CCA end). This is the only restriction for the arrangement of the tRNA molecules in the applied model. There are no similarly strict limits for the angle between the two tRNA molecules or the direction of the tRNA molecules in the ribosome. The 46 nucleotides of the protonated mRNA (12.9 kDa, about 20% of the tRNA–mRNA complex) were not taken into account, since no information about the configuration of the mRNA in the ribosome was available at that stage of data processing. The inclusion of the mRNA is likely to have a small influence on the coordinates of the tRNA molecules given here. A more detailed model taking into account the influence of the mRNA is presently being developed and will be published elsewhere.

The data analysis was performed as described above. The neutron scattering data confirmed that two tRNA molecules are bound to the ribosome (Stuhrmann et al., 1995).

For the pre-translocational state, the minimum R value was 1.56. The distance to the centre of mass was 57 Å. The coordinates of the position are shown in Table 3. To verify that the position is unique, a minimum map was calculated. Even up to 7.5% difference of the best R value there were still no other minima found (Wadzack, 1994).

The position of the post-translocational tRNA molecules is 12 (4) Å from the pre-translocational position. This coincides with the length of one codon and therefore with the movement by one codon during the elongational cycle. The distance to the centre of mass increased to 68 Å (see Table 3). As in the case of the pre-state, the minimum map did not show any other possible positions (Wadzack, 1994).

The calculated direction of the tRNA molecules was such that the anticodon stems were towards the 30S subunit and the CCA ends towards the 50S subunit, which is in agreement with the established biochemical view (see Fig. 5).

4. Conclusions

The method of spin-contrast variation or ‘spin-dependent’ SANS was successfully used to localize proteins and tRNA molecules in the 70S ribosome of the E. coli bacteria. To date, such structural studies have not been possible by conventional neutron scattering, as these labels are relatively small, and in the case of tRNA, weakly contrasted.

The positions of S6 and S10 were determined uniquely on the 30S subunit site of the 70S ribosome. Their radii of gyration are in agreement with the values found by other groups. The distance between these two proteins in 70S is the same as that determined in the 30S subunit. This could indicate that the 30S subunit does not change significantly while it associates with the 50S subunit to form the 70S ribosome, although further distance measurements are necessary to confirm this. The positions of the proteins are now used to combine the map of the proteins of the 30S subunit, as determined by neutron scattering (Capel et al., 1987) with the 70S model obtained from electron microscopy. A unique assignment of the proteins will follow as soon as other proteins are localized in 70S.

With the localization of tRNA molecules in a ribonucleoprotein particle, a first step was taken to directly access these molecules. From the intensity of forward scattering, it can be deduced that the ribosome binds two tRNA molecules in both elongational states. The translocational movement of the tRNA molecules by the length of one codon was detected. The orientation of the tRNA complex between the two ribosomal subunits was analysed and found to be in good agreement with that predicted from existing models. A detailed biochemical interpretation of this data has been given elsewhere (Wadzack, 1994).

A complicated experiment such as the spin-dependent neutron scattering of highly complex biological samples can only be achieved with the cooperation of many people. Therefore, we thank everyone who was involved, especially T. Niinikoski (CERN, Geneva), O. Schärpf (ILL, Grenoble) and W. Müller (GKSS, Geesthacht). The project was supported, in its initial stages, by the Bundesministerium für Forschung und Technologie.
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