Fluorescence Studies on the Location of L7/L12 Relative to L10 in the 50S Ribosome of *Escherichia coli*†

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ABSTRACT: The localization of the protein L7/L12 relative to protein L10 in the *Escherichia coli* ribosome was studied by fluorescence energy transfer. *N*-[7-(Dimethylamino)-4-methylcoumarinyl]maleimide, coupled to Cys-70 of L10, served as a donor for fluorescein which was attached to Lys-51 or to the N terminus of L7/L12. The binding of the fluorescein–L7/L12 dimers to a strong and a weak binding site in 50S ribosomes could be distinguished. Therefore, it was possible to measure the distances between Cys-70 of L10 and Lys-51 and the N terminus of each L7/L12 dimer. For L7/L12 in the strong binding site, these two distances are both about 43 Å, and for L7/L12 in the weak binding site, both are about 56 Å.

The acidic proteins L7/L12 play an important role in several of the ribosomal processes (Möller, 1974). Many studies have been performed to characterize these proteins. They have identical amino acid sequences, except for the presence of an acetylated amino-terminal serine in L7 (Terhorst et al., 1972). Four copies of the protein L7/L12 are present on the ribosome (Subramanian, 1975). Besides, L7/L12 forms a very stable complex with L10 in a 4:1 ratio (Pettersson et al., 1976). L7/L12 forms a strong dimer (Möller et al., 1972) which only dissociates into monomers under strongly denaturing conditions. In solution, the dimer has a strongly elongated structure (Wong & Paradies, 1974), and the two polypeptide chains probably have a shifted parallel alignment (Maassen et al., 1981). The crystal structure of the C-terminal part (residues 53–120) of L7/L12 has been determined (Leijonmarck et al., 1980), showing a compact, plum-shaped tertiary structure with a high content of secondary structure. In spite of all information available on the proteins L7/L12, still little is understood about their precise molecular function. This will be partially caused by the poorly defined localization of L7/L12 on the ribosome. By immunoelectron microscopy, L7/L12 was located either on the stalk (Strycharz et al., 1978), around the central protuberance (Tischendorf et al., 1975), or on both lateral protuberances (Boublik et al., 1976). The differences are in part explained by the observation of Spiess (1978) that the appendage is lost by precipitation of the ribosomes by ethanol. Stöffler et al. (1980) showed that ribosomes with a stalk have their L7/L12-specific IgG at the stalk, while in the absence of a stalk L7/L12 determinants are found around the central proturberance.

In order to get a better insight in the localization of L7/L12 on the 50S ribosomes, we have started distance measurements by fluorescence energy transfer (Schiller, 1975; Stryer, 1978; Fairclough & Cantor, 1978). We prepared for this purpose a number of fluorescent 50S ribosomes. In this study, we use a *N*-[7-(dimethylamino)-4-methylcoumarinyl]maleimide (DACM)[1] probe linked to the cysteine of L10 as a donor and a fluorescein residue coupled either to Lys-51 of L7 or to the N terminus of L12 as an acceptor. Details of the preparation and characterization of the fluorescent ribosomes are given in the preceding paper (Zantema et al., 1982).

Experimental Procedures

Ribosomes were isolated from *Escherichia coli* MRE 600 according to Gesteland (1966) and separated at low Mg^{2+} concentration on a sucrose gradient in a zonal rotor (Möller et al., 1970). Ribosomal 50S cores were deprived of L7/L12 (=P_0 cores) or deprived of L10, L7/L12, and part of L11 (=P_{37} cores) according to Hamel et al. (1972). The ribosomes were kept in GTPase buffer: 20 mM Tris-HCl, pH 7.6, 60 mM NH_4Cl, 10 mM $Mg(OAc)_2$, and 6 mM 2-mercaptoethanol. The specific attachment of fluorescent probes to L10 and L7/L12 is described in the preceding paper (Zantema et al., 1982).

Reconstituted 50S ribosomes were prepared by incubation of the different 50S cores and proteins in GTPase buffer for 10 min at 37 °C, followed by pelleting through a 15% sucrose solution in GTPase buffer in an SW 50.1 swing-out rotor equipped with 0.6-mL adaptors. P_{37} cores were first incubated with L11 in order to obtain cores lacking only L10 and L7/L12. The amount of reconstitution of the fluorescein–L7/L12's was determined from its fluorescein absorbance, while in the case of DACM–L10 we used reductively methylated DACM–[^{3}H]methyl-L10 in order to determine the degree of reconstitution. The ribosome concentration was determined from the absorbance at 260 nm by using for 50S ribosomes an equivalence of 39 pmol per absorbance unit. The fluorescence titrations of Figures 5 and 6 were performed by adding the fluorescein component to the cuvette. The content was mixed and incubated for 2 min at 37 °C, followed by fluorescence measurements at 20 °C.

Fluorescence measurements were performed with a Perkin-Elmer MPF-2A apparatus. Cuvettes were thermostated at 20 °C. Excitation and emission slits of 10 nm were used. The presented spectra, expressed in arbitrary units (au) per unit wavelength interval, are corrected for the apparatus sensitivity and for the contribution to the fluorescence of the same concentration of unlabeled 50S ribosomes (at the fluorescence maximum always less than 10%). If necessary, the fluorescence was corrected for the inner filter effect. Quantum yields were determined relative to quinine sulfate in 0.05 M sulfuric acid by using a quantum yield of 0.51 (Chen, 1973).

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[1] Abbreviations: DACM, *N*-[7-(dimethylamino)-4-methylcoumarinyl]maleimide; DACM–L10, DACM coupled to Cys-70 of L10; FPB–L7, 4-(4-formylphenoxy)butyrimidate reacted to Lys-51 of L7; DTE, 1,4-dithioerythritol; *R*(2/3), distance from Förster's theory by assuming an orientation factor of 2/3; EF-G, elongation factor G.

0006-2960/82/0421-3077$01.25/0

The fluorescence of the following four samples was measured in order to determine the energy transfer between donor and acceptor (Fairclough & Cantor, 1978): (a) L7/L12, L10–50 S; (b) fluorescein–L7/L12, L10–50 S; (c) L7/L12, DACM–L10–50 S; (d) fluorescein–L7/L12, DACM–L10–50 S. The first experiment gives the correction value for the other three and the second the fluorescence of fluorescein when excited at the DACM wavelength (=395 nm); this proved to be zero at 472 nm, i.e., the wavelength at the maximum fluorescence of DACM–L10–50 S. The ratio of the DACM fluorescence of the fourth and third samples reveals the energy transfer by $E = 1 - Q_T/Q_0$. (Q_0 and Q_T are the quantum yields without and with energy transfer, respectively.)

The relation between energy transfer and the distance R between the dyes is given by Förster's theory (Förster, 1965, 1967):

$$E = \frac{R_0^6}{R_0^6 + R^6}$$

in which R_0, the distance at which 50% energy transfer occurs, is given by

$$R_0 = (J\kappa^2 Q_0 n^{-4})^{1/6} \times 9.79 \times 10^3 \text{ (Å)}$$

in which J is the overlap integral $\int F(\lambda)\epsilon(\lambda)\lambda^4 \, d\lambda / \int F(\lambda) \, d\lambda$ (cm^3 M^{-1}), $F(\lambda)$ is the corrected fluorescence spectrum of the donor, and ϵ is the molar extinction coefficient of the acceptor. κ^2 is the orientation factor (see below), Q_0 is the quantum yield in the absence of an acceptor, and n is the index of refraction of the medium between the chromophores. The index of refraction is generally taken as 1.4 (Fairclough & Cantor, 1978), and possible deviation from this value will hardly affect the resulting R_0. The orientation factor κ^2 can vary from 0 to 4, depending on the relative orientation of the donor and the acceptor. When the donor and acceptor can randomly reorient at their location, the κ^2 is averaged to 2/3. In general, the chromophores will have a limited mobility. For this case, Dale & Eisinger (1974, 1975) have calculated the orientation factor for different principal orientations with variations over or in a certain cone. The cone angle of the movement is related to the emission anisotropy $[(I_\parallel - I_\perp)/(I_\parallel + 2I_\perp)]$. Thus, by measuring the emission anisotropy, it is possible to search for the extremes in the orientation factor, resulting in lower and upper limits of the determined distances. On the basis of an alternative theoretical approach, Haas et al. (1978) have presented calculations with similar practical applications.

When one donor has N acceptors, the resulting energy transfer is (Gennis & Cantor, 1972)

$$E = \frac{\sum_{i=1}^{N} (R_0/R_i)^6}{1 + \sum_{i=1}^{N} (R_0/R_i)^6}$$

By means of this relation, the resulting energy transfer can be calculated when the energy transfer to each site is known.

Results

The preparation and characterization of the fluorescent products of L10 and L7/L12 are described in the preceding paper (Zantema et al., 1982). L10 is modified with *N*-[7-(dimethylamino)-4-methylcoumarinyl]maleimide at the Cys-70 of L10. The maleimide ring is partly closed, partly open. The product is very fluorescent. When the protein is reconstituted in 50S ribosomes, it has a quantum yield of 0.25. L7/L12, bearing the fluorescein chromophore, is prepared by reacting fluorescein hydrazine with the formylphenoxy group, linked either for about 90% to Lys-51 of L7 or to the periodate-oxidized N terminus of L12. The fluorescein–L7/L12's are very weakly fluorescent, with quanta yields on the order of 0.01. The fluorescence of DACM shows a good overlap with the absorbance of the fluorescein–L7/L12's, as can be seen for fluorescein–FPB–L7 from Figure 1. The UV spectrum of the N-terminal fluorescein–L12 is similar to that of fluorescein–FPB–L7 except that the absorption maximum is 491 nm instead of 495 nm for fluorescein–FPB–L7. This shift results in a slightly different overlap integral. At the excitation maximum of the DACM, fluorescein is also excited, but due to the low quantum yield of the fluorescein–L7/L12, this does not disturb the DACM fluorescence seriously (see also Figure 2).

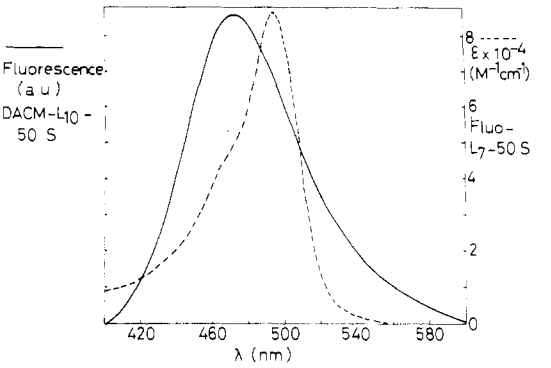

FIGURE 1: Demonstration of the overlap between the DACM–L10–50S fluorescence spectrum excited at 395 nm (—) and the absorption spectrum of fluorescein–FPB–L7 (---).

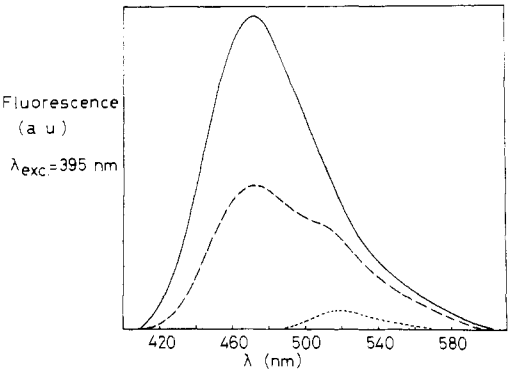

FIGURE 2: Typical fluorescent energy transfer experiment. Fluorescence spectra by excitation at 395 nm of (---) N-terminal fluorescein–L12–50 S, (—) DACM–L10–50 S, and (--) N-terminal fluorescein–L12 and DACM–L10–50 S. The concentration of 50 S was 0.36 μM, with a molar ratio of 1.53 N-terminal fluorescein–L12/50 S and 0.54 DACM–L10/50 S.

A typical result of an energy transfer experiment is shown in Figure 2 for DACM–L10–N-terminal fluorescein–L12 in 50S ribosomes. Clearly, the DACM fluorescence is quenched in the presence of N-terminal fluorescein–L12. Similar results were obtained for DACM–L10–fluorescein–FPB–L7 in 50S. By measurement of the fluorescence of DACM–L10–50S ribosomes with increasing amounts of fluorescein–L7/L12 bound (isolated by centrifugation), the results shown in Figure 3 are obtained. For both fluorescein–L7/L12 preparations, the decrease in fluorescence of DACM–L10 is linear, in agreement with the fact that the number of DACM molecules with a neighboring fluorescein increases on binding of fluorescein–L7/L12. The fluorescein–L7/L12–DACM–L10–50S particles (isolated by centrifugation) have bound at most one dimer (2 equiv) of fluorescein–L7/L12 per ribosome (see Figure 3). The saturation of a donor with one acceptor entity will result in a linear decrease of the donor fluorescence as is

Table I: Energy Transfer of DACM-L10 to Fluorescein-L7/L12 in 50S Ribosomes and the Resulting Distances (Å), Assuming an Orientation Factor of 2/3

	energy transfer	corrected energy transfer	$R_0(2/3)$	$R(2/3)$	range in $R(2/3)$[a]	distance[b]
fluorescein-FPB-L7 in the strong site	0.45 ± 0.05[c]	0.50 ± 0.09	44.9	45	42–48	45 ± 8
fluorescein-FPB-L7 in the weak site	0.20 ± 0.05[d]	0.22 ± 0.09	44.9	55	51–62	55 ± 10
N-terminal fluorescein-L12 in the strong site	0.62 ± 0.05[c]	0.62 ± 0.05	45.4	42	40–44	42 ± 7
N-terminal fluorescein-L12 in the weak site	0.18 ± 0.05[e]	0.18 ± 0.05	45.4	58	55–62	58 ± 10

[a] Based on the inaccuracy in the energy transfer. [b] Accuracy is based on accuracy in energy transfer measurements and the accuracy in the R_0 values as given in the last row of Table II; also see the text. [c] From Figure 3. [d] From solid circles of Figure 4. [e] From solid circles of Figure 5.

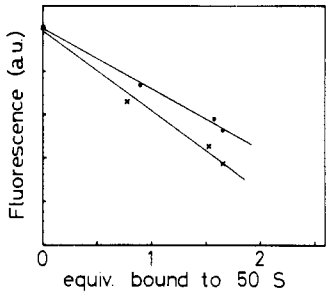

FIGURE 3: DACM fluorescence of DACM-L10-fluorescein-L7/L12-50S ribosomes, isolated by centrifugation through a sucrose cushion, as a function of the content of either fluorescein-FPB-L7 (●) or N-terminal fluorescein-L12 (×). For preparation of these samples, DACM-L10-50 S containing no L7/L12 was incubated with 0, 1.5, 3.0, and 5.0 equiv of fluorescein-FPB-L7 (●, from left to right) or 0, 2.5, 5.0, and 15.0 equiv of N-terminal fluorescein-L12 (×, from left to right).

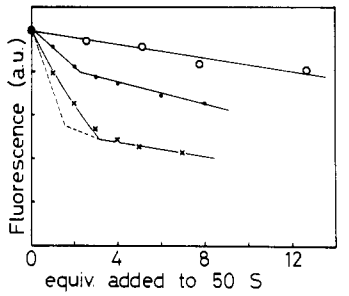

FIGURE 4: Effect on the DACM fluorescence of DACM-L10-50 S by various additions: (○) fluorescein amine effect on DACM-L10-50 S containing 0.63 DACM-L10/50 S and no L7/L12; (●) fluorescein-FPB-L7 effect on [^{14}C]methyl-L7, DACM-L10-50 S, isolated by centrifugation, containing 1.6 [^{14}C]methyl-L7/50 S and 0.64 DACM-L10/50 S; (×) fluorescein-FPB-L7 effect on DACM-L10-50 S containing 0.63 DACM-L10/50 S and no L7/L12. The drawn line through the ×'s and the dashed line are discussed in the text.

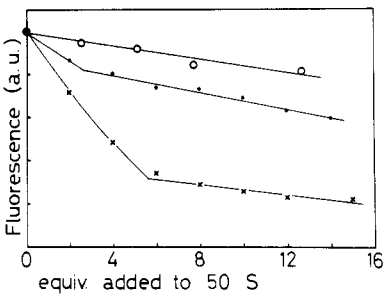

FIGURE 5: Change in the DACM fluorescence of DACM-L10-50 S by various additions: (○) fluorescein amine effect on DACM-L10-50 S containing 0.63 DACM-L10/50 S and no L7/L12; (●) N-terminal fluorescein-L12 effect on [^{14}C]methyl-L7, DACM-L10-50 S, isolated by centrifugation, containing 1.6 [^{14}C]methyl-L7/50 S and 0.64 DACM-L10/50 S; (×) N-terminal fluorescein-L12 effect on DACM-L10-50 S containing 0.63 DACM-L10/50 S and no L7/L12. The drawn line through the ×'s is in the text.

indeed observed (Figure 3). We have evidence that the binding of only one dimer instead of two is caused by the isolation procedure via centrifugation through a sucrose cushion [see Discussion and Zantema et al. (1982)]. By addition of fluorescein-L7/L12 to the cores, two dimers become bound, but the dimer bound to the weak site does not survive the centrifugation step (see Discussion). We therefore infer that the energy transfer observed in Figure 3 is from DACM on L10 to fluorescein on L7/L12 bound to the strong binding site.

The strong binding site can also be occupied by L7 without fluorescein, by incubating cores with unmodified L7 followed by pelleting through a sucrose cushion. This DACM-L10-50S particle with only the weak binding site for an L7/L12 dimer unoccupied can be used for measuring the change in DACM fluorescence as a function of the binding of fluorescein-L7/L12 to the weak binding site (see solid circles in Figures 4 and 5). Clearly we get a biphasic curve, a steep decrease caused by specific energy transfer followed by a smaller decrease. A similar slow decrease is obtained by addition of fluorescein amine to DACM-L10-50S particles, indicating an aspecific effect. As expected, the specific effect stops after approximately one dimer of fluorescein-L7/L12 has been bound. For N-terminal fluorescein-L12, this means that an excess of protein must be added because only part of the protein binds to cores. Table I shows the values for the energy transfer; these values were calculated on the basis of the results presented in Figures 3–5. For the calculation of the energy transfer to the strong binding site (from Figure 3), a saturation value of 1.6 L7 per ribosome was used, implying that 80% of the ribosomal particles participate. This percentage is in reasonable agreement with the L10 content of the ribosome: 0.60 DACM-L10 together with 0.1–0.15 unmodified L10. This latter value was estimated from EF-G-dependent GTPase of the P_{37} cores.

By addition of fluorescein-L7/L12 to DACM-L10-50S cores lacking L7/L12, we will occupy both the weak and the strong L7/L12 binding site. The resulting change in DACM fluorescence for these cases is shown by the × symbols in Figures 4 and 5. The drawn line, which coincides with the × symbols, is calculated on the basis of the energy transfer values mentioned in Table I and by assuming a random binding for the two L7/L12 dimers (see Discussion).

There are no indications for any exchange between the strong and the weak binding site. When DACM-L10-50S cores are reconstituted with one dimer of L7 in the strong site, followed by reconstitution with fluorescein-L7/L12 and subsequent centrifugation through a sucrose cushion, no binding of fluorescein-L7/L12 is present, indicating that fluorescein-L7/L12 has bound only in the weak site. When fluorescein-L7/L12-DACM-L10-50 S with one fluorescein-L7/L12 dimer in the strong binding site is incubated further with L7/L12, no change in the fluorescence occurs, indicating that no shift of fluorescein-L7/L12 from the strong

to the weak site occurs. Also experiments with [^{3}H]methyl-L7 and nonradioactive 50S particles show no exchange.

Discussion

In this study, we have determined the energy transfer in 50S ribosomes between fluorescent groups attached to Cys-70 of L10 and either Lys-51 of L7 or the N terminus of L12. The calculation of distances from these data is complex because of the situation of one donor with maximally four acceptor molecules. The mode of binding of the four L7/L12 molecules to the ribosome determines the interpretation of the data, and, therefore, we will first discuss the binding behavior, after which the calculated distances will be discussed. We assume that the modified L7/L12's behave like the unmodified ones, as we have no indications to the contrary.

Difference between the Two L7/L12 Dimer Binding Sites. The *E. coli* 50S ribosome has four molecules of L7/L12 bound to it (Subramanian, 1975). On the basis of the strong dimerization behavior of L7/L12 (Möller et al., 1972; Gudkov & Behlke, 1978; Luer & Wong, 1979), it is most likely that binding of L7/L12 occurs at two sites, each for one dimer. Recently, it has been published (Kar & Aune, 1981) that L7/L12 would be in an equilibrium between monomers, dimers, and tetramers. As pointed out by these authors, they are working with inhomogeneous L7/L12 preparations. This may be due to oxidized methionines, in line with their isolation procedure. Much stronger reducing conditions during isolation are required to keep L7/L12 intact. Because it is unclear whether there is any intact L7/L12 in their preparations, their results are no reason to change the view of the binding of two dimers to 50 S.

We have found that the number of copies of L7/L12 that can bind to P_0 cores, as determined by pelleting them through a sucrose cushion, is either two or four. L7/L12, prepared under nondenaturing conditions, always bind in four copies to P_0 cores in the binding assay via centrifugation through a sucrose cushion. For L7/L12, purified in 6 M urea, binding of 2 or 4 equiv to P_0 cores can occur. The difference seems to be quite subtle, as preparations which bind apparently only two copies (one dimer) show full activity in polyphenylalanine synthesis when P_0 cores are incubated with four copies of this L_7/L12. It has been clearly shown that two bound L7/L12 dimers are required for polyphenylalanine synthesis (W. Möller, P. I. Schrier, J. A. Maassen, A. Zantema, E. Schop, H. Reinalda, A. F. M. Cremers, and J. Mellema, unpublished results). Because L7/L12 preparations which bind only as one dimer to cores after centrifugation give full polyphenylalanine synthesis upon addition of two dimers, the second dimer still binds strongly. However, binding is not strong enough to survive the centrifugation step through a sucrose cushion. This property implies that the protein which binds apparently only as one dimer is changed, but based on the polyphenylalanine synthesis, the change is subtle, resulting in a slightly weaker, but functionally correct, binding. These results also indicate the existence of a strong and a weak binding site for the L7/L12 dimer. As a result of this situation, we can measure the energy transfer from DACM at Cys-70 of L10 to the fluorescein at L7/L12 in either the strong or the weak binding site in 50 S. The results (Table I) show a difference between the energy transfer to the strong and the weak binding site. These data exclude that the difference between the two binding sites is caused by a negative cooperativity resulting in a weaker second site after one of both sites is occupied. In such a case, the occupation with the first dimer will be for 50% in one and 50% in the other site, just as would be the case for the second dimer bound. Thus, no difference would be observed in energy transfer for the first and the second dimer bound. The fact that we do observe a difference means that the strong site has a location different from the weak site. However, the situation of the binding of the two dimers is still somewhat more complicated. In agreement with Lee et al. (1981), we find that the EF-G-dependent GTPase of P_0 cores with increasing amount of L7/L12 can be interpreted as random occupation of the two dimer binding sites, while one dimer bound is sufficient for full GTPase activity. Also, our polyphenylalanine synthesis results follow this interpretation, except that two dimers are necessary to give activity. This last result is in contrast to the results of Lee et al. (1981), and will be discussed elsewhere (W. Möller, P. I. Schrier, J. A. Maassen, A. Zantema, E. Schop, H. Reinalda, A. F. M. Cremers, and J. Mellema, unpublished results). The situation of random occupation is also supported by the results from titration experiments shown by the × symbols in Figures 4 and 5. When we would first occupy the strong site followed by the weak site, the energy transfer of DACM–L10 to fluorescein–FPB–L7 would follow the dashed line of Figure 4. This theoretical line is computed on the basis of the following: first, one dimer binds to the strong site with an energy transfer of 0.45 [because of the participiation of only 80% of the ribosomes (see Results), this is complete at 1.6 fluorescein–FPB–L7–50 S], followed by a binding to the second site with an energy transfer of 0.20. The resulting energy transfer is not a simple addition and results in a final value of 0.516 (see Experimental Procedures). Clearly, the measured values in Figure 4 do not fit the dashed line. The model of random occupation, however, has to deal with situations where one dimer occupies, statistically, one of the two available sites, resulting in the average energy transfer of 0.325, whereas if both sites are occupied the effective energy transfer becomes 0.516. The binomial distribution yields the number of ribosomes with one and two dimers bound and thus the resulting energy transfer. This slightly curved line is drawn in Figure 4 and fits very well the measured values. For N-terminal fluorescein–L12 (Figure 5), the same situation holds, but an extra complication is the fact that only part of this protein can reconstitute. On the assumption of a reconstitution of about 60% and the corresponding energy transfer values of Table I together with the same aspecific contribution as fluorescein amine for the unbound N-terminal fluorescein–L12, we get the curve in Figure 5. This curve fits very well the measured values.

The apparent contradiction we have to deal with is that based on binding studies performed via isolation of the reconstituted 50 S by pelleting through a sucrose cushion we bind a strong and a weak binding site, while in experiments in which L7/L12 is added without subsequent isolation of the 50S ribosomes, the results have to be explained by a random occupation of both sites. A straightforward explanation is that the on rates for the binding of L7/L12 dimers to both sites are equal while the off rates differ. In the case of short time experiments without isolation of the particles via centrifugation, the on rate determines the distribution over the two sites (solid circles in Figures 4 and 5). During isolation of the reconstituted 50 S by centrifugation through a sucrose cushion, the off rate and thus the difference in binding constants also becomes important; in other words, an equilibrium situation exists with the strong binding site being filled preferentially. The reliability of this statement may be demonstrated by the following results. By studying the polyphenylalanine synthesis activity of P_0 cores as a function of the amount of L7/L12 added, we use 1.6 pmol of ribosomes in a volume of 50 μL and

Table II: Uncertainty in the Orientation Factor and the Resulting Range of R_0 Values Obtained from the Emission Anisotropy of the Components

	emission anisotropy		range of κ^2 [a]	range of R_0 (Å) [a]	R_0 (Å) range from the half-height limit of $Q(r'/r)$ [b]
	donor	acceptor			
DACM-L10-fluorescein-FPB-L7-50 S	0.32	0.16	0.17-2.8	36-57	39-51
DACM-L10-N-terminal fluorescein-L12-50 S	0.32	0.24	0.12-3.1	34-59	39-52

[a] According to Dale & Eisinger (1974, 1975). [b] According to Haas et al. (1978).

find a maximal activity at the addition of 4 equiv of L7/L12, indicating a very strong binding. A rough estimation of the possible free concentrations results in an association constant of 10^{10} M^{-1}. The on rate probably will be diffusion controlled, therefore on the order of 10^7 M^{-1} s^{-1}, resulting in an off rate of 10^{-3} s^{-1}. This off rate clearly shows that in experiments in which P_0 cores are incubated with L7/L12 no real equilibrium is reached but that the on rate determines the site to which L7/L12 will be bound, while during isolation by centrifugation an equilibrium situation may be reached. This is also in agreement with the absence of any apparent exchange between L7/L12 bound to ribosomes and free in solution. Presently it cannot be decided whether the concept of a strong and a weak binding site is influenced by the way of handling and modification of L7/L12.

Distances. The calculations of the distances between the donor and acceptor molecules are based on the energy transfer values between the couples. These quantities are measured with DACM-L10-50 S saturated with fluorescein-L7/L12's in each of the two sites. For fluorescein-FPB-L7, we have a 90% modification of Lys-51, 6% of either Lys-95 or Lys-100, and 4% of undefined sites. Thus, the energy transfer of DACM-L10 to fluorescein at Lys-51 of L7 in the strong site is 0.45/0.9 = 0.50, while the remaining fluorescein may show either complete or no energy transfer, resulting in an extra uncertainty of 0.1. However, since L7/L12 is an elongated protein, with its N terminus bound to the ribosome (Van Agthoven et al., 1975), the fluorescein attached to either Lys-95 or Lys-100 will be far away from the DACM in L10, resulting in no energy transfer. As a result, only the remaining 4% of fluorescein will give an uncertainty in the energy transfer value of ±0.04, resulting in a final energy transfer of 0.50 ± 0.09 for the strong site and in a similar way 0.22 ± 0.09 for the weak binding site. For N-terminal fluorescein-L12, no corrections are needed because of the completely specific labeling at the N terminus.

The R_0 values shown in Table I can be calculated by assuming an orientation factor κ^2 of 2/3. The slight difference between both R_0 values is due to the small shift of the fluorescein-FPB-L7 absorption maximum (λ_{max} = 495 nm) relative to the N-terminal fluorescein-L12 maximum (λ_{max} = 491 nm), resulting in a slight difference in the overlap integral. We have observed no changes in the fluorescein spectra after binding fluorescein-L7/L12 to either the strong or the weak binding site. Therefore, the R_0 values for both cases are the same. The R_0 values are calculated by assuming one fluorescein per L7/L12 dimer. Actually both monomers contain a fluorescein, and the R_0 value from Table I will only be correct if one fluorescein is relatively close to the DACM at L10. When both fluorescein groups are at the same distance from the DACM, we will have an R_0 value which is $2^{1/6}$ = 1.12 times the one presented in Table I (Gennis & Cantor, 1972). Further experiments are needed to determine the actual situation, but it is clear that this may affect the interpretation by only 12%.

The R_0 values and the energy transfer data in Table I are used for the calculation of the distance R. The uncertainty in the energy transfer results in a variation in the distances shown also in Table I. Besides this uncertainty, the orientation factor represents another, even larger source of error. The real κ^2 is unknown and may vary from 0 to 4. As can be seen from the emission anisotropy values (Table II), the labels are not completely immobilized; therefore, the effective κ^2 will have smaller limits. The values of κ^2 for a series of orientations with a certain flexibility (expressed as locations over or in a cone) have been calculated by Dale & Eisinger (1974, 1975). These values were used for setting limits of κ^2, which are shown in Table II, together with the resulting R_0 range. In this range, the R_0 values have different probabilities, as shown by Hillel & Wu (1976). A similar result is obtained by calculations which use mixed polarizations obtained from the emission anisotropy data (Haas et al., 1978). The range of R_0 from those calculations is similar to the range obtained by the calculations of Dale & Eisinger (1974, 1975). Haas et al. (1978) also determined the limits of R_0 at half-height of the probability functions. The range of R_0 from these data is also given in Table II. These R_0 values can be used to calculate the limits of the distance. The highest probability corresponds to the $R(2/3)$ distance shown in Table I. When the inaccuracy in the energy transfer experiments shown in Table I and the possible variation in R_0 shown in Table II are taken into account, we obtain the inaccuracy in the distances, which are listed in the last row of Table I.

From these data, it is clear that the distance from DACM at Cys-70 of L10 to fluorescein at either the N terminus or the Lys-51 of L7/L12 is about the same. Based on the elongated structure of L7/L12 (Wong & Paradies, 1974) and with the assumption of a less extreme elongation for L10, these sets of distances suggest a binding of the N-terminal rather than the C-terminal part of L7/L12 to the protein L10. This is in agreement with studies on fragments of L7/L12 (Van Agthoven et al., 1975) and studies on oxidized L7/L12 (Gudkov et al., 1980). L7/L12 in the strong binding site is located somewhat closer to the DACM at Cys-70 of L10 than L7/L12 in the weak binding site.

Our data do not allow the construction of a definitive model. It is clear from immunoelectron microscopy that the stalk contains L7/L12, so at least one dimer is located in the stalk (Strycharz et al., 1978; Stöffler et al., 1980). The molecular weight of 50 000 of the stalk, as estimated from the electron microscopic dimensions (Lake, 1976) and the absence of a stalk in P_0 cores (Strycharz et al., 1978), suggests that both dimers are located in the stalk. It cannot be excluded, however, that the stalk also contains other ribosomal components which, upon removal of L7/L12, disappear from the stalk by a conformational change. Spiess (1978) reported that 50S ribosomes, precipitated by ethanol, lose their stalk, while Stöffler et al. (1980) showed for such particles binding of L7/L12-specific IgG around the central protuberance. These reports suggest that such ribosomes have lost the L7/L12 dimer bound

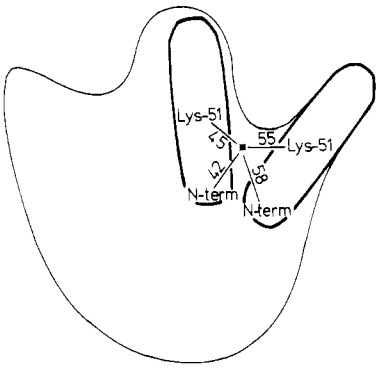

FIGURE 6: Hypothetical model of the location of the two L7/L12 dimers in the 50S ribosomal subunit including the measured distances in angstroms. The asterisk indicates the location of the DACM at Cys-70 of L10.

to the weak binding site, while the strong binding dimer is still present and is located in the central protuberance. In addition, the location of both dimers in the stalk, extending from L10, suggests limited contacts of L7/L12 with other ribosomal proteins. In contrast, cross-linking studies show next to L10 a neighborhood to L2, L5, L6, L11, and L14 (Traut et al., 1980). This also suggests that at least one of the dimers is located more inside the 50S body or can be arranged in a conformation directed toward the 50S body. Recent electron microscopy studies in our laboratory also favor a model with one dimer in the stalk and the other in the 50S body (W. Möller, P. I. Schrier, J. A. Maassen, A. Zantema, E. Schop, H. Reinalda, A. F. M. Cremers, and J. Mellema, unpublished results). Therefore, at this time, we favor the model shown in Figure 6. In order to make a final conclusion about the localization of the two dimers, we are now performing fluorescence energy transfer experiments to determine the distances between the two dimers.

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