Vol. 86, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 14, 1979 Pages 909-914

Internal Flexibility of Valyl-tRNA Synthetase from E. coli

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Received December 6, 1978

Summary: The values of the rotational correlation times of the native valyl-tRNA synthetase and the proteolytic modified enzyme are very close to those of the large fragment of molecular weight 70,000 that has a correlation time of 70 nsec, whereas the small proteolytic fragment has a correlation time of 15 nsec. This indicates that there is rotational freedom within the native valyl-tRNA synthetase corresponding to the biochemically active fragment of molecular weight 70,000. The structural model drawn from these results reveals that the valyl-tRNA synthetase is composed of two unequal, quasi-spherical parts covalently linked by a small peptide bridge. Mild tryptic hydrolysis breaks the covalent bridge between these quasi-spherical domains without changing the overall structure of the valyl-tRNA synthetase significantly.

Studies on the subunit structure of purified aminoacyl-tRNA synthetases have revealed different degrees of complexity, but three different classes are possible (see reviews 1-3): single polypeptide chains of molecular weights of 70,000-115,000, dimers and tetramers of apparently identical subunits each of molecular weight of 40,000-50,000, and dimers or tetramers with non-identical subunits. Useful information with respect to conformation and subunit composition of several synthetases can be obtained by mild proteolytic digestion and subsequent analysis of the products. In the case of valyl-tRNA synthetase the trypsin-modified form catalyzes the pyrophosphate exchange reaction with the same efficiency as the unmodified form, and is also capable of catalyzing the esterification of $tRNA^{val}$ with valine, however, with a lower K_m than for $tRNA^{val}$ (4, 5). By measuring the amount of fluorescence polarisation of conjugates of macromolecules with a fluorescent label, it is possible to obtain information about the form and structure of the two forms of valyl-tRNA synthetase and its two fragments, in particular

0006-291X/79/030909-06$01.00/0

to determine whether the modified or native enzyme form is rigid or flexible. We report here the results of fluorescence polarization measurements of the native and the modified valyl-tRNA synthetase, as well as from the large fragment of molecular weight 70,000, using 8-anilino-naphthalene-7-sulfonate (ANS) as the fluorescent protein conjugate.

Materials and Methods

Valyl-tRNA synthetase (VRS) was purified as described in (4-7), including the modified form. The two proteolytic fragments were purified by DEAE-cellulose chromatography in a linear gradient from 0.1 M KCl to 0.45 M KCl, in 0.15 M K_2HPO_4, pH 7.5. Further purification of the two fragments were achieved on BioGel A-0.5 m column chromatography (1.0 x 100 cm). Normally from 100 mg purified native synthetase 60 mg of the large fragment and 30 mg of the small fragment can be obtained in a homogeneous form as judged by polyacrylamide gel electrophoresis (8). Fluorescence measurements were made in a Perkin-Elmer model MPF III fluorimeter, equipped with a Hitachi 018-0054 polarization accessory in order to measure the polarization of fluorescence. Measurements of the ANS-labelled enzymes were made using polarized excitation at 380 nm and emission at 480 nm. The polarization was calculated according to

$$p = \frac{I_{VV} - I_{VH} \times G}{I_{VV} + I_{VH} \times G}$$

with $G = I_{HV}/I_{HH}$, where I is the fluorescence intensity, the first and second subscripts refer to the plane of polarization of the excitation and emission beams (V = vertical; H = horizontal). Fluorescence life times (τ) of ANS conjugates were measured at 22°C with a modified nanosecond fluorescence decay time apparatus from TRW, Inc. (California, USA) (9). An aliquot of a concentrated, aqueous solution of ANS was added sequentially to the enzyme solution. In order to remove aggregates before conducting polarization experiments, the solution of ANS conjugates were passed through a BioGel A-0.5 m column (1.5 x 75 cm). The molar ratios of ANS to protein, calculated from the absorbance at 280 nm and 340 nm (accounting for protein absorbancy at 340 nm) were 2.5 for ANS-VRS (native), 2.6 for ANS-VRS (nodified) and 1.2 for ANS-VRS (large fragment), whereas for the small fragment a value of 0.75 was applied.

Results

The results of the fluorescence polarization measurements are summarized in table 1, and are shown in figure 1 as isotherms, each corresponding to the change of viscosity on addition of sucrose. Furthermore, extrapolation of the linear part of these isotherms to the ordinate correspond to the values obtained for $1/P_o$ in table 1, where also the measured values of τ are listed.

Table 1.

Parameters of the fluorescence of the ANS-VRS-conjugates.

ANS-VRS conjugate	τ nsec	$\frac{1}{P_o}$	ρ_h (nsec)
ANS-VRS, native	7.2	2.49	68
ANS-VRS, modified	7.9	2.45	71
ANS-Frag,L	7.7	2.40	70
ANS-Frag,S	3.8	0.57	15.5

Measurements were performed in 0.015 M K_2HPO_4, pH 7.5, containing 0.1 M KCl.

ANS-Frag,L = the large fragment of VRS (molecular weight 70,000).

ANS-Frag,S = the small fragment of VRS (molecular weight 40,000).

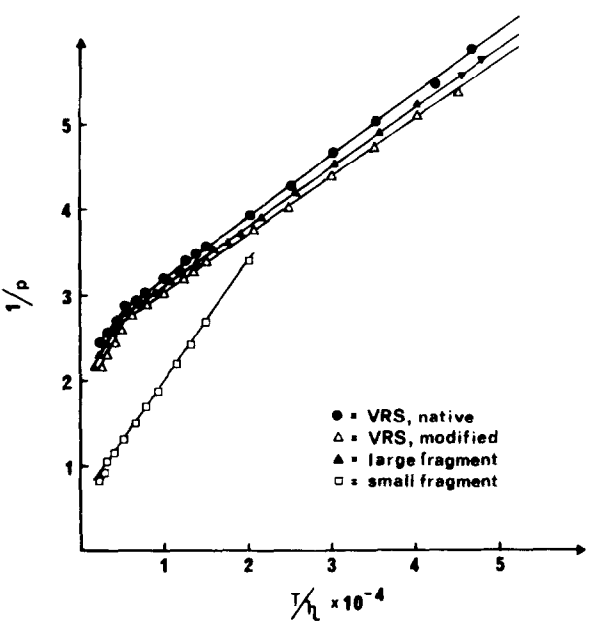

FIG. 1. Dependence of the reciprocal of the fluorescence polarization upon temperature, divided by viscosity for ANS-VRS, native; ANS-VRS, modified; ANS-Frag,L; and ANS-Frag,S. The initial buffer was 0.015 M K_2HPO_4, pH 7.5, 0.1 M KCl.

Using these values the relaxation time, ρH, was computed from the dependence of polarization on temperature according to the Perrin equation

$$(\frac{1}{P} - \frac{1}{3}) = (\frac{1}{P_o} - \frac{1}{3}) \ (1 + \frac{3\tau}{\rho_h})$$

where P_o is the limiting polarization at low temperature, ρ is the excited lifetime of the fluorophore. The relaxation time of a rigid sphere (ρ_o) can be computed according to

$$\rho_o = \frac{3\eta V}{R \cdot T}$$

where the molar volume, $V = \bar{v}_2 \cdot M$, and η is the viscosity of the solvent at temperature T. The life time of the ANS-native-VRS was found to be 7.2 nsec, for the modified ANS conjugate of VRS 7.9 nsec, for the large fragment, ANS-Frag L, 7.7 nsec, and for the small, 40,000 molecular weight fragment of VRS 6.8 nsec (Table 1). The values of the correlation time (ρ_h) of the ANS-VRS conjugates are considerably larger than the value, ρ_o, for an equivalent sphere of molecular weight 110,000 and partial specific volume of $\bar{v}_2 = 0.735 \pm 0.004$ ml·g^{-1} (8, 10) which is $\rho_o = 49$ nsec, whereas the value for the ANS conjugate of the large fragment is $\rho_o = 31.2$ nsec. The differences for the ANS-VRS native, ANS-VRS modified, and the ANS-Frag,L can be accounted for in a certain degree of asymmetry, having an axial ratio of about 3:1 for the native and 4:1 for the modified forms, assuming a degree of hydration of $S_1 = 0.25$ ml·g^{-1} and $S_2 = 0.35$ ml·g^{-1} (6) using the description of a prolate ellipsoid of revolution (11) which is consistent with other hydrodynamic measurements (4), including small angle X-ray scattering measurements in solution (10, 11). Considering the relaxation time of 70 nsec for the ANS-large fragment of VRS, which is close to the value of the modified and the native VRS, the value obtained for the ANS-conjugate for the small fragment, $\rho_h = 13.5$ nsec, is lower than that for a hydrated sphere, yielding a ratio of $\rho_h/\rho_o = 0.71$.

Discussion

The formula of the Perrin equation is only valid for the exponential fluorescence decay law. It was shown by Chen (9) and Wahl (12) that the fluorescence of 1-dimethylaminonaphtha-

lene-5-sulfonyl group in the case of the immunoglobulin may follow a more complicated decay law, and the above formula has to be replaced by the more general formula

$$\frac{1/\rho - 1/3}{1/\rho_0 - 1/3} = \frac{1}{\int_0^\infty \Phi(t)\exp(-Kt)dt}$$

with $K = \frac{R \cdot T}{V \cdot \eta}$. This indicates that the fluorescence polarization depends on the form of the decay curve. But in the case of all conjugates studied with respect to the native and the modified VRS, as well as the fragments, the exponential decay of anisotropy can be fit reasonably well by a straight line, and the slopes yield the relaxation times listed in table 1.

If the values of ρ_h for the ANS-Frag,L and the ANS-Frag,S were valid, not only for isolated fragments but also for independently rotating VRS-domains in situ, Perrin's equation can be applied for the mixture of molecules with different ρ_h-values. In that case, by applying this formula, ρ_h for the ANS-modified form would be 67.5 nsec and the value found for the native and modified forms were 68 nsec and 71 nsec, respectively. These calculations leave no doubt that the observed fluorescence polarization of the ANS-VRS forms is determined by Brownian rotation of structural domains within the VRS molecule which are similar to the large and small fragments isolated from the modified form of VRS that are connected by flexible bonds.

The value of ρ_h found for the large fragment is in good agreement with ρ_h calculated for a rigid ellipsoid of revolution having an axial ratio of 4:1 using the description of a prolate ellipsoid of revolution. But the experimentally measured value of ρ_h = 15.5 nsec for the small fragment of VRS is lower than that expected for a sphere of molecular weight 40,000 (ρ_o = 20.2 nsec) having a relaxation ratio of ρ_h/ρ_o = 0.77. This points to a certain lability of the structure of this small fragment that also is substantiated by its greater sensitivity to proteolysis and conformation changes with different pH (10, 11). Moreover, the independence of Brownian rotation of the two independent domains within the VRS molecule should not affect the behaviour of the enzyme particle as much in solution - this is only valid at concentrations of 5.0-8.0 mg/ml VRS -

because aggregation occurs (13). In particular, if there is a quasi linear or elongated arrangement of the two almost globular domains in the VRS molecule the molecule will behave in solution as an elongated ellipsoid of revolution; this is being substantiated by low angle X-ray scattering measurements in solution as well as by other hydrodynamic methods (10). But the certain degree of flexibility of parts within the domains of the VRS molecule makes it possible that the synthetase can change its form under the action of the cognate tRNA.

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