The anaerobic ribonucleotide reductase of Escherichia coli catalyzes the synthesis of the deoxyribonucleotides required for anaerobic DNA synthesis. The enzyme is an \( \alpha_2 \beta_2 \) heterotetramer. In its active form, the large \( \alpha \) subunit contains an oxygen-sensitive glycyl radical, whereas the \( \beta_2 \) small protein harbors a \([4Fe-4S]\) cluster that joins its two polypeptide chains. Formation of the glycyl radical in the inactive enzyme requires \( \text{S-adenosylmethionine (AdoMet)} \), dithiothreitol, \( K^- \), and either an enzymatic (reduced flavodoxin) or chemical (dithionite or 5-deazaflavin plus light) reducing system. Here, we demonstrate that AdoMet is directly reduced by the Fe-S center of \( \beta_2 \) during the activation of the enzyme, resulting in methionine and glycyl radical formation. Direct binding experiments showed that AdoMet binds to \( \beta_2 \) with a \( K_d \) of 10 \( \mu \)M and a 1:1 stoichiometry. Binding was confirmed by EPR spectroscopy that demonstrated the formation of a complex between AdoMet and the \([4Fe-4S]\) center of \( \beta_2 \). Dithiothreitol triggered the cleavage of AdoMet, leading to an EPR-silent form of \( \beta_2 \) and, in the case of \( \alpha_2 \beta_2 \), to glycyl radical formation. In both instances, 3 methionines were formed per mol of protein. Our results indicate that the Fe-S center of \( \beta_2 \) is directly involved in the reductive cleavage of AdoMet and suggest a new biological function of an iron-sulfur center, i.e. redox catalysis, as recently proposed by others (Staples, R. C., Ameyibor, E., Fu, W., Gardet-Salvi, L., Stritt-Etter, A. L., Schürmann, P., Knaff, D. B., and Johnson, M. K. (1996) Biochemistry 35, 11425–11434).

**Escherichia coli** uses different enzymes for the de novo synthesis of deoxyribonucleoside triphosphates (dNTPs) during aerobic and anaerobic growth (1). The aerobic ribonucleotide reductase, coded by \( \text{nrdAB} \) genes, has an \( \alpha_2 \beta_2 \) structure, with the large \( \alpha_2 \) protein carrying allosteric and catalytic sites and the small \( \beta_2 \) protein harboring two oxo-bridged diferric centers capable of forming and stabilizing a tyrosyl radical in their vicinity (2). During catalysis, the stable tyrosyl radical is thought to generate by long range electron transfer a transient thyl radical on the large protein which, together with two redox-active thios, catalyzes the reduction of the ribose group to deoxyribose (3). This aerobic enzyme was described already in 1960 and has become the prototype of a whole class of ribonucleoside diphosphate reductases (class I) that produce dNDPs in some bacteria and all higher organisms.

The anaerobic ribonucleoside triphosphate reductase of *E. coli* was discovered only in 1989 (4). It is the prototype for a group of class III anaerobic enzymes also found in other anaerobically growing microorganisms both in archaeabacteria and eubacteria (5). It was first believed to be a single large monomeric \( \alpha_2 \) protein, but subsequently, a second smaller homodimeric \( \beta_2 \) protein was found to be required for catalytic activity (6). The two proteins, coded by the \( \text{nrdD} \) and \( \text{nrdG} \) genes, respectively, are tightly bound to each other and form an \( \alpha_2 \beta_2 \) complex as shown by sucrose gradient centrifugation and kinetic experiments (7). The \( \beta_2 \) protein contains a \([4Fe-4S]\) cluster that links its two polypeptide chains, and the \( \alpha_2 \) protein harbors an oxygen-sensitive glycyl radical but no metal center (8, 10).

It is likely that both aerobic and anaerobic enzymes use redox-active cysteines as a source of electrons and catalyze the same radical chemistry as judged from studies with mechanism-based inhibitors and from stereochemical analysis of the reduction reaction (11–13). Both generate a stable free amino acid radical, as part of their protein structure, essential for activity: a tyrosyl radical in the case of the aerobic enzyme and a glycyl radical in the anaerobic one.

The activation of the aerobic reductase is reasonably well understood; generation of the tyrosyl radical is an oxygen-dependent process in which the iron center is involved (14–16). On the other hand, our knowledge concerning the formation of the glycyl radical of the anaerobic enzyme is limited. As prepared, the pure enzyme lacks the glycyl radical and is inactive. The radical is generated, and the enzyme is activated during anaerobic incubation with \( \text{S-adenosylmethionine (AdoMet)} \) together with NADPH, flavodoxin, flavodoxin reductase, \( K^- \), and

\(^1\) The abbreviations used are: AdoMet, S-adenosylmethionine; DTT, dithiothreitol; 5-DAF, 5-deaza-7,8-demethyl-10-methylisoalloxazine; Mes, 4-morpholineethanesulfonic acid.

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†† To whom correspondence should be addressed. Laboratoire d’Études Dynamiques et Structurales de la Sélectivité, Université Joseph Fourier, CNRS UMR 5616, Chimie-Recherche, BP 53, 38041 Grenoble Cedex 09, France, Tel: 33 04 76 51 44 67; Fax: 33 04 76 51 43 82; E-mail: Marc.Fontecave@ujf-grenoble.fr.

§§ A. Müller, E. Mulliez, K. F. Patel, D. B. Knaff, M. K. Johnson.

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Cleavage of AdoMet by the Fe-S Center of Reductase

**EXPERIMENTAL PROCEDURES**

**Materials**

All components of the anaerobic ribonucleotide reductase system were prepared as described previously (6, 7, 17–20). Reconstitution of the reductase with free iron has been described in a previous work (7). Pyruvate ferredoxin:oxidoreductase from *C. pasteurianum* was provided by J. M. Moulis (CENG, Grenoble, France) and ferredoxin IV from *Rhodobacter capsulatus* was provided by Y. Jouanneau (CENG, Grenoble, France). Protein concentrations were determined by the method of Bradford (23). AdoMet was from Boehringer Mannheim, and sodium dithionite was from Fluka. 5-Deaza-7,8-demethyl-10-methyl-

**Methods**

**Measurements of Enzyme Activity**—In the first step, the enzyme (0.32 µM), in a total volume of 20 µl, was degassed with argon, and 0.1 µl of cold water, and push through the filter. The filter was removed, blotted from underneath with Kleenex, and then centrifuged. After an additional 20 min, the closed tube was shifted to an anaerobic hood kept at +4 °C, received 0.9 µl of 50 mM Tris-HCl, pH 7.5, 20 mM KCl, 1 mM dithionite, and was transferred to the chamber of a syringe attached to a filter device containing a GSWP 01300 Millipore filter (25). Reduction of the iron-sulfur center for β2 or of the glycyl radical for αβ2. Recording conditions were as follows: microwave power, 0.1 milliwatt; modulation amplitude, 1 millitesla; frequency, 9.62 GHz; modulation amplitude, 1 millitesla; temperature, 20 K.

**Binding of AdoMet to β2**—A filter-binding assay used earlier to measure binding of allosteric effectors to ribonucleotide reductase (25) was adapted to the anaerobic conditions required for the present experiments. In the standard procedure, the enzyme in a volume of 90 µl of 50 mM Tris-HCl, pH 7.5, 5 mM DTT, 20 mM KCl was flushed at room temperature with moistened argon for 60 min on an anaerobic manifold, followed by the addition of 10 µl of an anaerobic solution of 25 mM dithionite to reduce the iron-sulfur center. Also, reduction by 5-DAF plus light was used, but with less reproducible results. After an additional 20 min, the closed tube was shifted to an anaerobic hood kept at +4 °C, received 0.9 µl of 50 mM Tris-HCl, pH 7.5, 20 mM KCl, 1 mM dithionite, and was transferred to the chamber of a syringe attached to a filter device containing a GSWP 01300 Millipore filter (25). Reduction of the iron-sulfur center for β2 or of the glycyl radical for αβ2. Recording conditions were as follows: microwave power, 0.1 milliwatt; modulation amplitude, 1 millitesla; frequency, 9.62 GHz; modulation amplitude, 1 millitesla; temperature, 20 K.

In the second step, 15 µl of the substrate mixture (giving a final concentration of 1.4 mM [3H]CTP (10 pmol), 1 mM ATP, 10 mM MgCl2) was added, and 0.1 µl of cold water, and push through the filter. The filter was removed, blotted from underneath with Kleenex, and then centrifuged. After an additional 20 min, the closed tube was shifted to an anaerobic hood kept at +4 °C, received 0.9 µl of 50 mM Tris-HCl, pH 7.5, 20 mM KCl, 1 mM dithionite, and was transferred to the chamber of a syringe attached to a filter device containing a GSWP 01300 Millipore filter (25). Reduction of the iron-sulfur center for β2 or of the glycyl radical for αβ2. Recording conditions were as follows: microwave power, 0.1 milliwatt; modulation amplitude, 1 millitesla; frequency, 9.62 GHz; modulation amplitude, 1 millitesla; temperature, 20 K.

In the third step, the protein solution received first sodium formate, 30 mM KCl was deaerated inside an anaerobic box during a 1-h reaction under anaerobic conditions, the EPR tube was frozen in liquid nitrogen and analyzed by EPR spectroscopy for its glycyl radical content.

**EPR Spectroscopy**—Low temperature EPR spectra were recorded on a Varian E109 (9.5 GHz) EPR spectrometer or on a Bruker ESP 300 EPR spectrometer, both equipped with an Oxford Instruments ESR 900 Helium Flow Cryostat. Double integrals of EPR signals were evaluated by using a computer online with the spectrometer. All of the 0.2-m1 samples were introduced into quartz EPR tubes under argon and sealed with a rubber septum.

**1-EPR Spectrum of the Glycyl Radical**—1-EPR spectrum of the glycyl radical was measured from an EPR tube with 0.5 mM AdoMet, 5 mM DTT, 5 mM sodium formate, 30 mM KCl, 20 µg of flavodoxin, 250 µg of flavodoxin reductase, 1.25 mM NADPH in 0.2 ml of 30 mM Tris-HCl, pH 7.5. After 10 min reaction under anaerobic conditions, the EPR tube was frozen in liquid nitrogen and analyzed by EPR spectroscopy for its glycyl radical content.

**2-EPR Spectrum of the Reduced Iron-Sulfur Cluster**—Proteins β2 or αβ2 (20–140 pmol) in 30 mM Tris-HCl, pH 8, were dosed in EPR tubes inside an anaerobic box at 4 °C for 1 h. Then the EPR tubes were transferred to an anaerobic manifold. Some of them received 5–10 mM dithionite exclusively, while others received first a deraferol solution of AdoMet (from 0 to 5 mM) immediately followed by dithionite. After a 40-min incubation at room temperature, the tubes were frozen in liquid nitrogen and analyzed by EPR spectroscopy.

For the power saturation experiment (Fig. 1D) the amplitude of the central component of the EPR signal (γ = 1.92) was measured as a function of the incident microwave power in the 40–4 dB (0.02–82 milliwatts) range at 10 K. The amplitude at the lowest power value was taken as 100%. At each power increment, the amplifier gain was changed to keep the signal amplitude constant under nonstirring conditions.

**Methionine Determination**—Methionine content was determined with a Pharmacia-LKB41511 Alpha Plus amino acid analyzer.

**Single Turnover Experiments**—All of the following procedures were done under strict anaerobic conditions. β2 or αβ2, in 30 mM Tris-HCl, pH 8, 30 mM KCl was dosed inside an anaerobic box during a 1-h reaction under anaerobic conditions.

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formation of methionine during the binding process. The smaller volume provided a higher sensitivity for the assays. In these experiments, we could also determine the amount of AdoMet bound to the filter from the loss of radioactivity in the filtrate.

All binding experiments were done as duplicates. With some practice, variation between duplicates was usually below 10%. The limitations of the method arise from a combination of two problems: the limited capacity of the filter to bind protein and the increasing background radioactivity at increasing concentrations of the labeled ligand.

RESULTS

Spectroscopic Properties of the \( \alpha_2\beta_2 \) Complex—In the following, \( \alpha_2\beta_2 \) defines solutions obtained by incubation of stoichiometric 1:1 amounts of each pure protein: the iron-free \( \alpha_2 \) large protein and the \( \beta_2 \) small protein (containing two irons and two sulfides per polypeptide chain) prepared separately from overproducing *E. coli* strains (7). Fig. 1 shows the light absorption spectrum of the oxidized enzyme (Fig. 1A) and the EPR spectrum after anaerobic reduction with either dithionite or photoreduced 5-DAF (Fig. 1B). Both spectra of \( \alpha_2\beta_2 \) were almost identical to those of \( \beta_2 \), reported previously (7). The power saturation properties (Fig. 1C) and the temperature dependence (not shown) of this EPR signal are characteristic of a \([4\text{Fe}-4\text{S}]^+\) center and close to those of \( \beta_2 \). Quantification of the EPR signal showed that the reduction was quantitative with 5-DAF as the reducing agent. With dithionite, the reduction yield did not exceed 50–70%. This then indicates that the properties of \( \alpha_2\beta_2 \) are exclusively due to the \([4\text{Fe}-4\text{S}]^+\) center and close to those of \( \beta_2 \).
Center, as shown from the appearance of an intense characteristic isotropic EPR signal at $g = 2.01$ (Fig. 1D). This explains why we earlier concluded that the reductase, when purified by dATP-Sepharose chromatography, contains a $[3\text{Fe-4S}^1]$ cluster (9).

Activation of $\alpha_2\beta_2$: Requirement of an Electron Source—Activation of $\alpha_2\beta_2$ involves the generation of a glycy radical on $\alpha_2$ during anaerobic incubation with AdoMet, K$^+$, DTT, and a reducing system. Photoreduced 5-DAF (19) could generate active enzymes with specific activities of up to 1000 nmol of dCTP/min/mg of protein. As shown in Fig. 2 (A and B), dithionite was also able to activate $\alpha_2\beta_2$. The maximal specific activity, obtained with a 12-fold molar excess of dithionite after a 30-min incubation, was 400. As shown above, both dithionite and 5-DAF converted the iron-sulfur center of $\alpha_2\beta_2$ to the EPR-active reduced $[4\text{Fe-4S}]^1$ form.

NADPH plus flavodoxin plus flavodoxin reductase also generates an active $\alpha_2\beta_2$ protein, with a specific activity of about 400 (19). Quantitation of the EPR signal of the glycy radical (Fig. 3) showed formation of up to one radical per $\alpha_2\beta_2$ after a 10-min reaction. However, this reducing system did not give rise to any discernible iron-based EPR signal during incubation with $\alpha_2\beta_2$ in the absence of AdoMet. This showed that the enzyme was not reduced to the $[4\text{Fe-4S}]^1$ form by flavodoxin.

The Cleavage of AdoMet to Methionine Is Catalyzed by the Iron-Sulfur Center—During activation of the enzyme, AdoMet is converted to 5'-deoxyadenosine and methionine (17). This reaction can be monitored by using $[\text{methyl}^{3}H]\text{AdoMet}$ and measuring the formation of $[\text{methyl}^{3}H]\text{methionine}$. Fig. 4 shows that $\alpha_2\beta_2$ catalyzes the reduction of AdoMet to methionine either by NADPH in the presence of flavodoxin and flavodoxin reductase or by photoreduced 5-DAF. DTT greatly stimulated the reaction (data not shown). No methionine could be detected when $\beta_2$ or the reducing agent was omitted from the activation mixture. As shown in Fig. 4, $\beta_2$ alone could support the reaction with 5-DAF as the reducing agent, with DTT again required for maximal activity. However, no methionine formation was observed with the flavodoxin system as a reducing reagent (data not shown). Thus, $\alpha_2$ had a clear stimulating effect. Since apo-$\beta_2$, the iron-depleted form, was totally
The complete system was as described under "Experimental Procedures" with 51 \( \mu \)g of \( \beta_2 \) and 13 \( \mu \)M AdoMet in a filtration volume of 0.15 ml. The tubes were kept in the anaerobic box at +4 °C and filtered after the indicated time periods. Methionine formation was determined on a portion of the filtrate (C). The radioactivity of a second portion was used to determine the amount of bound AdoMet (●).

inactive (data not shown) with either reducing system even in the presence of \( \alpha_2 \), the iron-sulfur center appeared to be the key component of the enzyme.

**Binding of AdoMet to Reduced \( \beta_2 \): Stoichiometry and Binding Constant**—Binding experiments with [methyl-\( ^3 \)H]AdoMet were done under anaerobic conditions by a filter binding assay (25), as described under "Experimental Procedures." The protein was first reduced on the anaerobic manifold with either DTT, a flux of electrons first generates the reduced form of the iron-sulfur center in the absence of AdoMet. After transfer to the dark, AdoMet was added, and the electron transfer from reduced \( \beta_2 \) to AdoMet was monitored in the absence of a continued reduction of \( \beta_2 \). Changes in the [4Fe-4S] cluster were observed by EPR spectroscopy, and the formation of methionine served to measure the one-electron reduction of AdoMet. Fig. 6 shows the time-dependent decay of the EPR signal of reduced \( \beta_2 \) and the parallel formation of methionine. Unexpectedly, the reaction occurred in two steps. In the first, the loss of the EPR signal and formation of one methionine per \( \beta_2 \) followed first order kinetics with the same rate constant (0.12 min\(^{-1}\)). In the second step, and with the same rate constant, an additional 1 mol of methionine were formed per mol of \( \beta_2 \) with no change of the iron-sulfur center detectable by EPR spectroscopy, since the solution then remained EPR-silent. In the absence of AdoMet, the EPR signal did not decay during 1 h of incubation in the dark. In the absence of DTT, little methionine (0.1 methionine/\( \beta_2 \)) was formed during the 60-min incubation.

The same experiment was also carried out with \( \alpha_2 \beta_2 \). In this case, EPR spectroscopy was also used to monitor the generation of the glycyl radical on \( \alpha_2 \). In less than 3 min, glycyl radical formation was complete. Methionine continued to be formed up to 10 min, when 3 methionines/glycyl radical had been generated, but then stopped (Fig. 9). At the earliest time point, the EPR signal of the the iron-sulfur center was no more detecta-
In the absence of DTT, less than 0.1 methionine/\( \beta_2 \) and less than 0.01 radical/\( \beta_2 \) were formed after 60 min, again showing the importance of DTT for both the reductive cleavage of AdoMet and radicalization of \( \beta_2 \).

**DISCUSSION**

The ribonucleotide reductase isolated from anaerobically grown *E. coli* cells is the prototype of class III ribonucleotide reductases (1). It consists of an \( \alpha_2 \beta_2 \) complex, with \( \beta_2 \) carrying an essential iron-sulfur center (7). Since \( \beta_2 \) after reconstitution binds only 2Fe-2S and 2S2 per polypeptide chain and in the reduced form exhibits an EPR signal characteristic of a [4Fe-4S]1 center, our present model is that the homodimer \( \beta_2 \) harbors a [4Fe-4S] cluster at the interface of its two polypeptide chains. However, this assignment mainly rests on EPR data and awaits confirmation from other spectroscopic studies.

Tight binding of \( \alpha_2 \) to \( \beta_2 \) is shown here to have only slight effects on the light absorption and the EPR properties of the iron-sulfur cluster of \( \beta_2 \). In fact, this iron-sulfur center seems to be unique in terms of its spectroscopic properties, which are currently under investigation. It is quite labile, especially in the reduced form, but can be significantly stabilized by the addition of DTT (7). This may, at least partly, explain the strong stimulating effect of DTT on the enzyme reaction. All of these unique features might be due to the fact that the [4Fe-4S] center is shared by two polypeptide chains, a situation that has been previously observed only in two cases: the iron protein of the nitrogenase (26) and the cluster FX of the photosystem I (27).

The anaerobic ribonucleotide reductase is a radical enzyme. This is a rather trivial statement, since all ribonucleotide re-
ductases known so far contain a free radical on one of their polypeptide chains (1). Generation of the radical at glycine 681 of the large component of the enzyme requires AdoMet, K+, DTT, and a source of electrons. The latter can either be a chemical reagent such as photoreduced 5-DAF or dithionite (Fig. 2) or an enzyme system consisting of NADPH plus flavodoxin plus flavodoxin reductase, with comparable activities (19).

In Scheme 1, we summarize our view of the mechanism of the reaction. A first version of this mechanism was published in 1993 but had at that time no experimental support. From the present work, we confirm this earlier speculation with experimental evidence for a direct electron transfer from the reduced cluster to AdoMet.

\[
\begin{align*}
(1) & \quad [\text{Fe-S}]_{\text{ox}} \rightarrow [\text{Fe-S}]_{\text{red}} \\
(2) & \quad [\text{Fe-S}]_{\text{red}} + \text{AdoMet} \rightarrow [\text{Fe-S}]_{\text{red}} \cdot \text{AdoMet} \\
(3) & \quad [\text{Fe-S}]_{\text{red}} \cdot \text{AdoMet} \rightarrow [\text{Fe-S}]_{\text{red}} + \text{methionine} + \text{Ado}^+ \\
(4) & \quad \text{Ado}^+ + \text{glyH} \rightarrow \text{gly}^+ + \text{AdH} \\
& \quad \text{Ado}^+ = 5'-\text{deoxyadenosyl radical} \\
& \quad \text{AdH} = 5'-\text{deoxyadenosine}
\end{align*}
\]

**Scheme 1. Proposed mechanism for the formation of the glycyl radical.**

Since enzyme activation and methionine formation absolutely require the presence of a reducing agent, we suggest that the active form of $\beta_2$ is the reduced one in which the cluster is in the EPR-active $[4\text{Fe}-4\text{S}]^2-$ state. This form accumulates when $\beta_2$ or $\alpha_2\beta_2$ is incubated with dithionite or photoreduced 5-DAF in the absence of AdoMet (Scheme 1, reaction 1). Enzymatically reduced flavodoxin, the physiological electron donor, is unable to reduce the iron-sulfur center to an EPR-detectable level in the absence of AdoMet. Nevertheless, it supports methionine and glycyl radical formation in the presence of AdoMet. A likely explanation for this apparent paradox is that, in the absence of AdoMet, the redox potential of the metal center is below $-350$ mV, high enough to allow electron capture from dithionite or 5-DAF but not from reduced flavodoxin. Binding of AdoMet and coupling of the reduction of the cluster to its reoxidation by AdoMet might result in an increase of the apparent redox potential of the cluster and drive the whole process. Whether AdoMet affects the redox potential of the iron-sulfur center is a fascinating hypothesis that remains to be studied.

The drastic and instantaneous change of the shape, but not the intensity, of the EPR signal of the reduced cluster upon the addition of AdoMet strongly suggests that, in the second step, AdoMet binds to $\beta_2$ (also in the presence of $\alpha_2$) to form an enzyme-AdoMet complex (Scheme 1, reaction 2). The large modification of the EPR signal, reflecting a change of the symmetry of the metal center, can be explained if AdoMet comes to a site in close proximity to that center or if AdoMet binding causes a significant conformational change of the polypeptide chain affecting the center.

The results from direct binding experiments support this interpretation. One mol of $\beta_2$ bound close to 1 mol of AdoMet with a $K_d$ value of 10 $\mu$M. Binding depended completely on the presence of the reduced cluster, thus explaining why early efforts to demonstrate binding of AdoMet to $\beta_2$ by methods involving equilibrium dialysis under aerobic conditions met with no success. DTT and $K^+$ were also required, pinpointing the earlier described requirements of enzyme activation for $K^+$ and DTT to the binding step. At first sight, it may seem unexpected that the homodimeric $\beta_2$ protein binds only one molecule of AdoMet and not two. This instead is in line with the proposal of a unique active center containing both the cluster and the AdoMet binding site at the interface of the two polypeptide chains.

For the third step, we have now evidence from single turnover experiments that the reduced iron-sulfur center of $\alpha_2\beta_2$ in the presence of DTT transfers electrons to AdoMet and induces its cleavage. Methionine was formed at the expense of the reduced cluster which, in parallel, was oxidized to an EPR-silent form. Cleavage of AdoMet is proposed to generate transiently one equivalent of a 5′-deoxyadenosyl radical and subsequently one stable glycyl radical (Scheme 1, Reaction 4). Since this reaction is fast, only the final glycyl radical and not the intermediate 5′-deoxyadenosyl radical could be detected by EPR spectroscopy. A puzzling observation was that three methionines were reproducibly formed per glycyl radical. That the reduced iron cluster is able to transfer electrons to AdoMet is further supported by the observation that electrons from the iron-sulfur cluster of $\beta_2$ were efficiently transferred to AdoMet with no need for $\alpha_2$; again, methionine was formed, while the EPR-active reduced cluster was oxidized to an EPR-silent form. Also in this case, as with $\alpha_2\beta_2$, 3 methionines/$\beta_2$ were generated. However, the reaction was much slower, showing that $\alpha_2$ binding to $\beta_2$ had a strong stimulatory effect. The rate of formation of the first equivalent of methionine was identical to the rate of oxidation of the cluster (Fig. 8), in agreement with reaction 3 of Scheme 1. Subsequently, two additional methionines were formed at the same rate, whereas the iron center remained EPR-silent. These results lead to the unusual conclusion that during reduction of both $\beta_2$ and $\alpha_2\beta_2$ the iron-sulfur cluster has the ability to store as many as three electron equivalents, which subsequently can be delivered at a sufficiently low redox potential to reduce three molecules of AdoMet. In the case of $\alpha_2\beta_2$, only the first electron serves to generate the glycyl radical, in agreement with reactions 3 and 4 of Scheme 1. This then explains why, as shown in Fig. 9, methionine formation continues, whereas the glycyl radical has reached its maximum.

We have also shown that $\alpha_2\beta_2$ can achieve multiple turnovers during reduction of AdoMet to methionine and thus behaves as an S-adenosylmethionine reductase. The catalytic properties of $\alpha_2\beta_2$ are due to the presence of the iron-sulfur cluster as apo$\beta_2$ in the presence of $\alpha_2$ or $\alpha_2$ alone was totally inactive. $\beta_2$ alone was by itself able to support the reaction, but less efficiently (Fig. 4). It would be interesting to understand the molecular basis for the potentiating effect of $\alpha_2$ on the reducing properties of the iron-sulfur center, since it may provide new insights into how the chemical reactivity of iron-sulfur centers can be modulated.

Reduction of AdoMet is a new biological function for an iron-sulfur protein, since there is no precedent for an iron-sulfur center that catalyzes such a redox reaction. The list of functions for this class of metal clusters has expanded recently and includes electron transport (photosynthesis, respiration, etc.); catalysis, but only in nonredox reactions such as dehydration reactions (e.g., aconitase and related systems); regulation of transcription (28); stabilization of protein structure (29); and stabilization of reactive intermediates (30). We now show that iron-sulfur proteins may also be involved in catalysis of redox reactions. Such a metal center might be required especially when the electron acceptor has to be reduced at a very low potential, as is the case with AdoMet. Sulfonium compounds are known to be very stable molecules that can be reduced at a redox potential of about $-1$ V in organic solvents, as shown by electrochemistry (31).

**Conclusion**—There are still a number of questions to solve and a number of unexpected observations to interpret, in par-
ticular the 3:1 stoichiometry for methionine formation that was observed for both $\beta_2$ and $\alpha_2\beta_2$. We believe that many answers and the understanding of the precise mechanism of the reaction will come from further investigation of the iron-sulfur cluster of the anaerobic ribonucleotide reductase. In fact, from many spectroscopic and functional observations, it seems to be unusual. It is also important to investigate the role of DTT, which appears to be a key component of the reaction. DTT serves to stabilize the iron-sulfur center but probably also has functional roles that remain to be identified. Nevertheless, this work provides the first demonstration that cleavage of AdoMet, release of methionine, and generation of the glycyl radical derive directly from the electron transfer from the reduced iron-sulfur cluster of the enzyme to AdoMet. This indicates that iron-sulfur clusters can also have redox catalytic functions, a concept that is currently emerging (32).

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