

# The pyridoxal-5'-phosphate-dependent catalytic antibody 15A9: its efficiency and stereospecificity in catalysing the exchange of the $\alpha$ -protons of glycine

Marrita M. Mahon<sup>a</sup>, Svetlana I. Gramatikova<sup>1,b</sup>, Philipp Christen<sup>b</sup>, Teresa B. Fitzpatrick<sup>a</sup>, J. Paul G. Malthouse<sup>a,\*</sup>

<sup>a</sup>Department of Biochemistry, University College Dublin, Belfield, Dublin 4, Ireland

<sup>b</sup>Biochemisches Institut der Universität Zürich, Zürich, Switzerland

Received 23 March 1998

**Abstract** <sup>13</sup>C-NMR has been used to follow the exchange of the  $\alpha$ -protons of [2-<sup>13</sup>C]glycine in the presence of pyridoxal-5'-phosphate and the catalytic antibody 15A9. In the presence of antibody 15A9 the 1st order exchange rates for the rapidly exchanged proton of [2-<sup>13</sup>C]glycine were only 25 and 150 times slower than those observed with tryptophan synthase (EC 4.2.1.20) and serine hydroxymethyltransferase (EC 2.1.2.1). The catalytic antibody increases the 1st order exchange rates of the  $\alpha$ -protons of [2-<sup>13</sup>C]glycine by at least three orders of magnitude. We propose that this increase is largely due to an entropic mechanism which results from binding the glycine-pyridoxal-5'-phosphate Schiff base. The 1st and 2nd order exchange rates of the pro-2S proton have been determined but we were only able to determine the 2nd order exchange rate for the pro-2R proton of glycine. In the presence of 50 mM glycine the antibody preferentially catalyses the exchange of the pro-2S proton of glycine. The stereospecificity of the 2nd order exchange reaction was quantified and we discuss mechanisms which could account for the observed stereospecificity.

© 1998 Federation of European Biochemical Societies.

**Key words:** Catalytic antibody; Pyridoxal-5'-phosphate; Stereospecificity; Glycine;  $\alpha$ -Proton exchange; NMR

## 1. Introduction

A wide range of catalytic antibodies have been produced which catalyse a diverse range of reactions with varying degrees of catalytic efficiency [1–3]. Most catalytic antibodies are formed by producing antibodies which bind transition state analogues. This approach is made more difficult when attempting to mimic enzymes which utilise cofactors. One of the earliest attempts at generating catalytic antibodies which bound a cofactor was reported by Raso and Stollar who generated polyclonal antibodies to a reduced aldimine hapten formed from 3-aminotyrosine and pyridoxal-5'-phosphate [4–6]. The antibodies formed bound the hapten but did not have any significant catalytic properties. A similar approach has been used to generate a monoclonal antibody which catalyses Schiff base formation between D-*p*-nitrophenylalanine and 5'-deoxy pyridoxal [7]. This procedure has also been

used to produce a monoclonal antibody, the catalytic antibody 15A9, which will catalyse in addition to Schiff base formation, transamination and  $\alpha$ -,  $\beta$ -elimination reactions [8,9]. It is this antibody that we have studied in the present work.

Pyridoxal-5'-phosphate-dependent enzymes catalyse a wide range of reactions in amino acid metabolism [10–12]. The pyridoxal-5'-phosphate cofactor is capable of catalysing a range of reactions involving amino acids and one of the main roles of the protein component of these enzymes is to direct catalysis down one of the many potential reaction pathways [12]. Dunathan has proposed that the course of the reaction will be determined by which of bonds from the  $\alpha$ -carbon to the  $\alpha$ -carboxylate, the  $\alpha$ -proton or the R-group lies together with the imine nitrogen atom in a plane orthogonal to the plane of the imine-cofactor p-electron system [13]. The majority of the reactions catalysed by pyridoxal-5'-phosphate-dependent enzymes proceed via loss of the  $\alpha$ -proton of the amino acid substrate making this a key step in catalysis [11]. The stereospecificity of the exchange of the  $\alpha$ -protons of L- and D-amino acids or of glycine is expected to depend on which of the  $\alpha$ -protons is orthogonal to the plane of the imine-cofactor p-electron system [14,15]. Recent NMR kinetic studies have quantified the stereospecificity of the tryptophan synthase and serine hydroxymethyltransferase catalysed exchange of the  $\alpha$ -protons of glycine [16–21]. In the present work we use the same approach to determine and quantify the stereospecificity of the exchange of the  $\alpha$ -protons of glycine when it is catalysed by the catalytic antibody 15A9. We compare our results with those obtained with tryptophan synthase, serine hydroxymethyltransferase and model compounds.

## 2. Materials and methods

### 2.1. Materials

[2-<sup>13</sup>C]glycine (99 atom %), <sup>2</sup>H<sub>2</sub>O (99.8 atom %) and 1,3-bis[tris-(hydroxymethyl)methylamino]propane were obtained from Aldrich Chemical Co. and Fluka Chemicals Ltd. respectively at Gillingham, Dorset, UK. All other chemicals used were obtained from Sigma Chemical Co., Poole, Dorset, UK.

### 2.2. Catalytic activity of the catalytic antibody 15A9

All measurements were performed in 0.05 M 1,3-bis[tris-(hydroxymethyl)methylamino]propane, 0.14 M NaCl, pH 7.0. The production of pyruvate from  $\beta$ -chloro-D-alanine (5–65 mM), in the presence of 1–5  $\mu$ M antibody and 1 mM pyridoxal-5'-phosphate, was measured in a coupled assay with lactate dehydrogenase and NADH. The concentration of antibody protein was determined using  $E_{280\text{mg/ml}} = 1.4$  [8] and  $M_r = 150\,000$ . The concentrations of antibody binding sites were calculated using  $k_{\text{cat}} = 50 \text{ min}^{-1}$  for  $\beta$ -chloro-D-alanine [9] and by assuming that there were 2 moles of binding sites per mole of protein.

\*Corresponding author. Fax: +49 (3531) 2837211.  
E-mail: J.Paul.G.Malthouse@ucd.ie

<sup>1</sup>Present address: Department of Molecular Biology, BCC-515, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA.

### 2.3. Determination of the apparent dissociation constant of the glycine-pyridoxal-5'-phosphate Schiff base

10–50  $\mu$ l aliquots of 0.1–1.0 M glycine were added to 0.7 ml of 50  $\mu$ M pyridoxal-5'-phosphate in 0.05 M potassium phosphate, pH 7.0 to give solutions containing 1.4–71.4 mM glycine. The absorbance at 440 nm was measured after each addition of glycine. The apparent dissociation constant was determined by fitting the experimental data to the following equation,  $\Delta A_{440\text{obs}} = (\Delta A_{440\text{max}} \cdot K) / (S + K)$ .

### 2.4. Preparation of [2-<sup>13</sup>C,pro-2S-<sup>2</sup>H]glycine

All solutions were prepared in 99.9 atom % <sup>2</sup>H<sub>2</sub>O. The serine hydroxymethyltransferase catalysed exchange of the pro-2S proton of [2-<sup>13</sup>C]glycine was initiated by adding 0.05 ml of 1.46 mM serine hydroxymethyltransferase to a 2.45 ml solution containing 76 mg [2-<sup>13</sup>C]glycine and 0.05 M potassium phosphate at pH 7.0. The solution of 2.5 ml contained 29.2  $\mu$ M serine hydroxymethyltransferase and 0.4 M [2-<sup>13</sup>C]glycine. When the exchange of the pro-2S proton was completed, the reaction was stopped by the addition of 0.5 ml of 1 M deuterium chloride to the sample. The pH was restored to 7.0 with the addition of 0.24 ml of [2H]potassium hydroxide. The precipitated enzyme was removed by centrifugation and the remaining solution assayed for serine hydroxymethyltransferase activity. No residual enzyme activity could be detected. The sample was then freeze-dried and dissolved in 1.0 ml <sup>2</sup>H<sub>2</sub>O to give a solution containing 0.97 M [2-<sup>13</sup>C,pro-2S-<sup>2</sup>H]glycine, 0.07 M KCl and 0.12 M potassium phosphate at pH 7.0.

### 2.5. NMR spectra

Spectra were recorded using a Bruker WP80 wide bore spectrometer operating at 20.115 MHz for <sup>13</sup>C-nuclei. Sample sizes were 0.9–1.0 ml with 10 mm diameter sample tubes. Spectral conditions were: 8000 time domain data points; 0.852 s acquisition time; spectral width 240 p.p.m.; 2 Hz exponential weighting factor; 11° pulse. Proton decoupling was not used.

### 2.6. NMR samples: determining the stereospecificity of exchange

All samples were made up in 99.8 or 99.9 atom % <sup>2</sup>H<sub>2</sub>O and contained 0.05 M 1,3-bis[tris(hydroxymethyl)methylamino]propane, 0.14 M NaCl, pH 7.1.

Antibody 15A9 catalysed exchange of the  $\alpha$ -protons of [2-<sup>13</sup>C]glycine were initiated by adding 0.045 ml of 1.0 M [2-<sup>13</sup>C]glycine to an 0.855 ml solution containing antibody 15A9. The final solution of 0.9 ml contained 26.7  $\mu$ M antibody binding sites, 1 mM pyridoxal-5'-phosphate and 0.05 M [2-<sup>13</sup>C]glycine.

All NMR spectra were recorded at 25  $\pm$  1°C. However, one sample (Fig. 1b–d) was maintained at 37°C in between recording NMR spectra by storing the NMR tube containing the sample in a water bath filled with distilled water at 37°C. The other sample (Fig. 1a, e–g) was kept at 25  $\pm$  1°C until our studies were completed. pH values were recorded at the end of the reactions with an electrode standardised in <sup>1</sup>H<sub>2</sub>O buffers before being transferred to <sup>2</sup>H<sub>2</sub>O.

The stereospecificity of the antibody 15A9 catalysed exchange of the  $\alpha$ -protons of glycine was determined by adding tryptophan synthase to the sample incubated with the catalytic antibody at 25  $\pm$  1°C and serine hydroxymethyltransferase to the sample incubated with the catalytic antibody at 37°C. Fifteen  $\mu$ M tryptophan synthase and 1.4  $\mu$ M serine hydroxymethyltransferase were incorporated into the appropriate samples. When adding serine hydroxymethyltransferase full anaerobic precautions were taken and 5 mM 2-mercaptoethanol and 0.2 mM tetrahydrofolic acid were incorporated into the sample [16].

### 2.7. NMR samples: antibody 15A9 catalysed exchange of the pro-2S proton of [2-<sup>13</sup>C]glycine

All samples were prepared in 99.8 atom % <sup>2</sup>H<sub>2</sub>O containing 0.05 M 1,3-bis[tris(hydroxymethyl)methylamino]propane, 0.14 M NaCl, pH 7.1. Exchange of the pro-2S proton of [2-<sup>13</sup>C]glycine was initiated by adding 0.045–0.36 ml of 1 M [2-<sup>13</sup>C]glycine to solutions containing 0.018–0.037 ml antibody. The final 0.9 ml samples contained 1 mM pyridoxal-5'-phosphate, 0.16–0.30  $\mu$ M antibody binding sites and 0.05–0.4 M [2-<sup>13</sup>C]glycine. The exchange was followed by initial rate measurements [19].

### 2.8. Antibody 15A9 catalysed exchange of the pro-2R proton of [2-<sup>13</sup>C,pro-2S-<sup>2</sup>H]glycine

All samples were prepared in 99.9 atom % <sup>2</sup>H<sub>2</sub>O containing 0.05 M

1,3-bis[tris(hydroxymethyl)methylamino]propane buffer and 0.14 M NaCl at pH 7.1. Exchange of the pro-2R proton of [2-<sup>13</sup>C,pro-2S-<sup>2</sup>H]glycine was initiated by the addition of 0.047–0.37 ml of [2-<sup>13</sup>C,pro-2S-<sup>2</sup>H]glycine to solutions containing 0.19–0.49 ml antibody. Buffer and pyridoxal-5'-phosphate were added to give final solutions of 0.9 ml containing 1 mM pyridoxal-5'-phosphate, 3.4–9  $\mu$ M antibody binding sites and 0.05–0.4 M [2-<sup>13</sup>C,pro-2S-<sup>2</sup>H]glycine. The exchange was followed by initial rate measurements [19]. In control samples in which the antibody was omitted, no exchange of the pro-2R of [2-<sup>13</sup>C,pro-2S-<sup>2</sup>H]glycine was detected.

## 3. Results and discussion

### 3.1. Exchange reactions catalysed by model compounds

Spectrophotometric studies showed that glycine and pyridoxal-5'-phosphate formed a Schiff base ( $\lambda_{\text{max}} = 410$  nm) which had an apparent dissociation constant of  $9.4 \pm 1.8$  mM at pH 7.0 and 25°C. However, the absorbance due to the Schiff base decreased slowly with time. With 0.1 mM pyridoxal-5'-phosphate and 0.4 M glycine the half life for the breakdown of the Schiff base was 13.9 days at pH 7.0 and 25°C. When this experiment was repeated using [2-<sup>13</sup>C]glycine no evidence for the exchange of the  $\alpha$ -protons of

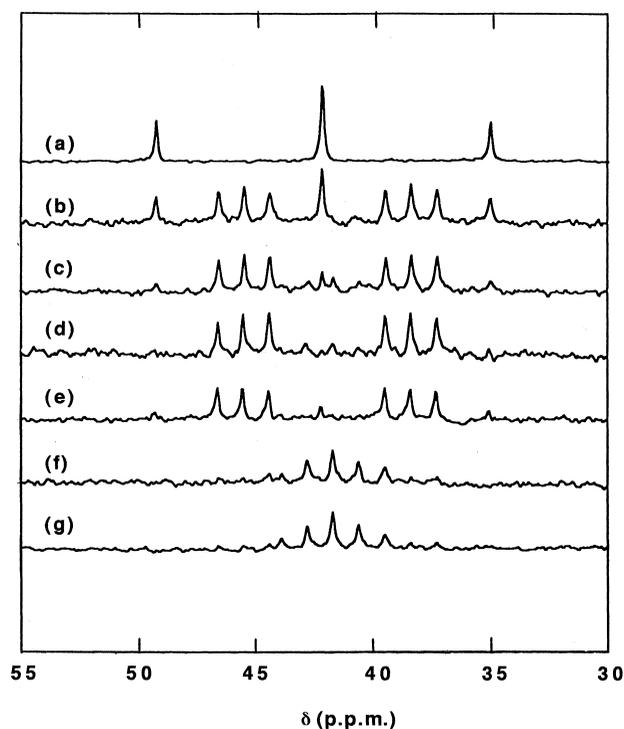


Fig. 1. Determination of the stereospecificity of antibody 15A9 catalysed exchange of  $\alpha$ -protons of [2-<sup>13</sup>C]glycine at pH 7.1. All samples contained: 0.05 M [2-<sup>13</sup>C]glycine, 1 mM pyridoxal phosphate, 0.14 M sodium chloride, 0.05 M 1,3-bis[tris(hydroxymethyl)methylamino]propane at pH 7.1. The samples used for spectra b and c also contained 26.7  $\mu$ M catalytic antibody and were incubated at 37°C for 10.8 and 30.2 days respectively before spectrum acquisition began. Spectrum d was recorded under anaerobic conditions 2 h after incorporating 1.4  $\mu$ M serine hydroxymethyltransferase, 0.2 mM tetrahydrofolic acid and 5 mM 2-mercaptoethanol into the sample c. Spectrum e was acquired 31.7 days after incubation with 26.7  $\mu$ M catalytic antibody at 25°C. Spectra f and g were recorded 2 h after the addition of 15  $\mu$ M tryptophan synthase to the sample used for spectrum e. 61440 transients were recorded for spectrum g while 16384 transients were recorded for spectra a–f. Acquisition parameters were as described in Section 2.

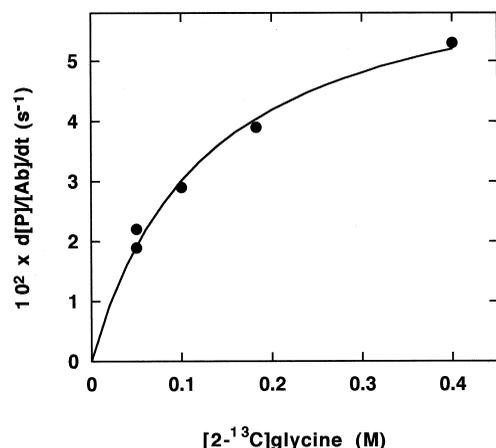


Fig. 2. Effect of the concentration of  $[2-^{13}\text{C}]$ glycine on the hydrogen-deuterium exchange rate of the pro-2S proton of  $[2-^{13}\text{C}]$ glycine catalysed by catalytic antibody 15A9 at pH 7.1.  $[2-^{13}\text{C}]$ glycine concentrations of 0.05–0.4 M and antibody concentrations of 0.158–0.314  $\mu\text{M}$  were used. All samples contained 1 mM pyridoxal phosphate, 0.05 M 1,3-bis[tris(hydroxymethyl)methylamino]propane and 0.14 M NaCl at pH 7.1. The continuous line was calculated using  $d[\text{P}]/[\text{A}_b]/dt = k_a[\text{S}_0]/([\text{S}_0] + K)$  and the fitted parameters  $k_a = 0.067 \pm 0.004 \text{ s}^{-1}$ ,  $K = 0.128 \pm 0.017 \text{ M}$  and  $k_a/K = 0.523 \pm 0.076 \text{ M}^{-1} \text{ s}^{-1}$ .

$[2-^{13}\text{C}]$ glycine was obtained. Therefore, we conclude that the rate of exchange of the  $\alpha$ -protons of  $[2-^{13}\text{C}]$ glycine must be much slower than the rate of breakdown of the Schiff base. By examining the NMR spectra obtained over a period of one half life for the breakdown of the Schiff base we estimate that the exchange rate of the  $\alpha$ -protons of 0.4 M  $[2-^{13}\text{C}]$ glycine in the presence of 0.1 mM pyridoxal-5'-phosphate must be  $< 0.000002 \text{ s}^{-1}$ . Increasing the concentration of pyridoxal-5'-phosphate to 10 mM enabled us to observe the signal at 55.6 ppm due to the  $^{13}\text{C}$ -enriched carbon of the Schiff base [22]. As expected, the rate of loss of the signal at 55.6 ppm was the same as the rate at which the absorbance due to the Schiff base at 410 nm decreased.

### 3.2. Determination of the stereospecificity of antibody 15A9 towards the $\alpha$ -protons of a glycine-pyridoxal-5'-phosphate Schiff base

In the absence of proton decoupling the  $^{13}\text{C}$ -enriched  $\alpha$ -carbon of a control sample of  $[2-^{13}\text{C}]$ glycine gives a characteristic triplet (Fig. 1a). This control sample does not contain the

antibody but it does contain the same concentration of pyridoxal-5'-phosphate as the samples of catalytic antibody. Replacement of one of the  $\alpha$ -protons of glycine with a deuterium results in a spectrum consisting of a pair of triplets (Fig. 1c–e) while replacement of both protons with deuterons gives a characteristic quintet (Fig. 1f, g). Therefore, we can use these spectra to follow the exchange of the  $\alpha$ -protons of glycine. We obtained no evidence for any exchange of the  $\alpha$ -protons of glycine in the control sample over the duration of our experiments. In the presence of the antibody there was  $\sim 64\%$  exchange of one of the  $\alpha$ -protons after incubation at  $37^\circ\text{C}$  for 11 days (Fig. 1b). The half life of exchange under these conditions was  $\sim 9$  days and after 30 days at  $37^\circ\text{C}$  there was  $\sim 84\%$  exchange of one of the  $\alpha$ -protons of  $[2-^{13}\text{C}]$ glycine (Fig. 1c). The sample was then made anaerobic and 1.4  $\mu\text{M}$  serine hydroxymethyltransferase in the presence of 0.2 mM tetrahydrofolate was added. Under these conditions, serine hydroxymethyltransferase will catalyse the exchange of the pro-2S proton of glycine with a half life of  $\sim 45$  min [21]. If the catalytic antibody catalysed the exchange of the pro-2R proton, we expect it to have catalysed the exchange of more than 75% of the remaining pro-2S protons after 2 h. However, the addition of serine hydroxymethyltransferase did not cause a detectable decrease in the intensity of the pair of triplets due to the glycine which had one of its protons exchanged after 2 h (Fig. 1d). This result suggests that the catalytic antibody has preferentially catalysed the exchange of the pro-2S proton of glycine. The unexchanged proton should be the pro-2R proton of glycine which should be preferentially exchanged by tryptophan synthase [16]. After incubating the catalytic antibody with  $[2-^{13}\text{C}]$ glycine for 32 days at  $25^\circ\text{C}$  there was 86% exchange of one of the  $\alpha$ -protons of glycine (Fig. 1e). Under these experimental conditions we calculate that 15  $\mu\text{M}$  tryptophan synthase will preferentially catalyse the exchange of the pro-2R proton of glycine with a half life of  $\sim 48$  min [19]. On adding 15  $\mu\text{M}$  tryptophan synthase there was rapid exchange of the non-exchanged proton to give a characteristic quintet due to  $[2-^{13}\text{C}, ^2\text{H}_2]$ glycine (Fig. 1f, g). This result confirms that catalytic antibody 15A9 in the presence of pyridoxal-5'-phosphate preferentially catalyses the exchange of the pro-2S proton of glycine. The accumulation of the transients of spectra f and g in Fig. 1 were both begun 2 h after adding tryptophan synthase. The only difference between these spectra is that spectra f and g resulted from the accumulation of 16384 and 61440 transients respectively.

Table 1

The kinetics and stereospecificity of the exchange of the  $\alpha$ -protons of  $[2-^{13}\text{C}]$ glycine catalysed by catalytic antibody 15A9, tryptophan synthase and serine hydroxymethyltransferase

Catalyst	pH	Proton exchanged	$k_{a(\text{fast})}$ ( $\text{s}^{-1}$ )	$K$ (M)	$k_a/K$ ( $\text{m}^{-1} \text{ s}^{-1}$ )	Proton exchanged	$k_{a(\text{slow})}$ ( $\text{s}^{-1}$ )	$K$ (M)	$k_a/K$ ( $\text{M}^{-1} \text{ s}^{-1}$ )	Stereospecificity of exchange	
										$k_{a(\text{fast})}/k_{a(\text{slow})}$	$(k_a/K)_{(\text{fast})}/(k_a/K)_{(\text{slow})}$
Pyridoxal-5'-phosphate	7.0	n.d.	$\leq 0.000002$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
15A9 antibody	7.1	pro-2S	0.067	0.13	0.52	pro-2R	$> 0.0076$	$> 0.4$	0.019	$< 9$	28
$\alpha_3\beta_2$ -tryptophan synthase <sup>a</sup>	7.0	pro-2R	1.7	0.054	32.0	pro-2S	0.0069	0.130	0.053	250	600
Serine hydroxymethyltransferase <sup>b</sup>	7.0	pro-2S	10	$\leq 0.01$	$\geq 1000$	pro-2R	0.0015	0.12	0.013	6700	$> 77000$

n.d., not determined.

<sup>a</sup>[19].

<sup>b</sup>[21].

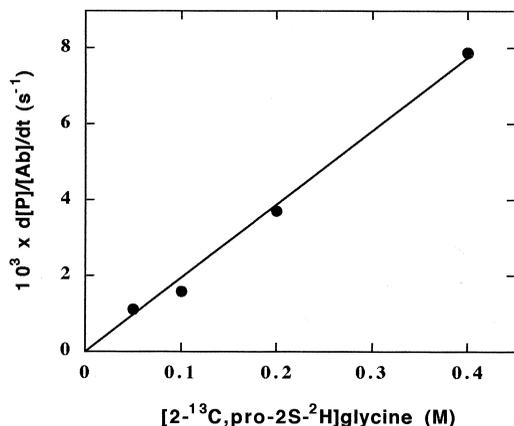


Fig. 3. Effect of the concentration of [2-<sup>13</sup>C, pro-2S-<sup>2</sup>H]glycine on the hydrogen-deuterium exchange rate of the pro-2R proton of [2-<sup>13</sup>C, pro-2S-<sup>2</sup>H]glycine catalysed by catalytic antibody 15A9 at pH 7.1. [2-<sup>13</sup>C, pro-2S-<sup>2</sup>H]glycine concentrations of 0.05–0.4 M and antibody concentrations of 3.4–9 μM were used. All samples contained 1 mM pyridoxal phosphate, 0.05 M 1,3-bis[tris(hydroxymethyl)methylamino]propane and 0.14 M NaCl at pH 7.1. The continuous line was calculated using  $d[P]/[A_b]/dt = (k_a/K)[S_0]$  and the fitted parameter  $k_a/K = 0.019 \pm 0.001 \text{ M}^{-1} \text{ s}^{-1}$ .

### 3.3. The effect of glycine concentration on the exchange rates of the α-protons of glycine by catalytic antibody 15A9

The exchange rates of the rapidly exchanged pro-2S protons of [2-<sup>13</sup>C]glycine showed saturation kinetics (Fig. 2) which allowed us to determine an apparent binding constant (*K*) as well as the 1st and 2nd order exchange rates. However, the exchange rates of the slowly exchanged pro-2R protons of [2-<sup>13</sup>C]glycine were directly proportional to the concentration of [2-<sup>13</sup>C]glycine (Fig. 3) and so while we could measure the 2nd order exchange rate we could only put limits on the values of the apparent binding constant and 1st order exchange rate for the slowly exchanged pro-2R proton (Table 1).

### 3.4. Comparison of the exchange rates and stereospecificities obtained with catalytic antibody 15A9 and those obtained with tryptophan synthase, serine hydroxymethyltransferase and model compounds

For antibody 15A9 the 1st order exchange rates for the rapidly exchanged proton of [2-<sup>13</sup>C]glycine were only 25 and 150 times slower than those observed with tryptophan synthase and serine hydroxymethyltransferase (Table 1). However, the 1st order exchange rates of the slowly exchanged protons of the 15A9 antibody were similar or greater than those of tryptophan synthase and serine hydroxymethyltransferase (Table 1). Therefore, the greater stereospecificity of the 1st order exchange rates of the rapidly exchanged protons with these enzymes is due to their ability to enhance the exchange rate of the rapidly exchanged protons and to reduce the exchange rates of the slowly exchanged protons. For the 2nd order exchange rates, the exchange rates of the slowly exchanged protons are similar (Table 1) and so specificity is largely determined by the exchange rates of the rapidly exchanged protons.

The greater stereospecificity of the enzymes is largely due to their greater efficiency in catalysing the exchange of the rapidly exchanged protons (Table 1). For the enzymes it is expected that evolution will have resulted in them having acid-

base catalysts capable of catalysing the exchange of the rapidly exchanged proton. In tryptophan synthase the active site lysine residue is thought to be the catalytic base [23] while in serine hydroxymethyltransferase some other group is thought to be the catalytic base [24]. But, no such selection process is expected to occur when the catalytic antibody was produced and so it is not surprising that its stereospecificity is ~20–100-fold less than that of the enzymes. However, this does not explain why the catalytic antibody preferentially catalyses the exchange of the pro-2S proton. It has been argued that for optimal catalytic efficiency, the α-carbon bond to be cleaved should be orthogonal to the plane of the imine-cofactor π-electron system [13,15,19]. The stereospecificity of the antibody could be explained if the glycine-pyridoxal-5'-phosphate Schiff base is bound such that the pro-2S proton of glycine is closer than the pro-2R proton to this optimal position. Alternatively, the antibody could possess a base which preferentially catalyses the exchange of the pro-2S proton. If exchange is catalysed by a base on the antibody then we would expect the rapidly exchanged pro-2S proton to point towards the protein. However, if exchange is not catalysed by a base on the antibody then we would expect that the rapidly exchanged pro-2S proton would point towards the solvent while the slowly exchanged pro-2R proton would point towards the protein and so be partially shielded from solvent. It has been argued that pyridoxal-5'-phosphate-dependent enzymes evolved from a common ancestor where the pyridoxal-5'-phosphate adopts an extended conformation with the Cα-H bond orthogonal to the plane of the imine-cofactor ring and pointing toward the surface of the protein to allow protein-assisted deprotonation at Cα [25]. Due to the low stereospecificity of the 15A9 antibody we would not like to speculate as to whether the rapidly exchanged pro-2S proton of glycine points towards or away from the protein surface.

The catalytic antibody catalyses the exchange of the pro-2S and pro-2R protons of glycine at a rate which is 34000- and > 3800-fold faster than the corresponding model compound (Table 1). As we have discussed, the rapidly exchanged proton could be the one exposed to solvent or it could be pointing towards the protein and its exchange catalysed by a protein base. These mechanisms could explain why the 15A9 antibody catalyses the exchange of the pro-2S proton more rapidly than the pro-2R proton. However, they do not explain why the slowly exchanged pro-2R proton of the glycine-pyridoxal-5'-phosphate Schiff base is exchanged at least 3800 times faster in the presence of the catalytic antibody than in its absence. It has been shown that the loss of rotational energies on binding can lead to large rate enhancements [26] and it has also been suggested that the binding of NADH by lactate dehydrogenase could promote catalysis by altering the distribution of conformations in the cofactor [27]. Likewise, we suggest that binding of the glycine-pyridoxal-5'-phosphate Schiff base by the catalytic antibody should stop rotation about the bonds formed between the α-amino group and the α-carbon of the glycine moiety as well as the C-4 to C-4' bond of the cofactor. This binding could optimise exchange by ensuring that the imine-cofactor π-electron system is planar and that the α-protons are perpendicular or nearly perpendicular to the plane of the imine-cofactor π-electron system. We propose that this entropic mechanism could explain how the 15A9 antibody catalyses the exchange of the pro-2R and pro-2S protons of glycine.

*Acknowledgements:* We thank Forbairt for Basic Research Awards for M.M.M. and T.B.F. This work was part of the COST-D7 'Molecular Recognition Chemistry' programme of the EU and was supported by Grant nos. SC/92/103 and SC/94/047 from EOLAS and Forbairt respectively.

## References

- [1] Shokat, K.M. and Schultz, P.G. (1991) *Methods Enzymol.* 203, 261–287.
- [2] Driggers, E.M. and Schultz, P.G. (1996) *Adv. Protein Chem.* 49, 261–287.
- [3] Benkovic, S.J. (1992) *Annu. Rev. Biochem.* 61, 29–54.
- [4] Raso, V. and Stollar, B.D. (1975) *Biochemistry* 14, 591–599.
- [5] Raso, V. and Stollar, B.D. (1975) *Biochemistry* 14, 584–591.
- [6] Raso, V. and Stollar, B.D. (1973) *J. Am. Chem. Soc.* 95, 1621–1628.
- [7] Cochran, A.G., Pham, T., Sugawara, R. and Schultz, P.G. (1991) *J. Am. Chem. Soc.* 113, 6670–6672.
- [8] Gramatikova, S.I. and Christen, P. (1997) *J. Biol. Chem.* 272, 9779–9784.
- [9] Gramatikova, S.I. and Christen, P. (1996) *J. Biol. Chem.* 271, 30583–30586.
- [10] Davis, L. and Metzler, D.E. (1972) *Enzymes*, 3rd Edn., Vol. 3, pp. 33–74.
- [11] Hayashi, H. (1995) *J. Biochem.* 118, 463–473.
- [12] John, R.A. (1995) *Biochim. Biophys. Acta* 1248, 81–96.
- [13] Dunathan, H.C. (1966) *Proc. Natl. Acad. Sci. USA* 55, 712–716.
- [14] Dunathan, H.C. and Voet, J.G. (1974) *Proc. Natl. Acad. Sci. USA* 71, 3888–3891.
- [15] Dunathan, H.C. (1971) *Adv. Enzymol. Relat. Areas Mol. Biol.* 38, 79–134.
- [16] Malthouse, J.P.G., Milne, J.J. and Gariani, L.S. (1991) *Biochem. J.* 274, 807–812.
- [17] Malthouse, J.P.G., Milne, J.J., Fitzpatrick, T.B. and Mahon, M.M. (1996) *Biochem. Soc. Trans.* 24, 134.
- [18] Malthouse, J.P.G., Fitzpatrick, T.B. and Mahon, M.M. (1998) *Biochem. Soc. Trans.* 26, 66.
- [19] Milne, J.J. and Malthouse, J.P.G. (1995) *Biochem. J.* 311, 1015–1019.
- [20] Milne, J.J. and Malthouse, J.P.G. (1996) *Biochem. J.* 314, 787–791.
- [21] Fitzpatrick, T.B. and Malthouse, J.P.G. (1998) *Eur. J. Biochem.* 252, 113–117.
- [22] Dalling, D.K., Grant, D.M., Horton, W.J. and Sagers, R.D. (1976) *J. Biol. Chem.* 251, 7661–7668.
- [23] Lu, Z., Nagata, S., M Phie, P. and Miles, E.W. (1993) *J. Biol. Chem.* 268, 8727–8734.
- [24] Schirch, D., Delle Fratte, S., Iurescia, S., Angelaccio, S., Contestabile, R., Bossa, F. and Schirch, V. (1993) *J. Biol. Chem.* 268, 23132–23138.
- [25] Christen, P., Kasper, P., Gehring, H. and Sterk, M. (1996) *FEBS Lett.* 389, 12–14.
- [26] Page, M.I. and Jencks, W.P. (1971) *Proc. Nat. Acad. Sci. USA* 68, 1678–1683.
- [27] Young, L. and Post, C.B. (1996) *Biochemistry* 35, 15129–15133.