J Biomol NMR (2012) 52:57–64
DOI 10.1007/s10858-011-9583-4

ARTICLE

# Amide temperature coefficients in the protein G B1 domain

**Jennifer H. Tomlinson · Mike P. Williamson**

Received: 13 September 2011 / Accepted: 6 October 2011 / Published online: 11 November 2011


**Abstract** Temperature coefficients have been measured for backbone amide $^{1}H$ and $^{15}N$ nuclei in the B1 domain of protein G (GB1), using temperatures in the range 283–313 K, and pH values from 2.0 to 9.0. Many nuclei display pH-dependent coefficients, which were fitted to one or two $pK_a$ values. $^{1}H$ coefficients showed the expected behaviour, in that hydrogen-bonded amides have less negative values, but for those amides involved in strong hydrogen bonds in regular secondary structure there is a negative correlation between strength of hydrogen bond and size of temperature coefficient. The best correlation to temperature coefficient is with secondary shift, indicative of a very approximately uniform thermal expansion. The largest pH-dependent changes in coefficient are for amides in loops adjacent to sidechain hydrogen bonds rather than the amides involved directly in hydrogen bonds, indicating that the biggest determinant of the temperature coefficient is temperature-dependent loss of structure, not hydrogen bonding. Amide $^{15}N$ coefficients have no clear relationship with structure.

**Keywords** Temperature coefficient · Chemical shift · Hydrogen bond · pH · Protein G

J. H. Tomlinson · M. P. Williamson (✉)
Department of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, UK
e-mail: m.williamson@sheffield.ac.uk

*Present Address:*
J. H. Tomlinson
Institute of Molecular and Cellular Biology, University of Leeds, Leeds LS2 9JT, UK

## Introduction

NMR as a structural method has a problem compared with X-ray crystallography, that there are far fewer experimental data measurements available for structure calculation. It is therefore important that NMR spectroscopists make the best use of the data that there are, particularly with the increasing emphasis on faster and more automated structure calculations (Williamson and Craven 2009). A good example of this is the recent explosion of interest in chemical shifts, which has led to marked improvements in the speed and accuracy of structure calculations. Here, we look again at a parameter that has been studied for a long time, namely the temperature dependence of the chemical shift in amides in proteins, to define in more detail what its origins are, and whether it can provide useful structural information.

Temperature coefficients were first studied in 1969 (Ohnishi and Urry 1969), when it was shown that the chemical shifts of amide protons involved in an intramolecular hydrogen bond change less with temperature than amide protons that are hydrogen bonded only with solvent. The measurement has been used extensively since then for identifying amides involved in hydrogen bonding, but mainly for peptides rather than proteins (reviewed in Williamson and Waltho 1992), because some of the techniques that have proved useful in proteins, such as solvent exchange rates of amide protons, are not accessible for peptides.

The status of temperature coefficients was discussed in a landmark paper by Andersen et al. (1997), who showed that for many peptides, there is a strong correlation between the temperature coefficient and the secondary shift (the difference in chemical shift between its value in the peptide and in the random coil). The importance of this

observation is that it demonstrates that for many peptides, the temperature coefficient is mainly describing the temperature-dependent loss of folded structure, and only indirectly characterising hydrogen bonding, although proper appreciation of the relationship permits a useful analysis of hydrogen bonding (Daley et al. 2004).

The same is not necessarily true in proteins, which in most cases remain almost completely folded over the range of temperatures in which they have a linear shift change. For proteins, temperature coefficients have been shown to be a useful measure of hydrogen bonding, with values more positive (smaller) than about −4.5 ppb/K indicating a likely hydrogen-bonded amide, and values larger than −4.5 indicating an amide hydrogen bonded only to water (Baxter and Williamson 1997; Cierpicki and Otlewski 2001; Cierpicki et al. 2002).[1]

The change in chemical shift was ascribed to a lengthening of the average hydrogen bond length with temperature (Baxter and Williamson 1997). For hydrogen-bonded amides, the lengthening arises from thermal expansion of the hydrogen bond length, while for non-hydrogen-bonded amides, it arises from lengthening of the hydrogen bond to water. Because this latter bond is weaker and less well defined, its length increases more than an intramolecular hydrogen bond, and the shift therefore changes more.

Cierpicki et al. (Cierpicki and Otlewski 2001; Cierpicki et al. 2002) also noted that for amides with an intramolecular hydrogen bond length of less than approximately 3.0 Å, there was a tendency for the shorter and stronger hydrogen bonds to have a larger temperature coefficient, presumably because the secondary chemical shift due to hydrogen bonding is greater, and so the same fractional change in shift gives rise to a larger gradient.

Here, we discuss data on temperature coefficients in the B1 domain of staphylococcal protein G (GB1). The data provide new information for three reasons. First, there are high resolution crystal structures of GB1 at both low (Tomlinson et al. 2010b) and high (Gallagher et al. 1994) pH, allowing us to assess the dependence of shift on hydrogen bond length with high accuracy. Second, we analyse the effect of pH on temperature coefficients, based on a detailed understanding of structural and dynamic changes in GB1 with pH (Tomlinson et al. 2010a, b). Change in pH in GB1 affects hydrogen bonding interactions involving only four carboxylate sidechains and is a relatively small perturbation, and therefore allows us to isolate different effects on temperature coefficient. Third, we present details of $^{15}$N temperature coefficients, which have not previously been described.

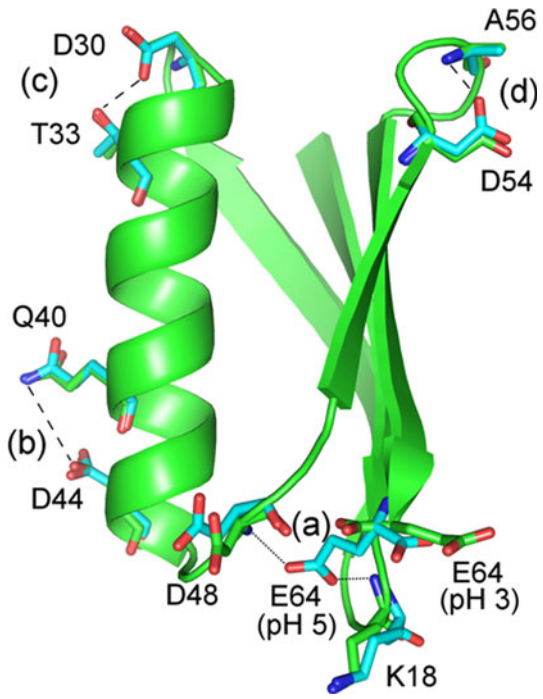


**Fig. 1** The structure of GB1 domain, showing titrating sidechain interactions that are lost at low pH. The pH3 structure is in *green*, and residues from the high pH structure in *blue*. Hydrogen bond interactions are indicated by (**a**) through (**d**) as described below, and by *dashed lines*, except for the two hydrogen bonds formed by E64 which are *dotted*. **a** At neutral pH, the C-terminal E64 sidechain forms two hydrogen bonds: one to K18 backbone HN and one to D48 backbone HN. These are lost at low pH and the conformation of E64 alters and becomes more disordered, as does that of the adjacent loops 17–19 and a smaller effect on 48–49. **b** There is a sidechain–sidechain hydrogen bond between D44 and Q40 which is lost at low pH. This leads to a shortening at the C terminus of the helix, which finishes at N45 at neutral pH but N43 at low pH, with some rearrangement in the loop 43–47. **c** The N-cap hydrogen bond from D30 sidechain to T33 HN at the N-terminal end of the α-helix is lost at low pH. This produces only a very localised structure rearrangement. **d** The hydrogen bond from D54 sidechain to A56 HN is lost at low pH. This again results in only a localised change in structure

GB1 has no histidines except in the His tag. Structural changes between pH 2 and 9 are therefore limited to protonation of acidic sidechains and the C terminus. $pK_a$ values for all titrating groups have been published (Tomlinson et al. 2009), as has a detailed discussion of structural changes over this pH range (Tomlinson et al. 2010a, b). There are five hydrogen bonds involving carboxylates that are disrupted at low pH (Fig. 1).

## Materials and methods

GB1 domain was expressed and purified as a $^{15}$N, $^{13}$C or $^{15}$N-labelled His-tagged protein, as described (Tomlinson

[1] Temperature coefficients are almost always negative (a decrease in chemical shift value as the temperature is increased). Here we describe a 'smaller' value of a temperature coefficient as one in which the numerical or absolute value is smaller: thus a coefficient of −4.5 ppb/K is smaller than one of −8 ppb/K.

et al. 2010b). It was dissolved in 33 mM sodium phosphate, 33 mM deuterated sodium acetate, 33 mM orthoboric acid (substituted by 33 mM sodium citrate at pH values less than 4), 1 mM sodium azide, and 10% $D_2O$. Two samples were used: 300 μM $^{15}$N-labelled and 1 mM double labelled. Consistent shifts were obtained from both samples. Sample pH was altered by buffer exchange with freshly prepared buffer at the desired pH to avoid changes in salt concentration during titrations. HSQC spectra were collected every 5 K from 283 to 313 K and at pH values every 0.5 pH unit from 2.0 to 9.0, on Bruker DRX-500 and 600 spectrometers. Chemical shift assignments were based on Tunnicliffe et al. (2005), supplemented with standard triple resonance experiments, particularly at low pH where overlap made assignment by simple titration difficult. Spectrometer temperature was calibrated using methanol (Findeisen et al. 2007). Nitrogen chemical shifts were calculated from the proton shifts (Wishart et al. 1995).

Spectra were processed in *Felix* (Felix NMR Inc., San Diego, CA). Crosspeak positions were downloaded to a text file and temperature coefficients were obtained by fitting to a straight line over the complete temperature range. pH-dependent coefficients were fitted to the equations:

$$\delta = \frac{\delta_{\text{acid}} + \delta_{\text{base}} 10^{(\text{pH}-\text{pK}_a)}}{1 + 10^{(\text{pH}-\text{pK}_a)}}$$

for a single $pK_a$, or

$$\delta = \frac{\delta_a 10^{-2\text{pH}} + \delta_b 10^{-(\text{pH}+\text{pK}_{a1})} + \delta_c 10^{-(\text{pK}_{a1}+\text{pK}_{a2})}}{10^{-2\text{pH}} + 10^{-(\text{pH}+\text{pK}_{a1})} + 10^{-(\text{pK}_{a1}+\text{pK}_{a2})}}$$

for two $pK_a$s, or to the equivalent equation for three $pK_a$s (Joshi et al. 2001). An *F* test was used to judge which model produced the best fit, and the simplest consistent model was used in each case.

## Results and discussion

### Relationship between temperature coefficient and hydrogen bonding

Amide $^1$H chemical shifts were measured at a range of temperatures and fitted to derive linear temperature coefficients, at a range of pH values between 2 and 9. There is relatively little change in temperature coefficient over this pH range, as reported previously for other proteins (Baxter and Williamson 1997; Cierpicki and Otlewski 2001). GB1 contains no histidines (except in the His tag), implying that essentially all the changes over this pH range reflect protonation of acidic sidechains and the C terminus (Fig. 1).

As expected, temperature coefficients are in general smaller (in absolute value, i.e closer to zero) for hydrogen bonded amides than for non-hydrogen bonded amides. As reported by Cierpicki et al. (2002), for protons involved in hydrogen bonds, there is a correlation between the amide proton temperature coefficient and the length or strength of hydrogen bonds (Fig. 2a). One consequence of this is that amides in very strong hydrogen bonds often have values of temperature coefficient more negative than −4.5 ppb/K (Fig. 2a). The correlation with hydrogen bond length is not very strong. The poor correlation is unlikely to be due to inaccuracies in the crystal structure coordinates, since the structure was determined at 1.2 Å resolution. There are also several structures at higher pH. Comparison using these, and temperature coefficients at the relevant pH

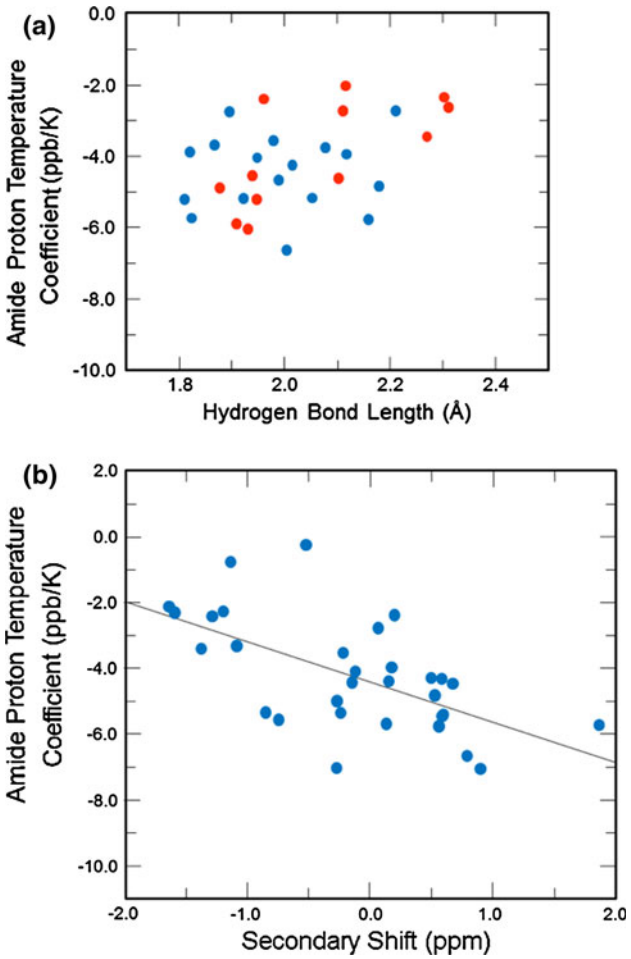


**Fig. 2** Correlation between amide proton temperature coefficients in GB1 and measures of hydrogen bond strength. **a** Hydrogen bond length, pH 3. Protons were added to the crystal structure in standard geometry. Data are shown for all amides involved in hydrogen bonds in regular secondary structure. *Blue*: β sheet, *red*: α helix. Similar plots for hydrogen bond energy and for data at higher pH look similar, though with slightly more scatter. **b** Secondary shift, determined at pH 6.0. The correlation coefficient is 0.60. In both cases, inclusion of data for amides in hydrogen bonds but not in regular secondary structure produces increased scatter

values, does not improve the correlation. We therefore conclude that the correlation between temperature coefficient and hydrogen bond strength is genuinely weak: in other words, the value of the coefficient is not a good measure of the strength of a hydrogen bond.

The strongest correlation that we were able to find was with the secondary shift (the difference in shift between protein and random coil; Schwarzinger et al. 2001), shown in Fig. 2b. Such a correlation has been reported before, most notably by Andersen et al. (1997). In this paper they show that for *peptides*, the correlation can be remarkably good, and indicates a temperature-dependent global unfolding of the peptide. The relationship with fraction of peptide folded is quantitative. The largest typical slope for the correlation of temperature coefficient with secondary shift in peptides is approximately 20 ppt/K [= ppb/K divided by ppm], which corresponds to the loss of about 10% of the folded population over 10 K. For *proteins*, Andersen et al. (1997) conclude that the proportion of protein unfolding with temperature is not enough to give rise to a significant change in chemical shift, and thus that the correlation cannot be due to thermal unfolding. It is therefore suggested to arise from a thermally induced loss of structure: possibly sidechain rotations or backbone vibrations. They report an average slope for this correlation in proteins of −2.2 ppt/K, with a range of −1.4 to −4.1. GB1 has a slope of −1.2, and is thus very much at the low end of the range.

The thermal stability of GB1 is large. Despite a fairly typical unfolding free energy of 6.6 kcal $mol^{-1}$ at 25°C, it unfolds at the unusually high temperature of 87.5°C (Alexander et al. 1992). It therefore has a very small change in stability with temperature. Calculations based on these figures suggest that the fraction of protein unfolded at 313 K is still less than 0.1%, in agreement with Andersen's comment that the temperature coefficient cannot be due to global unfolding. Cordier and Grzesiek (2002) used the temperature dependence of *J* coupling across hydrogen bonds to derive a thermal expansion coefficient for amide hydrogen bonds of about $1.7 \times 10^{-4}$ $K^{-1}$. Over 10°C this would correspond to a thermal expansion of $1.7 \times 10^{-3}$ or 0.17%. This can be compared with the slope of −1.2 ppt/K shown in Fig. 2b, which by comparison to Andersen's estimates corresponds to a loss of about 0.6% of structure over 10°. The approximate agreement between these two figures suggests that the change in chemical shift of amide protons with temperature in folded proteins can be explained simply as thermal expansion of the hydrogen bond, as suggested previously (Baxter and Williamson 1997). The data further suggest that the unusually small gradient of the correlation in GB1 (−1.2 ppt/K, Fig. 2b) may be due to the unusually small change in stability of GB1 with temperature.

The analysis above shows that the overall pattern of change in chemical shift with temperature is quantitatively predictable. However, detailed examination of individual values in comparison with crystal structures does not give any useful pattern of behaviour. We therefore looked at changes in temperature coefficients with pH, because a related analysis of chemical shifts as a function of pH had been able to provide an essentially complete understanding of the origins of the chemical shift changes (Tomlinson et al. 2010b).

## Temperature coefficients as a function of pH

Temperature coefficients for amide protons were measured at different pH values between 2 and 9 and fitted to one, two or three $pK_a$ values (Fig. 3). Residues showing changes in temperature coefficient of more than 1 ppb/K are listed in Table 1. We have previously determined the $pK_a$ values for almost all titrating groups in GB1 (Tomlinson et al. 2009).

There are four backbone amides that hydrogen bond to carboxylate sidechains in GB1. All of these show large changes in temperature coefficient with pH. For three of the four, the fitted $pK_a$ matches the $pK_a$ of the carboxylate. However, this is not true for the T33–D30 hydrogen bond ($pK_a$ of D30 = 2.78, fitted $pK_a$ 4.15). Furthermore, one might expect that the temperature coefficient would become smaller (less negative) as the pH is raised above the carboxylate $pK_a$, and the hydrogen bond strength correspondingly increases. This is only true for two of the four amides. These results suggest that the temperature coefficient does not depend primarily on the strength of the hydrogen bond.

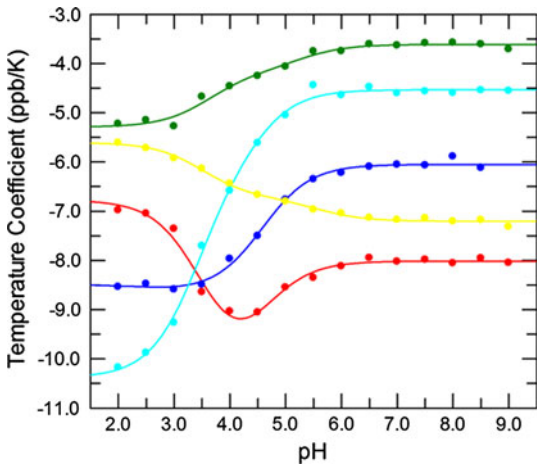


**Fig. 3** Examples of the fitting of $^1H$ temperature coefficients to $pK_a$ values. For all these residues, a statistical analysis showed that two $pK_a$ values were required. Residues are T19 (*blue*), T33 (*green*), E35 (*yellow*), D48 (*red*) and G49 (*cyan*)

**Table 1** The residues in GB1 with changes in $^{1}$H temperature coefficients over the pH range 2.0–9.0 and the $pK_a$s affecting them

| Residue | $pK_a$ affecting the change in temperature coefficient | Magnitude of the change from low to high pH (ppb/K) | Low pH temperature coefficient (ppb/K) | High pH temperature coefficient (ppb/K) |
|---|---|---|---|---|
| A7 | 5.51 ± 0.22 | −1.58 | −8.42 | −10.00 |
| G17 | 3.85 ± 0.17 | −1.77 | −3.86 | −5.61 |
| K18 | 4.31 ± 0.44 and 4.66 ± 0.26 | −0.76[a] and +3.72 | −7.25 | −8.01 at pH 4.0, −4.29 at pH 9.0 |
| T19 | 4.61 ± 0.12 | +2.52 | −8.54 | −6.12 |
| L20 | 3.27 ± 0.50 and 4.51 ± 0.34 | +2.10 and +2.56 | −9.83 | −5.43 |
| K21 | 3.73 ± 0.25 | −1.60 | −7.29 | −9.16 |
| T24 | 3.72 ± 0.25 | +1.25 | −3.61 | −2.41 |
| T33 | 4.15 ± 0.19 | +1.53 | −5.23 | −3.80 |
| A34 | 5.02 ± 0.22 | +1.30 | −3.40 | −2.14 |
| E35 | 3.51 ± 0.46 | −1.70 | −5.61 | −7.31 |
| V37 | 3.60 ± 0.20 | +1.59 | −2.20 | −0.75 |
| N43 | 3.40 ± 0.14 | +2.48 | −2.73 | −0.36 |
| D44 | 3.24 ± 0.51 | −1.33 | −4.72 | −6.05 at pH 4.5[b] |
| D48 | 3.52 ± 0.27 and 4.55 ± 0.35 | −2.07 and +1.00 | −6.98 | −9.05 at pH 4.5, −8.05 at pH 9.0 |
| G49 | 3.39 ± 0.30 and 4.53 ± 0.67 | +4.18 and +1.66 | −10.17 | −4.55 |
| D54 | 2.86 ± 0.40 | −1.05 | −0.26 | −1.27 |
| D55 | 4.03 ± 0.25 | −1.21 | −5.71 | −6.97 |
| A56 | 4.26 ± 0.17 | −1.76 | −5.91 | −7.44 |
| V62 | 3.20 ± 0.42 | +1.05 | −4.17 | −3.12 at pH 4.5[b] |
| E64 | 2.82 ± 0.32 and 4.76 ± 0.38 | +2.17 and −1.16 | −6.16 | −4.53 at pH 3.5 and −5.54 at pH 9.0 |

Other residues do not change by >1.0 ppb/K over this pH range

[a] Although the observed temperature coefficient change for the first $pK_a$ affecting K18 is below the 1.0 ppb/K cutoff, the data fit to a total change of −3.39 ppb/K and so are included in this study

[b] The temperature coefficient increases with pH after pH 4.5 but the total increase is less than 1.0 ppb/K and is therefore below the threshold considered significant for this study

Furthermore, it is striking that the largest changes to temperature coefficient were not for the amides hydrogen bonding to carboxylates, such as K18 and D48, but to the adjacent residues T19, L20 and G49. This behaviour is particularly unexpected because the structures at low and high pH show that these adjacent residues do not hydrogen bond to the carboxylate at either pH. Therefore we can infer that the changes to temperature coefficients for these residues do not arise directly from changes in hydrogen bonding. The most reasonable explanation for this behaviour is that the residues concerned become more easily disordered or mobile at low pH as a consequence of change in temperature. K18 is at the end of the first $\beta$-strand, and thus the residues before K18 are fixed by interstrand hydrogen bonding; in contrast, the loop from K18 to L20 is relatively unrestrained. Similarly, D48 is close to the end of the long helix and V47 forms a hydrogen bond back to the helix, whereas G49 is not involved in hydrogen bonds. Thus, the most obvious characteristic of the residues with large changes to their temperature coefficients is that they are unrestrained by hydrogen bonds and can therefore

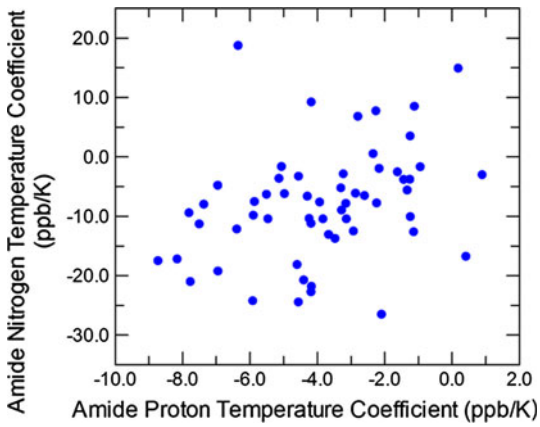


**Fig. 4** Correlation of $^{15}$N versus $^{1}$H temperature coefficients, pH 6

**Table 2** The residues in GB1 with changes in $^{15}N$ temperature coefficients over the pH range 2.0–9.0 and the $pK_a$s affecting them

| Residue | $pK_a$ affecting the change in temperature coefficient | Magnitude of the change (ppb/K) | Low pH temperature coefficient (ppb/K) | High pH temperature coefficient (ppb/K) |
|---|---|---|---|---|
| A7 | 3.50 ± 0.09 | −3.78 | −11.91 | −14.37 |
| M8 | 4.68 ± 0.03 | +6.80 | −10.41 | −3.61 at pH 5.0[a] |
| D9 | 3.56 ± 0.02 | +18.65 | −9.14 | −9.10 |
| T10 | 4.35 ± 0.07 | −3.99 | −6.58 | −9.91 |
| Y11 | 4.48 ± 0.05 | −5.73 | −18.54 | −23.68 |
| K12 | 4.34 ± 0.06 | +5.13 | −2.34 | −7.74 |
| L13 | 4.60 ± 0.04 | −8.14 | 5.76 | −2.83 |
| I14 | 3.42 ± 0.14 | +7.81 | −12.87 | −5.06 |
| N16 | 3.31 ± 0.07 and 4.89 ± 0.09 | −8.79 and +5.82 | 0.18 | −2.45 |
| G17 | 3.06 ± 0.03 | −11.10 | −10.63 | −20.77 |
| K18 | 3.58 ± 0.08 and 4.91 ± 0.11 | −12.61 and +5.65 | −0.31 | −14.23 at pH 3.5, −6.06 at pH 9.0 |
| T19 | 4.03 ± 0.01 | +22.19 | −23.52 | −1.34 |
| L20 | 4.97 ± 0.05 | +5.42 | −27.08 | −22.24 |
| K21 | 4.16 ± 0.04 | −7.09 | −14.22 | −21.33 |
| G22 | 3.61 ± 0.06 | −5.69 | −21.38 | −11.01 |
| E23 | 4.64 ± 0.03 | +10.46 | −20.72 | −10.26 |
| T24 | 3.97 ± 0.03 | +10.00 | −6.63 | 3.80 |
| T25 | 3.44 ± 0.16 and 4.58 ± 0.26 | −7.38 and −4.05 | 18.69 | 7.55 |
| V29 | 4.19 ± 0.05 | −6.30 | −3.00 | −18.07 |
| A32 | 3.17 ± 0.04 | +8.16 | 7.18 | 15.22 |
| T33 | 3.94 ± 0.05 | +5.96 | −12.05 | −6.64 |
| K39 | 4.42 ± 0.06 | −4.84 | −8.21 | −12.82 |
| Q40 | 4.75 ± 0.09 | +5.67 | −12.81 | −10.09 |
| D44 | 3.25 ± 0.12 and 4.12 ± 0.07 | −4.22 and +8.63 | −7.34 | −11.56 at pH 3.5, −2.92 at pH 9.0 |
| V47 | 4.75 ± 0.05 | +5.76 | −11.38 | −5.82 |
| D48[b] | 3.37 ± 0.52 and 4.82 ± 0.11 | −4.93 and +4.95 | −19.39 | −22.21 at pH 3.5, −18.73 at pH 9.0 |
| D55 | 4.40 ± 0.03 | −10.16 | 0.67 | −7.54 |
| A56 | 4.11 ± 0.04 | +7.89 | −7.91 | −1.54 |
| T57 | 3.88 ± 0.4 | +7.12 | −10.55 | −3.60 |
| F60 | 3.63 ± 0.05 | +5.99 | −29.97 | −24.32 |
| V62 | 3.20 ± 0.13 | +4.00 | −1.87 | 2.13 |
| T63 | 4.46 ± 0.03 | +9.44 | −17.17 | −8.34 |
| E64 | 4.40 ± 0.02 | +18.52 | −18.95 at pH 3.5[a] | −0.43 |

Other residues do not change by >4.0 ppb/K over this pH range

[a] The temperature coefficient increases below pH 3.5 but as the total change is below the cutoff these data are not included

[b] The observed temperature coefficient changes for the titration of D48 in both directions fall slightly below the cutoff level used in this study. However, both titrations fit to chemical shift differences greater than the cutoff so these data were included in the analysis

change conformation more readily as the temperature is raised. We conclude that the temperature coefficient is primarily not a measure of hydrogen bonding (strikingly, even the loss of a hydrogen bonding carboxylate does not necessarily perturb the coefficient much), but is a measure of the capability of the residue to become unstructured as the temperature is raised.

Analysis of the other amides hydrogen bonding to carboxylates confirms this theory. For the hydrogen bond between A56 amide and D54 sidechain, changes to temperature coefficients are smaller and limited to the N-terminal side of the hydrogen bond, because A56 is part of a shorter and more structured loop, with the amides of residues T57-T59 all forming hydrogen bonds to backbone carbonyls. The hydrogen bond from T33 amide to D30 sidechain is a helix N-capping interaction, and its removal has very little effect on the mobility of T33 and surrounding residues, because they are held in place by other helical interactions. Consequently, changes in temperature coefficients are again small.

The largest changes in temperature coefficients all fit to this pattern. After G49, L20 and T19, the next largest

change is for N43, which is likely to be related to the disruption of the two C-terminal residues of the helix (D44 and N45) at low pH (Tomlinson et al. 2010a) (Fig. 1). This is followed by E64, which is much less restricted in its mobility at low pH.

### Temperature coefficients for $^{15}N$

Temperature coefficients were measured for $^{15}N$ nuclei as a function of pH. They span a wide range, from approximately −28 to +26 ppb/K. There is only a very weak correlation of $^{15}N$ temperature coefficients with $^{1}H$ coefficients (Fig. 4), and no correlation with hydrogen bond location or strength.

$^{15}N$ temperature coefficients were fitted to one, two or three $pK_a$ values, in a similar way to $^{1}H$ coefficients (Table 2). The majority of backbone $^{15}N$ nuclei have a significant pH dependence (33 out of 58 with a pH-dependent change greater than 4 ppb/K). There is a general tendency for $^{15}N$ coefficients to be affected by the same $pK_a$ as $^{1}H$ coefficients, indicating that factors that affect $^{1}H$ coefficients also affect $^{15}N$ coefficients, but there is very little relationship between the magnitudes of the changes. We have previously observed that while $^{1}HN$ chemical shifts vary in a fairly predictable way with hydrogen bonding, $^{15}N$ shifts are far less predictable (Tomlinson et al. 2010b). This is at least as true for temperature coefficients, which for $^{15}N$ are essentially uninterpretable. For $^{1}H$, the largest pH-dependent changes in coefficient were for L20 and G49, adjacent to two of the amides that have hydrogen bonds to titrating carboxylates. For $^{15}N$, these residues have small changes in temperature coefficient that do not reflect the $pK_a$ of the relevant carboxylate. Unlike the nitrogen chemical shifts (Tomlinson et al. 2010b), the nitrogen temperature coefficients of carboxylate residues or of residues immediately following carboxylates do not show pH-dependent changes that reflect the titration of that carboxylate side chain. Some of these residues exhibit no change in temperature coefficient over the pH range studied while others do exhibit a change but that change does not reflect the $pK_a$ of the side chain with which they are associated. This indicates that, unlike the chemical shifts themselves, the nitrogen temperature coefficients are not influenced by titrating side chains through covalent bonds. They also show little discernable effect from hydrogen bonding, and are therefore of little value for characterising protein structure or dynamics.

## Conclusions

$^{1}H$ temperature coefficients in proteins are determined mainly by local melting of the structure, and not by the strength of hydrogen bonds to the amide, although as described previously there is a tendency for (a) intramolecularly hydrogen-bonded amides to have a smaller (less negative) coefficient, and (b) strongly hydrogen-bonded amides to have a larger coefficient, because the same fractional loss in structure gives rise to a larger change in shift. The origins and applications of temperature coefficients in proteins and peptides are therefore closely related. Despite having high-resolution structures at low and high pH, and a detailed understanding of chemical shifts in GB1 (Tomlinson et al. 2010b), we have been unable to interpret individual coefficients in any useful way. $^{15}N$ temperature coefficients have almost no discernable pattern.

**Acknowledgments** We thank the Biotechnology and Biological Science Research Council (UK) for funding a studentship to JHT.

## References

Alexander P, Fahnestock S, Lee T, Orban J, Bryan P (1992) Thermodynamic analysis of the folding of the streptococcal protein G IgG-binding domains B1 and B2: why small proteins tend to have high denaturation temperatures. Biochemistry 31: 3597–3603

Andersen NH, Neidigh JW, Harris SM, Lee GM, Liu Z, Tong H (1997) Extracting information from the temperature gradients of polypeptide NH chemical shifts. 1. The importance of conformational averaging. J Am Chem Soc 119:8547–8561

Baxter NJ, Williamson MP (1997) Temperature dependence of $^{1}H$ chemical shifts in proteins. J Biomol NMR 9:359–369

Cierpicki T, Otlewski J (2001) Amide proton temperature coefficients as hydrogen bond indicators in proteins. J Biomol NMR 21: 249–261

Cierpicki T, Zhukov I, Byrd RA, Otlewski J (2002) Hydrogen bonds in human ubiquitin reflected in temperature coefficients of amide protons. J Magn Reson 157:178–180

Cordier F, Grzesiek S (2002) Temperature-dependence of protein hydrogen bond properties as studied by high-resolution NMR. J Mol Biol 715:739–752

Daley ME, Graether SP, Sykes BD (2004) Hydrogen bonding on the ice-binding face of a $\beta$-helical antifreeze protein indicated by amide proton NMR chemical shifts. Biochemistry 43:13012–13017

Findeisen M, Brand T, Berger S (2007) A $^{1}H$ NMR thermometer suitable for cryoprobes. Magn Reson Chem 45:175–178

Gallagher T, Alexander P, Bryan P, Gilliland GL (1994) Two crystal structures of the B1 immunoglobulin-binding domain of *Streptococcal* protein G and comparison with NMR. Bichemistry 33: 4721–4729

Joshi MF, Sidhu G, Nielsen JE, Brayer GD, Withers SG, McIntosh LP (2001) Dissecting the electrostatic interactions and pH-dependent activity of a family 11 glucosidase. Biochemistry 40:10115–10139

Ohnishi M, Urry DW (1969) Temperature dependence of amide proton chemical shifts: the secondary structures of gramicidin S and valinomycin. Biochem Biophys Res Comm 36:194–202

Schwarzinger S, Kroon GJA, Foss TR, Chung J, Wright PE, Dyson HJ (2001) Sequence-dependent correction of random coil NMR chemical shifts. J Am Chem Soc 123:2970–2978

Tomlinson JH, Ullah S, Hansen PE, Williamson MP (2009) Characterization of salt bridges to lysines in the protein G B1 domain. J Am Chem Soc 131:4674–4684

Tomlinson JH, Craven CJ, Williamson MP, Pandya MJ (2010a) Dimerization of protein G B1 domain at low pH: a conformational switch caused by loss of a single hydrogen bond. Proteins: Struct Funct Bioinf 78:1652–1661

Tomlinson JH, Green VL, Baker PJ, Williamson MP (2010b) Structural origins of pH-dependent chemical shifts in protein G. Proteins: Struct Funct Bioinf 78:3000–3016

Tunnicliffe RB, Waby JL, Williams RJ, Williamson MP (2005) An experimental investigation of conformational fluctuations in proteins G and L. Structure 13:1677–1684

Williamson MP, Craven CJ (2009) Automated protein structure calculation from NMR data. J Biomol NMR 43:131–143

Williamson MP, Waltho JP (1992) Peptide structure from NMR. Chem Soc Rev 21:227–236

Wishart DS, Bigam CG, Yao J, Abildgaard F, Dyson HJ, Oldfield E, Markley JL, Sykes BD (1995) $^{1}H$, $^{13}C$ and $^{15}N$ chemical shift referencing in biomolecular NMR. J Biomol NMR 6:135–140