# NAG (*N*-Acetylglucosamine) Binding Reduces the Conformational Flexibility of Lysozyme

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NAG (*N*-acetylglucosamine) binding to lysozyme, unlike 2′-CMP binding to ribonuclease-A, dramatically reduces the conformational flexibility of the protein, as revealed by the thermal variation of $^{13}C$ chemical shifts.

$^{13}C$ Chemical shifts in proteins frequently have a substantially greater dependence on temperature than those in smaller molecules, presumably because the proteins are more likely to have alternative conformations whose excess free energies are of the order of thermal energies. Thus $\Delta\delta(^{13}C)/\Delta T$ for small molecules is typically 0 to +0.006 p.p.m./K,[1] whereas for proteins it can commonly exceed ±0.012 p.p.m./K. Are these alternative conformations few or many?

We have measured the temperature dependence of 36 $^{13}C$ resonances in H.E.W. lysozyme, at pD 3.2, each of which can be resolved and reliably traced over a temperature range of 32—50 °C. We have also observed similar shifts at pD 5.0.

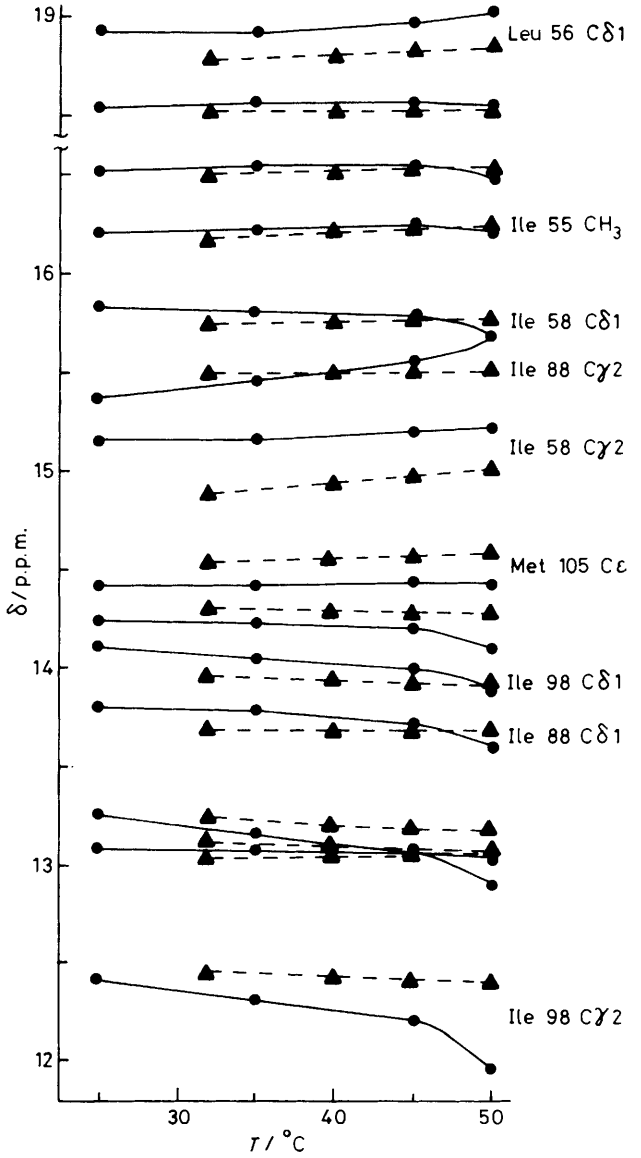


**Figure 1.** Temperature dependence of the $^{13}C$ chemical shifts of lysozyme in the absence (●) and presence (▲) of NAG.

For comparison, we have data for 15 resonances of ribonuclease-A at pD 5.5 over the range 25—44 °C. The lysozyme resonances, partly shown in Figure 1, have a root mean square (r.m.s.) temperature variation of 0.0074 p.p.m./K, whereas those of ribonuclease-A vary by only 0.0042 p.p.m./K. Referencing was by the method of Walters and Allerhand.[2]

However, the corresponding r.m.s. temperature coefficient for the lysozyme–NAG (*N*-acetylglucosamine) complex, pD 3.2, is only 0.0032 p.p.m./K, less than half that of the free protein and similar to that expected for small molecules. In contrast, when 2′-CMP is bound to ribonuclease-A (pD 5.5) the coefficient increases slightly, to 0.0049 p.p.m./K. Another way of expressing the lysozyme data is that with NAG bound, only 3 resonances out of the 36 have a coefficient exceeding 0.005 p.p.m./K, whereas the free protein has 14 such resonances.

The observations on lysozyme concern a substantial proportion of its resonances, especially those of methyl groups, and thus permit general conclusions. They show that the temperature dependence of the shifts is not a general protein property or even a general effect of ligand binding, but rather that about half of it arises from some specific aspect of molecular flexibility that is removed upon binding of NAG. In support of this we have assigned many of the resonances,[3-4] exploiting existing proton assignments where appropriate.[5]

We note that, of these, the ones with coefficients exceeding 0.005 p.p.m./K only in the absence of NAG arise from Ile 98 Cγ2 and Cδ1, Ile 88 Cγ2 and Cδ1, Ile 58 Cδ1, Leu 56 Cδ1, Trp 108 Cε2, Trp 63 Cε2, Tyr 23 Cζ, and Tyr 53 Cζ. All these atoms lie in or reasonably close to the active site cleft, though most are not directly bound to NAG.

Phillips and Blake[6] have already noted the unusual mobility of atoms at the edges of the lysozyme cleft, in the crystalline state. It now seems probable that much of the mobility observed in solution arises from the presence of one or a very few alternative conformations which are excluded upon binding of NAG in either anomeric form.[7]

We thank the S.E.R.C. for access to the Bruker WH400 spectrometer, and for a post-graduate research assistantship (L. Y. L.).

*Received, 21st January 1983; Com. 100*

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