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Response to 'Group additivity calculations of the thermodynamic properties of unfolded proteins in aqueous solution: a critical comparison of peptide-based and HKF models'

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Hakin and Hedwig on page 253–264 of this issue compare their model for predicting the thermodynamic properties of unfolded proteins with that of Amend and Helgeson (2000) (*Biophys. Chem.* **84**, 105–136). Hakin and Hedwig use experimentally measured heat capacities ($C_P°$) and volumes ($V°$) to $\sim$100°C of small aqueous peptides to extract temperature coefficients for all 20 common amino acid side chains (R groups), the peptide backbone (CHCONH), and the two terminal groups (NH_3^+ and $CHCOO^-$). In contrast, Amend and Helgeson (2000) regressed experimental data for a variety of side chain analogs, polyglycines, and fully denatured proteins to obtain the standard molal thermodynamic properties and revised Helgeson–Kirkham–Flowers (HKF) equation of state parameters for all of the side chains, terminal groups, and the protein backbone. The two approaches use similar group additivity algorithms and both permit calculation of $C_P°$ and $V°$ as a function of temperature. However, our approach also permits calculation of Gibbs free energies, enthalpies, and entropies at temperatures and pressures well beyond those so far investigated in the laboratory. Nevertheless, two assertions made by Hakin and Hedwig concerning our biochemical application of the revised HKF equations of state require comment in this response. The first is their notion that our group contributions should be equally applicable to small peptides as well as unfolded proteins. The second is whether Hakin and Hedwig's 'peptide-based model' can in fact be used to accurately predict the thermodynamic properties of unfolded proteins.

In their comparison, Hakin and Hedwig criticize our approach because it does a poor job of predicting $C_P°$ and $V°$ of several tri-, tetra-, and pentapeptides. This comes as no surprise, because our approach was designed to permit calculation

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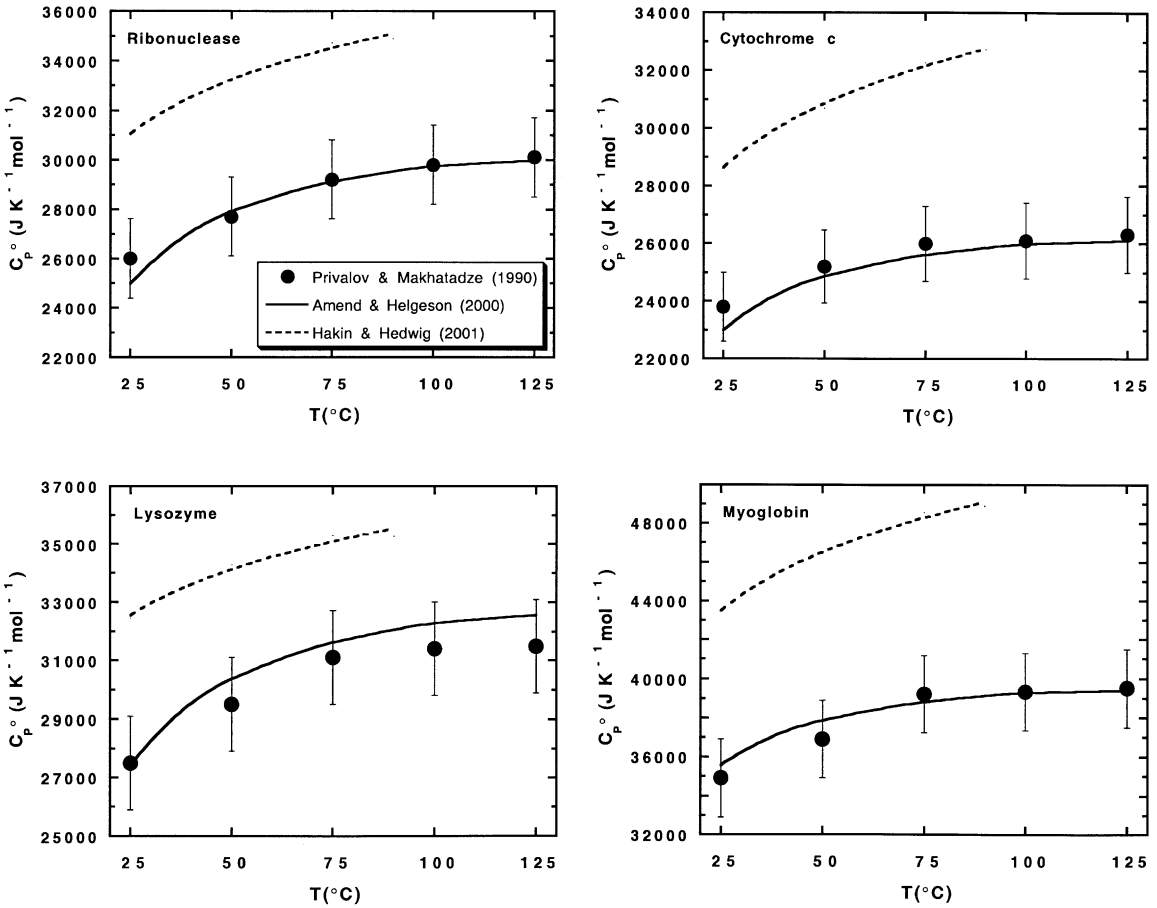

Fig. 1. Comparison of experimental and calculated values of C_P° as a function of temperature at P_{SAT} for four unfolded proteins. The symbols depict experimental data, but the curves were generated using group additivity algorithms of Amend and Helgeson (—) and Hakin and Hedwig (----).

of the thermodynamic properties of unfolded proteins, not small peptides. We disagree with Hakin and Hedwig's assertion that for our scheme to have 'any general predictive utility,... it should be applicable also to small polypeptides.' It is an admirable goal of Hakin and Hedwig, and one that we share, to seek a single method for computing internally consistent thermodynamic properties of amino acids, short and long peptides, and unfolded and native proteins. However, we are acutely aware of the dangers in predicting from the same group contributions the thermodynamic properties of both small and large biomolecules. Such predictions are invariably subject to high uncertainties, and we therefore restricted our approach to optimize predictions of the thermodynamic properties of unfolded proteins.

The second issue that needs to be addressed here is the ability of Hakin and Hedwig's peptide-based model to predict the thermodynamic properties of unfolded proteins at elevated temperatures. In Fig. 1, we critically compare the computational results of Amend and Helgeson (2000) and Hakin and Hedwig (2001) (Biophys. Chem. 89 (2–3) 253–264) (including their earlier work referenced therein) with experimental heat capacities to 125°C of ribonuclease, cytochrome *c*, lysozyme, and myoglobin (Privalov and

Makhatadze, 1990, *J. Mol. Biol.* **213**, 385–391). It can be seen in this figure that the calculations based on the approach taken by Hakin and Hedwig result in large discrepancies, both with the experimental data and the curves generated from the revised HKF equation of state. Note that their predicted heat capacities are consistently higher than the experimental values, differing by as much as $\sim$10,000 J K^{-1} mol^{-1} in the case of myoglobin. We find it curious that Hakin and Hedwig made a 'critical comparison' between their model and ours, but not between their model and the experimental data for unfolded proteins. Although Hakin and Hedwig have shown that their approach is state-of-the-science for small peptides, we maintain that our approach is state-of-the-science for unfolded proteins.