ELSEVIER

Thermochimica Acta 298 (1997) 37–46

thermochimica acta

Heat capacity measurements from 10 to 300 K and derived thermodynamic functions of lyophilized cells of *Saccharomyces cerevisiae* including the absolute entropy and the entropy of formation at 298.15 K

Edwin H. Battley[1], Robert L. Putnam, Juliana Boerio-Goates*

Department of Chemistry, Brigham Young University, Provo, UT 84602, USA

Received 20 November 1996; received in revised form 30 January 1997; accepted 30 January 1997

Abstract

Heat capacity measurements using an adiabatic calorimeter have been made from 7 to 310 K on a carefully prepared specimen of lyophilized cells of *Saccharomyces cerevisiae* (yeast). From these measurements, a value of 1.304 J K^{-1} g^{-1} has been obtained for the absolute entropy of yeast cells at 298.15 K, based on third-law calculations. Chemical analysis of the cells yielded an empirical chemical formula for the cellular stoichiometry, which has been expressed as an ion-containing carbon mole, (ICC-mol). A value of 34.167 J K^{-1}·ICC-mol^{-1} for the absolute entropy of this mass of cells and of −151.46 J K^{-1}·ICC-mol^{-1} for the entropy of formation has been calculated. The absolute entropy/g of the yeast cells falls within the range of those for simple biological molecules like sugars and amino acids and more complex biopolymers like proteins. We conclude that the thermodynamic effect of cellular organization in the dried cells is negligible. © 1997 Elsevier Science B.V.

Keywords: Entropy; Heat capacity; S. cerevisiae; Yeast

1. Introduction

Heat capacity measurements have been made on a number of biological molecules of various degrees of complexity including sugars, amino acids, proteins and nucleic acids. Absolute entropies for these substances, based on the application of the third law of thermodynamics to the heat capacity measurements, have also been reported [1]. However, no such measurements have ever been made on whole, dried cells. Here, we report heat capacities obtained on a carefully prepared sample of lyophilized cells of *Saccharomyces cerevisiae* (yeast), which are used to obtain an estimate of the third-law entropy of the material as a function of temperature. A calculation is also made of the entropy of formation, ΔS_f, for the dried cells, given an elemental analysis of the cellular material.

Cells can be regarded as having some of the properties of precipitates in that they are insoluble [2] and a close approximation to their entropy per unit mass can be obtained using lyophilized cells, if cellular integrity is not destroyed by lyophilization. Entropy changes will occur when these cells are suspended in water and hydrated to form functional cells. We estimate the entropy difference, S(hydrated) − S(anhydrous), between hydrated and dried cells to be very small

*Corresponding author. Tel.: 801-378-2302; fax: 801-378-5474.

[1]Department of Ecology and Evolution, State University of New York, Stony Brook, New York 11794-5245.

PII S0040-6031(97)00108-1

when the entropy is expressed *per unit mass*, such as per gram of material. The reaction: anhydrous cell + $nH_2O(l)$ → hydrated cell would have a non-zero entropy change, particularly when expressed per 'mole' of cell. These estimates are based on the observation by Dunitz [3] of typical entropy contributions of various kinds of waters of hydration and the stoichiometries of hydrated biopolymers [4]. Also, heats of combustion (ΔH_c^0) values for hydrated biomass are less exothermic than those for dried biomass by only 0.1–0.2% [5], and it is reasonable that the entropy differences would be of the same magnitude.

2. Experimental

2.1. Sample preparation

The composition of yeast cells differs depending on how they have been cultured. This is because many microorganisms take every advantage to acquire energy. If the conditions for growth are limiting except for the quantity of substrate, these organisms tend to accumulate storage materials for subsequent use. This phenomenon is called oxidative, or fermentative assimilation [2]. However, it was pointed out by Duclaux [6] that cellular storage products are internal substrates that become used when the supply of external substrate becomes exhausted. Battley [2] reasoned that these substances should not be considered permanent parts of the structural fabric of the cells. Therefore, one of the requirements for a sample of yeast cells that is to be lyophilized for use in entropy determinations is that it contains no glycogen or trehalose, the two principal forms of storage products in this yeast genus. A commercial preparation of *S. cerevisiae* containing the least initial quantity of storage substances was found to be Red Star Universal Compressed Yeast (General Foods Corp., Milwaukee, Wisconsin 53218.) This product is grown commercially on a solution of clarified molasses with added mineral nutrients. It is then washed twice by being passed through a separator (a type of zonal centrifuge) during concentration while being harvested from the fermentor, after which it is compressed. Of this product, 100 g were suspended in 100 ml of water and incubated at 30°C for 16 h to ferment away any residual carbohydrate. An aliquot of this suspension was then tested by the Durham tube method [7] to determine if any residual, fermentable materials were present. When no further metabolic activity was observed, the cells were centrifuged from suspension (this was then the third wash) and lyophilized according to the method of Battley and DiBiase [8]. The lyophilized cells were ground to a fine powder using a mortar and pestle, after which the powder was held *in vacuo* over concentrated H_2SO_4. This process was repeated until a sufficient quantity of lyophilized cell powder was obtained for the heat capacity measurements.

Because powders of this type do not have good thermal relaxation characteristics in an adiabatic calorimeter, the sample was pressed into pellets using a hydraulic press at room temperature and 21 MPa for 30 s. Prior to loading into the calorimeter, the pellets were dried overnight in a drying oven at 60°C because thermogravimetric measurements indicated that the pellets had picked up a small amount of moisture during the pelletizing process.

The dried cells, taken from the calorimeter after the heat capacity determinations were made, had a slightly more grey aspect than those that were lyophilized but not used in the calorimeter. An aliquot of the former was rehydrated by suspension in a liquid medium consisting of 1.5% malt extract (Difco), 0.5% yeast extract (Difco), and 0.5% glucose, dissolved in distilled water. Because most of the cells had become clumped during preparation for the heat capacity determinations, a 60 ml glass stoppered bottle, half-filled with ~250 μm diameter glass beads was filled to capacity with the suspension and hand shaken for 10 min. This broke up most of the clumps into individual cells, although a few clumps of 3–4 cells still remained. Microscopic examination indicated that the suspension consisted of a mixture of cells that appeared completely normal, others that were obviously damaged, and cellular debris. A Neubauer counting chamber (a type of microscope slide with which a microscopic count can be made of the number of particles suspended in a known, small volume of fluid) was used to make an initial cell count of both normal and damaged cells. Then, a count of only the viable cells was made by adding an aliquot of the suspension to a liquified 1.5% agar medium at 45°C and carrying out a pour plate dilution series in which each Petri plate had a 10X dilution. Following soli-

dification of the agar and incubation at 25°C, individual living cells grew into colonies that could be counted on the plates at a given dilution. This resulted in the estimation that not more than 2% of the cells were viable. Such a low count is to be expected because the lyophilization procedure [8] that was used to dry the cells for the heat capacity measurements could not make use of substances such as bovine serum albumin or casein that coat the cells during this procedure and tend to preserve them. In addition, the cells contained no carbohydrate storage substances, and the processes of lowering the cells to 7 K and of rehydrating them for a viability study may also have destroyed many cells. On the other hand, those cells that were viable formed similar sized colonies in the pour plates during the same time period as identical cells that had not been lyophilized or lowered to 7 K, indicating that the experimental treatment had no apparent effect on their ability to grow if they were not otherwise damaged. These are, of course, qualitative observations. An increase in viability might be obtained with experimentation on different ways of preparing the cells for the heat capacity measurements.

2.2. *Heat capacity measurements*

The calorimetric measurements reported here were obtained with an adiabatic calorimeter operating in a pulsed heat mode. The general characteristics of the calorimetric system used for these measurements have been described previously by Boerio-Goates [9]. Since the publication of that paper, modifications to the cryostat include the replacement of several of the home-built adiabatic shield control units with commercial nanovolt amplifiers and temperature control circuitry. Also, the computer data acquisition system described in the original paper has been replaced by a PC-based system employing LabVIEW® (National Instruments, 6504 Bridge Point Parkway, Austin, TX 78730-5039), a program for data acquisition systems developed by National Instruments Experimental procedures and data analysis have not changed from those reported previously.

Pellets with a total mass of 31.9003 g *in vacuo* were loaded into the calorimeter which has an internal volume of 70 cm^3. Following evacuation and the addition of helium exchange gas, the calorimeter was sealed using a gold gasket pressed onto a knife edge. Small amounts of Apiezon-T grease were added to the wells built into the calorimeter for the thermometer/heater assembly and the adiabatic shield thermocouples to improve thermal conduction. The heat capacity of the empty calorimeter, thermometer/heater and other addenda represented approximately 20% of the total measured heat capacity at the lowest temperatures, about 50% near 90 K, and less than 40% above 200 K.

3. Results

Heat capacity measurements were obtained from 7 to 310 K. Because the heat capacity showed hysteresis in the region near 250 K, the experimental results are presented in Table 1 in chronological order. One can infer the approximate size of the temperature increment employed in the measurement from the difference between successive entries in a series. The complete set of results is shown in Fig. 1. A small region of anomalous heat capacity was observed between 230 and 270 K. To characterize this region we collected four sets of data. An enlargement of this section of Fig. 1 is plotted in Fig. 2 so that the details of each series are more easily seen. From this Figure, it is apparent that each series taken individually generates a smooth curve. However, when the data points are regarded collectively, it can be seen that the heat capacity is not reproducible. The first of the four data sets (Series 3) showed no signs of the anomalous heat capacity, but these measurements were taken after the sample had been cooled only to 245 K. The remaining three series (Series 5, 7, and 11) exhibit a small maximum, although each shows it at a different temperature and with a different height above the baseline heat capacity. These measurements were taken after the sample had been cooled to 230 K to accomplish a complete conversion to the low-temperature phase.

While the C_p values were not reproducible in this region, the total enthalpy required to heat across the transition from 231 to 272 K was reproducible to better than 0.1%, our expected limit of precision. Since it is known [10] that both cellular water and membranes undergo structural transitions at temperatures below the freezing point of pure water, it is likely

Table 1
Experimental heat capacities of lyophilized *S. cerevisiae* cells

Series 1															
T (K)	290.86	296.03	301.14	306.26											
C_p (J K^{-1} g^{-1})	1.266	1.288	1.311	1.334											
Series 2															
T (K)	279.77	284.30	289.44	294.56	299.68	304.81	309.93								
C_p (J K^{-1} g^{-1})	1.222	1.241	1.262	1.284	1.306	1.327	1.350								
Series 3															
T (K)	247.33	251.77	256.90	262.03	267.16	272.29	277.42								
C_p (J K^{-1} g^{-1})	1.095	1.115	1.134	1.157	1.179	1.199	1.216								
Series 4															
T (K)	282.55	292.22	296.76												
C_p (J K^{-1} g^{-1})	1.235	1.275	1.294												
Series 5															
T (K)	235.30	239.69	244.81	249.92	255.04	260.17	265.31	270.44	275.58	280.71	285.84	290.97	296.08	301.20	306.30
C_p (J K^{-1} g^{-1})	1.047	1.068	1.092	1.117	1.140	1.156	1.176	1.194	1.209	1.228	1.248	1.269	1.290	1.313	1.336
Series 6															
T (K)	211.06	215.40	220.54	225.67	230.80	235.93									
C_p (J K^{-1} g^{-1})	0.948	0.966	0.987	1.008	1.030	1.052									
Series 7															
T (K)	238.70	243.12	248.22	253.30	258.42	263.58	268.72	273.87	279.02						
C_p (J K^{-1} g^{-1})	1.058	1.079	1.110	1.150	1.161	1.173	1.190	1.204	1.222						
Series 8															
T (K)	162.18	166.35	171.45	181.64	186.74	191.84	196.95	202.08	207.20	212.33	217.47				
C_p (J K^{-1} g^{-1})	0.739	0.757	0.780	0.827	0.849	0.871	0.892	0.913	0.933	0.954	0.975				
Series 9															
T (K)	67.76	71.59	76.46	81.33	86.23	91.16	96.11	101.09	106.09	111.10					
C_p (J K^{-1} g^{-1})	0.339	0.358	0.382	0.405	0.428	0.450	0.472	0.494	0.515	0.536					
Series 10															
T (K)	116.20	121.28	126.33	131.39	136.45	141.53	146.61	151.70	156.79	161.88	166.97				
C_p (J K^{-1} g^{-1})	0.557	0.577	0.598	0.618	0.638	0.657	0.677	0.697	0.718	0.738	0.760				

Table 1
(Continued)

Series 11												
T (K)	218.48	222.83	227.96	233.08	238.20	243.32	248.41	253.53	258.68	263.84	268.97	274.10
C_p (J K^{-1} g^{-1})	0.977	0.995	1.017	1.038	1.062	1.091	1.125	1.143	1.153	1.167	1.187	1.205
Series 12												
T (K)	53.47	62.02	66.75	71.54	76.37	81.24	86.14	91.07	96.03			
C_p (J K^{-1} g^{-1})	0.264	0.310	0.334	0.358	0.382	0.405	0.427	0.450	0.472			
Series 13												
T (K)	7.86	8.45	9.19	9.99								
C_p (J K^{-1} g^{-1})	0.009	0.011	0.012	0.014								
Series 14												
T (K)	10.85	11.75	12.74	15.10	16.29							
C_p (J K^{-1} g^{-1})	0.017	0.020	0.024	0.037	0.042							
Series 15												
T (K)	12.95	15.47	16.70	18.16	19.74							
C_p (J K^{-1} g^{-1})	0.025	0.038	0.044	0.052	0.061							
Series 16												
T (K)	23.69	25.53	27.81	30.28	32.99	35.94						
C_p (J K^{-1} g^{-1})	0.085	0.096	0.110	0.126	0.143	0.161						
Series 17												
T (K)	26.01	28.26	30.78	33.53	36.53	39.82	43.42	47.37				
C_p (J K^{-1} g^{-1})	0.099	0.113	0.129	0.146	0.165	0.184	0.206	0.229				
Series 18												
T (K)	152.86	157.79	162.71	167.80	172.89	177.98	183.08	188.18	193.29	198.40		
C_p (J K^{-1} g^{-1})	0.701	0.720	0.740	0.762	0.784	0.808	0.831	0.853	0.874	0.895		
Series 19												
T (K)	193.37	198.48	203.59	208.71	213.82							
C_p (J K^{-1} g^{-1})	0.874	0.894	0.916	0.936	0.959							

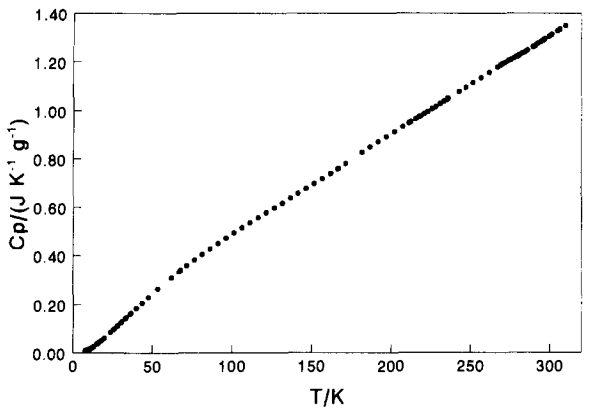

Fig. 1. Heat capacity of lyophilized cells of *S. cerevisiae* as a function of temperature.

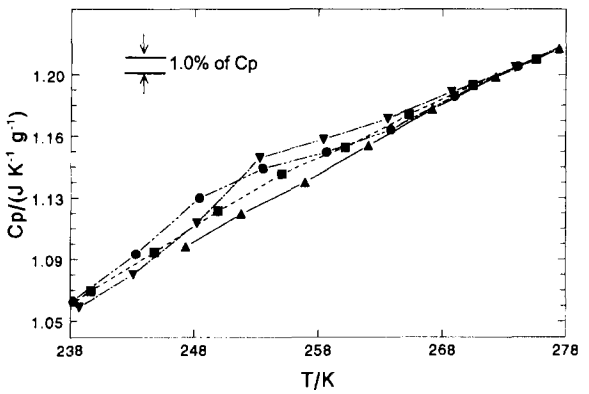

Fig. 2. Enlargement of the heat capacity curve of *S. cerevisiae* cells in the region of the anomalous heat capacity attributed to water. ▲ – Series 3; ■ – Series 5; ▼ – Series 7; • – Series 11. The lines between the points are drawn to aid the eye in differentiating the data from different series, but are not intended to represent smoothed heat capacities.

that one or both of these phenomena are responsible for the observed behavior in C_p.

When the heat capacity curve is inspected on a large plot the appearance of a glass transition with a very small ΔC_p (about 1% of the total background) centered at 180 K is also evident. This feature was so subtle that it could not be detected initially. A reinvestigation of this region (Series 18) was made. The curve generated by the second set of data was parallel to the first, but lower by about 0.2%. Because the change in C_p at the transition was just at the level of our detectability, we did not pursue further studies in this region.

The points in Series 1–4, 6, 8–10, and 12–17 have been fitted to a smooth curve using orthogonal polynomials. A non-linear plot of C_p/T vs. T^2 was obtained at the lowest temperatures of our experiments (7–13 K), indicating that the data did not follow the Debye-T^3 law. However, to calculate the entropy and enthalpy increments from 0 K to the temperature at which we began our fitting routine, it was assumed that the data point at 7.86 K did follow a simple $C_p = aT^3$ relationship. Appropriate integration of the Debye expression from 0 to 7.86 K coupled with similar integrations from 7.86 K to various upper temperature limits of the orthogonal polynomials yielded values for the thermodynamic functions. These are reported in Table 2 at rounded temperatures. To emphasize that these functions were generated by integration of heat capacities from 0 K, we adopt the notation for the heading of the columns $S_T - S_0$, $H_T - H_{298}$ and $-\{G_T - H_{298}\}/T$, for the entropy, enthalpy and Gibbs free energy function, respectively. Later in the text, however, we refer to the entropy of the yeast cells simply as S.

A typical determination of the composition of dried cells usually takes the form of a combustion analysis of the percentages of C, H, N, and ash, with O being calculated as the residual percentage of the dry wt. This results in an empirical formula representing what is considered to be the organic, energy-containing substance of the cells in terms of C, H, O, and N. However, it has recently become apparent that a determination of the oxygen directly gives a greater percentage of this element [11]. This is because elements other than C, H, and N contribute greater weight to the ash as oxides and salts than the contribution they make as a part of the cellular fabric. P and S are not usually included in these formulations because their oxides are a part of the ash formed on combustion. On the other hand, these elements are found in significant quantities as a part of the cell structure. In addition, ions have never been included in representations of cells because they do not play a significant role in the material balance of cellular reactions, and are therefore ignored. Nevertheless, P, S, and ions do contribute to the entropy of cells, and should be included as a part of the biomass of which an entropy is to be determined. A chemical analysis of the yeast cells used in

Table 2
Thermodynamic functions of *S. cerevisiae*

T (K)	C_p (J K^{-1} g^{-1})	$S_T - S_0$ (J K^{-1} g^{-1})	$H_T - H_{298}$ (J g^{-1})	$-\{G_T - H_{298}\}/T$ (J K^{-1} g^{-1})
10	0.014	0.004	−201.21	20.12
15	0.035	0.013	−201.09	13.419
20	0.063	0.027	−200.84	10.069
25	0.093	0.044	−200.45	8.062
30	0.124	0.064	−199.91	6.728
35	0.155	0.085	−199.21	5.777
40	0.186	0.108	−198.36	5.067
45	0.215	0.132	−197.36	4.517
50	0.244	0.156	−196.21	4.080
60	0.299	0.205	−193.49	3.430
70	0.350	0.255	−190.25	2.973
80	0.399	0.305	−186.50	2.636
90	0.445	0.355	−182.28	2.380
100	0.489	0.404	−177.61	2.180
110	0.531	0.452	−172.51	2.021
120	0.572	0.500	−166.99	1.892
130	0.612	0.548	−161.06	1.787
140	0.651	0.595	−154.75	1.700
150	0.691	0.641	−148.04	1.628
160	0.731	0.687	−140.93	1.568
170	0.774	0.732	−133.41	1.517
180	0.819	0.778	−125.45	1.475
190	0.863	0.823	−117.04	1.439
200	0.904	0.869	−108.20	1.410
210	0.944	0.914	−98.96	1.385
220	0.985	0.959	−89.32	1.365
230	1.026	1.003	−79.27	1.348
240	1.066	1.048	−68.81	1.334
250	1.107	1.092	−57.94	1.324
260	1.149	1.136	−46.66	1.316
270	1.189	1.180	−34.96	1.310
273.15	1.201	1.194	−31.20	1.309
280	1.226	1.224	−22.88	1.306
290	1.264	1.268	−10.442	1.304
298.15	1.299	1.304	0.000	1.304
300	1.308	1.312	2.412	1.304

this study has been reported by Battley [12]. Here, the percentages of C, H, O, N, and P were determined by direct analysis, with the percentages of S, K, Mg, and Ca being taken from the literature, to account for 99.97% of the total dry wt. The cellular substance can then be represented by a formula, analogous to an empirical formula for molecules, as follows:

$$C_{3.820}H_{6.161}O_{2.127}N_{0.605}P_{0.045}S_{0.012}K_{0.083}Mg_{0.010}Ca_{0.003} \quad (1)$$

This formula is not particularly convenient to deal with, and Battley [13] adopted the use of a unit carbon formula (UCF), the concept of which has become generally accepted.

Formula (1) then becomes:

$$CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001} \quad (2)$$

Formula (2) represents a mass of cellular fabric having a unit-carbon formula weight (UCFW) of 26.202 Da.

Such a biomass is also called a 'carbon mol' of cells (C mol), and this acronym is in greater use in the literature than UCFW because it is easier to pronounce or use. There is some convenience in this, and its use will be adopted here. It is apparent that there can be three forms of a UCF. One is expressed in terms of only C, H, O, and N [13], one in terms of C, H, O, N, P, and S [11], and one that also includes the ions that are a part of the cell mass [12]. This latter can be called an ion-containing carbon mol, or ICC-mol, and is what is represented by formula (2), which includes ions (K, Mg, and Ca) with proportions greater than 0.001 mol relative to 1.000 mol of C, all of the others being trace elements. According to the data of Reed and Nagodawithana [14], the ions with proportions less than this constitute only about 0.020% of the total dry wt. and only 0.05% of the formula wt (ICC-mol). We consider them to be insignificant with respect to their contribution to the entropy of the cellular mass. Although ions do not play a significant role in the energetics of cellular reactions in terms of a mass balance, they do contribute to the absolute entropy of a cell and to its entropy of formation.

One ICC-mol times the entropy/g gives the entropy/ICC-mol. A value of 1.304 J K^{-1} g^{-1} was measured for the entropy of yeast cells at 298.15 K and 1 bar as indicated in Table 2. From the chemical composition analysis given above, the entropy of dried *S. cerevisiae* cells at 298.15 K is calculated to be 34.167 J K^{-1}·ICC-mol^{-1}. Given the composition analysis we can also calculate the entropy of formation, ΔS_f, of these cells by means of the following equation:

$$\Delta S_f = S - 5.74nC - 65.34nH - 102.57nO - 95.81nN - 41.09nP - 31.80nS - 64.18nK - 32.68nMg - 41.42nCa \quad (3)$$

where S is the entropy at 298.15 K of one ICC-mol of dried *S. cerevisiae* cells and n is the quantity of each atom in the formula. The constants 65.34, 102.57, and 95.81 are one-half of the molar entropies of $H_2(g)$, $O_2(g)$, and $N_2(g)$, respectively. The other constants are the molar entropies of solid graphite, white phosphorus, rhombic sulfur, potassium, magnesium, and calcium. They all apply to conditions at 298.15 K and 1 bar, and all have the dimensions of J K^{-1} mol^{-1} [15]. However, we have chosen not to signal standard conditions by using S^0 or ΔS_f^0 on quantities pertaining to the yeast cells since they are not pure materials. Using Eq. (3), ΔS_f for dried *S. cerevisiae* cells is calculated to be -151.46 J K^{-1}·ICC-mol^{-1}.

4. Discussion

These heat capacity measurements represent the first attempt to measure a third-law entropy for whole, dried cells. Given the variety of macromolecules and cellular structures contained in the biomass, it is somewhat surprising to see such a simple heat capacity curve. The anomaly observed below 270 K probably arises from the presence of residual water or it, like the small glass transition, may arise from structural changes in one or more of the biopolymers which make up the cell. The presence of the glass transition, however, serves as a reminder that the material under study is not in a true equilibrium state, and any measure of its entropy by integration of the heat capacity can only be regarded as a lower bound of the actual true value.

Andronikashvili et al. [4] have asked the question "at what level does the entropy of the native biological substance become measurably less than the entropy of its simple, but not ordered elements." Their results suggest that for appropriately hydrated DNA molecules, the decrease in entropy upon organization into the macromolecular structure relative to a random mixture of hydrated nucleotides arises primarily from the loss of freedom of the water molecules which are immobilized through structured hydrogen bonding in the helical or coiled states. Hutchens et al. [16] in their studies of proteins observed that the entropies of anhydrous proteins can be approximated as the sum of the entropies of the constituent amino acids minus an entropy contribution for each peptide bond formation. These results suggest that the organization of macromolecules relative to their simple monomeric components does not lead to a significant entropy decrease.

It is of interest to see if cellular organization results in a more measurable decrease in the entropy of the dried cellular material relative to the simple monomeric materials from which the cells are derived than is observed in biopolymers. Table 3 shows the entropies at 298.15 K of a number of biological molecules.

Table 3
Standard Entropies at 298.15 K of Selected Biologically Important Molecules. All values are taken from Reference [1] unless noted otherwise

Compound name	Molecular formula	S^0_{298} (J K^{-1} g^{-1})
D-lactic acid	$C_3H_6O_3$	1.593
Methionine	$C_5H_{11}NO_2S$	1.551
L-valine	$C_5H_{11}NO_2$	1.527
DL-alanyl glycine	$C_5H_{10}N_2O_3$	1.460
L-alanine	$C_3H_7NO_2$	1.450
Creatine	$C_4H_9N_3O_2$	1.445
Proline	$C_5H_9NO_2$	1.426
L-serine	$C_3H_7NO_3$	1.419
Succinic acid	$C_4H_6O_4$	1.417
Glycine	$C_2H_5NO_2$	1.379
Glycyl glycine	$C_4H_8N_2O_3$	1.365
Anhydrous bovine chymotrypsinogen A	$C_{1077}H_{1736}N_{304}O_{343}S_{12}$	1.350
Anhydrous bovine zinc insulin	$C_{508}H_{752}N_{130}O_{150}S_{12}$	1.315
Yeast cells [a]	—	1.304
Aspartic acid	$C_4H_7NO_4$	1.278
L-tyrosine	$C_9H_{11}NO_3$	1.181
α-D-glucose [b]	$C_6H_{12}O_6$	1.161
Sucrose [c]	$C_{12}H_{22}O_{11}$	1.146
Adenine	$C_5H_5N_5$	1.118
Guanine	$C_5H_5N_5O$	1.060

[a] Value reported in this work.
[b] [9].
[c] [17].

(The data for entries in these tables have been adapted from Ref. [1], except where noted). They range from a high of 1.59 J K^{-1} g^{-1} for lactic acid to a low of 1.06 J K^{-1} g^{-1} for guanine. The value of 1.304 J K^{-1} g^{-1} obtained for the yeast falls in the middle of this range, and it is quite close to the values obtained for native anhydrous proteins such as bovine serum albumin, bovine zinc insulin, collagen, and α-chymotrypsin which have values of 1.328, 1.315, 1.289, and 1.350 J K^{-1} g^{-1}, respectively [13]. It appears, then, that if there is an entropy associated with organization of the anhydrous cellular material, it is too small to be measured. The primary contributions to the entropy must arise then from thermal excitation of intra- and intermolecular energy levels of the cellular components which are relatively unchanged from the pure macromolecules.

While no other experimental values of the entropy of a cell has been reported, Battley [18] has developed an indirect method to calculate the entropy of a cell from an estimate of the ΔG and ΔH for the combustion of CHON-containing materials. These thermochemical quantities are obtained from data developed by Erickson and Patel [19,20] and have yielded a value for the room-temperature entropy of *Escherichia coli* cells of about 3 J K^{-1} g^{-1}. Given the above observations, even though *E. coli* and *S. cerevisiae* cells are quite different biologically, we would expect the entropy of cells in general to be almost the same, thus there is some disagreement between the experimentally obtained value and the value that has been calculated indirectly. Since, the calorimetric results give a lower bound to the 'true' entropy, the differences may be a result of an unresolved zero-point entropy in the third-law measurement. They may, however, arise from some unknown problem when indirect method is used.

5. Conclusions

The entropy of dried yeast cells derived from calorimetric measurements is within the range of entropies observed for a number of biologically important molecules, including simple monomeric species like sugars and amino acids, and more com-

plex macromolecules like proteins. This result, coupled with similar observations on proteins and nucleic acids, suggest that one might reasonably estimate the entropies of complex anhydrous cellular structures or biopolymers as weighted sums of the entropies of their various constituents, if the compositions and constituent entropies are known. It also suggests that the actual method of preparation of our cells, and their exact composition, with the exception of water content, might not significantly affect the final entropy/g. However, since the entropy derived from the calorimetric measurements relies on an application of the third law of thermodynamics, and because cells are not perfectly crystalline materials, the value reported here should be regarded as a lower bound to the true absolute value.

We tend to agree with Morrison that "Nearly all the manifest visual and mechanical intricacy of organisms, like their apt behavior, turns out to be without quantitative thermodynamic importance. Morphology and ecology are... only small secondary properties of a fundamentally thermodynamic system." [21].

References

[1] E.S. Domalski, W.H. Evans, and E.D. Hearing, J. Phys. Chem. Ref. Data, 13 (1984), Supplement 1, p. 286.
[2] E.H. Battley, Energetics of Microbial Growth, Wiley Interscience, New York, 1987, p. 261.
[3] J.D. Dunitz, Science, 264 (1994) 670.
[4] E.L. Andronikashvili, G.M. Mrevlishvili, G.Sh. Japaridze, V.M. Sokhadze and P.A. Talishvili, J. Non-Equilib. Thermodyn., 14 (1989) 23.
[5] U.v. Stockar, L. Gustafsson, C. Larsson, I. Marison, P. Tissot and E. Gnaiger, Biochim. Biophys. Acta, 1183 (1993) 221.
[6] E. Duclaux, Traité de Microbiologie, III, Masson et Cie., Paris, 1900.
[7] H.G. Schlegel, Allegemeine Mikrobiologie, Georg Thieme Verlag, Stuttgart, 1969, p. 242.
[8] E.H. Battley and G. DiBiase, Biotechnol. Bioeng., 22 (1980) 2417.
[9] J. Boerio-Goates, J. Chem. Thermodyn., 23 (1991) 403.
[10] J.M. Lyons, J.K. Raison, and P.L. Steponkus, in: J.M. Lyons, D. Graham, and J.K. Raison (Eds.), Low Temperature Stress in Crop Plants: The Role of the Membrane, Academic Press, New York, 1979, pp. 1–24.
[11] E.H. Battley, Appl. and Environment. Microbiol., 61 (1995) 1655.
[12] E.H. Battley in: P. Gallagher and R.B. Kemp (Eds.), (Ed.), Handbook of Thermal Analysis and Calorimetry, Vol. 3, Elsevier, Amsterdam, in press.
[13] E.H. Battley, Physiol. Plantarum, 13 (1960) 192.
[14] G. Reed and T.W. Nagodowithana, Yeast Technology, 2nd edn., Van Nostrand Reinhold, New York, 1991, p. 273.
[15] D.D. Wagman, William H. Evans, V.B. Parker, R.H. Schumm, I. Halow, S.M. Bailey, K.L. Churney, and R.L. Nuttall, J. Phys. Chem. Ref. Data, 11 (1982), Supplement 2, p. 392.
[16] J.O. Hutchens, in: H.A. Sober (Ed.), Handbook of Biochemistry The Chemical Rubber Co., Cleveland, 1970, p. B-61.
[17] R.L. Putnam and J. Boerio-Goates, J. Chem. Thermodyn., 25 (1993) 607.
[18] E.H. Battley, Pure and Appl. Chem., 65 (1993) 1881.
[19] L.E. Erickson and S.A. Patel, Crit. Rev. Biomed. Eng., 8 (1982) 311.
[20] S.A. Patel and L.E. Erickson, Biotechnol. Bioeng., 23 (1981) 2051.
[21] P. Morrison, Rev. Mod. Phys., 36 (1964) 517.