

Water Binding in Legume Seeds

Received for publication December 15, 1986 and in revised form June 15, 1987

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ABSTRACT

The physical status of water in seeds has a pivotal role in determining the physiological reactions that can take place in the dry state. Using water sorption isotherms from cotyledon and axis tissue of five leguminous seeds, the strength of water binding and the numbers of binding sites have been estimated using van't Hoff analyses and the D'Arcy/Watt equation. These parameters of water sorption are calculated for each of the three regions of water binding and for a range of temperatures. Water sorption characteristics are reflective of the chemical composition of the biological materials as well as the temperature at which hydration takes place. Changes in the sorption characteristics with temperature and hydration level may suggest hydration-induced structural changes in cellular components.

A large proportion of Angiosperm seeds are orthodox in that they can survive desiccation. As desiccation of these seeds proceeds there is no evidence of large structural changes in their membranes (15, 19, 23) and it has been suggested that the lack of major conformational changes in membranes upon drying is an essential characteristic of desiccation tolerance (5, 13).

Because there are limited structural changes induced by the addition or removal of water in seeds, and because seeds can be wetted and redried with no apparent damaging effect, they are an appropriate material for a thermodynamic study of hydration in biological tissues. Such studies can contribute to our understanding of how water binds to biomolecules and how water binding affects the physical state of the water as well as the biomolecules. This paper examines water binding in some orthodox seeds.

The concept of bound water has become a point of confusion. Here we define bound water as water associated with a matrix, that has thermodynamic and/or motional properties that differ from bulk solution water (12, 21). For instance, freezable water was not detected at temperatures as low as -70°C in soybean cotyledons with moisture contents less than 38% water (13). It is believed that there are at least three types of interstitial water: water that is very strongly bound, water that is weakly bound and water that is loosely associated with polymer surfaces (for reviews, see Refs. 7, 12, and 21). Each of these different 'types of water' probably display different structural characteristics (7). Because bound water can be defined in thermodynamic terms (*i.e.* the strength of its association with a surface), assaying bound water by thermodynamic methods is appropriate.

A convenient method for studying the thermodynamics of hydration is through the use of water sorption isotherms. Water

sorption isotherms have been made for many biological tissues (see Refs 2 and 12 for review of proteins; Ref. 16 for review of seeds; Ref. 9 for review of foods; Ref. 22 for erythrocyte membranes; and Ref. 4 for *Artemia* cysts). A mathematical model developed to describe water sorption which is applicable to a wide range of systems makes provisions for differing energies of sorption sites (6). Included in the D'Arcy/Watt model are terms to describe sites where individual water molecules bind strongly, sites where water binds weakly and sites where water condenses as a collection of molecules (multimolecular sorption). The D'Arcy/Watt model is therefore a composite of isotherms represented by

$$W = \frac{KK'(p/p_0)}{1 + K(p/p_0)} + c(p/p_0) + \frac{kk'(p/p_0)}{1 - k(p/p_0)}, \quad (1)$$

where W is the amount of water sorbed per gram of tissue and p/p_0 is the relative vapor pressure. The first term relates to sorption of strong binding sites, the second term to weak binding sites and the third term to multimolecular sorption or water binding to already adsorbed water molecules. The five parameters have thermodynamic significance: K' is an expression of the number of strong binding sites, K relates to the attraction of these sites for water, c to the number and strength of weakly binding sites, k' to the number of multimolecular sorption sites, and k to the water activity of multimolecular water (6).

An alternative treatment of isotherm data is the comparison of sorption at two temperatures via the Clausius-Clapeyron equation (2, 14, 22, 26), where it can be shown that

$$\Delta H = R \times \frac{(T_1 \times T_2)}{(T_2 - T_1)} \times \ln(aw_1/aw_2), \quad (2)$$

with ΔH being the differential enthalpy (in J/mol water) given a constant water content, aw_1 and aw_2 are the relative vapor pressures at the lower and higher temperatures, T_1 and T_2 . R is the ideal gas constant (8.3143 J/degree/mol). Calculated enthalpies reflect properties of the polymer surface-water interaction and will therefore reflect the strength of water binding as well as any conformational changes or swelling effects (14).

It is also useful to calculate free energy (ΔG) and differential entropy (ΔS) changes with hydration.

$$\Delta G = R \times T \times \ln(aw) \quad (3)$$

$$\Delta S = (\Delta H - \Delta G)/T, \quad (4)$$

where aw is the relative vapor pressure at temperature T .

A shortcoming of many of the water sorption isotherms reported in the literature is that they were made at nonphysiological temperatures ($>35^{\circ}\text{C}$). Also, interpretation of the physiological significance of isotherm parameters is lacking with a few notable exceptions (4, 20, 21). Nonetheless, the role of bound water in biological systems is becoming increasingly apparent. It has been reported that the structure of bound water contributes to the conformation of macromolecules, and vice-versa (for review, see Refs. 7 and 26). Thermal anomalies observed in the structure of

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bound water at about 15, 31, and 45°C probably affect macromolecular structure as evidenced by anomalies in enzyme reactions, membrane functions and physiological activities (reviewed in Ref. 7). The binding of water is, therefore, likely to have temperature dependencies.

We have measured the water sorption characteristics of 5 legume seeds within a temperature range of 5 to 35°C in order to characterize the strength of water binding and the number of binding sites for tissues of different compositions under different temperature regimes.

MATERIALS AND METHODS

Sorption Isotherms. Determinations were made for soybean (*Glycine max* L.), pea (*Pisum sativum* L.), fava bean (*Vicia faba* L.), cowpea (*Vigna sinensis* Enal), and peanut (*Arachis hypogaea* L.). Whole axes or pellets of cotyledons (0.5–1.0 mm) were equilibrated at 5, 15, 25, and 35°C over various saturated salt solutions according to methods described earlier (26, 27). To check whether the grinding of the cotyledons affected the sorption characteristics, whole cotyledons of pea and soybean were also equilibrated at 5 and 25°C in parallel tests and showed no significant difference when compared with the ground counterparts (25). In all cases, desorption curves were used, the initial moisture content being elevated to about 0.35 g H₂O/g dry weight. Water content is expressed on a dry weight basis. Dry weights were determined by heating material at 95°C for 5 d, a time sufficient to achieve a constant weight.

Values used in van't Hoff analyses were determined by a curvilinear interpolation of the isotherms.

Sorption Model. Characteristics of the three regions of water sorption were estimated by fitting the data for each region of the isotherm to a term in the D'Arcy/Watt equation using linear least square analysis. Data collected at relative humidities between 20 and 45% were used to fit the middle term. These relative humidities were chosen because of the nearly linear shape of the isotherm in this region. Data collected at relative humidities less than 20% and greater than 65% were used in the least squares fit of the first and third term, respectively.

The number of sorption sites and strength of water binding were determined from the D'Arcy/Watt parameters using equations and constants derived earlier (3, 6). The strength of water binding onto strong sorption sites ($\Delta H_{int}(s)$), the number of strong sorption sites, the number of weak sorption sites, and the number of multimolecular sites are given by Eqs. 5, 6, 7, and 8, respectively.

$$\Delta H_{int}(s) = \ln(K/Q) \times R \times T \quad (5)$$

$$\text{No. sites } (s) = K' \times N/M \quad (6)$$

$$\text{No. sites } (w) = c \times N/(M \times f(\Delta H_{int}(w))) \quad (7)$$

$$\text{No. sites } (m) = k' \times N/M \quad (8)$$

where Q is the molecular partition coefficient for primary sorption sites ranging from 0.8 to 1.25 depending on temperature (3), R is the ideal gas constant, T is the temperature, $f(\Delta H_{int}(w))$ is a temperature related function of the strength of weakly binding sites (ranging from 0.997 to 1.001 (dimensionless) for temperatures between 5 and 35°C [3]), N is Avogadro's number, and M is the gram mol wt of water (3, 6), and s , w , and m identify strong, weak, and multimolecular binding.

RESULTS

Isotherms. Moisture sorption isotherms for axis and cotyledon tissues from pea, soybean, cowpea, fava bean and peanut were measured at temperatures between 5 and 35°C. Isotherms for 5°C and 25°C are given as illustrations in Figure 1. The curves

follow the expected reverse sigmoidal shape and can be divided into three regions: a "knee" at low relative humidities (0–20%), a linear region at intermediate relative humidities (20–65%) and a sharp upswing at high relative humidities (70–95%).

The extent of water sorption varied with the species and tissue type. Cotyledons with high lipid content sorbed less water than did cotyledons with high starch content (Fig. 1, A and C). Peanuts (25% carbohydrate, 45% lipid) absorbed the least water, while fava bean, pea, and cowpea (68% carbohydrate, 2% lipid) adsorbed more water at a given relative humidity. Soybean (38% carbohydrate, 20% lipid) had intermediate sorption characteristics. The differences in moisture sorption among species are most apparent in the first two regions of the isotherms. Embryonic axes often showed different sorption characteristics than their associated cotyledons (Fig. 1, B and D). For example, moisture contents of fava bean, cowpea, and pea axes were lower in the second region of the isotherm than the corresponding cotyledon tissue. In both axis and cotyledon tissue, larger amounts of water were sorbed at lower temperatures (Fig. 1).

The differential heats of sorption (ΔH) for soybean and pea seeds, calculated from the sorption data using the Clausius-Clapeyron equation at different water contents, are shown in Figure 2. These curves may be interpreted as showing three regions with different strengths of water binding. The calculated value of ΔH varied with temperature. In soybean cotyledons (Fig. 2A), at water contents lower than 0.08 g H₂O/g dw, ΔH is very negative, ranging from –30 to –80 kJ/mol in the temperature range of 5 to 15°C and –20 to –36 kJ/mol in the temperature range of 25 to 35°C. At moisture contents between 0.09 and 0.20 g/g, ΔH is only slightly different at lower temperatures and ranges between –2.4 and –5.6 kJ/mol. A third level of binding may occur at water contents between 0.22 and 0.30 g/g with ΔH values ranging from –1.0 to –0.36 kJ/mol regardless of the temperature. Similar trends were found for pea (Fig. 2, C and D) as well as in other seed tissues (Table I). The ΔH values calculated for axes are generally more negative than those calculated for cotyledons (Table I).

Free energy (ΔG) can also be calculated from the isotherm data (Eq. 3); representative values associated with hydration of soybean tissues at 15°C are shown in Figure 3B. The free energy increases monotonically with increasing hydration. Differential entropy, (ΔS), calculated by Eq. 4 (Fig. 3C), is roughly inverse to differential enthalpy (Fig. 3A).

Sorption Model. Quantification of the sorption characteristics of different species and tissue types at different temperatures was also accomplished by fitting the data to the D'Arcy/Watt sorption model. Unlike most sorption models, this analysis provides for more than one type of sorption site. The sorption characteristics of the strong, weak and multimolecular sorption sites each contribute to the overall shape of the isotherm and can be separated into three different functions by the D'Arcy/Watt equation (Eq. 1). This is shown for soybean cotyledons at 25°C in Figure 4. Sorption onto strong binding sites (region 1) accounts for about 4 to 6% of the water sorbed and is completed at relative humidities less than 15% (Fig. 4). Sorption onto weak sites (region 2) is described as a linear function (as in the Langmuir model when the sorbate has a low affinity for the sorbent) and accounts for at most 10% of the sorbed water. The multimolecular sorption of water (region 3) becomes increasingly important at relative humidities greater than 60% and represents as much as 85% of the total sorbed water at relative humidities above 90%.

The separate components of the D'Arcy/Watt sorption curves (Fig. 4) can be estimated mathematically. The sorption coefficients calculated from a least-squares fit of the equation contain information about the relative strength at which water is sorbed to sorption sites, and also the relative number of sites available

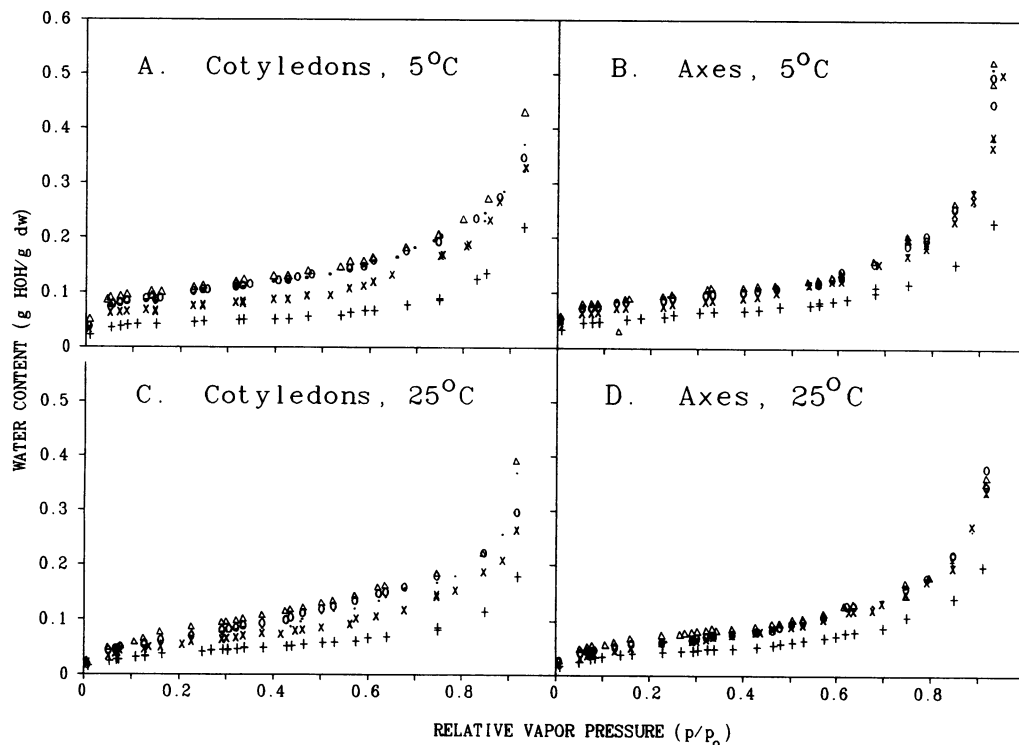


FIG. 1. Samples of sorption isotherms for cotyledon and axis tissues from pea (\cdot), peanut (+), soybean (\times), cowpea (O), and fava bean (Δ) seeds. A and C are isotherms of ground cotyledons at 5 and 25°C, respectively. B and D are isotherms of unground axes at 5 and 25°C, respectively.

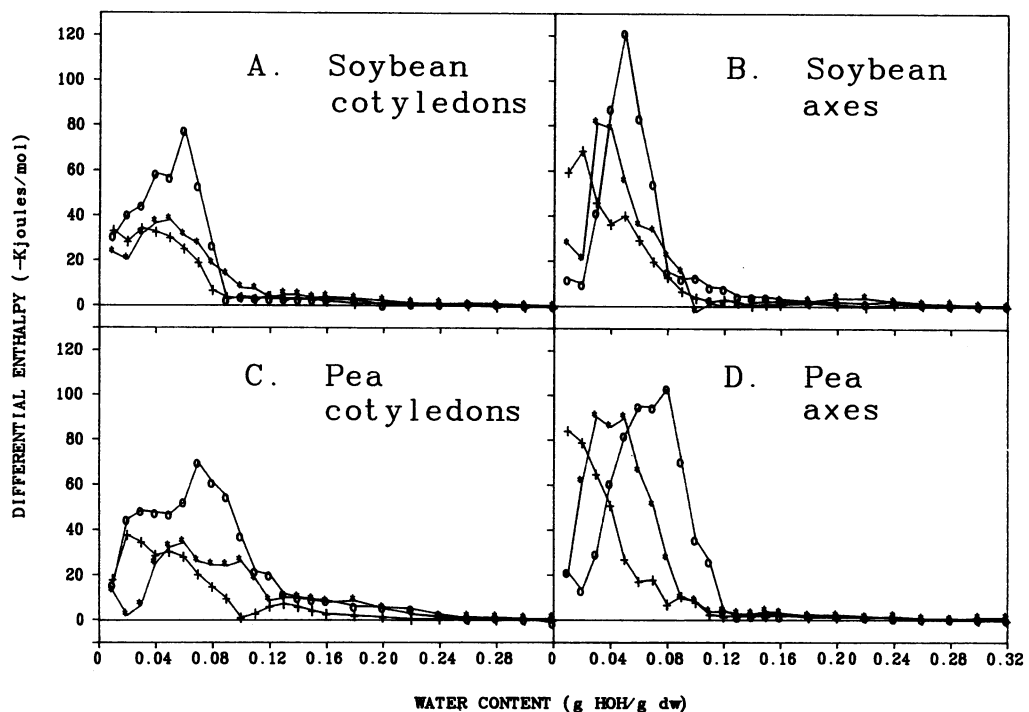


FIG. 2. Differential heats of water sorption (ΔH) onto soybean and pea tissues under different temperature regimes. A and C are data from ground cotyledons of soybean and pea, respectively. B and D are data from whole axes of soybean and pea, respectively. Van't Hoff analyses were performed on isotherms at (O) 5 to 15°C, (*) 15 to 25°C and (+) 25 to 35°C.

(3, 6). Calculated values for those parameters are listed in Table II for the five different species and tissue types at different temperatures.

The parameters K and K' describe the relative strength of sorption and the number of binding sites, respectively, within region 1 (6). In 4 out of 5 seed species, K seems to be relatively constant among cotyledon tissues ranging from 51 to 79 (dimensionless) at temperatures between 5 and 25°C, sometimes dropping to lower values at 35°C. Substituting these parameters into Eq. 5, the integral enthalpy of water binding for the first binding region, $\Delta H_{int}(s)$, was determined to be between -11.8 and -7.4 kJ/mol in cotyledon tissue (Table III). As with the differential

enthalpy parameter, the calculated values of K (Table II) and $\Delta H_{int}(s)$ (Table III) for axis tissues are generally greater than those calculated for cotyledon tissues. The affinity values for axis tissues tend to be lower at higher temperatures.

K' describes the number of strong sorption sites as in Eq. 6. The number of sites consistently decreases with increasing temperature (Table III). A reduction of the number of strongly binding sites is also suggested by the shift of the region 1 water binding to lower water contents at higher temperature ranges (Fig. 2, Table I). Binding sites of the first binding region tend to be more responsive to temperature in axes than in cotyledons.

The coefficient c is related to the strength of weak binding and

Table I. Differential Heats of Water Sorption Calculated for Three Regions of Water Binding in 5 Legume Seeds

Data are calculated using van't Hoff analyses of isotherms at temperatures between 5 and 35°C and represent the average value per region.

Tissue	Temperature	Region					
		1		2		3	
		Moisture range	ΔH	Moisture range	ΔH	Moisture range	ΔH
	°C	g H ₂ O/g dry wt	kJ/mol	g H ₂ O/g dry wt	kJ/mol	g H ₂ O/g dry wt	kJ/mol
Soybean cotyledon	5-15	<0.09	51.5	0.09-0.24	3.03	0.20-0.30	0.92
	15-25	<0.08	31.4	0.09-0.24	3.83	0.26-0.31	0.34
	25-35 ^a	<0.08	31.8	0.09-0.18	4.98		
Soybean axes	5-15	<0.11	68.6	0.12-0.24	3.30	0.26-0.34	0.65
	15-25	<0.10	46.0	0.11-0.24	2.52	0.26-0.30	0.83
	25-35	<0.09	27.6	0.09-0.24	1.68	0.26-0.32	0.46
Pea cotyledon	5-15	<0.12	49.0	0.13-0.26	8.16	0.26-0.30	0.74
	15-25	<0.11	27.6	0.12-0.24	7.45	0.24-0.32	0.73
	25-35	<0.09	25.5	0.10-0.20	3.14	0.22-0.30	0.71
Pea axes	5-15	<0.11	71.1	0.12-0.26	2.27	0.26-0.36	0.22
	15-25	<0.10	53.6	0.11-0.26	3.15	0.26-0.36	0.49
	25-35	<0.09	28.0	0.10-0.26	2.25	0.26-0.30	0.29
Cowpea cotyledon	5-20	<0.11	45.2	0.12-0.24	3.68	0.26-0.33	0.53
	20-35 ^a	<0.08	26.4	0.09-0.20	9.87	0.22-0.28	0.82
Cowpea axes	5-15	<0.10	74.9	0.11-0.22	4.94	0.23-0.36	0.64
	15-25	<0.09	48.1	0.10-0.22	2.14	0.24-0.36	0.51
	25-35	<0.08	34.0	0.09-0.26	2.12	0.28-0.34	0.47
Fava bean cotyledon	5-20 ^a	<0.12	46.0	0.13-0.24	3.22	0.26-0.32	0.48
	20-35	<0.12	26.4	0.13-0.24	5.02		
Fava bean axes	5-15	<0.11	76.1	0.13-0.28	2.04	0.26-0.38	0.67
	15-25	<0.12	47.3	0.13-0.22	2.46	0.24-0.28	0.83
	25-35	<0.09	27.6	0.10-0.20	2.13	0.22-0.34	0.75
Peanut cotyledon	5-20	<0.05	29.3	0.06-0.13	1.37	0.14-0.18	0.46
	20-35	<0.055	38.5	0.07-0.11	2.85	0.12-0.15	0.36
Peanut axes	5-20	<0.07	48.1	0.08-0.15	2.84	0.16-0.20	0.37
	20-35	<0.06	23.4	0.07-0.15	2.70	0.16-0.20	0.33

^a Difficult to define three discreet regions.

the number of weakly binding sorption sites (Eq. 7). Because hydrogen bonds dominate in weak water-tissue interactions, a constant $\Delta H_{\text{int}(w)}$ of -2.0 kJ/mol was assumed (Table III). In making this assumption, we follow the scheme of RUPLEY *et al.* (21) and CARERI *et al.* (3). This may be an unfair assumption, and if it is in error, it would be reflected in the estimates of weakly binding sites (Table III). However, from the data in Table III it seems apparent that the numbers of weak sites vary without a particular trend among species, tissues, and temperatures.

The D'Arcy/Watt parameters k and k' give the water activity and number of binding sites for water clusters sorbing to tissue. In many instances, k is slightly greater than 1 (Table II and III). The number of these types of sorption 'sites,' estimated by k' (Table III), generally decrease with increasing temperature.

With the exception of peanut cotyledons, the total number of binding sites (Table III) is greater at lower temperatures. This is consistent with the higher moisture contents observed in isotherms of seed tissues at lower temperatures (Fig. 1) and with the observations that macromolecular surfaces generally sorb more water at lower temperatures (17).

DISCUSSION

We have compared the water sorption characteristics of five legume seeds at different temperatures. In each case, sorption isotherms followed the typical sigmoidal shape consistent with

the D'Arcy/Watt model involving two sorption sites plus a region of multimolecular sorption. The calculated differential heats of sorption (ΔH) are consistent with the interpretation of three levels of strength of water binding. We suggest that the three regions of water binding are general characteristics of viable legume seeds. The calculated numbers of sites and intensities of binding should be relevant to interpretations of physiological activities in the seeds (25-28).

Isotherms as Functions of Chemical Composition. Moisture isotherms of heterogeneous biological materials represent the integrated hygroscopic properties of the numerous cellular constituents. The moisture absorbing capacity of legume seeds may be related to the carbohydrate and lipid content of the seed: cotyledons of seeds we have tested with high lipid content sorbed less water per g dry weight than cotyledons with high carbohydrate levels. Similar trends have been noted for other seeds (18, 20). The lipid region can be assumed to be simply unavailable to the water, hence the lower sorption per g dry weight (18). This trend is reflected in the number of strong plus weak binding sites per gram of tissue, estimated by the D'Arcy/Watt equation (Table III). Cotyledons which have high starch content (pea, fava bean, cowpea) have between 6.2 and 5.8×10^{21} sorption sites/g tissue at 25°C, and the more fatty soybean and peanut have lower numbers (3.7 and 2.9×10^{21} sites/g tissue, respectively).

The moisture sorbing capacity of the axes differ from their

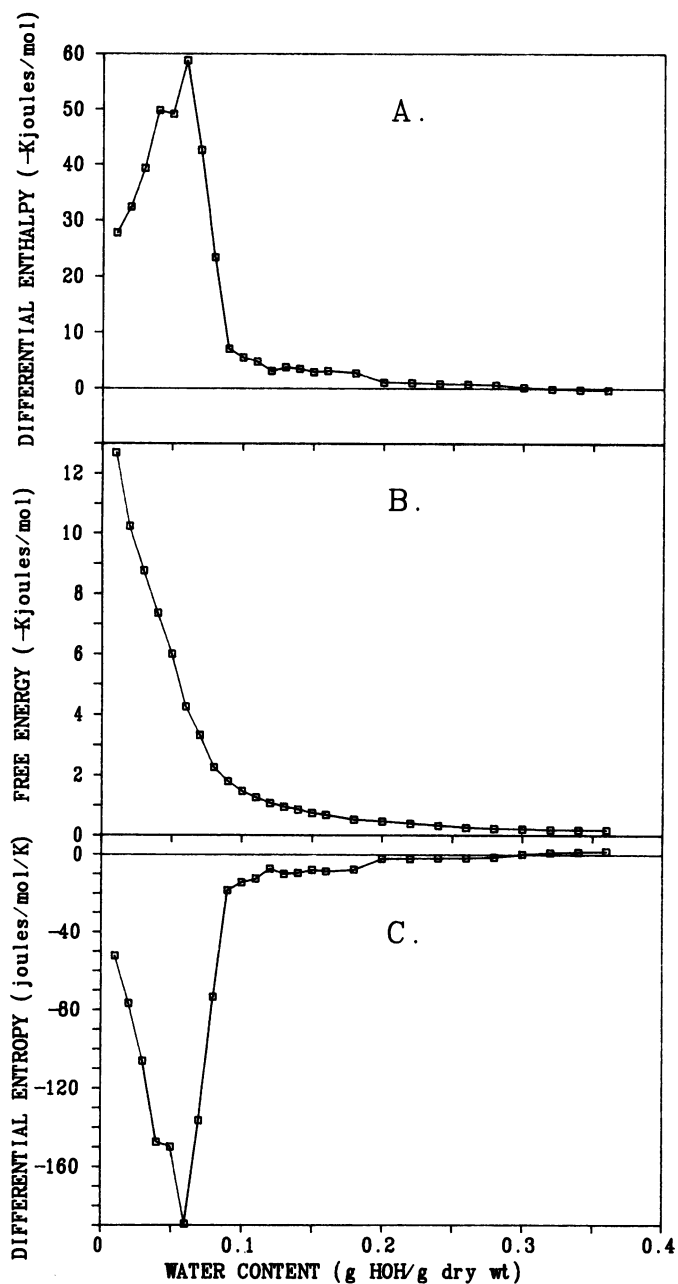


FIG. 3. Thermodynamic parameters of water sorption onto soybean cotyledons as a function of water content. A is differential heat of sorption (ΔH) for the temperature range of 5 to 25°C. B is the free energy (ΔG) of sorption at 15°C, and C is the differential entropy (ΔS) of sorption at 15°C.

corresponding cotyledons (Fig. 1). Starchy cotyledons have more water sorbing capacity than axes from the same seed. When compared to the corresponding cotyledons, pea axes had fewer strong binding sites, cowpea axes and fava bean had fewer weak binding sites (Table III). Because the sorption characteristics among axes of different species tend to be similar, we suggest that there are commonalities in the chemical composition of legume axes.

Isotherms as Measures of Water Binding in Tissue. Sorption isotherms have proven to be a convenient method to assay the physical state of water in relatively dry tissues (3, 4, 12–14, 20, 21). The application of Clausius-Clapeyron theory to determine the strength of water sorption has inherent weaknesses because of (a) uncertainties of water content determinations at very low

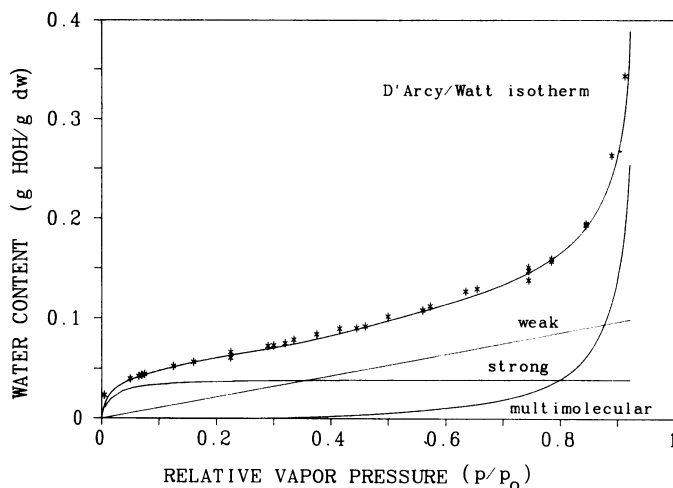


FIG. 4. A sorption isotherm of ground soybean cotyledons at 25°C illustrating its fit to the D'Arcy/Watt model of sorption. Curves drawn represent the individual terms of the equation as well as the composite isotherm equation. The r^2 value for this regression is 0.998.

relative humidities, (b) magnification of calculation errors from reciprocal/semi-log plots, and (c) dependence of the theory on equilibrium conditions (errors introduced by hysteresis). Nevertheless, the method is useful because, with relatively simple techniques, it permits estimation of the affinities of water binding. A second method to analyze sorption isotherms uses the D'Arcy/Watt model, and this permits estimation of both the abundance of binding sites and their affinities. Both interpretations correspond well with other methods used to determine the condition of water in biological materials (21).

The parameters K , K' , c , and k' estimated by least squares fit for legume tissues (Table II) correspond well with parameters estimated for proteins and DNA. For example, K' ranges from 0.024 g H₂O/g dry weight for egg albumin to 0.114 g/g for lysozyme, K ranges from 7 (dimensionless) for skin collagen to 200 for DNA, c ranges from 0.028 g H₂O/g dry weight for zein to 0.183 g/g for collagen, k' ranges from 0.0062/g H₂O/g dw for keratin to 0.124 g/g for DNA (3, 6, 14). Our values of K , calculated from isotherms of axes tissue at 5°C, are higher than those reported above. However, most of the earlier studies used temperatures of 35–40°C and our data consistently show that K is reduced by high temperatures.

There is a discrepancy in the estimated values of k , the water activity of multimolecular sorption. For proteins, k values are usually less than 1.0, though Luscher-Mattli and Ruegg (14) did report a few instances where calculated values of k exceeded 1. A water activity greater than 1 is clearly unlikely, so these values may indicate a limitation in the curve fitting routine. The high calculated k values (close to 1.0) do indicate that the multimolecular sorption is very weak, indeed. In addition, the increasing value of k with increasing temperature suggests that the water activity also increases with temperature.

Structural Changes Associated with Water Sorption. Knowledge of structural changes induced by the addition of water to dry macromolecules is important not only for our understanding of enzyme activation at different water activities, but also for our understanding of water as the 'structural cement' of biomolecules. Although large structural changes are not observed with the desiccation of phospholipid bilayers in seeds (15, 23) or proteins from erythrocyte membranes (22), there are indications of minor changes in bilayer (23) and protein (14, 21) structures when most of the interstitial water is removed. (See Ref. 25 for review.)

Perhaps an important distinction is not so much that a con-

Table II. Parameters Calculated from Application of D'Arcy/Watt Equation to Sorption Isotherms of Axis and Cotyledon Tissues of 5 Legumes at Temperatures from 5 to 35°C

K is logarithmically related to strength of binding and is dimensionless. K' , c , and k' are terms linearly related to the number of binding sites and are expressed in g H₂O/g dry weight.

Tissue	Temperature	Sorption Sites					r^2 of Fit
		Strong		Weak		Multi-molecular	
	°C	K	K'	c	k	k'	
Soybean cotyledon	5	62.9	0.070	0.068	0.97	0.019	0.993
	15	55.5	0.049	0.071	1.01	0.018	0.998
	25	58.0	0.040	0.072	1.05	0.009	0.998
	35	19.8	0.043	0.076	1.07	0.008	0.997
Soybean axes	5	334.4	0.062	0.093	1.01	0.015	0.996
	15	136.7	0.048	0.102	1.03	0.012	0.993
	25	153.5	0.037	0.101	1.03	0.011	0.996
	35	80.5	0.025	0.116	1.04	0.011	0.996
Pea cotyledon	5	67.1	0.086	0.096	0.99	0.014	0.998
	15	51.3	0.066	0.105	1.02	0.015	0.998
	25	55.1	0.057	0.114	1.05	0.009	0.996
	35	55.3	0.039	0.110	1.07	0.005	0.997
Pea axes	5	149.2	0.082	0.084	1.02	0.013	0.974
	15	139.7	0.053	0.118	1.03	0.011	0.966
	25	144.0	0.035	0.137	1.04	0.010	0.996
	35	39.6	0.027	0.143	1.06	0.008	0.997
Cowpea cotyledon	5	76.8	0.088	0.097	1.02	0.011	0.998
	20	78.8	0.042	0.142	1.01	0.009	0.998
	35	59.4	0.034	0.139	1.06	0.005	0.997
Cowpea axes	5	227.3	0.075	0.085	1.01	0.017	0.998
	15	206.3	0.052	0.108	1.03	0.012	0.997
	25	218.5	0.044	0.106	1.03	0.013	0.997
	35	20.3	0.039	0.098	1.04	0.010	0.997
Fava bean cotyledon	5	62.6	0.082	0.108	1.02	0.013	0.999
	20	53.9	0.054	0.130	1.03	0.009	0.998
	35	17.9	0.050	0.115	1.05	0.009	0.998
Fava bean axes	5	227.2	0.071	0.073	1.02	0.014	0.995
	15	88.1	0.060	0.079	1.04	0.014	0.994
	25	76.4	0.050	0.088	1.04	0.011	0.998
	35	23.2	0.042	0.078	0.99	0.020	0.996
Peanut cotyledon	5	134.5	0.040	0.036	1.04	0.007	0.982
	20	114.1	0.029	0.057	1.06	0.004	0.994
	35	135.9	0.014	0.077	1.08	0.002	0.997
Peanut axes	5	284.8	0.045	0.046	1.03	0.007	0.998
	20	89.9	0.034	0.050	1.01	0.011	0.998
	35	34.3	0.029	0.058	1.04	0.006	0.999

formational change occurs or does not occur with hydration, but that the ability to undergo a structural change is affected by hydration. Such a distinction is relevant to the study of protein denaturation (10, 13) and enzyme activity (1, 21) at different water contents. At very low water contents, proteins and phospholipids are held rather rigidly, with very limited mobility (10, 19) and perhaps the conformational changes necessary for activity or denaturation are inhibited.

The ability to undergo structural changes is related to the motional or thermal properties of the system and therefore to the free energy. When molar free energy ($-\Delta G$) is equal to RT , the average thermal energy of a mole of molecules (2.5 kJ/mol at 15°C), there is enough thermal energy for a reaction to occur spontaneously. In soybean cotyledons $-\Delta G = 2.5$ kJ/mol at the onset of region II of water binding (Fig. 4). Accordingly, reactions may be facilitated when all of the strong binding sites are filled.

The decrease in the number of multimolecular binding sites

with rising temperature (Table III) may indicate that higher temperatures induce a tighter packing of the polymer chains, or an increase in structural order (14). It has been suggested that the final stage of 'wetting' of proteins is the hydration of hydrophobic groups (21). Since a large portion of the surface of the protein molecule is hydrophobic, a certain amount of water must interact with these nonpolar groups even if it does not bind *per se*. When hydrophobic groups are 'wetted,' water molecules form cage-like structures around the nonpolar moieties (7, 24), and it is suggested that stronger water-water bonds are created in the vicinity of the hydrophobic groups (11, 24). The strengthening of the hydrophobic interaction is probably associated with an increase in the structuring of water (8). It is well known that hydrophobic interactions become stronger at higher temperatures (24). Our data in Tables II and III frequently indicate stronger water-water interactions and also a presumably tighter molecular packing in the third hydration region with increasing

Table III. *Water Binding Characteristics Determined from Parameters of D'Arcy/Watt Equation Applied to Isotherms of Axis and Cotyledon Tissues of 5 Legumes at Temperatures between 5 and 35°C*

Sorption characteristics include the integrated values of water binding (ΔH_{im}) and also the numbers of strong, weak, and multimolecular sorption sites. Values are calculated using D'Arcy/Watt parameters listed in Table II and Eqs. 5 to 8.

Tissue	Temperature °C	Strength of Water Binding onto Sorption Sites			Number of Sorption Sites ^d				
		Strong ^a	Weak ^b	Multimolecular ^c	Strong	Weak	Multimolecular	Strong + weak	Total
Soybean cotyledon	5	10.1	2	0.97	2.33	2.28	0.62	4.61	5.23
	15	9.7	2	1.01	1.63	2.38	0.60	4.01	4.61
	25	9.8	2	1.05	1.34	2.40	0.31	3.74	4.05
	35	7.1	2	1.07	1.45	2.55	0.25	4.00	4.26
Soybean axes	5	14.0	2	1.01	2.06	3.12	0.51	5.19	5.69
	15	11.9	2	1.03	1.61	3.41	0.39	5.02	5.41
	25	12.2	2	1.03	1.25	3.36	0.36	4.61	4.97
	35	10.7	2	1.04	0.83	3.89	0.35	4.72	5.08
Pea cotyledon	5	10.2	2	0.99	2.89	3.21	0.47	6.10	6.57
	15	9.6	2	1.02	2.21	3.52	0.49	5.73	6.22
	25	9.7	2	1.05	1.92	3.80	0.31	5.72	6.03
	35	9.7	2	1.07	1.31	3.68	0.16	4.99	5.14
Pea axes	5	12.1	2	1.02	2.75	2.80	0.45	5.55	6.00
	15	12.0	2	1.03	1.78	3.94	0.38	5.72	6.10
	25	12.1	2	1.04	1.18	4.58	0.35	5.77	6.11
	35	8.8	2	1.06	0.92	4.79	0.27	5.70	5.97
Cowpea cotyledon	5	10.6	2	1.02	2.94	3.23	0.37	6.18	6.55
	20	10.5	2	1.01	1.42	4.76	0.32	6.18	6.49
	35	10.0	2	1.06	1.15	4.64	0.17	5.79	5.96
Cowpea axes	5	13.1	2	1.01	2.49	2.84	0.57	5.33	5.90
	15	12.9	2	1.03	1.75	3.61	0.39	5.36	5.75
	25	13.1	2	1.03	1.47	3.56	0.44	5.02	5.47
	35	7.1	2	1.04	1.32	3.27	0.34	4.59	4.93
Fava bean cotyledon	5	10.1	2	1.02	2.75	3.62	0.44	6.37	6.81
	20	9.6	2	1.03	1.79	4.34	0.30	6.13	6.43
	35	6.8	2	1.05	1.68	3.85	0.29	5.53	5.82
Fava bean axes	5	13.1	2	1.02	2.38	2.45	0.47	4.83	5.30
	15	10.9	2	1.04	1.99	2.63	0.46	4.62	5.08
	25	10.5	2	1.04	1.68	2.96	0.36	4.60	4.97
	35	7.5	2	0.99	1.42	2.61	0.65	4.02	4.68
Peanut cotyledon	5	11.8	2	1.04	1.35	1.19	0.23	2.54	2.76
	20	11.4	2	1.06	0.97	1.89	0.12	2.86	2.98
	35	12.0	2	1.08	0.47	2.58	0.07	3.06	3.13
Peanut axes	5	13.6	2	1.03	1.51	1.55	0.23	3.06	3.29
	20	10.8	2	1.01	1.14	1.67	0.38	2.81	3.19
	35	8.5	2	1.04	0.98	1.93	0.20	2.92	3.12

^a Integral heat of sorption in $-kJ/mol$. tissue $\times 10^{21}$.

^b Assumes $-2 kJ/mol$ (3, 21).

^c Water activity (dimensionless).

^d Number of sites/g dry weight

temperatures. We suggest that the third hydration level, the multimolecular sorption of water, consists of hydrophobic interactions.

We have tried to ascertain the potential for conformational changes in legume seed tissues with hydration. Assuming that conformational changes may be a function of temperature as well as water content, we measured the sorption characteristics at several temperatures and observed a difference in the calculated numbers of binding sites and heats of sorption in regions 1 and 3. Since ΔH is actually a composite function which incorporates the strength of water binding and other factors related to structural changes of the macromolecule (14), the differences in ΔH values at different temperatures suggest that temperature dependent conformational changes may occur with hydration

(Tables I and III). These data suggest a greater unfolding of polymers at lower temperatures and/or a greater refolding of polymers at higher temperatures. This is substantiated by increases in the numbers of primary and multimolecular sorption sites with decreasing temperature.

The analysis of sorption isotherms from a variety of tissues at several different temperatures consistently suggest at least three types of bound water. Further, the presented data indicate a strong influence of temperature on the binding of water to seed tissues. Quantifying these changes by van't Hoff or D'Arcy/Watt analyses shows us that the differences in sorption isotherms with temperature may be due to changes in hydrophilic and hydrophobic interactions. The structural changes which might occur during hydration of macromolecules are temperature-dependent

as well. The collective influences of temperature on sorption dynamics and on structural characteristics underscore the importance of temperature in the physiology of desiccated systems.

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