MEASUREMENT OF GRAM-POSITIVENESS

WALTER BORZANI

Department of Chemistry, Escola Politécnica, University of São Paulo, São Paulo, Brazil

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Several papers have been published (Kennedy and Barbaro, 1953; Barbaro and Kennedy, 1954; Bartholomew and Finkelstein, 1954; Kennedy and Woodhour, 1956; Barbaro et al., 1956) on methods of comparing degrees of gram-positiveness and the influence of certain factors on the quantity of crystal violet retained by the microorganisms. These papers compare the degrees of gram-positiveness by measuring the quantities of dye adsorbed by a given mass of cells.

In experiments carried out with methylene blue and yeasts (Borzani and Vairo, 1958), it was verified that the adsorption of a dye by a microorganism follows a physico-chemical law (Freundlich’s law) and logical measures of the degree of gram-positiveness could probably be derived if the adsorption of crystal violet by microorganisms also followed Freundlich’s law.

Later (Borzani and Vairo, 1959) attention was called to the fact that only adsorption of crystal violet by gram-positive microorganisms follows physico-chemical laws; the dye adsorption by gram-negative cells does not follow any law. Also, it was demonstrated that it is not correct to compare degrees of gram-positiveness by measuring the quantities of dye retained by a given mass of cells, and that Freundlich’s law and Langmuir’s law do not provide a method to compare degrees of gram-positiveness. Thus it appeared that the correct way to make comparisons was to measure the quantity of crystal violet adsorbed by a given area of cells.

Freundlich’s law and Langmuir’s law considered in papers by Borzani and Vairo (1958, 1959) show:

\[
\frac{C_i - C_f}{C_f} = KPC
\]

Freundlich’s law

\[
\frac{PCC_f}{C_i - C_f} = \frac{1}{a} + \frac{b}{a} \cdot C_f
\]

Langmuir’s law

where \(C_i\) is the initial dye concentration, \(C_f\) is the dye concentration at the equilibrium point, \(C\) is the cell concentration and \(P\) is the percentage of dead cells (\(P = 100\) per cent in the cases studied).

These adsorption laws do not provide a method for measuring degrees of gram-positiveness, not only because they consider the quantities of dye retained by a given mass of cells, as stated in a previous article (Borzani and Vairo, 1959), but also because the values of \(K\), \(n\), \(a\), and \(b\) vary greatly with experimental variations of \(C\), \(C_i\), and \(C_f\). In fact, values of \(K\), \(n\), \(a\), and \(b\) obtained in five identical experiments of methylene blue adsorption by dead yeast cells (Vairo, personal communication), presented in table 1, obtained in experiments in this laboratory, show that the coefficients of the tested adsorption laws cannot be used as measures of the specific adsorption of the dye.

The measurement of the degree of gram-positiveness proposed in a previous paper (Borzani and Vairo, 1959) as the quantity of crystal violet adsorbed by a given area of cells, does not offer any advantage over the older methods, which calculate the gram-positiveness by the quantity of dye adsorbed by a given mass of cells. In fact, if:

\[
m_1 = \text{mass of microorganism I};
\]

\[
m_2 = \text{mass of microorganism II};
\]

\[
S_1 = \text{total area of microorganism I};
\]

\[
S_2 = \text{total area of microorganism II};
\]

\[
\sigma_1 = \text{specific area of microorganism I} = \frac{S_1}{m_1};
\]

\[
\sigma_2 = \text{specific area of microorganism II} = \frac{S_2}{m_2};
\]

\[
M_1 = \text{mass of dye adsorbed by microorganism I};
\]

\[
M_2 = \text{mass of dye adsorbed by microorganism II};
\]

the relation \(R_m\) between the degrees of gram-positiveness of microorganisms I and II, calculated as the mass of dye adsorbed by a given mass of cells will be:

\[
R_m = \frac{M_1/m_1}{M_2/m_2}
\]
and the relation $R_s$ between the degrees of gram-positiveness of microorganisms I and II, calculated as the mass of dye adsorbed by a given area of cells will be:

$$R_s = \frac{M_1}{S_1} = \frac{M_2}{S_2}$$

But, by the definition of specific area, it follows that:

$$R_s = \frac{R_m \sigma_2}{\sigma_1}$$

and as $\sigma_2/\sigma_1$ is constant for two given microorganisms, this last equation shows that the variations of $R_s$ are proportional to the variations of $R_m$. In other words, the inconvenience pointed out in a previous paper (Borzani and Vairo, 1959) regarding the measurement of degrees of gram-positiveness by the quantity of dye adsorbed by a given mass of cells, does not disappear when the mass of cells is substituted by the area of the microorganisms.

Based on known experimental facts it seems then logical to define the degree of gram-positiveness of a microorganism as the relation between the mass of crystal violet adsorbed and the area of the adsorbing microorganism, all the measurements being made under standardized experimental conditions.

After the fixation of experimental conditions, namely dry cell concentration, initial dye concentration, time and temperature of dyeing, pH of the cell suspension, etc., if $M$ is the mass of crystal violet adsorbed by the microorganism, and $S$ is the total area of the adsorbing cells, the degree of gram-positiveness of the microorganism, in the selected experimental conditions, will be $M/S$.

The comparison of degrees of gram-positiveness could be made only if they were measured under the same experimental conditions.

The following example elucidates the definition proposed above.

Freundlich's curves of the adsorption of crystal violet by *Saccharomyces cerevisiae* and *Sarcina lutea* (Borzani and Vairo, 1959) show that when the dry cell concentration is 0.30 g per L (arbitrary selected value), the quantities of adsorbed crystal violet are 23.3 mg per L and 55.0 mg per L, respectively. The average cell areas of *S. cerevisiae* and *S. lutea*, calculated from direct measurements of the cell dimensions, are $110 \mu^2$ and $4.5 \mu^2$, respectively. The number of cells per g of dry matter, calculated from direct counting, is $1.11 \times 10^9$ for *S. cerevisiae* and $1.23 \times 10^9$ for *S. lutea*. Then, the specific areas, in $\text{cm}^2$ per g of dry matter, will be $3.74 \times 10^4$ for *S. cerevisiae* and $5.5 \times 10^3$ for *S. lutea*.

The degrees of gram-positiveness are calculated as follows:

*S. cerevisiae*:

$$\frac{23.3}{0.3 \times 3.74 \times 10^4} = 2.08 \times 10^{-3} \text{mg/cm}^2$$

*S. lutea*:

$$\frac{55.0}{0.3 \times 5.5 \times 10^3} = 3.33 \times 10^{-4} \text{mg/cm}^2$$

It is clear from the above that if the experimental conditions are fixed, a scale of gram-positiveness can be easily made.

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**SUMMARY**

A new method is proposed for the measurement of the degrees of gram-positiveness of microorganisms. The degree of gram-positiveness is defined as the relation between the mass of crystal violet adsorbed and the total area of the adsorbing cells, all the measurements being made.
under standardized experimental conditions. Two examples show the possibility of establishing a scale of degrees of gram-positiveness.

REFERENCES


