Chemical analysis of the surface of microorganisms by X-ray photoelectron spectroscopy


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1. SUMMARY

A collection of microorganisms, including a microfungus and various yeasts and bacteria has been analyzed by X-ray photoelectron spectroscopy (XPS). A correlation is observed between the N/P atomic concentration ratio of the cell surface and the cell electrophoretic mobility measured at pH 4, indicating that the dehydrated surface analyzed by XPS is representative of the cell surface in contact with water.

Deprotonation of phosphate groups plays a predominant role in the development of the cell negative charge, and carboxylic groups are not involved appreciably; a partial neutralization is allowed by protonation of free amino groups of proteins.

These results advocate a broader use of XPS in order to understand physicochemical properties (electrostatic charge, hydrophobicity, ion binding) of the surface of cells, which are of prime importance in various processes occurring in nature and technology.

2. INTRODUCTION

X-ray photoelectron spectroscopy (XPS) of solids provides an elemental analysis of the outermost 2–5 nm of the surface. This method has been extensively used during the last 15 years, in the fields of heterogeneous catalysis and material sciences [1–3]. However, there have been few and limited attempts to apply it to the study of cell surfaces [4–9]. This is probably due to the necessity of dehydrating the sample, which raises questions concerning the representativity of the surface analyzed. Encouraging results have been obtained by studying a few yeasts [9]. The study of a much broader collection of microorganisms presented in this paper shows that XPS provides significant information on the true surface of the cells and helps to understand their physicochemical properties.

3. MATERIALS AND METHODS

The microorganisms examined are listed in the legend of Fig. 1. The growth media and the culture conditions have been described elsewhere [10]. Cells were washed by successive centrifugations and resuspensions in distilled water.

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The X-ray photoelectron spectrometer was a VG3 MK2 with an MgK radiation. The samples were prepared by freezing a cell pellet in liquid nitrogen, drying under vacuum (268°K), loading the cell powder in a stainless steel trough [9] and pressing in order to minimize surface roughness. The nitrogen 1 s peak and the phosphorus 2p peak were recorded in less than 1.2 h in order to reduce surface degradation by the X-ray beam. The atomic concentration ratio has been deduced from the peak intensity ratio by using the sensitivity factors computed by Wagner [11].

The electrophoretic mobility was determined on suspensions of freshly harvested cells adjusted at different pH as described elsewhere [9]. The characteristic parameter which is usually obtained from mobility curves versus pH is the isoelectric pH [9]. However, its accurate determination is often difficult because the mobility curves flatten in the range pH 2–3 due to the high ionic strength. The value of mobility at pH 4 was found more adequate for the characterization of the surface charge.

4. RESULTS AND DISCUSSION

Fig. 1 shows that there is a good correlation (r = 0.81) between the mobility at pH 4 measured on fresh cells and the N/P concentration ratio determined by XPS on freeze-dried cells. Comparison between circles 7 and 8 in Fig. 1 shows that the yeast strain MUCL 28733 cultivated in malt extract 12% instead of glucose 5% + yeast extract 2% are characterized by a lower N/P ratio and a higher mobility. This stresses that the surface varies not only according to genera and species but also to culture conditions.

The predominant role of phosphate in determining the surface charge has been recognized by several authors for yeasts [14,16] and bacteria [17,18]. Compared to previous studies, the data presented here have been obtained on whole cells and not only a concentration of protonated nitrogen. In fact the latter should give a peak at 402 eV [13] and may be responsible for a small contribution which cannot be separated easily from the main nitrogen peak. The observation of a good correlation involving total nitrogen indicates that protonated nitrogen represents a small but constant fraction of total nitrogen of the cell surface. It demonstrates the influence of proteins in making the surface less negative.
rather than on isolated cell walls and they refer specifically to the outermost surface of the cell wall. Moreover, the broad set of microorganisms examined here shows that the same correlation is followed by yeasts (circles in the figure) and bacteria (squares), when considered as distinct sets of samples.

Due to the low concentration of the carboxylic groups with respect to other forms of carbon and oxygen, it is not possible to determine their specific contribution in the C1s and O1s peaks. It may be surprising that a satisfactory correlation is found in Fig. 1, without invoking carboxylic groups. This suggests that they are of minor importance for the surface charge of microorganisms or that their surface concentrations does not vary appreciably from one species to another. Actually, the study of the surface charge of polystyrene of latexes [19] has shown that deprotonation of strongly acidic groups creates a negative surface potential which provokes a strong increase of the apparent pKa (several units) of other acid groups; this effect is well documented in the literature of colloid chemistry.

From our data and from these theoretical considerations, the following picture appears concerning the molecular mechanisms which are responsible for the development of the cell surface charge (near pH 4): deprotonation of phosphate plays a predominant role; the amino groups of proteins allow for a partial neutralization of the charge by formation of zwitterion configurations (R-POa-, R'-NH+); the dissociation of carboxylic groups is repressed by the negative potential and the latter do not contribute to the surface charge.

To our knowledge, this is the first report of a relationship between the composition of the surface of various microorganisms and one of its physicochemical properties. It demonstrates that the surfaces probed in the two different states, dehydrated and hydrated, are intimately related. This gives great confidence in the potentiality of XPS which could be applied more widely to the study of biological surfaces: it could be extended to plant and animal cells. It should be used to understand better the physicochemical properties of the cell surfaces such as the electrostatic charge, surface energy, hydrophobicity, ion binding and immunogenic response. This opens promising avenues in the investigation of biological surfaces in connection with processes which are of prime importance in nature, health care and many fields of technology: flocculation, adhesion or anchorage to a support, fouling, etc.

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